Studies in Isothermal Heat Conduction Calorimetry

I. The 2-Drop Calorimeter and Educational Applications

II. The Quartz Crystal Microbalance/Heat Conduction Calorimeter (QCM/HCC) and Applications in Studying the Thermodynamic and Rheological Properties of Polymer and Protein Thin Films at a Solid/Gas Interface

A Thesis

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by

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In Memoriam

Sister Maria Josita Feighan, I.H.M.
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Heat conduction calorimetry is a well-developed technology that can be used on a variety of levels ranging from high school and undergraduate laboratories to diverse applications in industry and research. Both the educational and research aspects of heat conduction calorimetry are explored in this study.

The 2-Drop Calorimeter was designed as an educational project in collaboration with researchers at Dow Chemical, Midland, MI and Lund University, Lund, Sweden. Calorimetry Sciences Corporation has since marketed a commercial model. Several educational applications have been explored spanning the physical, biological and chemical sciences. The calorimeter design is described along with the calibration procedure. Six different experiments are discussed which provide experience in measuring heats from a variety of reactions and processes including acid-base titrations, vaporization and insect metabolism.

The Quartz Crystal Microbalance/Heat Conduction Calorimeter (QCM/HCC) is a new instrument coupling quartz crystal mass detecting capabilities with heat conduction calorimetry. Both thermodynamic and rheological properties of thin films are studied as a function of vapor activity. The instrument is capable of simultaneously measuring mass (+10ng), thermal (+50nW), and motional resistance (+0.5Ω) changes in a thin film.
(0.5 – 9 µm) upon interaction with a solvent vapor. The results of the sorption experiments allow for the determination of sorption enthalpies, sorption isotherms, partition coefficients, and diffusion coefficients.

Studies have been conducted on the cycloaliphatic poly (ether urethane) polymer, Tecoflex. Several films (0.75 – 8.5 µm) were exposed to water and ethanol vapor. The sorption/desorption properties were evaluated for their thermodynamic and rheological properties. Comparisons are given for thin versus thick films and for water sorption versus ethanol sorption.

Protein hydration/dehydration studies of lysozyme and myoglobin have been accomplished using the QCM/HCC. Comparisons have been made between sorption enthalpies, isotherms and diffusion coefficients. The protein films were very sensitive to the direction of hydration with results differing when the protein was hydrated from higher vapor activities versus the lower vapor activities. The presence of sodium phosphate buffer in the myoglobin film influenced the hydration results. Separate hydration studies were conducted on a sodium phosphate buffer film with results suggesting the formation of hydrates in the phosphate buffer film.
Chapter 1. Introduction

1.1 Heat Conduction Calorimetry

The measurement of heat, defined as calorimetry, is of paramount importance in understanding chemical processes. Nearly all chemical, biological and physical processes involve heat thus rendering calorimetry as an indispensable thermodynamic tool to these disciplines (Brown, 1998; Calvet & Prat, 1963; Kemp, 1999). Different types and classifications of calorimeters have evolved in the efforts to measure the heat involved in a variety of processes and reactions. Three groups of criteria are used to classify calorimeters: (1) the principle of measurement, (2) the mode of operation, (3) and the type of construction (Hemminger & Sarge, 1998).

The Quartz Crystal Microbalance/Heat Conduction Calorimeter (QCM/HCC) as the name indicates is a form of heat conduction calorimetry. For the first classification criterion listed above, the principle of measurement in the QCM/HCC is defined as the heat-exchange principle. This method is characterized by the measuring of the temperature difference between the sample and surroundings. The method is based on the Tian-Calvet type calorimeter where the thermal events taking place in the reaction cell are permitted to flow freely to the surroundings, an aluminum heat sink in this case. The heat must first flow through thermal sensors; typically thermopiles are used for this purpose. The voltage potential produced by the thermopiles is proportional to the thermal power (Calvet & Prat, 1963; Wadsö, 1997).

The mode of operation, also described as the temperature conditions of the calorimeter, for the QCM/HCC can be classified as static. The temperature is not scanned but instead a constant temperature environment between the system and
surroundings is maintained. This categorizes the measurements as being isothermal. It is noted however that an ideally isothermal environment is difficult to establish. Temperature gradients occur when the heat flow to be measured first occurs in the reaction vessel. Thermal gradients can also occur because of undesired heat leaks in the construction of the calorimeter (Hemminger & Sarge, 1998).

The QCM/HCC was constructed following a twin or differential measuring principle. Identical sample and reference chamber are utilized and subject to the same conditions. The only difference between the two is that the sample chamber also contains the material being studied. The difference between the sample and reference chambers is monitored so that the measured heat effects are the result of the sample being studied (Giraldo & Moreno, 2000; Wadsö, 1997). Further details of the QCM/HCC construction and operation are given in Chapter 3.

1.2 Calorimetry in Education

One aspect of the research presented here involved a collaborative project to develop heat conduction calorimetry experiments that could be used in undergraduate physical chemistry courses. Analysts report that typically physical chemistry laboratories are unpopular in part due to the number of theoretical concepts that students must know prior to and during the experiments (von Nagy-Felsobuki, 1991). According to Piaget’s development of intelligence, when students approach a subject for the first time they are in the concrete operational stage of intellectual development. In order to intellectually operate at a formal level, students need relevant laboratory experiences (von Nagy-Felsobuki, 1991). On their first exposure to the concepts of thermodynamics, students cannot always see what is being measured (Crosby, 1988). This is where it is important
to have a curriculum that includes well-developed thermodynamic experiments in the laboratory.

Teaching physical chemistry may seem to be a daunting task in today’s world. Educators surmise that students find physical chemistry to be “too hard, too abstract, and worst of all, esoteric and irrelevant (Crosby, 1988; Schwenz & Moore, 1993).” Educators in the field of physical chemistry struggle to make the curriculum relevant to everyday experiences of physical chemistry. In any discipline, it is advantageous to step back, survey the current trends and techniques and to analyze whether the current educational process uses these current techniques and methods of analysis. According to a symposium sponsored by the American Chemical Society, there is a growing concern about the content of physical chemistry courses and laboratories. Because a relatively small number of students choose to take physical chemistry, updating instrumentation and the curriculum does not always get first precedence (Schwenz & Moore, 1993).

In planning a physical chemistry laboratory curriculum, there are many topics vying for attention that would benefit students, topics ranging from spectroscopic techniques, kinetics and computational studies. The area of thermodynamics is no exception and could use updating. Calorimetry has been a long-standing pillar in the determination of thermodynamic values such as heat capacity, specific heat and enthalpy changes. The isothermal heat conduction calorimeter described here has been utilized in the physical chemistry laboratories at Drexel University for the past six years. Students have had the opportunity to measure the heats from a variety of reactions and chemical processes. In addition, the students become acquainted with the inner workings of a calorimeter and
learn some aspects of concern in designing calorimeters. The calorimeter and its applications will be described in Chapter 2.

1.3 Quartz Crystal Microbalance/Heat Conduction Calorimetry

1.3.1 History of the QCM/HCC

The Quartz Crystal Microbalance/Heat Conduction Calorimeter (QCM/HCC) is a relatively new, unique instrument that allows simultaneous thermodynamic and rheological properties of thin films to be studied as a thin film is exposed to varying gas phase solvent activities.

The technique was created through the efforts of Dr. Allan Smith during a sabbatical at the University of Lund, Lund, Sweden. The technique was created to devise a method to measure the enthalpy of sublimation of solids with low vapor pressures at ambient pressures. Dr. Smith sought to develop a method that would simultaneously measure mass and thermal events. Coupling quartz crystal microbalance technology with existing heat conduction calorimetry brought this desire to realization.

The instrumentation has provided an avenue to probe sorption studies of thin films with a broad range of applications. Dr. Smith has continued in the development of this technology and has begun the construction of a second-generation instrument involving this technology.

1.3.2 Development of Instrumentation

Dr. Hamid Shirazi, a former Drexel graduate student, under the direction of Dr. Allan Smith, continued work in understanding the behavior of the instrumentation and its sensitivity (Shirazi, 2000). Hamid explored the use of an impedance analyzer to probe the behavior of the film-coated QCMs and also as a means of calibrating the
thermopiles. Many improvements were made in automating the instrumentation and data collection. Several LabView VI’s were written to control the operation and data collection of the QCM/HCC, mass flow controllers were added for the gas introduction, and several applications were explored ranging from polymer sorption, protein hydration, pharmaceutical measurements, to hydrogen sorption in palladium, and the formation of self-assembled monolayers (Smith & Shirazi, 2000; Smith *et al.*, 2002). In conjunction, Mr. Jun Tian explored the use of the QCM/HCC in studying the organic vapor sorption behavior of C\textsubscript{60} films and C\textsubscript{60}-piperazine films (Tian, 2002).

1.4 Thesis Overview

**Chapter 2** involves an educational aspect of calorimetry and the utilizing of a specially constructed “2-Drop” heat conduction calorimeter in the undergraduate physical chemistry laboratories. A brief survey of the current status of calorimetry in education is discussed. At Drexel University and in collaboration with Dr. Lars Wadsö of the University of Lund, Lund, Sweden and Dr. Thomas Hofelich of the Dow Chemical Company, Midland, MI, heat conduction calorimeters were designed and student experiments were explored. In addition to the original designs, Calorimetry Sciences Corporation, CSC, has manufactured a commercial 2-Drop Calorimeter. The company provided us with a commercial model to further explore its use as an educational tool. The basic design of the student calorimeters is discussed followed by a description of the calibration process and six experiments utilized by both the Drexel-built and the CSC 2-Drop Calorimeters.

**Chapter 3** focuses on the Quartz Crystal Microbalance/Heat Conduction Calorimeter (QCM/HCC). A physical description of the instrumentation is given along
with an overview of the QCM/HCC experimental controls and data acquisition. A special word of gratitude is given to Mr. Jun Tian for the schematic diagrams of the QCM/HCC apparatus and setup. A few modifications and updating of equipment are also explained. The chapter is then dedicated to describing the mass and thermal sensing devices in the QCM/HCC, quartz crystal microbalances and thermopiles. Some background history and theory is outlined introducing quartz crystals and their use as microbalances. A portrayal of film-coated QCMs is given along with the equations describing their operation as mass sensing devices. Likewise, a general background is then given to explain the theory of thermopiles and their use as heat sensing devices. A description follows of the equations used to relate the voltage potential from the thermopiles to the thermal power resulting from the measured chemical processes.

Chapter 4 includes a survey of the thermodynamic and rheological properties that are accessible from the data analysis of QCM/HCC studies. The chapter is divided into three parts. The first section is devoted to the portrayal of sorption processes in thin films. Following this, a description of the thermodynamic analysis of the QCM/HCC data is given. Four avenues of data analysis are discussed in this section including the determination of sorption enthalpies, sorption isotherms, partition coefficients and diffusion coefficients. The final section of the chapter explains the rheological properties of thin films that are probed when using quartz crystal microbalances, namely the shear storage modulus, $G'$, and the shear loss modulus, $G''$.

Chapter 5 describes the application of the QCM/HCC in sorption studies of the cycloaliphatic poly(ether urethane) polymer, Tecoflex®. Several different film thicknesses ranging from 0.75 µm to 8.5 µm, were compared for their sorption of
ethanol and water vapor independently. This work was a collaborative effort comparing original studies with studies done by Dr. Hamid Shirazi, a former Drexel graduate student, and several undergraduate researchers including Jason Riggs, Rebecca Mason, Anna Ayrapetova and Betty Jacobs. Introductory background is given describing other polymer QCM studies and the properties of Tecoflex. The experimental parameters are outlined followed by graphs of the QCM/HCC data. The data is then analyzed and sorption enthalpies, isotherms, partition coefficients and diffusion coefficients are compared. Our attempts at analyzing the shear storage and shear loss moduli are described. Avenues that we explored in this area include DSC studies of Tecoflex, cold temperature QCM frequency measurements and the exploring of two methods known as the “Simple Three-Step Method” and the “Δf-ΔR” technique. The DSC studies were possible through the time and generosity of Dr. Andrew McGhie of the Laboratory for Research on the Structure of Matter (LRSM) at the University of Pennsylvania. The Simple Three-Step Method and Δf-ΔR techniques are the result of the comprehensive work on polymer coated QCMs by Dr. Ralf Luckum et al. On a recent visit to Drexel University, Dr. Ralf Lucklum of Otto-von-Guericke-University, Magdeburg, Germany, generously spent time in describing his studies and provided some helpful suggestions with our studies. He also provided us with a copy of an Excel spreadsheet, ZYSYN_QP.xls, which he devised and uses to model film-coated QCM responses.

Chapter 6 includes hydration studies of the proteins lysozyme and myoglobin using the QCM/HCC. The chapter begins with a general introduction of protein hydration studies. Prior work involving lysozyme hydration is described and a recent analysis of the diffusion coefficients is presented. The second part of the chapter deals with
hydration studies of the protein myoglobin. The parameters for the experiments are outlined and the initial data is presented in graphic form. A thermodynamic analysis of the sorption processes then follows. The chapter finishes with a sorption study of the buffer used in the myoglobin solution, sodium phosphate buffer. The effects of the buffer on the protein sorption studies are shown. A discussion of the possible hydrate formation of the buffer concludes the chapter.

Chapter 7 is a conclusion to the studies presented here. Some future experimental possibilities in the above-mentioned studies are described.
List of References


Tian, J. (2002). Comparative Solubility Studies of C$_{60}$ and C$_{60}$-Piperazine and Applications of the Quartz Crystal Microbalance/Heat Conduction Calorimeter. Doctor of Philosophy, Drexel University.


Chapter 2. 2-Drop Heat Conduction Calorimeter

2.1 The 2-Drop Calorimeter in the Physical Chemistry Curriculum

Investigative attempts at explaining and measuring heat ranks among one of the earliest scientific phenomena explored by researchers. In the early 18\textsuperscript{th} century, endeavors to describe and quantify heat occupied much of the scientific effort at that time. In fact, at the start of the 18\textsuperscript{th} century, scientists had no established methods for measuring the quantity of heat yet, by the end of that century the calorimeter was recognized as a premier instrument in scientific investigations (Armstrong, 1964). Calorimetry soon became an emerging technique and researchers in the 18\textsuperscript{th} and early 19\textsuperscript{th} centuries spent much effort exploring combustion and the nature of heat. By the mid-19\textsuperscript{th} century, calorimetry became an established area of research thus moving it out of the forefront of research as newer trends and questions in science were explored. Although not new and novel, at this point, calorimetry became the backbone of thermodynamic techniques for its contributions in explaining chemical reactions and heats (Armstrong, 1964). Much of the existing thermodynamic data has been in a large part provided by studies in calorimetry and rests on the shoulders of early researchers in this field such as Cavendish, Fahrenheit, Lavoisier and Petit and Dulong (Armstrong, 1964).

Through the years, researchers have expanded calorimetric instruments and techniques to include a variety of applications with studies performed over diverse physical conditions. This is evidenced through the efforts of the International Union of Pure and Applied Chemistry (IUPAC) in the recent publishing of The Handbook of Thermal Analysis and Calorimetry (Brown, 1998; Kemp, 1999). In this eventual four-
volume work, the application of calorimetry to chemical reactions, phase changes, materials, solutions, proteins, plants, animals and humans is discussed. Scientists recognize that almost all physical, chemical and biological processes produce or consume heat. Because thermodynamics is one of the foundations of physical chemistry, it is important for educators to provide students with relevant laboratory experiences. To emphasize the role of physical chemistry in the development of research, educators note that students are helped when courses can be constructed around instrumental techniques (Atkins, 1992).

In first year chemistry courses, students are taught a variety of heat related topics such as heat capacity, specific heat, work, changes in enthalpy, and the thermodynamic laws (Jasien & Oberem, 2002; von Nagy-Felsobuki, 1991). In a recent study, researchers evaluated undergraduate and post-baccalaureate students as well as pre- and in-service teachers in regards to their understanding of heat transfer and thermal equilibrium (Jasien & Oberem, 2002). The results of the study revealed that across the board, the students’ and teachers’ working knowledge and application of heat transfer and thermal equilibrium was lacking. Many involved with the study were confused in three main areas (Jasien & Oberem, 2002): (1) a definition of thermal equilibrium, (2) a physical basis for heat transfer and temperature change, and (3) the relationship between specific heat, heat capacity, and temperature change.

This study evaluating students’ and teachers’ concepts of heat and temperature comes at a critical time in the broader picture of calorimetry. Researchers, reviewing certain trends in calorimetry, note that there is a rapid decline in calorimetry practitioners while the number of calorimetry users has increased dramatically (Mathot,
2000; Wadsö, 1997). The problem is not isolated to calorimetry and it is noted that in general there is a decrease in analytical disciplines at universities and research institutes (Mathot, 2000). This problem can be seen as stemming from two sources: (1) company and government funding has not been as generously allocated to the development of analytical techniques in recent years, (2) the ease of use of thermal analytical and calorimetric commercial instruments has had a “paralyzing effect, causing developments to stagnate.” In his comprehensive analysis of the situation, Mathot highlights the field of education one of the premier ways to stop the decline of practitioners in thermal analysis and calorimetry (Mathot, 2000).

However, in the area of education, a sobering reality exists. In a recent article analyzing the teaching of calorimetry and thermal analysis, Bernhard Wunderlich, a prominent calorimetrist in the U.S., commented that in a recent survey “out of 10,000 students, only 6% took a course in thermal analysis and only 21% had heard about thermal analysis in courses on polymers, materials, physical chemistry, or even analytical chemistry (Haines & Lever, 1999).” Analysts state that chemists, pharmacists, materials scientists, and polymer scientists among others should be educated in the fundamentals of thermogravimetry, differential scanning calorimetry (DSC), and thermomechanical and dynamic mechanical analysis (Haines & Lever, 1999). Haines and Lever note that on the education front, thermal methods are rarely included in more “popular” journals and that few analytical texts have full up-to-date chapters on thermal analysis and calorimetry.

Traditionally, any educational experiments involving calorimetry involved adiabatic calorimetry. Although useful, calorimetric techniques are much more diverse in
industry and research and generally involve not adiabatic but heat conduction methods.

A general overview of some recent (previous 20 years) calorimetry experiments in Journal of Chemical Education include:

Adiabatic Calorimetry

- A Styrofoam cup adiabatic calorimeter interfaced to a computer and used to determine heats of formation (Wong et al., 2001)

- An adiabatic bomb calorimetry experiment comparing the heats of combustion of naphthalene and azulene with theoretical computational values (Salter & Foresman, 1998)

- A Styrofoam cup calorimeter to determine the enthalpy of decomposition of hydrogen peroxide (Marzzacco, 1999)

- A bomb calorimeter constructed from a camera flash bulb to measure the heat of combustion of aluminum (Hornyak, 1961)

- Solution calorimetry and an application of the acid-base reactions of the amino acid glycine (Ramette, 1984)

- A modified adiabatic, isothermal calorimeter to determine heats of vaporization of organic compounds (Arnett & Oancea, 1975)

Simulation or Computational Methods

- A method to estimate heat capacity of complex inorganic solids in the event that calorimetric equipment or data is unavailable (Qiu & White, 2001)

- A method used in undergraduate and graduate courses to develop a thermodynamic basis for DSC signals obtained from protein unfolding and a method to simulate DSC data for better understanding and interpretation (Deal & Hurst, 1997)

Differential Scanning Calorimetry

- A DSC experiment to analyze the gel to liquid-crystalline phase transition of a phospholipid bilayer system designed for physical/biochemistry courses (Ohline et al., 2001)

- A DSC experiment to determine chemical purity. This was devised to expand students’ exposure to melting point measurements (Brown, 1979)
Laser Based Methods

- A laboratory experiment coupling laser spectroscopy and calorimetry to indirectly measure the heat of a reaction, the complexation of Cd\(^{2+}\) and Mg\(^{2+}\) with EDTA (Schneider et al., 2002)

- A modified procedure to determine heat capacity of liquids using thermal lens calorimetry (Seidman & Payne, 1998)

Calorimetric experiments are being explored and applied in educational settings as evidenced above, however, the above examples include a search of over the past twenty years in the Journal of Chemical Education. Adiabatic calorimetry has seen the greatest applications in the educational setting, but this does not reflect the type of calorimetry, heat conduction, most commonly used in industry and research. Recent interest in providing students with laser based techniques and DSC experiments does show potential to keep students abreast of calorimetric techniques.

In an effort to contribute to the education of students in the field of calorimetry, we have designed and successfully utilized an isothermal heat conduction calorimeter with physical, chemical, and biological applications (Wadsö et al., 2001). The design of the instrument and the educational applications resulted from collaborative efforts with Dr. Lars Wadsö of the University of Lund, Lund Sweden, and Dr. Thomas Hofelich of Dow Chemical Co., Midland MI. This 2-Drop Calorimeter was designed so that only microliters of reagents were needed to measure the heat effects of different processes. Since this work, developers at Calorimetry Sciences Corporation (CSC) have designed a commercial student 2-Drop Calorimeter, Model 2100/2200. Researcher at CSC generously supplied us with a commercial model to utilize and continue in the development of educational applications. A description of the instrumentation and experiments are given below.
2.2 Development of the 2-Drop Calorimeter

2.2.1 Design and Building of the 2-Drop Calorimeter

The original designs for the 2-Drop Calorimeter were drawn by Dr. Thomas Hofelich of the Dow Chemical Company. He sought to design a calorimeter that could be used to determine the heats of reaction of hazardous chemicals. Because of the potential dangers involved, he wanted to design a calorimeter that would use only microliters of solution. Dr. Lars Wadsö also contributed to the design of the calorimeter and contributed student experiments involving the heat capacity of solids, cement hydration and insect metabolism. From his engineering perspective, he has explored the necessity of calorimetric studies of building materials.

At Drexel University, Dr. Allan Smith and Hamid Shirazi had three student models built and pursued experiments involving heats of adsorption, reaction, vaporization and insect metabolism. These calorimeters have been routinely used in the undergraduate physical chemistry laboratories for the past six years and have provided students with experience in measuring heats from a variety of chemical processes.

A schematic of the Drexel-built 2-Drop Calorimeter instrument drawn by Dr. Lars Wadsö is shown in Figure 2-1.
Figure 2-1. A schematic diagram of the 2-Drop Calorimeter. A) syringe, B) insulation jacket, C) syringe holder, D) U-shaped holder, E) glass ampoule, F) sample aluminum cup, G) reference aluminum cup, H) heat sink, I) thermocouple plate or thermopile (Wadsö et al., 2001)

The calorimeter consists of an aluminum heat sink (1” x 4.5” x 4.5”), mounted to this is an aluminum U-shaped bracket (1/8”), containing a 5/8” x 4” slot for the syringe holder hardware. For the experiments presented here, a 1 ml syringe (Popper) was used. The aluminum sample and reference cups are 0.53” o.d., 0.46” i.d. and 0.47” in height. The thermopiles were purchased from Melcor, (Trenton, NJ), Model # OT 1.2-66-FO. The thermopiles were bonded directly to the aluminum heat sink with thermal epoxy (Melcor, TCE-002). The thermopiles were wired in series to provide a differential signal produced from the sample and reference sides. The voltage potential generated in the reference cell is subtracted from the potential generated in the sample cell so the final potential corresponds to the net process being studied (Giraldo &
Moreno, 2000). The electrical wires from the thermopiles were connected to a terminal strip (Newark, #65F1120) mounted on the aluminum heat sink. A schematic of this is shown in Figure 2-2.

**Figure 2-2.** A schematic diagram of the 2-Drop Calorimeter’s electrical components. a) thermopiles, b) sample aluminum cup, c) reference aluminum cup, d) 1kΩ resistor and copper leads, e) heat sink, f) terminal board. The thermopiles are differentially coupled to provide a net signal.

The aluminum cups were mounted to the thermopiles via thermal grease (Melcor, TG-001). For the thermopile calibration, a 1 kΩ resistor (Newark, Farnell # 613-708) was mounted to the side of the sample aluminum cup. The resistor was connected to the terminal strip with copper wire (Anaconda, 36 HML). The 2 ml glass ampoules are
placed in the sample and reference aluminum cups for the experiments. The whole unit is housed in a polystyrene box to insulate the calorimeter from the surroundings (Hofelich, 1997).

The electrical connections from the terminal strip of the calorimeter are connected to the pins of a terminal strip, which is then linked to a PC via a computer interface cable. Data from the calorimeter is processed through a Data Translation data acquisition card and students control and monitor the measurements through a program written using VEE Pro 6 (Agilent Technologies). A sample diagram is shown in the student lab in Appendix A.

2.2.2 Commercial Models

Calorimetry Sciences Corporation (CSC) has designed commercial 2-Drop Calorimeter Models (2100/2200) for hazardous waste studies and educational applications. Dr. Greg Nelson of CSC generously sent a Model 2200 2-Drop Calorimeter for our use to test the hardware/software and to develop potential student experiments. This 2-Drop Calorimeter can be seen on their web site (CSC, 2002).

The commercial model is similar to the home built model in that thermoelectric plates are used as the thermal sensors. The heat conduction calorimeter contains both sample and reference sides with the thermopiles differentially coupled to provide the net thermopile signal from the reaction of interest. Calibration is performed electrically as a heat pulse which can be run before and/or after an experiment. An added feature includes thermoelectric plates used as heating/cooling devices so that titration experiments could be performed at temperatures ranging from 5°C to 50°C.
The calorimeter is equipped with a mechanical titrant delivery system to deliver specified aliquots of titrant ranging from 1, 2, 5, 10, or 25 µL per injection. The reaction vessels are 2 ml glass vials (12x32 mm) with crimp top septum seals. A magnetic stir bar (3mm x 6mm) can be used and a 100 µL syringe is employed to deliver the titrant.

An appealing feature of the CSC 2-Drop Calorimeter is the ability to measure thermodynamic parameters for ligand binding reactions. From the titration information the binding constant $K$, the enthalpy change $\Delta H$, entropy change $\Delta S$, and the moles, $n$, involved in a reaction can all be determined.

2.3 Experiments Used with the 2-Drop Calorimeter

The 2-Drop Calorimeter experiments that have been used with the Drexel-built models are described in Appendix A. This student handout describes heat conduction calorimetry and the instrumentation used in the lab. The theory behind the calibration is then given and the theory of each experiment is described along with pertinent equations. Following this, the procedures for each experiment are listed as well as the method for the data analysis and description of the data.

2.3.1 Calibration of the Calorimeter

The thermopiles are the important heat sensors used in the 2-Drop calorimeter presented above. In heat conduction calorimetry, the heat is allowed to flow freely from the reaction vessel. Thermopiles placed between the reaction vessel and the heat sink measure the heat generated or absorbed from the reaction vessel. Depending on the construction of the calorimeter, the measured amount of heat often differs from the actual amount of heat involved in the chemical process. Some of the produced thermal
power may reach the heat sink by other paths than the thermopiles such as through mechanical supports and electrical leads (Wadsö, 2000). A calibration method that closely mimics the heat flow patterns of an actual experiment is necessary for accurate determination of the thermal power.

Because calibration methods are often automated, students do not always gain an experiential knowledge or appreciation of the importance of the calibration. Using the Drexel – built 2-Drop calorimeter, students perform an electrical calibration of the instrument. Typically a 1.5-volt battery is used in conjunction with the resistor fastened to the side of the sample aluminum cup, a known amount of heat is produced by the Joule effect (Guan & Kemp, 2000). From the electrically generated thermal power, the steady-state signal from the thermopiles is then measured and the calibration coefficient $\varepsilon$ (Watts/volt) is calculated using Equation A-10 found in Appendix A.

A description of the calibration process is more fully developed in the student lab handout in Appendix A. In Chapter 3 there is also an in-depth discussion on thermopiles and calibration issues. Once students determine the calibration coefficient, the value is used as the proportionality constant between the output voltage potentials of the thermopiles and the generated thermal power. The calibration coefficient is used to convert the thermopile voltages to the thermal power.

2.3.2 Acid - Base titration

Using the 2-Drop calorimeter, students determined the $\Delta H$ of an acid-base reaction. In the student laboratories, typically the titration of tris(hydroxymethyl)aminomethane (THAM) with hydrochloric acid (HCl) was used as well as sodium hydroxide (NaOH) and HCl. The overall reactions and equations used to determine the enthalpy are shown
in Appendix A. Typical results have also been published in an article describing the applications of the calorimeter in educational laboratories (Wadsö et al., 2001).

In an effort to incorporate the CSC 2-Drop Calorimeter, acid–base titrations were done in our laboratory to test the CSC software and to gain familiarity with the instrument. Initially some software problems existed which were eventually corrected.

An example is shown of a thermal titration of THAM and HCl. A few items need to be considered when using microcalorimeters for acid-base reactions such as: (1) the initial concentrations of the reactants need to be large enough so that a strong thermal signal is produced, (2) for the 2 ml glass ampoules used here, at least 300 µL of reagent should be placed in the ampoule so that each drop from the syringe reaches the reagent. Stirring is also desirable and is available in the CSC model, (3) a large enough syringe size is needed to deliver adequate amounts of titrant to neutralize the acid-base reaction, and (4) strong acids are corrosive to stainless steel syringe needles and cause the production of hydrogen gas as well as rusting.

Figure 2-3 displays the results from a titration of 1M THAM with 0.1 M HCl. Parameters for the titration are shown in Table 2-1. The data was analyzed in a software package provided by CSC, Bindworks. The software allows the user to modify a baseline and to assign intervals for the integration of the thermal peaks. It is this integration of the thermal peaks, which yields the heat in Joules. A description of this is given in Appendix A as well as in Chapter 3. When integrating the thermal peaks using Bindworks, a separate plot opens which displays the heat from each injection. This plot is not shown for this titration because of some software glitches. It
is not possible to rescale the axis in Bindworks. The heats evolved from each injection in this experiment were beyond the defined axes in Bindworks and therefore not shown.

![Bindworks plot](image)

**Figure 2-3.** A Bindworks plot of the thermal titration of 1M THAM and 0.1M HCl using the CSC 2-Drop Model. The initial sharp peak is a calibration heat pulse.

**Table 2-1.** Parameters for the titration of 1M THAM and 0.1M HCl using the CSC 2-Drop Calorimeter.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data intervals</td>
<td>0.5 sec</td>
</tr>
<tr>
<td>Experiment duration</td>
<td>165 min</td>
</tr>
<tr>
<td>Temperature</td>
<td>25°C</td>
</tr>
<tr>
<td>Injection time</td>
<td>10 min.</td>
</tr>
<tr>
<td>Injection interval</td>
<td>10 min.</td>
</tr>
<tr>
<td>Number of injections</td>
<td>15</td>
</tr>
<tr>
<td>Injection size</td>
<td>5 µL</td>
</tr>
<tr>
<td>Stirrer</td>
<td>on</td>
</tr>
<tr>
<td>Calibration heat pulse</td>
<td>before 1st injection</td>
</tr>
<tr>
<td>Volume in vial</td>
<td>600 µL HCl 0.1M</td>
</tr>
<tr>
<td>Volume in syringe</td>
<td>100 µL THAM 1M</td>
</tr>
</tbody>
</table>
When analyzing the data in the student laboratories, to integrate the peaks, the software package Grams was used. A description of the integration is given in Appendix A. For the above titration, upon integration of the peaks and factoring in the last four peaks as being the heat of dilution of the excess base, the $\Delta_rH$ was $-53.4 \text{ kJ/mol}$. This is a 12.7% error from the literature value of $-47.4 \text{ kJ/mol}$ (Buschmann & Schollmeyer, 1999; Eatough et al., 1974). Errors may have resulted when defining a baseline for the integration of the thermal peaks, also, some corrosion of the syringe needle may have factored in to the error, and some of the heat may have been generated from the dilution of the acid-base and not part of the neutralization process.

The CSC calorimeter has not been utilized in the student laboratories but the same reaction has been used with the Drexel built models. Results are generally within 10% of the literature value and can be seen in (Wadsö et al., 2001).

### 2.3.3 Heat of Vaporization

The Drexel-built 2-Drop calorimeter has been used in the laboratories to determine the $\Delta_{\text{vaporization}}H$ of volatile organic solvents. Typically the students would use hexane, methanol, ethanol, or isopropanol. The syringe would be filled with about 50 $\mu$L of solvent and after attaining thermal equilibrium, the students would release one drop of solvent. The ensuing endothermic peak indicated that vaporization was occurring. Students would then perform three trials and use the average heat of vaporization from the drops of solvent released. Generally results were within 10 – 15% with some error occurring from release of extra solvent from the syringe and the lab temperature being colder than 25°C. A detailed description of the experiment and the data analysis is given in Appendix A.
2.3.4 Laser Power Meter

In this experiment, students explore the use of thermopiles as laser power meters. A 5 mW He-Ne laser is used and directed at the graphite-blackened bottom of the sample aluminum cup. The steady-state output voltage is recorded and using the calibration coefficient, students can then calculate the thermal power generated from the laser. A comparison can then be made between the amount of light actually absorbed and the amount reflected. Again, a detailed description is included in Appendix A.

2.3.5 Heat of Zeolite Water Sorption

The heat of water vapor sorption of zeolites has been measured using the Drexel-built 2-Drop calorimeter. Zeolites, commonly referred to as molecular sieves, have a porous structure with the internal surface area being comprised of nearly several hundred square meters per gram of zeolite.

When measuring the heat of adsorption, the students placed a small vial of water in the calorimeter to introduce water vapor to the atmosphere. After thermal equilibrium was attained, the students introduced a few milligrams of zeolites to the sample aluminum cup via a glass tube protruding through a small hole in the top of the polystyrene housing. Immediately the zeolites produced large exothermic signals. An example can be seen in Figure 2-4. The exothermic adsorption does not reach completion in one lab period. Generally, a defined time-period was used (e.g. 10 minutes) and students calculated the heat of adsorption that occurred during that time period. The enthalpy of adsorption for the zeolites is found per kilogram of zeolite.
Figure 2-4. The thermal power generated when a few milligrams of zeolites are exposed to water vapor. These results were measured using the Drexel-built 2-Drop calorimeter. The initial large exothermic signal is due to the introduction of the zeolites into the calorimeter chamber.

The background and equations for this experiment are also found in Appendix A. In the time span of ~ 1 hour, the $\Delta_{\text{adsorption}} H$ of water by the zeolites measured $\sim -213 \text{ kJ/kg}$. In performing this experiment, students are able to see the magnitude of the heat effects upon water sorption in molecular sieves.

2.3.6 Insect Metabolism

Calorimetry of living organisms has been in existence for well over 200 years. Lavoisier and Laplace in 1780, used an ice calorimeter to measure the metabolism and heat production of guinea pigs. It was Lavoisier who declared, “La vie est donc une combustion (Lamprecht & Schmolz, 1999).” In other words, “Life is a slow combustion maintained through respiration.” Calorimetry of animals has been reported
on a wide range of species spanning insects of 1mg to large domestic animals such as cows and horses more than 500 kg.

The use of biological calorimetry has forced a delineation of terms between quantitative and analytical calorimetry. Quantitative calorimetry refers to the classical thermodynamic research in determining enthalpy, entropy and heat capacity while analytical calorimetry is the term used to determine whether heat producing processes are occurring. Analytical results are then evaluated as to their time profile (Lamprecht & Schmolz, 1999).

Using the Drexel-built 2-Drop calorimeter, students have measured the heat generated as a small insect is permitted to crawl in the sample aluminum cup. An example of a woodlouse moving about in the sample cup is shown in Figure 2-5.

![Figure 2-5](image-url)  

**Figure 2-5.** The thermal power generated by a woodlouse in the Drexel-built 2-Drop Calorimeter.
Although when looking at the literature of calorimetric studies done on living organisms, many researchers refer to the plot of the calorimeter signal as a function of time as a “thermogram,” this term is no longer used for this purpose. The term thermogram has become more specialized and defines changes of enthalpy or heat capacity as a function of temperature. The term is also reserved for infrared thermo-camera images. Instead of thermogram, the currently used nomenclature is “power-time curve” and other acceptable terms include: “heat flow versus time”, “heat flux versus time”, and “heat dissipation curve (Lamprecht & Schmolz, 1999).”

The heat generated from the insect was generally categorized as the heat of metabolism. Independently, students calculated the $\Delta_{\text{combustion}}H$ of glucose as a measure of metabolic activity. From the enthalpy of glucose combustion and the heat generated by the insect, students found the possible moles of glucose metabolized by the insect during its measured activity in the calorimeter. Specific equations are shown in Appendix A, Equations A-16, A-17.

2.3.7 Barium Chloride Complexation with 18-Crown-6 Ether

Using the CSC 2-Drop Calorimeter, we pursued a titration experiment involving the complexation of barium chloride with 18-crown-6 ether. This $\text{Ba}^{2+}$ binding to 18-crown-6 has been proposed as a chemical test reaction for titration microcalorimeters (Bäckman et al., 1994; Buschmann & Schollmeyer, 1999). One appealing reason to try the titration was the fact that the Bindworks software from CSC offered the possibility of determining the equilibrium-binding constant, $K$, through a fitting procedure in the software. Previous to software developments, binding constants were found through
tedious calculations involving calculations of the concentrations of the various complexes (Christensen et al., 1972; Eatough et al., 1972a; Eatough et al., 1972b).

Data for a trial of 1M BaCl$_2$ being titrated into 0.1M 18-crown-6 ether is shown in Figure 2-6 and the parameters for the experiment are described in Table 2-2.

![Figure 2-6](image)

**Figure 2-6.** Titration of 1M BaCl$_2$ and 0.1M 18-crown-6 ether using the CSC 2-Drop Calorimeter. The data is shown as pictured in the software, Bindworks. The first large peak is the heat generated in the calibration heat pulse.
Table 2-2. Parameters for the titration of 1M BaCl$_2$ and 0.1M 18-crown-6 ether.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data intervals</td>
<td>0.5 sec</td>
</tr>
<tr>
<td>Experiment duration</td>
<td>65 min</td>
</tr>
<tr>
<td>Temperature</td>
<td>25°C</td>
</tr>
<tr>
<td>Injection time</td>
<td>10 min.</td>
</tr>
<tr>
<td>Injection interval</td>
<td>3 min.</td>
</tr>
<tr>
<td>Number of injections</td>
<td>15</td>
</tr>
<tr>
<td>Injection size</td>
<td>5 µL</td>
</tr>
<tr>
<td>Stirrer</td>
<td>on</td>
</tr>
<tr>
<td>Calibration heat pulse</td>
<td>before 1$^{st}$ injection</td>
</tr>
<tr>
<td>Volume in vial</td>
<td>300 µL 0.1M 18-C-6</td>
</tr>
<tr>
<td>Volume in syringe</td>
<td>100 µL 1M BaCl$_2$</td>
</tr>
</tbody>
</table>

When using Bindworks, data for this reaction was within the axes limits for the graph depicting the integrated heat yielding the heat per injection. This is displayed in Figure 2-7. The software also displays the numerical values associated with each point on the graph, however the text font is very small and in this version is not able to be resized.

Figure 2-7. The integrated heat per injection for the titration of 1M BaCl$_2$ and 0.1M 18-C-6. Values calculated using the software, Bindworks.
From the above titration, the $\Delta_r H$ was determined to be $-42.4$ kJ/mol. This value is larger than the generally reported values which range between $\sim -29$ to $33$ kJ/mol (Buschmann et al., 1998; Buschmann & Schollmeyer, 1999). Because of some further work needed in using the software Bindworks, the binding constants were not determined for this titration. In the software, parameters are input for the titration and the software simulates a binding curve. This curve is then fit to the data and the binding constant is extracted from the parameters. Typically literature values for the binding constant for this reaction range from $\sim \log 3.45$ to $\log 3.87$ (Buschmann & Schollmeyer, 1999).

In his comprehensive studies of this macrocyclic titration, Buschmann notes that reported reaction enthalpies are very close to each other, however there is considerable variation in the binding constants. He attributes this to four possible factors: (1) the influence of the ligand and salt concentrations, (2) the influence of the solution’s ionic strength, (3) the influence of the solution pH values, and (4) the influence of the anion used (Buschmann & Schollmeyer, 1999).

This titration involving the complex formation between the crown ether and the alkaline earth cation can also be extended to include other alkali and alkaline cations (Ozutsumi & Ishiguro, 1992; Vasil'ev et al., 1994). The Bindworks software has the potential to add the aspect of students being able to determine the equilibrium binding constants. This titration has not yet been used at Drexel in the student laboratories.

2.4 Conclusion

The 2-Drop Calorimeter has been used in physical chemistry laboratories at Drexel
University for six years now. Several advantageous aspects to incorporating this laboratory:

1. Students are exposed to calorimetric techniques. In research, titration calorimetry has emerged as a widely utilized tool in studying protein ligand binding.

2. In the home-built model, students see the thermopiles, heat sink, reaction vessels, and wiring connections. The students have also participated in trouble-shooting techniques and solved problems when sample cups were not in good thermal contact, wires may have been touching and short-circuiting or good mixing was not occurring in the titrations. The lab has provided good experiential learning.

3. A variety of types of reactions and chemical processes are used in the lab with students measuring: $\Delta_{\text{vaporization}}H$, $\Delta_{\text{reaction}}H$, $\Delta_{\text{metbolism}}H$, $\Delta_{\text{adsorption}}H$, the power generated from a laser and the heat of adsorption of water.

4. Students perform the calibration of the instrument and learn about calibration techniques in calorimetry.

5. Because only microliters of solvents are needed, the lab is generally cost effective.

6. In performing these experiments, students were introduced to the International Union of Pure and Applied Chemistry’s (IUPAC) website and explored the correct nomenclature for terms related to thermal analysis and calorimetry.
List of References


Hofelich, T. (1997). 2 Drop Calorimeter Construction and Parts


Chapter 3. Introduction to the Quartz Crystal Microbalance / Heat Conduction Calorimeter (QCM/HCC)

3.1 Overview of the QCM/HCC Instrumentation

The quartz crystal microbalance/heat conduction calorimeter (QCM/HCC) is designed for very sensitive and simultaneous mass and heat flow measurements of a thin film as it interacts with a solvent vapor in a controlled gas phase. Several pieces of equipment are necessary for the control, measurement, data acquisition and processing. A schematic diagram of the instrumentation is shown in Figures 3-1 and 3-2 (Tian, 2002).

The two sensitive mass detectors are 5 MHz, AT cut, quartz crystal microbalances, (Maxtek P/N 149211-1, model SC-501-1) with one QCM having the sample film coated on it while the second QCM remaining unchanged and acting as a reference. Each QCM is 0.33 mm thick, and 2.45 cm in diameter with each side having a 15 nm chromium layer onto which a 160 nm thick gold electrode is vacuum deposited. The active surface is the 12.9 mm diameter top gold electrode on which a thin film is coated. The bottom electrode, 6.6 mm diameter, however, is the region exposed to the rf electric potential. This 0.32 cm² area is the mass sensitive area of the QCM. Therefore, the piezoelectric active area is not identical to the electrode area. The quartz oscillations are maximum at the center of the gold electrode; the oscillations then decay toward the edges of the QCM (Lucklum & Hauptmann, 1997).
Figure 3-1. Schematic diagram of the QCM/HCC including the vapor flow and data acquisition setup (Tian, 2002).
Figure 3-2. Schematic diagram of the control and data acquisition of the QCM/HCC (Tian, 2002).
Four thermopiles (Melcor, Trenton, OTO.8-66-OF, Part # 430009-01) each 1 cm$^2$, are used to measure the heat flow from the sorption processes. Two of the four thermopiles are connected in series to form one heat flow sensor that is in direct, thermal contact with two D-shaped brass electrodes upon which the reference QCM is placed. The D-shaped electrodes serve in applying the rf voltage to the QCM. The sample side is constructed in the same fashion.

The D-shaped brass electrodes and thermopiles are contained in a Kel-F housing. Two identical Kel-F units are used for the sample and reference chambers. These are tightly fit into a circular opening in the aluminum heat sink. The aluminum heat sink is then capped with an aluminum cylinder. This aluminum cap contains two Kel-F circular inserts (1 inch in diameter) each having a Kalrez o-ring (DuPont, AS-568A, K#016), which interface with the top of the QCM. Two openings on both the sample and reference sides are bored through the Aluminum cap. A Kel-F insert fits in the bored hole of the Aluminum. The Kel-F has two openings; one is 1/16” o.d. to provide the tubing for the gas inlet. The other is 1/8” o.d. for the gas outlet. The Teflon tubing is introduced through this Kel-F piece. The top aluminum is then clamped to the bottom. The calorimeter chamber can be seen in the diagram shown in Figure 3-3.
Figure 3-3. The configuration of the QCM/HCC calorimetric chamber (Tian, 2002).

The whole aluminum heat sink/calorimeter is placed in a stainless steel/copper chamber and tightly clamped for an air/water tight seal. This unit is then placed in a constant temperature bath (Tronac 1250). The temperature is controlled to $\pm 0.0001^\circ C$.

The sample QCM is driven by a Maxtek, PLO-10, Phase Lock Oscillator (Santa Fe Springs, CA), while the reference QCM is driven by a regular feed-back oscillator without the phase-locking capabilities (Lucklum et al., 1999). An HP53513A frequency counter is utilized to count and determine the frequency of both the sample and reference sides. The HP5313 is a two-channel counter and collects data via a GPIB
board interfaced to a Macintosh computer. The differential of the sample and reference thermal signals are fed to a DC low noise – preamplifier (Nanovoltmeter, Model N15, EM Electronics, UK). The gas flow is controlled by two Unit 8100 Mass Flow Controllers. Nitrogen (BOC, grade 5.0) is typically used as the carrier gas. One mass flow controller sends the carrier gas to a glass gas bubbler. The vapor leaving the gas bubbler travels downstream to a mixing tee. At this point, carrier gas from the second mass flow controller is introduced. The mixed gas flow proceeds through a 1.5 m length of tubing to ensure adequate mixing. At the end of this mixing, the tubing is then routed to a tee which splits the flow and directs equal concentrations of the gas to the sample and reference chambers. A RH-100 Relative Humidity Meter (Sable Systems, NV) is set-up downstream from the sample chamber and is used to check the accuracy of the applied water vapor in an experiment.

Several LabView (National Instruments) virtual instruments have been written by Dr. Hamid Shirazi to aid in the control of the experiments and the collection of data (Shirazi, 2000).

### 3.2 Updating and Modifications to the Instrumentation

#### 3.2.1 Nanovoltmeter

Because of the small voltages produced by the thermopiles, a low noise preamplifier is used to amplify the voltages. An EM Electronics Nanovoltmeter Amplifier was purchased. This amplifier has 12 input ranges from $\pm 100 \, \text{nV}$ to $\pm 30 \, \text{mV}$. Another added feature was the offset control giving maximum offsets of $\pm 1 \, \mu\text{V}$, $10 \, \mu\text{V}$, $100 \, \mu\text{V}$, $1 \, \text{mV}$, $10 \, \text{mV}$, and a 0 position (Electronics, 2000).
The amplifier was calibrated with the help of Rebecca Mason, an undergraduate researcher working in our laboratory. To achieve the calibration, known voltages within a given range of one of the amplifier settings were produced using a Precision Power Source (Power Designs, Model 2005). Appropriate resistors were added to produce the small voltages needed. These small voltages were fed to the preamplifier and the corresponding output voltages were measured via a precision multimeter (HP 34401A). A plot was then constructed of the output voltage versus the input voltage and a linear regression was performed to determine the slope. The slope then gave the appropriate gain for that setting. This was done for several of the settings and the appropriate gains were changed in the LabView VI. The gains for the most sensitive voltage settings were inferred from the readings in the higher ranges. Figure 3-4 displays the linear regression of the input and output voltage plots measured at 10 mV and 3 mV.

Figure 3-4. Linear regression analysis of input versus output voltage plots for calibration of the low noise Nanovoltmeter preamplifier. The slope of the line is the appropriate gain for the particular setting. a) Measurements done at the 10 mV setting, b) Measurements done at the 3 mV setting.
3.2.2 Phase Lock Oscillator (PLO)

Quartz crystal microbalances are sensors composed of piezoelectric (vide infra) quartz. In general, sensors operate on the principle that a measurable quantity can be transformed into an electrical value such as a voltage, current or frequency. In the studies presented here, the quartz resonator is used as a frequency-determining element in a feedback loop of an oscillator circuit. A simplified oscillator feedback circuit with a quartz resonator is shown in Figure 3-5 (Hewlett-Packard, 1997).

![Feedback AMP](Image)

**Figure 3-5.**  A simplified oscillator feedback circuit using a crystal resonator. The quartz resonator has a small amount of energy fed back to it, which causes it to oscillate. The oscillations then stabilize the generated frequency at the quartz’s resonant value (Hewlett-Packard, 1997).

The resonator shown above actively controls the frequency. The oscillator circuit uses an electrical excitation to keep the sensor at its mechanical resonance. The circuit then outputs an electrical signal with the frequency of the mechanical resonance (Benes et al., 1995). This technology of using piezoelectric crystals for frequency control has become so common and widespread with uses ranging from watches to computers and cell phones, it is estimated that over 1.3 billion resonator units are in use worldwide (Benes et al., 1995)!
An added feature to our QCM/HCC instrumentation was the purchasing of a new oscillator driver, a Maxtek PLO-10 Phase Lock Oscillator (Santa Fe Springs, CA). With this instrument we are able to probe the rheological properties of thin films and monitor changes in these properties upon vapor sorption. An important relationship lies in the fact that because of the piezoelectric effect (vide infra) the electrical properties of the oscillator are intimately related to the mechanical properties of the film-coated QCM. A more detailed discussion of the relationship between the electrical and mechanical properties will be presented in the next section.

This electro-mechanical relationship is well established and is often shown in terms of the Butterworth-Van Dyke (BVD) equivalent circuit. The equivalent circuit consists of two branches, a parallel branch depicting the static characteristics and a series branch representing the motional characteristics. A diagram of the BVD equivalent circuit is shown in Figure 3-6 (Arnau et al., 2001a; Bandey et al., 1999; Lu, 1984):

![Figure 3-6](image)

**Figure 3-6.** A Butterworth-Van Dyke (BVD) equivalent circuit used to depict the relationship between the electrical and mechanical properties of a QCM (Lu, 1984).
The series motional branch of the equivalent circuit has three components: (1) a motional capacitance, C, representing the mechanical elasticity of the oscillating body, (2) a motional inductance, L, which gives a measure of the oscillating mass, and (3) an equivalent resistance, R, corresponding to the mechanical energy losses due to internal friction and energy dissipation to the surrounding structures (Lu, 1984).

The parallel static branch has one component, the parallel shunt capacitance, $C_o$. This capacitance stems from the electrodes on the quartz plate plus the stray capacitance due to the crystal holder and cables (Vig, 2002). One goal of the oscillator circuitry is to eliminate the effects of the shunt capacitance. When the shunt capacitance is eliminated, the resonant frequency of the QCM is the point where there is zero phase between the crystal current and voltage (Maxtek, 2000). A plot of the QCM at its resonant frequency is depicted in Figure 3-7.

**Figure 3-7.** The resonant frequency of a QCM. At the series resonant frequency, the real part of the admittance reaches a maximum. The phase angle between the crystal current and voltage is zero at this resonant frequency (Lucklum, 2002).
When the shunt capacitance, $C_o$, is effectively cancelled, the inductive reactance, $\omega L$, cancels the capacitive reactance, $1/\omega C$, with $\omega$ defined as the angular frequency, $2\pi v$. At resonance, only the motional resistance, $R$, remains visible in the circuit. This is depicted in Figure 3-8 (Lee et al., 2002).

![Figure 3-8. An electrical equivalent circuit for a quartz crystal resonator. a) quartz crystal resonator, b) Butterworth-Van Dyke equivalent circuit, c) compensated circuit, d) compensated circuit at resonance (Lee et al., 2002).](image)

At resonant frequency, the magnitude of the current is directly proportional to the crystal conductance. The current is then converted to a voltage, which is proportional to the crystal conductance. The resistance is found by taking the reciprocal of the conductance at that frequency (Lee et al., 2002). It is this motional resistance, $R$, which provides a view of the energy loss within the system. This measured damping effect is closely related to the physical properties of the film cast on the QCM.

When a viscoelastic or lossy film is present on the QCM, the $C_o$ introduces a current with a phase shift that is in quadrature with the applied voltage (Arnau et al., 2001b; Lee et al., 2002). The QCM data is distorted by the presence of this shunt capacitance.
This additional phase shift changes the magnitude and frequency at which the current to voltage (or admittance) phase shift is zero (Lee et al., 2002). At this point, the series resonant frequency of the motional arm does not accurately describe the frequency of the oscillator.

The PLO, now added to our system, contains a phase detector. The phase difference between the crystal current and voltage is continuously monitored. The phase detector sends an output signal to an integrator where positive or negative phase errors cause the integrator output to correspondingly rise or fall. The integrator output remains the same if the phase error is zero. The integrator output is then connected to the PLO internal oscillator, a voltage controlled oscillator (VCO) which then drives the crystal. Three situations can arise when comparing the actual frequency, \( f \), with the resonant frequency, \( f_r \) (Maxtek, 2000):

1. If \( f < f_r \), then the current leads the voltage and the phase = 90\( ^\circ \). Therefore, the integrator output rises causing the VCO frequency to increase.

2. If \( f > f_r \), then the current lags the voltage and the phase = -90\( ^\circ \). Therefore, the integrator output falls causing the VCO frequency to decrease.

3. If \( f = f_r \), there is no phase error between the current and the voltage. Therefore, the integrator output remains steady as well as the VCO output.

The phase detector aids in keeping the VCO frequency locked on to the QCM resonant frequency. When the VCO is locked on, the in-phase component (there is no out of phase component at this point) of the crystal current is converted to a dc voltage. This output voltage is proportional to the crystal conductance. The output scaling for the conductance voltage is 100 volts per siemen (ampere/volt). This output conductance voltage is inversely proportional to the sum of the crystal resistance plus
the crystal drive voltage source resistance, $R_{\text{source}} = 20 \, \Omega$. The motional resistance is calculated from (Maxtek, 2000):

$$R_{\text{crystal}} = \frac{100}{\text{Conductance}} - R_{\text{source}} \quad \text{Equation 3-1}$$

where the resistance is in Ohms (volts/ampere), and the conductance is in siemens (ampere/volt). The motional resistance traces upon vapor sorption will be shown for our polymer and protein data in Chapters 5 and 6.

### 3.2.3 Refinements in the Gas Flow

In order to better monitor and control our gas flow through the QCM/HCC two modifications were introduced, the addition of a relative humidity meter and the installation of stainless steel tubing. A relative humidity meter, RH-100 (Sable Systems, Las Vegas, NV) was purchased and introduced to our gas flow stream. The RH meter was added downstream from the sample and reference chambers, measuring the water vapor content after it had interacted with the sample, film-coated QCM. Benefits of the RH meter include the ability to verify that the applied water vapor activity was the same as the actual amount reaching the sample, film-coated QCM and to trace the water vapor interaction with the film. It is interesting to note that in our protein studies, hysteresis in the water sorption of the film is also recorded in the gas flow stream by the RH meter. The protein films did not always desorb the same amount of water that was sorbed. These effects will be discussed in Chapter 6.

In our gas flow system we now use all stainless steel tubing, 1/16” o.d. This eliminated any questions regarding possible water permeation through some of the
Teflon tubing which was used for the gas flow stream. The effects of water permeation were considered negligible because of the positive pressure present due to the gas flow through the tubing. After installing the stainless steel tubing however it was noticed that there was a 1% reduction in the amount of water vapor in the gas stream as measured by the RH meter.

3.3. Quartz Crystal Microbalance as a Mass Sensing Device

3.3.1 Properties of Quartz Crystal Resonators

Quartz is an appealing choice to use as a stable resonator for several reasons. On a practical level, quartz is a single crystal form of SiO$_2$, which is abundant in nature and is easily grown, inexpensively and in large quantities with a high level of purity and perfection. It has a low solubility in all solvents except fluoride etchants. Quartz crystals are piezoelectric (vide infra) and can be cut to have a zero temperature coefficient. In addition they also exhibit low intrinsic losses, that is, they exhibit a high quality factor, $Q$ (Vig, 2002). For example, a $10\,\Omega$, $5\,\text{MHz}$ quartz crystal has a quality factor, $Q$, of approximately 120,000. The bandwidth of this particular crystal, which is the crystal resonant frequency divided by $Q$, would be 42 Hz (Maxtek, 2001).

3.3.1.1 Piezoelectricity

Primary among the qualities of quartz is that it exhibits the piezoelectric effect. Piezo stems from the Greek word “piezin” meaning “to press.” In 1880, the Curie brothers first described piezoelectricity when they noticed the generation of an electrical charge that occurred when a weight was placed on a quartz crystal. The magnitude of the charge was proportional to the weight. In fact, for a piezoelectric solid, it was shown that an electric charge can be generated by any strain such as bending, shear, tension,
compression, or torsion of the solid (Cady, 1946; Hewlett-Packard, 1997). In 1881, quartz was also shown to undergo the converse piezoelectric effect. When an external electric field was applied, lattice strains in the solid forced a mechanical deformation to occur. When the voltage was reversed, the strains were likewise reversed (Vig, 2002).

An applied electric field to a quartz resonator produces a periodic perturbation (stress), which in turn results in an elastic deformation (strain). This strain travels across the solid as a transverse or longitudinal wave with a certain phase velocity (Janshoff et al., 2000). Acoustic waves, traveling in a solid, span a frequency range of 14 orders of magnitude. These range from $10^{-2}$ Hz exhibited as seismic waves to $10^{12}$ Hz resulting from thermoelastic excited phonons. The oscillation of acoustic quartz resonators occurs in the narrow range of $10^6$ – $10^9$ Hz (White, 1998).

Piezoelectricity can be found in a large number of crystals; however, quartz provides a distinct blend of identified mechanical, electrical, chemical and thermal properties which makes quartz commercially appealing (Janshoff et al., 2000). Although there are 32 crystal classes, only 20 of these exhibit the piezoelectric effect. One requirement for piezoelectricity is that the crystals lack a center of symmetry. A force deforming a crystal lattice separates the centers of gravity of the positive and negative charges in the crystal thus producing a surface charge (Lu & Czanderna, 1984; Vig, 2002).

An important relationship exists between the mechanical properties of the oscillation and equivalent electrical components. This relationship will be more fully developed in the following sections.
3.3.1.2 Types of Crystal Cuts

The physical properties of quartz crystals (e.g. etching rates, thermal expansion coefficient, and temperature coefficient) are highly anisotropic, which means that their properties vary greatly depending on the crystallographic direction. The optic axis is the main axis of quartz growth. When quartz is cut to produce resonators, the optic axis is labeled the Z axis in an orthogonal X, Y, Z coordinate system. This Z-axis has a three-fold symmetry evidenced by the physical properties repeating every $120^\circ$ as the crystal is rotated about the Z axis. The X axes bisect the angles adjacent to the prism faces and the Y axes are perpendicular to the prism faces (Hewlett-Packard, 1997). The delineation of axes and typical crystal cuts can be seen in Figure 3-9.

Figure 3-9. Axes orientations for quartz crystal cuts. Z is the optic axis. A quartz crystal having 6 sides has three separate X axes and three Y-axes identified at $120^\circ$ intervals about the Z axis (Fox, 2002).
Mason has compiled an extensive list of the different types of crystal cuts and the ensuing properties. Also included in his treatment are many diagrams depicting the orientation of each cut along the X, Y, Z coordinate axes (Mason, 1964).

Crystal cuts are identified by two-letter names. The “T” in a name indicates a temperature-compensated cut. The AT-cut was the first temperature-compensated cut discovered. The BT, DT, FT, IT, and RT-cuts are other cuts along the temperature coefficient locus. The SC-cut, a stress compensated cut, and the AT-cut crystal units are the highest stability crystal oscillators employed (Vig, 2002). AT-cut crystals typically have a stability of change in frequency with respect to its resonant frequency, $\Delta f/f_0 \approx 10^{-8} \text{ Hz}$ (Janshoff et al., 2000).

The mode of vibration of a crystal depends on the cut. The AT-cut is $35^\circ 15'$ off the Z axis along the Y plane and vibrates in the thickness shear mode. These resonators are often referred to as thickness shear mode resonators (TSM), quartz crystal resonators (QCR), or the commonly used name, quartz crystal microbalances (QCM). Other resonators with varying modes of wave propagation include: FPW – flexural plate wave resonators, SAW – surface acoustic wave resonators, APM – acoustic plate mode and SH-APM – shear horizontal acoustic plate mode resonators. The characteristics of these techniques have been reviewed in the literature (Buttry & Ward, 1992; Grate et al., 1993; Janshoff et al., 2000). Some of the modes of motion exhibited by a quartz resonator are seen in Figure 3-10.
Figure 3-10. Possible modes of motion for quartz resonators. The thickness shear fundamental mode applies to the studies shown here using QCMs (Vig, 2002).

After cutting and shaping the quartz crystal, metal electrodes are applied by vacuum deposition to the quartz wafer. A variety of geometries can be used, however, circular quartz plates with circular electrodes are the most commonly used. Thickness shear mode resonators (TSM) a.k.a. quartz crystal microbalances (QCM) are utilized in this study.

3.3.1.3 Quartz Crystal Resonators

The quartz crystal microbalance or quartz crystal unit is a quartz wafer with electrodes applied on the top and bottom surfaces. The quartz wafer is also named the “blank” or “crystal plate.” A schematic diagram can be seen in Figure 3-11 (Martin et al., 1994).
Figure 3-11. Quartz Crystal Unit. a) top view of a quartz unit, b) side view of quartz unit, metal electrodes on top and bottom of quartz wafer (Martin et al., 1994).

When an alternating potential is applied to the top and bottom electrodes, mechanical vibrations are excited in the piezoelectric quartz. Although several modes may be excited in quartz, it is the shear vibrational modes which predominate in an AT-cut quartz crystal resonator. The shear displacement occurs parallel to the quartz surfaces. A cross sectional view of a quartz resonator is depicted in Figure 3-12 (Martin et al., 2000; Martin et al., 1991).

Figure 3-12. Cross-sectional view of a QCM resonator. A shear deformation is created by the potential, V. (a) film, (b) electrodes, (c) quartz wafer, (d) acoustic wave (Deakin & Buttry, 1989; Martin et al., 1994).
The quartz is characterized by its thickness, $h_q$, density, $\rho_q$, and the shear elastic constant, $c_q$. A mass layer on the quartz is identified by the film’s thickness, $h_f$, density, $\rho_f$, and the film storage and loss moduli, $G'(\omega)$ and $G''(\omega)$” (vide infra). Each resonant mode corresponds to a unique standing shear-wave pattern (eigenmode) across the quartz thickness. Resonances occur at frequencies where the quartz thickness is an odd multiple of half the acoustic wavelength (Martin et al., 2000).

The resonant frequency of an AT-cut quartz crystal is intrinsically dependent on the quartz thickness. A decrease in the quartz thickness results in an increase in the quartz resonant frequency. For example, a 5 MHz QCM has a thickness of 330 µm while a 30 MHz crystal has a thickness of 55µm (Janshoff et al., 2000).

3.3.2 Relationship Between QCM Mechanical and Electrical Properties

QCM resonators are sensitive to the accumulation of a mass because the displacement maxima occur at the crystal faces. The above would correspond to an ideal mass layer on the quartz surface. An “ideal mass layer” is defined as being infinitesimally thin while imposing a finite mass per unit area on the QCM surface. An “ideal layer” is sufficiently thin and rigid so that the shear acoustic wave traveling though the layer has a negligible phase shift (Cernosek et al., 1998). Deviations from an ideal mass layer will be discussed towards the end of this chapter.

The relationship between the mechanically vibrating quartz crystal and the electrical equivalent circuit can be seen in Figure 3-13 (Buttry & Ward, 1992; Muramatsu et al., 1988; Vig, 2002).
As can be seen in the diagram, the spring, mass and damping element (dashpot) correspond to the capacitor, inductor, and resistor in the circuit. The driving force is akin to the voltage, the displacement of the mass corresponds to the charge on the capacitor, and the velocity is related to the current.

The oscillating properties of a QCM can be understood by looking at a simple LCR circuit. If a switch on the LCR circuit is open, the capacitor remains charged. However, when the switch is closed, the capacitor discharges through the inductor. A magnetic field is established around the inductor because the inductor opposes the current as the current is increasing. Once the capacitor is completely discharged, the current drops to zero. The magnetic field around the inductor begins to decrease and induces an electromotive force in the inductor opposite in direction to the initial current. The current continues to flow until the magnetic field is gone and the capacitor is once again fully charged. The charged capacitor at this point is opposite in polarity from its previous state. If $R = 0$, this process can repeat in opposite directions indefinitely.
resulting in electrical oscillation. Oscillation damping occurs when $R>0$, this corresponds to energy losses in the mechanical model due to friction (Buttry & Ward, 1992).

A mechanically vibrating quartz crystal and the circuit shown above are equivalent because both can be described by the same form of differential equations (Buttry & Ward, 1992), (Vig, 2002).

\[
F = m \left( \frac{d^2x}{dt^2} \right) + r \left( \frac{dx}{dt} \right) + \left( \frac{1}{c_m} \right) x \tag{Equation 3-2}
\]

\[
V = L_1 \left( \frac{d^2q}{dt^2} \right) + R_1 \left( \frac{dq}{dt} \right) + \left( \frac{1}{C_1} \right) q \tag{Equation 3-3}
\]

**Equation 3-2** represents the mechanical motion where $F$ is the driving force, $m$ is the mass, $r$ is a dissipation factor, $c_m$ is the elasticity, and $x$ is the displacement. **Equation 3-3** displays the equivalent circuit components where $V$ is the voltage, $L_1$ is the inductance, $R_1$ is the resistance, $C_1$ is the capacitance, and $q$ is the charge. The two equations are related through the electromechanical coupling coefficient for a lossless quartz, $K_q^{\omega_2}$, where $F = K_q^{\omega_2} V$ (Buttry & Ward, 1992; Muramatsu et al., 1988).

### 3.3.3 Extracting Mechanical Information from Electrical Properties

#### 3.3.3.1 Acoustic Wave Propagation

In order to extract the mass and shear modulus information of a film cast upon a quartz resonator, the resonator is often depicted as a sensor comprised of different parts namely a piezoelectric layer with an attached non-piezoelectric layer. The piezoelectric
layer, the quartz, generates and also detects an acoustic wave which propagates through both of these layers. A cross-sectional picture of a film-coated QCM can be seen above in Figure 3-12. Other layers can be added for measurements done in liquid (Kanazawa, 1999).

A change occurring in the acoustic wave amplitude and phase of the quartz crystal, indicates a physical property change in the non-piezoelectric layer adhered to the resonator, the film (Lucklum & Hauptmann, 1997). A simplified view includes three possible cases. In the first case when an rf potential is applied to a bare QCM, the crystal oscillates at its resonant frequency. The second case is due to the effect of a thin rigid film on the QCM. A thin rigid film causes the frequency of the acoustic wave to decrease however the wave is not subject to a phase shift. In the third case, a viscoelastic film is on the QCM. A viscoelastic film causes not only a decrease in the frequency of the acoustic wave but also causes a phase shift and attenuation.

3.3.3.2 Acoustic Load Impedance, $Z_L$

One goal in relating the electrical properties of a quartz resonator to the mechanical properties is to convert the electrical admittance, $Y_{el}$, to the acoustic load impedance, $Z_L$. A description of these terms follows in the ensuing paragraphs. Two general methods exist to accomplish this purpose: (1) using a transmission line analogy in relationship to the BVD equivalent circuit (2) using a physics based approach that involves solving wave equations of motion and piezoelectric equations for each layer (Kanazawa & Gordon, 1985; Lee et al., 2002; Reed et al., 1990).

The physics based approach is a tedious and time consuming process in which proper boundary conditions must be applied, as well as the determination of the strain,
polarization, charge density and potential at each point on the crystal surface (Buttry & Ward, 1992; Kanazawa, 1999). From the wave equation, it is possible to calculate the electrical admittance, $Y_{el}$, of the resonator, which in turn yields calculated information of the equivalent circuit parameters.

In the equivalent circuit method, instead of calculating the electrical admittance, $Y_{el}$, it is measured and is then used in an equation which relates this value to the mechanical property of a film, the surface acoustic load impedance, $Z_L$. Several models have been employed with the most common one being the transmission line model (TLM), applied to a Butterworth Van-Dyke equivalent circuit (Bandey et al., 1999; Behling et al., 1999; Johannsmann, 1999). The transmission line model allows for the separation of the acoustic wave propagating outside the quartz crystal to be separated from the acoustic wave propagating inside the quartz crystal. This one-dimensional model extracts the relevant acoustic information and embodies this in one parameter, the acoustic load impedance, $Z_L$. This complex value, $Z_L$, gives a measure of the mechanical properties at the quartz – film interface.

When referring to the BVD equivalent circuit in Figure 3-6, an added feature is necessary to account for a viscoelastic material on a QCM. The presence of an acoustically thick viscoelastic coating on the QCM introduces the complex motional impedance, $Z_2$, created by the surface load. This term is shown in the modified BVD circuit in Figure 3-14 (Bandey et al., 1999; Martin et al., 2000; Smyth et al., 2001).
Figure 3-14. A modified BVD equivalent circuit with an added complex impedance, $Z_2$, to characterize an acoustically thick viscoelastic load on the QCM surface. The mechanical/piezoelectric properties of the unloaded crystal are shown in the motional arm, $R_1$, $L_1$, $C_1$. The effects of the film are $R_2$, $L_2$, and $C_2$ (Martin et al., 2000; Smyth et al., 2001).

From the above, it can be seen that the total motional impedance in the electrical circuit, $Z_m$, is composed of two additive parts, the motional impedance of the unperturbed quartz crystal, $Z_1$ and the motional impedance created by the surface load, $Z_2$ where:

$$Z_m = Z_1 + Z_2$$

Equation 3-4

$Z_1$, the motional impedance of the unperturbed quartz depends on the frequency and quartz constants. $Z_2$, the motional load impedance is a function of the surface acoustic impedance and reflects any changes in the acoustic load (Lucklum & Hauptmann,
Near resonance, the motional impedance of the unloaded quartz, $Z_1$ is simplified to:

$$Z_1 = R_1 + i\omega L_1 + \frac{1}{i\omega C_1}$$  \hspace{1cm} \text{Equation 3-5}$$

where $R_1$, $L_1$, and $C_1$ are the motional elements in the BVD equivalent circuit as discussed above. When the QCM is oscillating at its series resonant frequency, (for the case of a bare QCM or a thin rigid film) the reactive imaginary parts of Equation 3-5 are zero (Martin et al., 2000). At this point the acoustic load impedance, $Z_L$, is best reflected in measurements of the motional resistance, $R$.

For the motional impedance due to a viscoelastic mass loading, $Z_2$, a linear approximation can be assumed for small loads\(^1\) (Lucklum et al., 1997; Martin et al., 2000):

$$Z_2 \approx \frac{h_q}{4C_q K_q^2 c_q} Z_L$$  \hspace{1cm} \text{Equation 3-6}

with $h_q$ being the effective height of the quartz including the electrodes, $c_q$ is the piezoelectric stiffened elastic constant for the quartz crystal and $K_q^2$ is the electromechanical coupling factor for a lossy quartz.

The measurable property, the electrical admittance, $Y_{el}$, of an oscillating QCM is equal to the inverse of the electrical impedance, $Z_{el}^{-1}$, and likewise a function of the

\(^1\)A small load being defined as $Z_L/Z_q << 2 \tan(\alpha/2)$ (Lucklum et al., 1997). Where $Z_L$ is the acoustic load impedance, $Z_q$ is the characteristic quartz impedance, $\alpha$ is the wave phase shift in the quartz.
quartz density, $\rho_q$; the piezoelectrically stiffened elastic modulus, $c_q$; the effective quartz viscosity, $\eta_q$; the effective quartz thickness, $h_q$; the static capacitance, $C_o$; and the acoustic load impedance, $Z_L$ (Lucklum et al., 2001):

$$Z_{el} = Y_{el}^{-1} = f (\rho_q, c_q, \eta_q, h_q, C_o, Z_L) \quad \text{Equation 3-7}$$

Two cumbersome equations from this correlation include the relationship between the electrical admittance, $Y_{el}$, and the acoustic load impedance, $Z_L$ (Lucklum & Hauptmann, 2000a; Martin et al., 2000):

$$Y_{el} = \frac{1}{Z_{el}} = \frac{i\omega C_o}{1 - \frac{K^2}{\alpha} \frac{2 \tan(\alpha_q/2) - iZ_L/Z_q}{1 - iZ_L/Z_q \cot \alpha_q}} \quad \text{Equation 3-8}$$

and upon rearrangement, the equation yields a specific form for the acoustic load impedance, $Z_L$:

$$Z_L = iZ_{cq} \frac{(Y_{el} - i\omega C_o) \frac{\alpha}{K^2} - 2Y_{el} \tan(\alpha_q/2)}{Y_{el} - (Y_{el} - i\omega C_o) \frac{\alpha}{K^2} \cot \alpha} \quad \text{Equation 3-9}$$

where $Z_{cq}$ is the acoustic impedance of quartz, $K^2$ is the quartz electromagnetic coupling coefficient, $\alpha$ is the acoustic phase shift across the crystal, and $C_o$ is the static
capacitance. A glossary of terms and their units is listed in Appendix B. Equations for each of these are listed in Appendix C.

The effect of the acoustic load impedance, $Z_L$, in relation to the film coated QCM can be visualized through two simplifying approximations (Lucklum & Hauptmann, 2000b; Lucklum et al., 2001):

\[
\frac{\Delta f_r}{f_o} = -\frac{\Im(Z_L)}{N\pi Z_{cq}} \quad \text{Equation 3-10}
\]

\[
\frac{\Delta R}{2\omega L_q} = \frac{\Re(Z_L)}{\pi Z_{cq}} \quad \text{Equation 3-11}
\]

where $Z_q$ is the intrinsic quartz impedance, $L_q$ is the motional inductance of the crystal, and $N$ is the harmonic number of the frequency. A shift in the resonant frequency is caused by the imaginary part of the acoustic load impedance while the real part of $Z_L$ affects the damping of the oscillator.

### 3.3.3.3 Gravimetric Measurements and the Sauerbray Equation

When a single coating is present on the QCM, the acoustic load impedance, $Z_L$, can be shown as a function of the density of the film, $\rho_f$, the thickness of the film, $h_f$, and the complex shear modulus, $G$ (Behling et al., 1998; Lucklum et al., 1997):

\[
Z_L = i\sqrt{\rho_f G} \tan\left(\omega h_f \sqrt{\frac{\rho_f}{G}}\right) \quad \text{Equation 3-12}
\]
This equation can be transformed to show two main contributions:

\[
Z_r = i \omega \rho_f h_f \frac{\tan \varphi}{\varphi} = i \cdot M \cdot \nu \tag{Equation 3-13}
\]

where \( \varphi \) is the acoustic wave shift inside the film coating the QCM. This value is the acoustic wave phase difference between the QCM surface and the outer film surface. This phase shift is given by:

\[
\varphi = \omega h_f \sqrt{\frac{\rho_f}{G}} \tag{Equation 3-14}
\]

In Equation 3-13 the acoustic load impedance is shown as two distinct parts, a mass factor \( M \), and an acoustic factor, \( \nu \), where each is defined as (Lucklum & Hauptmann, 2001):

\[
M = \omega \rho_f h_f \tag{Equation 3-15}
\]

\[
\nu = \frac{\tan \varphi}{\varphi} \tag{Equation 3-16}
\]

From the above equations, the mass factor \( M \), measures the coating mass and is shown to be independent of the shear modulus. The acoustic factor, \( \nu \), relies heavily on the material properties of the film coating. The acoustic phase shift inherent in \( \nu \) is a complex quantity encompassing the wave propagation and damping in the film coating.
When a film on the surface of a QCM is thin and rigid, it oscillates in unison with the QCM and there is no detectable acoustic wave phase shift, $\phi$. In this gravimetric region, the real part of the acoustic load, Equation 3-11, is very small, therefore, any damping of the electrical admittance is negligible. From Equation 3-13, when $\phi$ is very small, $\tan \phi \sim \phi$, leading to the acoustic factor approximately equal to one, $\nu \sim 1$ (Behling et al., 1998). In the limit of $\phi$ being very small, the acoustic load can be calculated solely from the mass factor, $Z_L = iM$. At this point the acoustic load and the electrical admittance are independent of the shear modulus, $G$. Combining this mass factor relationship and the approximation in Equation 3-10, the frequency shift is related to the coating mass by (Behling et al., 1998):

$$\Delta f = \frac{-2f_o \rho_t h_f}{Z_{cq}}$$

Equation 3-17

where the areal mass density of the coating is:

$$\rho_t h_f = \frac{m}{A}$$

Equation 3-18

Equation 3-17 is of the same form as the Sauerbrey equation (Sauerbrey, 1959):

$$\frac{\Delta f}{f_o} = -\frac{2f_o \Delta m}{A\sqrt{\rho_q c_q}}$$

Equation 3-19
where $\Delta m$ is the change in mass, $A$ is the piezoelectrically active area, $\rho_q$ and $c_q$ are terms for the quartz density and the piezoelectric stiffened elastic constant respectively. The square root term in the denominator is equal to $Z_{cq}$. In 1959, Sauerbrey used this relationship to show that the resonant frequency of an oscillating circular quartz resonator is inversely proportional to the thickness of the quartz. Therefore, an increase in the thickness of a coating on a QCM results in a frequency decrease (Smith & Shirazi, 2000). This is the principle behind the mass measurements used in our studies with the QCM/HCC. To determine the mass change in our vapor sorption experiments, the Sauerbrey equation is rearranged and of the form:

$$\Delta m = -\left(\frac{\Delta f}{C}\right) \cdot A_{\text{meas}}$$  \hspace{1cm} \text{Equation 3-20}$$

where the mass, $m$, is determined in $\mu$g, $C$ is a constant encompassing the quartz constants and is equal to $56.6 \text{ Hz} \mu\text{g}^{-1}\text{cm}^2$, and $A_{\text{meas}}$ is the area of the film that was exposed to the adsorbate vapor. In our instrumentation the experimental area is $1.9793 \text{ cm}^2$.

3.3.3.4 Deviations from the Gravimetric Regime

Complications arise when a film exhibits viscoelastic characteristics. Viscoelastic materials exhibit a lower shear modulus, $G$, than elastic materials (vide infra). The lower the shear modulus, the greater the acoustic phase shift, $\phi$, and the greater the deviation of $\tan \phi$ from $\phi$ (Behling et al., 1998). In this case, the quartz resonator frequency shift cannot be interpreted as a shift due solely to a mass change. The acoustic phase shift is very dependent on the coating thickness and the probing
frequency. Because of this complexity, many quartz resonators are used to qualitatively determine whether a film is viscoelastic; determining exact values for the shear modulus and mass is another story. Knowledge of the viscoelastic effects on four film parameters, is needed: the film height, $h_f$, the film density, $\rho_f$, the shear storage modulus $G'$ and the shear elastic modulus $G''$. From Equations 10 and 11, the experimental data available includes the real and imaginary parts of the acoustic load impedance, $\text{Re}(Z_L)$ and $\text{Im}(Z_L)$. The problem is underdetermined because there are only two input parameters available to try to extract four output parameters (Hillman et al., 2001).

It is at this point that researchers step out into open territory. Several models exist to capture the different possibilities of film thicknesses, density and shear modulus (Arnau et al., 2001a; Etchenique & Weisz, 1999; Johannsmann, 1999; Lucklum & Hauptmann, 2001; Muramatsu et al., 1995). To describe the system, the BVD equivalent circuit is equipped with an addition impedance term as seen in Figure 3-14. In many of the discussions, three possible film situations arise: an acoustically thin film, a semi-infinite film and an acoustically thick film (Hillman et al., 2001; Martin et al., 2000). An acoustically thin film poses the least difficulty; the acoustic wave phase shift is negligible and the film can be treated as responding in the gravimetric regime. However, accurate knowledge of the $h_f$ and $\rho_f$ is still required. When a film coating is considered semi-infinite, the acoustic wave attenuation is large ($h_f > 2\delta$, where $\delta$ is the acoustic decay length). The acoustic wave decays before it ever reaches the outer film boundary, leaving some of the film unsampled by the acoustic wave. If a film is acoustically thick, the acoustic phase shift across the film is considerable but the attenuation of it is not ($h_f < 2\delta$). In this case, the shear displacement starting at the
quartz/film interface propagates through the film, is reflected at the outer film surface, and then propagated back to the inner surface. This reflection occurs with sufficient amplitude so as to cause interference with the outgoing wave (Hillman et al., 2001). In each of these cases models have been formulated to try to fit data and extract the parameters $h_f$, $\rho_f$, $G'$, and $G''$.

In our QCM/HCC measurements, the question of gravimetric and non-gravimetric measurements upon vapor sorption will be discussed. In our polymer studies, the polymer Tecoflex was used. Upon vapor sorption it is known that polymers undergo relaxation and swelling. Because of this, the $h_f$ and $\rho_f$ values change. At the present time, we do not have a method to characterize these processes during sorption. There are some methods and models, which are applicable to our instrumentation, and these will be discussed in Chapters 4 and 5.

3.4 Thermopiles as Thermal Sensing Devices

Because calorimetry involves the measurement of heat, the thermal sensor is an important feature. Thermopiles are commonly used in calorimeters as a means of converting thermal energy to electrical energy. In heat conduction calorimetry, the heat produced or absorbed in a reaction vessel is allowed to freely flow through a thermopile to a heat sink (aluminum in our instrumentation) surrounding the reaction vessel. The thermopile between the reaction vessel and the heat sink is key to capturing the heat effects of the reaction. Traditionally, thermopiles have been used as heat pumps operating under the Peltier effect. This effect is responsible for the absorption and release of heat due to an electric current flowing through two dissimilar conducting materials. In calorimetric applications, the inverse-Peltier effect is utilized; a
temperature difference between two conducting materials produces a voltage which is then measured as a means of probing the heat flow during a reaction.

### 3.4.1 Thermopile Theory

A thermopile is one heat-sensing unit made from the combination of several semiconductor thermocouple plates. Thermocouple plates are also commonly known as Peltier plates. The basic theory states that the thermopile potential, \( U \), is proportional to the heat flow through the thermopile, \( P \). In a calorimeter, this leads to a very small temperature difference between the reaction vessel and the heat sink, \( \sim 10^{-3} \) K for a heat flow of 100 µW, therefore, the conditions are essentially isothermal (Bäckman et al., 1994). Thermopiles used as heat sensors in isothermal heat conduction calorimeters need to have one side of the plate maintained at a fixed reference temperature while the other side of the thermopile is set up as the measuring junction (Pollock, 1985). For the QCM/HCC studies presented here, the reference temperature is 25°C in most of the applications.

In 1850, Lord Kelvin combined the thermoelectric effects reported by Seebeck in 1821 and Peltier in 1834. He also predicted and experimented on what is now known as the Thomson effect (Nicholas & White, 2001). This fusing of the three effects has led to the “Three Laws of Thermoelectricity” and the operating principles of thermopiles rest upon these three thermoelectric effects: the Seebeck, Peltier, and Thomson Effects. In their basic form, the three thermoelectric principles are based on the properties of electrons interacting with a metal ion lattice. The energy of the electrons are dependent on the type of metal and the temperature of the metal. This dependence of electron flow is shown in the following description of the three effects.
3.4.1.1 Seebeck Effect

The Seebeck effect occurs when there is a temperature difference between two dissimilar conductors joined in a circuit. This temperature difference causes a current to flow through the two conducting materials (Pollock, 1971). Before looking at the junction of two dissimilar metals, it is helpful to observe how this effect occurs in one homogeneous conducting element. A single conducting element (such as a wire) exposed to a temperature gradient is shown in Figure 3-15 (Nicholas & White, 2001):

\[\text{Cold} \quad \text{Hot}\]

**Figure 3-15.** The Seebeck effect shown in a single conducting material. Electrons at the hot end have a higher kinetic energy and vigorously diffuse towards the cold end of the material. The flow of electrons is due solely to the temperature gradient (Nicholas & White, 2001).

At the hot end of the conducting material, the electrons possess a higher kinetic energy thus inducing a frenzied motion of the electrons. The electrons spontaneously diffuse towards the cold end of the material. The cold electrons do diffuse towards the hot end, but not in as rapid a manner. As the hot electrons move towards the cold end, they carry heat with them. The cool electrons take up the heat from the hot parts. The high thermal conductivity of metals lies in this principle of free electron diffusion (Nicholas & White, 2001). In this migration process, the cold end of the wire can develop a
surplus of electrons thus producing a change in the voltage along the wire. This voltage or electric potential change due to the redistribution of electrons is the Seebeck effect.

The change in voltage produced by the migration of electrons is proportional to the temperature gradient. This is shown through the mathematical relationship (Nicholas & White, 2001):

\[ dE_s = e(T) \, dT \]  \hspace{1cm} \text{Equation 3-21}

where the change in Seebeck voltage is \( dE_s \), the temperature gradient is denoted by \( dT \), and \( e(T) \) is the Seebeck coefficient. The Seebeck coefficient is different for every metal because it depends on the electronic properties of the metal. From Equation 3-21 it is easily seen that when \( dT = 0 \), the change in Seebeck voltage, \( dE_s \) is also equal to zero.

In thermopile applications, the Seebeck effect is shown in relation to two dissimilar conductors joined to form a circuit. A schematic of two conducting materials, labeled A and B, are shown in Figure 3-16:

![Figure 3-16](image.png)

\textbf{Figure 3-16.} The Seebeck effect in two dissimilar conducting materials, A and B. The metals and the corresponding junctions are at different temperatures with \( T < T + \Delta T \). The potential is measured by the voltmeter, V, in the circuit (Jäckle, 2000).
The metals and their junctions, A and B, are at different temperatures, T and T+ΔT where T < T+ΔT. In Figure 3-16, the electrons flow from A to B at the colder junction. The Seebeck effect is not affected by the contact potential between the two dissimilar conducting materials. The contact potential is measured by the difference in work functions when two different materials are brought close enough that electron transfer is possible. The contact potential does not require a temperature difference and therefore is not the same as the Seebeck effect (Pollock, 1971; Pollock, 1985).

3.4.1.2 Peltier Effect

Unlike the Seebeck effect, which can be seen in one conducting material, the Peltier effect comes alive when two dissimilar materials (wires) are brought together at a junction. When two combined wires are at the same temperature and a current is passed through, the electrons move from the metal where they have a higher chemical potential to the wire with the lower chemical potential. The magnitude of the potential difference depends on the types of materials used and the temperature at the junction. Electrons from the region of higher energy, upon crossing the junction to the lower energy region, will release some of their spare energy to the metal lattice. This extra energy is seen as heat. Likewise, at the other junction, electrons leave the metal with the lower chemical potential and travel to the wire with the higher potential. Because they are lacking in energy in this higher potential region, the electrons take in energy from the lattice to reach thermal equilibrium. This is evidenced as a cooling of the temperature (Nicholas & White, 2001). The Peltier effect can be seen schematically in Figure 3-17.
The Peltier Effect. When a current is applied across two dissimilar conducting materials bound together at a junction, a flow of electrons results. Electrons traveling across a junction may emit or absorb heat causing local heating or cooling (Pollock, 1985).

This junction effect uses an electric current to carry heat from one end to another. Utilizing this concept, Peltier was able to freeze a droplet of water from a thermocouple he built using antimony and bismuth. Today, Peltier devices are often built using doped semiconductors. These devices are commonly used to cool solid-state electronic circuits such as those used in computers (Pollock, 1985).

In the QCM/HCC, the inverse Peltier effect is relied upon for the detection of heat events. When a temperature difference exists across a thermopile plate with one end being kept at the reference temperature and the other end in contact with a reaction of interest, an output voltage is generated due to the temperature difference.

Where the Peltier effect describes changes in the heat at the junctions of the conducting material, the Thomson effect describes the heat content of the individual conductor. This effect will be explored next.
3.4.1.3 Thomson Effect

The last in this trio of thermoelectric properties is the Thomson effect and although it is important, it has the smallest magnitude of the three effects (DiSalvo, 2002). This effect involves a temperature gradient but is different from the Seebeck effect. In the Thomson effect, an existing temperature gradient is manipulated by the flow of an electric current. In a single conductor, an electric current can carry electrons from the lower temperature to the higher temperature region. The cooler electrons take some of the kinetic energy from the lattice thus cooling that portion and eventually attaining thermal equilibrium (Nicholas & White, 2001). The process is reversible depending on the current, making it possible to move the hotter electrons to the cooler area and accordingly warming this region of the conductor.

In Figure 3-18 the Thomson effect is described where, (a), a single conducting metal is displayed (Pollock, 1971). As a section of the material is heated at \( T_2 \), a temperature gradient is induced on either side of the heated portion. The two points shown, \( P_1 \) and \( P_2 \), on either side are of equal temperature with \( T_1 < T_2 \). If the conducting material is made into a circuit as shown in (b), electrons will flow in the direction of the current. In the above figure, the electrons flow in the direction from \( P_1 \) to \( P_2 \). Electrons moving from \( P_1 \) towards the heated region of \( T_2 \) are traveling against the area of greater temperature gradient (\( P_1 \)). In this case the electrons will absorb energy and likewise increase their kinetic energy. Electrons moving in the same direction as the temperature gradient from the heated region \( T_2 \), towards the cooler area, \( P_2 \), will give energy to the surroundings and decrease in their kinetic energy (Pollock, 1985). From Figure 3-18 (b), it can be seen that heat will be absorbed at \( P_1 \) while heat
will be liberated at \( P_2 \). These changes in the conductor heat content are known as the Thomson effect.

\[ T_1 \quad T_2 \quad T_1 \]
\[ \downarrow \quad \downarrow \quad \downarrow \]
\[ P_1 \quad P_2 \]

\[ \text{Conductor} \quad \text{Conductor} \]
\[ \text{Current Direction} \quad \text{Current Direction} \]

\[ \text{Heat Sink} \quad \text{Heat Sink} \]

**Figure 3-18.** The Thomson Effect. a) A single conducting element heated at \( T_2 \). At points 1 and 2, \( (P_1 \text{ and } P_2) \) the temperature, \( T_1 < T_2 \). b) A current is applied to the single conducting material. Heat is absorbed at \( P_1 \) where the electron current is opposite in direction to the heat flow. Heat is liberated at \( P_2 \) where the direction of the electron current is the same as the heat flow (Pollock, 1971).
The Thomson effects are equal and opposite in a single conductor and therefore cancel out. This canceling out gave birth to the Law of Homogeneous Conductors which states that the application of heat to a single homogeneous conductor cannot in itself maintain a thermoelectric current. It is when two dissimilar materials are paired together that the Thomson effects are unequal and a resulting current is seen (Pollock, 1971).

### 3.4.2 Thermopile Material Properties

The semiconducting material, bismuth telluride, Bi$_2$Te$_3$, is the thermoelectric material used most often in thermoelectric applications such as thermopiles. This semiconductor can be doped so that individual blocks have distinct negative characteristics, that is, an excess of electrons, N, or positive characteristics, that is, a deficiency of electrons, P. Because bismuth telluride’s thermoelectric properties are at a maximum between −10 and 130°C, it is widely used in thermoelectric applications (Ferrotec, 2002).

A gauge for good thermoelectric properties of materials is based on each material’s figure of merit, $Z$. This is shown in the relationship (Mzerd et al., 1995):

$$Z = \frac{e_s^2}{\rho \kappa}$$  \hspace{1cm} \text{Equation 3-22}

where $e_s$ is the Seebeck coefficient also known as the thermoelectrical power, $\rho$ is the electrical resistivity, and $\kappa$ is the thermal conductivity. When a current is applied across a junction, at equilibrium, heat is generated from two sources: thermal conductance and Joule resistive heating. Some of this excess heat is absorbed to compensate for the heat
generated. Each thermoelectric material is characterized by this ability to generate and absorb heat with this heat balancing being labeled as the material’s “figure of merit”. When two thermoelectric materials are joined, the Peltier coefficient of the junction is directly proportional to the magnitude of the difference between the Seebeck coefficients of the two materials (DiSalvo, 2002). When designing the most efficient thermoelectric device, it is best to have two materials whose figure of merit, $Z$, is as large as possible (DiSalvo, 2002; Jäckle, 2000). This can be difficult because a conducting material’s properties, such as the thermopower, electrical conductivity, and thermal conductivity, are intertwined, and changing one property oftentimes mean a change in the other properties. As a starting point, materials are chosen for their thermoelectric power, or Seebeck coefficient (DiSalvo, 2001). If two conducting materials have $G_c$ values that are large and opposite in magnitude, they are potential candidates for a thermoelectric device.

The thermopiles used in our QCM/HCC instrumentation are bismuth telluride semiconductors with the second electrically conductive material being copper. Usually each end of the semiconductor N or P-doped pellet is soldered to plated copper which also serves as the connection path to the data collecting instruments. The key concept in using semiconductor materials lies in the fact that heat is moved or pumped in the direction of charge carrier movement through the circuit (Ferrotec, 2002). An example of the charge flow is shown for an N-type and P-type semiconductor (Tellurex, 2002):
Figure 3-19. A schematic diagram of the charge carrier flow and heat flow in an N-type and P-type semiconducting pellet. a) In an N-type semiconductor, electrons are the charge carrier and are responsible for the Peltier effect. b) In P-type semiconducting elements, the charge carriers are known as “holes”. The hole current flows in an opposite direction from the electron flow (Tellurex, 2002).

In an N-type semiconducting element, electrons are the charge carriers and primarily responsible for the Peltier effect. When a DC voltage source is applied, the electrons are repelled by the negative pole and attracted to the positive pole. From the diagram, the electrons then flow in the clockwise direction with heat being absorbed at the bottom of the plate and released at the top of the plate. In a P-type semiconducting material, the hole flow carriers the positive charge. The holes are repelled by the positive end of the DC source and flow towards the negative end of the DC source. The flow in this case proceeds in the counter-clockwise direction. The heat in this case is drawn towards the negative end of the voltage source.
From the above diagram, it would seem possible to construct a thermoelectric device with only one type of semiconducting material. A few problems arise when attempting to use one type of material. One semiconducting pellet cannot pump an appreciable amount of heat; so many semiconducting pellets are often used in a device, typically up to 254 pellets. If using one type of semiconducting material, e.g. all N-type pellets, two possible types of connections are: (1) connecting the pellets in parallel thermally and electrically, (2) connecting the pellets in parallel thermally but in series electrically. In the first case for the pellets connected in parallel electrically, problems arise because of the small voltages and high current involved with each pellet’s capabilities. One semiconducting pellet can draw five amps or more with only 60 mV applied. In a typical configuration, this would mean the device would draw more than 1000 amps when only 60 mV are applied. In the second case, the semiconducting pellets are wired in series electrically but in parallel thermally. In this case, the connections between pellets introduce thermal shorting that decrease the performance of the device (Tellurex, 2002).

Researchers have found that the best configuration is to arrange the N and P-type pellets in a couple. A plated copper tab is then used to form a junction between them. Using these methods, a series circuit is constructed which allows for the heat to be moved in the same direction through each N and P-type pellet. An example of three joined thermocouples is shown in Figure 3-20 (Tellurex, 2002).
Figure 3-20. N and P-type semiconducting pellets connected as a couple with a plated copper tab forming the junction. The pellets are coupled in series electrically and in parallel thermally. The free end of the P-type pellet is connected to the positive voltage potential while the free-end of the N-type is connected to the negative end. It is the charge carrier inherent in each semiconducting material that determines the heat flow (Tellurex, 2002).

In the figure, the free end of the N-type semiconductor is connected to the negative pole of the voltage potential while the free end of the P-type semiconductor is connected to the positive pole of the voltage potential. It is significant to note that the charge carriers inherent in each semiconducting material are responsible for the flow of heat. Therefore, when looking at the flow of electrons, these charge carriers are repelled by the negative potential but attracted to the positive potential. The electrons flow through the N-type material through the copper tab and through the holes within the P-type pellet eventually ending up at the positive pole (Tellurex, 2002). Even though the electrons are flowing through the P-type pellets, they do not carry heat at this point because the hole carriers are responsible for this in the P-type material. In this
configuration, the heat and charge carrier flows are in the same direction allowing for appreciable amount of heat to flow through the material. Common thermoelectric devices utilize 254 alternating N and P-type pellets connected in this fashion. If used as a heat pumping device, the unit can run on a 12 to 16 V DC supply while drawing only 4 – 5 amps (Tellurex, 2002).

When connecting multiple semiconducting couples, it is important to electrically insulate the flow of charge. This is done in the manufacturing process and is shown schematically in Figure 3-21 (Melcor, 2002).

**Figure 3-21.** A cross section of a typical multi-couple configuration used in thermoelectric devices. Bismuth telluride is used as the N and P-type semiconducting pellets. Plated copper is used as the electrical conductor between the pellets. Ceramic substrates are used on both top and bottom, serving both as a layer of insulation and as a mechanical support for the pellets. (Melcor, 2002).
To mechanically hold the device together, typically a ceramic substrate is used as an interface between the thermocouples and the interacting body. Ceramic materials are chosen for this task because of the combined properties of mechanical strength, electrical resistivity, and thermal conductivity.

3.4.3 Tian Equation

When used in a calorimeter, thermopiles are placed in close contact with the reaction vessel. In our QCM/HCC instrumentation, the thermopiles are in direct thermal contact with the brass electrodes upon which sits the QCMs. The second sides of the thermopiles are in contact with a large aluminum heat sink. Any heat effects due to vapor sorption by a thin film on a QCM travel through the thermopiles to the heat sink. When at steady state, the heat flow from the event is directly proportional to the thermopile potential, $U$. The thermal power is (Wadsö, 1997):

$$ P = \varepsilon U \quad \text{Equation 3-23} $$

with $\varepsilon$ being the calibration constant (W/v). In an ideal situation, $\varepsilon$, is the quotient between the average thermal conductance of the thermocouples forming the thermopile, $G / \text{WK}^{-1}$, and the Seebeck coefficient for the thermocouple material, $e / \text{mVK}^{-1}$ (Wadsö, 1995):

$$ \varepsilon = \frac{G}{e} \quad \text{Equation 3-24} $$
Therefore, from Equation 3-23, the rate of heat absorbed or released, $P$, is related to the heat quantity, $dQ$, during a time $dt$:

$$dQ = \varepsilon UdtdQ = \varepsilon Udtd$$

Equation 3-25

Similarly, for an ideal situation, the heat flow rate is (Bäckman et al., 1994):

$$\frac{dQ}{dt} = G_c n \Delta T$$

Equation 3-26

where $G_c$ is the thermal conductance of an individual thermocouple, $n$ is the number of thermocouples used to compile the thermopile, and $\Delta T$ is the temperature difference over the thermopile. The thermopile potential is then shown by:

$$U = n e \Delta T$$

Equation 3-27

where $e$ is the Seebeck coefficient and as stated above, is a material constant for the thermocouples. In a non-steady state process such as in a fast reaction, it becomes necessary to account for the time constant of calorimeter. In a non-steady state process, the thermopile potential is related to the thermal power through the relationship:

$$P = \frac{dQ}{dt} + C\left(d \frac{dU}{dt}\right)$$

Equation 3-28
when Equation 3-28 is combined with Equations 3-26 and 3-27, the relationship is shown as the Tian equation (Bäckman et al., 1994):

\[
P = \varepsilon \left[ U + \tau \left( \frac{dU}{dt} \right) \right]
\]

**Equation 3-29**

with \( \tau \) being the time constant of the instrument and is equivalent to:

\[
\tau = \frac{C}{G \varepsilon n}
\]

**Equation 3-30**

In **Equation 3-30**, \( C \) is the heat capacity of the reaction vessel, its contents in addition to the heat capacity of half of the thermopile. The time constant, \( \tau \), can be thought of as the time it takes for the thermal signal to go from zero to 63% of the maximum output value when a constant thermal power is applied to the reaction cell (Wadsö, 1998). The time constant for the QCM/HCC is ~ 53 seconds.

In our sorption experiments using the QCM/HCC, the sorption events reach a steady state and **Equation 3-23** is employed to convert the thermopile potential, \( U \), to the thermal power, \( P \). The calibration coefficient, \( \varepsilon \), is found using an electrical calibration procedure and will be discussed in the next section.

### 3.4.4 Calibration of Thermopiles

When measuring heats in the microwatt range, it is important that the thermal sensing device is correctly calibrated. Calibration in this sense is a measure of the watts being measured per output voltage of the thermopile sensor. In the Tian equation
above, the calibration coefficient is denoted, \(\varepsilon\), and in units of Watt/volt this term is akin to the thermopile detection sensitivity, \(S = 1/\varepsilon\), measured in volts/Watt. Because of the small quantities of heat involved, an incorrect calibration can cause large deviations in the experimental results.

Many commercial calorimeters employ electrical calibration techniques where a known power is generated electrically and the heat output is then measured. Electrical calibrations are convenient because of the accuracy of the heat generated and the ease of use. A word of caution is frequently noted however; serious calibration errors can arise if the heat flow pattern of the calibration method is different from that of an actual experiment (Briggner & Wadsö, 1991). Several factors can bring about these errors, such as, a non-ideal design of the reaction vessel and heater, the position of the heater and the thermal conductance of the reaction vessel (Wadsö, 2000). In an ideal situation, where the heat flow passes uninterruptedly and through a direct path through the thermopiles and to the heat sink, the ideal thermopile sensitivity, \(S_{id}\), is approximated as (Briggner & Wadsö, 1991):

\[
S_{id} = \frac{\varepsilon}{G_c}
\]

Equation 3-31

where \(\varepsilon\) is the Seebeck coefficient and \(G_c\) is the thermal conductance per thermocouple plate as defined above. From the above equation it is noted that \(S_{id}\) is independent of the number of thermocouples in the thermopile for a given type of thermocouple plate.

Depending on the construction of the microcalorimeter, it is possible that a good portion of the thermal power produced from the reaction of interest will not pass
through the thermopile. Instead, the thermal power may reach the heat sink by avenues other than through the thermopile, such as through the mechanical supports, electrical leads, and air gaps. In fact, the heat flow passing through the thermopile may be even less than 80% of that produced in the reaction (Bäckman et al., 1994). The experimental thermopile sensitivity, $S_{\text{exp}}$, is defined as the calorimeter’s response to a given heat flow rate. Therefore, it is possible to get an estimate of the fraction of heat flow, $f_h$, that does not pass through the thermopile:

$$
 f_h = \frac{(S_{\text{id}} - S_{\text{exp}})}{S_{\text{id}}} 
$$

\textbf{Equation 3-32}

An electrical calibration that closely imitates the heat flow of a reaction can eliminate such systematic errors (Nicholas & White, 2001). It is noted that for an electrical calibration, the calibration heater should be in close thermal contact with the content of the reaction vessel (Wadsö, 2000). Ingemar Wadsö has explored various test reactions as possible chemical calibration reactions (Kemp & Lamprecht, 2000; Wadsö, 2000). These have been shown to provide a good correlation between the heat released in a reaction and the calibration procedure. Wadsö does note that dependable test reactions are lacking in the areas of sorption processes such as adsorption and absorption of gases and solutes on solid materials (Wadsö, 2000).

In our QCM/HCC studies we utilize an electrical calibration procedure for our gas sorption studies on thin solid films. Our calibration procedure does meet the requirement that the calibration heater is in close thermal contact with the content of the reaction vessel. In our case the calibration is performed on the reaction vessel itself, the
QCM. As the Maxtek PLO drives the QCM oscillation, it generates a known power in the crystal. The voltage output of this power can then be measured to determine the calibration coefficient, $\varepsilon$, in units of Watts/volt. The electrical calibrations performed this way exactly match the heat flow of each experiment.

Using the Maxtek Phase Lock Oscillator, an output voltage is given which is proportional to the conductance. The power generated in the crystal by the oscillator is a well-defined function of the conductance voltage. A general form of the equation used to calculate the power generated in the crystal is:

$$P_{\text{crystal}} = (V_{\text{soc}})^2 \left(G_t \left(1 - \frac{G_1}{G_s}\right)\right)$$  \hspace{1cm} \text{Equation 3-33}

where $V_{\text{soc}}$ is the open circuit crystal drive voltage, $G_t$ is the total conductance of the crystal in series with the source resistance and is shown by:

$$\frac{1}{G_t} = \frac{1}{G_c} + \frac{1}{G_s}$$  \hspace{1cm} \text{Equation 3-34}

$G_t$ can also be expressed as being equivalent to the conductance voltage divided by a conversion constant $K$, or $V_{\text{cond}}/K$ where $K = 100$ v/siemen. $G_s$ is the source conductance equivalent to 1/source resistance. For the PLO, the source resistance is 20 $\Omega$, therefore $G_s = 0.05$ siemens. $G_c$ is the crystal conductance or $1/$crystal resistance. The power generated in the crystal is 0 Watts when the $V_{\text{cond}}$ is at 0 volts or 5 volts. At 5 volts, $V_{\text{cond}}$ output represents a crystal with 0 resistance.
To determine the power generated in the crystal, the following equation is used, based on the general form shown above:

$$P_{\text{crystal}} = (V_{\text{soc}})^2 \left( \frac{V_{\text{cond}}}{100 \text{v/siemen}} \right) \left( 1 - \frac{V_{\text{cond}}}{5V} \right)$$  \hspace{1cm} \text{Equation 3-35}

where $V_{\text{soc}}$ is the open circuit crystal drive voltage, $V_{\text{cond}}$ is the conductance voltage. For the low powered PLO, $V_{\text{soc}} = 0.020 \text{ v}$ and for the regular powered PLO, $V_{\text{soc}} = 0.125 \text{ v}$.

Calibration of the thermopiles is routinely performed in our measurements with the procedure frequently being performed before and after each experiment. Actual data for some of the calibrations will be shown in the Chapters 5 and 6 regarding polymer and protein films.

3.5 Conclusion

In this chapter the QCM/HCC instrumentation has been introduced and discussed. The two sensitive sensors, a quartz crystal microbalance for the mass measurements and semiconductor thermopiles for the heat sensors are shown with their basic properties and theory behind their sensing capabilities.

The use of QCM’s as mass sensing devices is a well-established technology. An added, thin, rigid film on a QCM oscillates with the QCM but decreases its initial resonant frequency. The Sauerbray relationship describes this effect and allows for the determination of the mass. Complexities arise when a film is thick and viscoelastic. In this case, the shear storage and loss moduli change upon vapor sorption.
acoustic wave through the film can be phase shifted and attenuated. The simple mass relationship does not apply in this case. Some studies into this area were presented.

With the addition of a phase lock oscillator for driving the crystal, two additional pieces of information are obtained: the motional resistance and the power generated in the crystal upon oscillation. The PLO yields an output voltage proportional to the conductance of the oscillating quartz and film. From this output voltage we are able to extract the motional resistance of an oscillating film and monitor how this changes upon vapor sorption. The PLO allows us to determine the power generated in the crystal. Using this value, we are able to conveniently calibrate the thermopiles used to measure the heat effects.

The basic operating principles behind thermopiles have been presented. They find widespread use in calorimetric applications. For our thermal signals, the Tian equation is employed to convert the thermopile output voltage into the thermal power, \( P \).
List of References


Chapter 4. Thermodynamic and Rheological Properties of Thin Films Interacting with Solvent Vapors Studied Using the QCM/HCC

4.1 Sorption Processes

The QCM/HCC allows for very sensitive and simultaneous mass (typically $\pm 10$ ng), thermal ($\pm 50$ nW), and motional resistance ($\pm 0.5 \Omega$) measurements of vapor sorption by a thin film. Studies in this area of solid/gas phase interactions of thin films find applications in the biological and chemical sensor area (Grate & Abraham, 1991), microlithography of silicon chips (Buchold et al., 1999; Buchold et al., 1998), food preservation (Matveev et al., 2000; Schiraldi et al., 1999), pharmaceutical coatings and drug delivery studies (Ford & Willson, 1999; Sharp et al., 2001; Thompson, 2000), wear-resistant coatings and paints (Cannon & Pethrick, 1999; Petersen et al., 1999), and the effects of sorption upon pipeline linings, seals and flexible hoses (Hilic et al., 2001).

In the applications presented here, the solid is in the form of a thin film generally between 1 - 9 $\mu$m. The thin film is referred to as the adsorbent or solvent. The interacting sorbed gas molecules are the adsorbate or solute particles. The sorption of vapor molecules from the gas phase into an adsorbent thin film on a QCM is shown schematically in Figure 4.1 (Grate, 2000).

![Figure 4-1. Vapor sorption on a thin film. a) QCM, b) adsorbent thin film (1 – 9 $\mu$m), c) adsorbate vapor introduced in the chamber containing the QCM and adsorbent film (Grate 1991.)](image-url)
When using the QCM/HCC to study these interactions, the adsorbate vapor activity that is in contact with the thin film is controlled and scanned throughout an experiment. By varying the vapor activity, it is possible to measure: (1) the repeatability of the sorption/desorption processes, (2) the rheological effect the sorption process has on the film and (3) the magnitude of the heat effects.

In a thin film, the distribution of the moles of gas molecules, \( n_a \), adsorbed per gram of solid is a function of the pressure (\( P \)) and the temperature (\( T \))(Adamson & Gast, 1997).

\[
n_a = f(P, T) 
\]

Equation 4-1

The experiments presented here using the QCM/HCC were done under isothermal conditions; the temperature is constant and the amount of sorbed vapor is dependent on the controlled vapor pressure that comes in contact with the thin film. Several factors influence the uptake of gaseous vapors by a thin film including the thermodynamics of the mixing interaction and the many-chain relaxation effects in polymer-like films due to osmotic stresses (Sharp et al., 2001). Using the QCM/HCC, we are able to probe the thermodynamics of vapor sorption, and from the uptake of the vapor, we can determine the diffusion coefficient. Also, by measuring the motional resistance of the film upon vapor sorption, we are able to gain insights into the relaxation effects taking place in the polymer film.

Sorption can be classified into two general categories: physisorption and chemisorption. Some of the results of polymer relaxation upon vapor sorption include a
lowering of the glass transition temperature, plasticization, and swelling. These effects will be discussed briefly here.

4.1.1 Physisorption

Physisorption (short for physical adsorption) of gas molecules below their critical temperature are generally characterized by van der Waals interactions, such as dispersion or dipole interactions, with the solid surface. These interactions are distinguished by the size and nature of the adsorbent-adsorbate interactions as well as the interactions between adsorbate molecules. Two factors concerned with this type of interaction include the degree of heterogeneity of the surface and the level of translational and internal degrees of freedom possessed by the adsorbed molecules (Adamson & Gast, 1997). Although the range of Van der Waals interactions is considerably far-reaching, the interaction energies are weak. When a particle is physiosorbed, adsorption occurs because of the nonspecific intermolecular forces responsible for the condensation of a vapor to a liquid. The released energy is expected to be of the same order of magnitude as the enthalpy of condensation (Atkins, 1998).

Molecules being physisorbed to a surface undergo a process named accommodation. The small energies of the gaseous adsorbate interacting with the thin film adsorbent are absorbed as vibrations in the absorbent lattice and dissipated as thermal motion. A molecule bouncing on the surface of the adsorbent eventually loses energy and finally adsorbs to the surface. It is noted however, that the surface structure of a molecular solid (such as a polymer) adsorbent may be altered during the process of physisorption. This altering of the surface structure is not seen on a surface of high surface energy, refractory solids (for example metals, metal oxides, and carbon black) but may be seen
on surfaces such as polymers (Adamson & Gast, 1997). Equilibrium is rapidly attained in physical adsorption, limited only by mass transport rates in the gas phase or by a porous solid. Physisorption is also reversible with the reverse occurring when the surrounding pressure is lowered. This type of sorption is what appears to be seen in our studies of sorption by polymer films as shown in Chapter 5.

4.1.2 Chemisorption

Chemisorption (or chemical adsorption) interactions are characterized by much greater enthalpy magnitudes than those seen in physisorption processes. Molecules involved in chemisorption adsorb to a surface by forming a covalent bond and finding sites that maximize their coordination with the substrate. Because of the stronger interaction between adsorbate and adsorbent, the distance between the adsorbing molecule and the surface is usually shorter than that observed in physisorption (Atkins, 1998). Although some surface diffusion or mobility may occur, the adsorbate is generally localized at a particular site. As with physisorption, chemisorption may alter the surface structure of the adsorbent during the adsorption process. A chemical reaction occurring at the surface of the absorbent, in contrast to physisorption, may even modify the surface of a molecular solid as well as cause surface restructuring of refractory solids (Adamson & Gast, 1997). Chemisorption is not characterized by one type of rate and may occur quickly or slowly. The rate behavior of a slow chemisorption process gives some insight into the presence of the corresponding activation energy. Usually chemisorbed gases are difficult to remove and desorption usually brings about chemical changes. In the hydration studies of proteins presented in Chapter 6, the enthalpy of hydration at lower water vapor activities is greater than the
enthalpy of vaporization for water. The enthalpy values in this case are not attributed to chemisorption but to water binding to the charged or polar groups on the protein surface.

4.1.3 Plasticization

Organic or water vapor being sorbed in a film will cause some slight structural and relaxation effects in the properties of the film. Sorbed vapors and liquids act as plasticizers in some materials, especially in polymer and proteins. In their classic work on this topic, Sears and Darby define a plasticizer as “a material incorporated in a polymer to increase the polymer’s workability, flexibility, or extensibility (Sears & Darby, 1982).” In their comprehensive description of plasticization, the authors point out that humans have long relied on plasticization technology from using water to plasticize clay for pottery and tablets, to soaking rawhide in water in order to swell, shape and tool it before hardening. In fact, the plasticizing effect of water is necessary in controlling the hydrogen bonding in proteins, which otherwise would have a dry, tough consistency. Industrial and consumer needs have pushed plasticizer technology so much so that rarely is a polymer used alone. Most applications of polymers include some plasticizer to gain specific flexibility, damping effects, and resistance to mechanical shocks.

Two types of plasticization may occur, external or internal. External plasticization is so named because the material added to the polymer does not chemically combine with it. Internal plasticization occurs when the polymer itself has been chemically modified to produce the desired plasticized effects (Sears & Darby, 1982). Both types of plasticization bring about three main effects in the polymer material, namely a lowering
of: the melt viscosity, the second-order transition, and the elastic modulus. Our studies of protein and polymer films using the QCM/HCC fall into the category of external plasticization.

A few theories have surfaced in trying to describe the plasticizing effect on polymers. On the molecular level it is believed that select bonds between molecules are weakened or even broken upon plasticization, while other bonds actually remain strong. With the weakening and breaking of some bonds, the intermolecular spaces (free volume) increase allowing for a greater flexibility of the material. This has led to the “free volume theory” which started as a description to explain what lies between the molecules and atoms, that is, “nothing.” As Sears and Darby point out, this “nothing” between molecules has since been measured, subdivided into types, utilized in explaining flow phenomena, and even placed into thermodynamic equations (Sears & Darby, 1982)!

The free volume, \( V_f \), existing in a crystal, glass, or liquid is often defined as the difference between the free volume at absolute zero and the volume for the crystal, glass, or liquid at a given temperature. This is shown in Equation 4-2:

\[
V_f = V_t - V^o
\]

where \( V_t \) is the specific volume (cc/g) at a temperature, \( t \), and \( V^o \) is the specific volume at absolute zero. Problems arise in trying to determine the free volume at absolute zero. Measurements are not easily made in this region. As a noncrystallizing liquid or rubber is cooled, a slow, linear volume decrease is seen with decreasing temperature. Once the
glass transition temperature, $T_g$, has been reached, the material becomes glassy and the volume decrease continues however, the amount of change per °K is smaller than above the $T_g$. Because no mass is removed, the volume change is attributed solely to a decrease in the space between the atoms and molecules. To determine the free volume at absolute zero, extrapolating the volume decrease of the liquid/rubbery volume to absolute zero leads to a specific volume, $V^o_L$, which is too small to describe the material. Likewise, extrapolating the volume decrease of the glassy portion leads to a specific volume, $V^o_G$, that is too large. The theory states that the hypothetical, real specific volume, $V^o$, lies between these two extrapolated specific volumes (Gregory, 1995) (Forrest & Jones, 2000). At absolute zero, it is believed that instead of an ideal lattice structure, there exists some “frozen in” free volume. Therefore, at absolute zero, the free volume has two contributing factors: (1) anharmonic oscillations of the atoms and (2) frozen in imperfections and holes. The linear coefficient of expansion, $\alpha$, defined as the change in length per unit length per °C, is most affected by the anharmonic atomic oscillations, with additional contributions from the “frozen in” free volume. The coefficient of expansion, $\alpha_o$, of an ideal material cannot be measured but it can be assumed that it is less than the coefficient of expansion of the glass, $\alpha_G$. Above the $T_g$, the material is characterized as being rubbery with the molecules possessing enough energy to move, bend and rotate. These molecular motions produce an increase in the free volume with the coefficient of expansion of the rubber/liquid, $\alpha_L$, on the order of $10^{-3}$ cc/cc · °K. This $\alpha_L$ is an increase of 2-3 times that of the $\alpha_G$ (Sears & Darby, 1982).
Increased molecular motion of polymers occurs when there is an increase of the hole free volume. Plasticization can be seen as a study of ways to increase the free volume which stems from three contributing factors, namely: (1) chain end motions, (2) side chain motions, (3) main chain motions. In the studies presented here, a compatible lower molecular weight compound is sorbed into the thin film and acts as if it is increasing the three motions mentioned above.

Plasticization is complex but generally can be accounted for in six steps with some of these steps broken even further into substeps. In general these steps are (Sears & Darby, 1982): (1) wetting (2) solvation and penetration of the surface, (3) diffusion and initial swelling, (4) disassociation and freeing of polar groups; dissolution of the amorphous region, (5) structure breakdown, diffusion and dissolution of some of the crystalline regions, (6) reestablishment of the structure.

Some generalization can be drawn about good plasticizers, mainly that (Roland, 1998; Sears & Darby, 1982): (1) the smaller the plasticizer molecule, the more effective the plasticizer is in lowering the $T_g$, (2) the plasticizer efficiency is proportional to the $T_g$ of the plasticized polymer, meaning the higher the $T_g$ of the material, the greater the effect of the first small amounts of the plasticizer while increasing amount become less effective and (3) a good plasticizer is a bad solvent, in other words the smaller the affinity between plasticizer and polymer in comparison to the polymer-polymer affinity, the more efficient the plasticizer.

In our studies of vapor sorption of polymer and protein films, the recorded change in motional resistance gives a glimpse of the relaxation and free volume effects occurring in the film upon vapor sorption.
4.1.4 Glass Transition Effects

For most liquids, when cooled, some type of solid is formed, formally defined as a material with a non-zero shear modulus. This solid phase is most often crystalline in structure and characterized by a repeated unit cell throughout the material thus giving long range order to the crystal. The transition from the liquid to the crystalline state occurs at the melting temperature, $T_m$, and is labeled a first order phase transition (Forrest & Jones, 2000).

Of course not all materials fit neatly into this category. In some cases, upon cooling, a material may also have a non-zero shear modulus, yet possess no long-range order among the molecules. This type of material is best described as a glass rather than as a crystal. A glassy state occurs when either the constituent molecules lack an intrinsic order inhibiting crystallization, or the cooling rate is too high thus rendering insufficient time for the molecules to achieve the long-range order characteristic of crystals (Plazek & Ngai, 1996).

The glass transition region of amorphous polymers is simply defined as a liquid melt or rubbery material becoming a glass (without crystallization) upon either a temperature decrease or extraction of the solvent (adsorbate in our studies presented here). This is usually characterized by a few tell-tale signs such as: (1) an increase in viscosity by almost 15 orders of magnitude, (2) a discontinuity in the coefficient of thermal expansion, $\alpha$, (3) a discontinuity or peak in the heat capacity, $C_p$, (4) a sizeable decrease (~3 orders of magnitude) in the shear modulus, $G$ (vide infra) (Aklonis & MacKnight, 1983; Donth, 2001). A simple definition such as this is deceiving and many comprehensive works have discussed the topic of the glass transition in attempting to
clear up the many misunderstandings and define the sometimes ambiguous terms (Aklonis & MacKnight, 1983; Donth, 2001; Ferry, 1980; Plazek & Ngai, 1996).

Although the glass transition resembles a second order phase transition, investigators are hesitant to label it as a true phase transition. A phase transition occurs at a single temperature whereas a glass transition occurs over a region rather than at a single temperature and it is often dependent on the experimental time-scale as well as the sample’s thermal history. The transition is seen more as a kinetic transition because the translational degrees of freedom due to diffusion cannot be equilibrated on the time scale of the experiment. Kinetically, these contributions to the thermodynamic quantities are “frozen out” (Aklonis & MacKnight, 1983; Forrest & Jones, 2000). A schematic picture of the glass transition range is shown in Figure 4-2 (Menard, 1999):

![Figure 4-2](image)

**Figure 4-2.** A schematic plot of an ideal dynamic mechanical analysis scan of a polymer sample. As the temperature is increased the corresponding changes in the shear storage modulus, $G'$, a measure of the molecular relaxations of the polymer material, is plotted. The transitions are labeled as: 1) $\delta$ transition - local motions, 2) $\gamma$ transitions - bending and stretching motions, 3) $\beta$ transition - relaxation of side groups, 4) $\alpha$ or $T_g$ region, gradual main chain relaxation, 5) rubbery region, large scale chain movements, 6) melt, $T_m$ characterized by chain slippage (Menard, 1999).
The relaxation process that takes place in the liquid-glass transition is named the $\alpha$-relaxation and is characterized as the coordinated motion of 50 – 100 carbon atoms and the associated substituent groups about the chain axis. To add to the complexity, it is believed that sub-$T_g$ relaxations also occur. These sub-$T_g$ relaxations are labeled as $\beta$, $\gamma$, and $\delta$, in order of decreasing temperature. Each of these names the motions of progressively smaller molecular units with lower activation energies (Fried, 1996).

Glass transitions are measured by a variety of methods; however, it is difficult to compare data of various research groups because each experimental method yields a different value for the $T_g$. And to make matters even more complicated, when using the same method, different results may be obtained depending on the time scale of the measurement (Aklonis & MacKnight, 1983). Common methods used to measure the glass transition include: (1) dilatometric methods where the thermal expansion coefficient, $\alpha$, is found by measuring the displacement of a confining fluid surrounding the polymer, (2) differential scanning calorimetry, DSC, where a polymer sample is heated at a defined rate and the $T_g$ is marked by a discontinuity in the heat capacity, $C_p$, (3) measuring the temperature dependence of the viscosity using creep or stress relaxation techniques, (4) measuring abrupt changes in the refractive index, (5) measuring an abrupt line width narrowing in an NMR scan.

In our studies using the QCM/HCC, the relationship between the change in QCM oscillation frequency and the corresponding change in mass is dependent on the density, thickness and rigidity (the shear storage and loss moduli, $G'$, $G''$) of the film. As a polymeric film sorbs a gaseous adsorbate, the properties of the film change. It is important to know whether the film is in a glassy/rigid state thus falling in the
gravimetric regime of the QCM measurements or whether the film has crossed over into a rubbery state, the non-gravimetric regime of the QCM. The variables that affect the glass transition pertaining to our experimental procedure include the $T_g$ dependence on the frequency at which the measurements are made and the effect of adsorbate concentration introduced to the film. These two aspects, frequency and adsorbate concentration, and their effect on the $T_g$ will be discussed briefly. Certainly the temperature at which the $T_g$ is measured is important, however, our measurements using the QCM/HCC are made under isothermal conditions so the temperature is held constant.

4.1.4.1 $T_g$ Dependence on the Probing Frequency

The time scale used in measuring the $T_g$ of a polymer greatly influences the resulting $T_g$ value. A simple example exists in looking out an antique glass window. Often the bottom of the window appears to be much thicker than the top. Over the time span of decades of years, gravity has played an effect and the glass has acted as a viscous liquid within this long time frame. On the time scale of a day however, no viscous flow is observed and the glass appears as a rigid, hard material. When very short time ranges are used, the polymer molecules may not have enough time to reorient themselves. They react to a stress by distorting molecular distances and the high energy involved in this leads to a corresponding high modulus or measure of rigidity. Longer time ranges permit the polymer to reorient the chain segments and relieve the strains imposed by the stress. The strained chains relax to lower energy conformations and cause a decrease in the measured modulus (Aklonis & MacKnight, 1983). Without stating the time scale of the measurement, reported $T_g$ values can be misleading.
Most of the above-mentioned methods used to measure the $T_g$ are obtained with the polymer being perturbed at relatively low frequencies (e.g. 10 Hz to the kHz range) (Brodt et al., 1995; Ferry, 1980; Fitzgerald et al., 1998). The quartz crystal microbalance, used in our measurements, oscillates at a much higher frequency, 5 MHz. The film cast on the QCM is subject to this oscillatory motion which induces a shear stress in the film. The shear modulus, $G$, is a measure of the rigidity or elasticity of the film in response to the shear stress (vide infra). This marked increase in the probing frequency does have an influence on the film and increases the $T_g$ (Donth, 2001). It is noted that generally a one-decade frequency increase in the measurement technique is equivalent to an approximate 6 – 7°C increase in the $T_g$. The temperature increase depends on the corresponding activation energy for the relaxation process (Gregory, 1995; Sperling, 1990). An example of this is the $T_g$ of the polymer polyisobutylene (PIB). The $T_g$ for PIB films when measured at low frequencies is –68°C but the $T_g$ when measured at 100 MHz using a surface acoustic wave device (SAW) is 40°C (Martin et al., 1994).

In using the QCM to measure sorption properties of polymer and protein films, it is helpful to note the $T_g$ values at low frequency so that a starting approximation can be made for the $T_g$ at 5 MHz.

4.1.4.2 $T_g$ Dependence on Amount of Sorbed Adsorbate

As a diluent is added to a polymer, the polymer $T_g$ noticeably decreases. This effect is even more pronounced when the diluent has an even lower $T_g$ than the polymer (Plazek & Ngai, 1996). An example of the diluent influencing the $T_g$ includes the hydration of polyamides, the polymer used in Nylon. The $T_g$ of several polyamides
(Nylon6, Nylon 6,6, Nylon 4,6) with no added water is $\sim 67^\circ C$. When water is added to a hydration level of $0.10 \text{ g}_\text{water}/\text{g}_\text{polymer}$, the $T_g$ is lowered to $\sim -33^\circ C$. This is an $80 – 100^\circ C$ change in the $T_g$ in a relatively small hydration range (Gregory, 1995).

In his comprehensive review on the hydration effects and glass transition behavior of proteins, Gregory shows that proteins follow this same dependence namely that upon hydration a protein sample will undergo a decrease in $T_g$ (Gregory, 1995). The protein lysozyme is used as an example and data is presented for lysozyme in both the denatured and native states. A graph is presented showing that at a specific temperature, upon increasing hydration, the protein sample changes from the rigid state to a flexible state. For lysozyme at 298K, this transition occurs at a hydration of $0.12 \text{ g}_\text{water}/\text{g}_\text{protein}$. Water in this case does act as a plasticizer on proteins in the same way as it acts on polymers. Hydration water provides alternative conformational pathways by adding mobile hydrogen bond donors and acceptors. As a result, the barriers between conformations states are decreased and conformational transitions are permitted to occur at lower temperatures (Gregory, 1995).

Vapor induced softening could lead to non-gravimetric responses of the QCM (Domack & Johannsmann, 1996; Martin et al., 1994). In using the QCM/HCC it is important to trace how the added diluent is affecting the thin film under study. Because the QCM/HCC operates at 5 MHz while most $T_g$ values are measured at much lower frequencies (e.g. 10 Hz), the higher frequency measurement increases the initial $T_g$ value, but the addition of a plasticizer has the opposite effect and lowers the $T_g$. These two opposing forces factor into the QCM/HCC measurements. The measured motional
resistance provides a window for us to observe the effects of a plasticizer on the properties of the thin film being studied.

4.1.5 Swelling Effects

The effects of swelling in polymer samples have seen considerable research such as (Chuang et al., 2000; Hilic et al., 2001; Meier et al., 1999; Sharp et al., 2001; Sirard et al., 2001) to name just a few studies. Upon vapor sorption, a polymer often undergoes a volume expansion defined as a swelling. It is difficult to make generalizations about polymer sorption and swelling because these processes are specific for each type of polymer and depend on the processing conditions. Researchers note that changes in the processing of a polymer such as film thickness and annealing sequence may cause as much as a 20% change in the sorption and swelling of a particular polymer (Buchold et al., 1999). Swelling of a polymer is determined by three factors: (1) the mean pore density in the polymer film, or the number of sorption sites, (2) the mean pore volume which gives a probability of whether a molecule of a certain volume will fit in a pore, and (3) an affinity between the polymer and adsorbing gas molecule (Buchold et al., 1999).

As a polymer swells, changes in the polymer shear modulus occur and affect the QCM mass measurements of a thin polymer film. Some models of vapor sorption of a polymer film depict three distinct regions within the film: a swollen plasticized region at the interface where vapor sorption occurs, (2) a swelling front that propagates through the polymer, and (3) a glassy region where the solvent front has not yet reached (Sharp et al., 2001).
Because of the difficulty in directly measuring the shear modulus from quartz resonator studies, some investigators have chosen to estimate the volume increase in a polymer film to determine the swelling and the effect this has on the gravimetric measurements of quartz resonators such as QCM and surface acoustic wave, SAW, devices (Grate et al., 1992; Grate & Zellers, 2000; Martin et al., 1994).

Upon plasticization of a polymer by an absorbed gaseous diluent, the fractional free volume, \( f \), of the polymer adsorbent/diluent adsorbate at a temperature, \( T \), is defined as (Ferry, 1980):

\[
 f(T, v_1) = f_2(T) + \beta' v_1 \quad \text{Equation 4-3}
\]

where \( f_2 \) is the polymer free volume, \( v_1 \) is the volume fraction of diluent, and \( \beta' \) is a parameter relating the diluent volume fraction to the free volume. The volume fraction is calculated using the following assumption (Grate & Zellers, 2000):

\[
 V_v/V_s \sim V_v/(V_s + V_v) \quad \text{Equation 4-4}
\]

where \( V_s \) is the volume of the adsorbent polymer phase and \( V_v \) is the volume of the adsorbate liquid vapor. Because the fractional free volume, \( f \), is not additive it is dependent on the volume composition (Ferry, 1980):

\[
 f = f_1 v_1 + f_2 v_2 + k_v v_1 v_2 \quad \text{Equation 4-5}
\]
where the subscript 1 refers to the diluent or sorbed vapor in this case and the 2 refers to the polymer film, free volumes are denoted by $f$, and volume fractions are shown as $v$. In studies where very small amounts of diluent are added to the film such as in our QCM/HCC measurements, the parameter $\beta'$ is given by:

$$\beta' = f_1 - f_2 + k_v'$$  \hspace{1cm} \textbf{Equation 4-6}

Because the last two terms in \textbf{Equation 4-6} are small, $\beta'$ is essentially equal to $f_1$ and is reported to be in the range of 0.1 – 0.3 (Ferry, 1980).

To relate relaxation data measured at a temperature $T$ to the $T_g$ range, the classic Williams-Landel-Ferry (WLF) equation is used. Doolittle applied a similar approach in investigating viscosity. Because viscosity is so closely related to the mobility of a material, the viscosity is influenced by the free volume and swelling. It is noted that as the free volume increase, the viscosity decreases (Ferry, 1980). Doolittle’s treatment actually ends up in an identical form to the WLF equation and is shown as:

$$\log a_T = -\frac{B}{2.303f_g} \left( \frac{T - T_g}{f_g/\alpha_f + T - T_g} \right)$$  \hspace{1cm} \textbf{Equation 4-7}

where $a_T$, is a shift factor used to fit data taken relative to some standard temperature, to a master curve, $f_g$ is the fractional free volume at $T_g$, $B$ is a constant close to unity, $\alpha_f$ is the thermal expansion of the fractional free volume above $T_g$ relative to the total
volume. This value is equal to the polymer thermal expansion coefficient if the expansion is due entirely to the increasing free volume. Following this approach, several investigators have used this equation to determine the effects of diluent concentration on the polymer modulus as shown in Equation 4-8 (Grate & Zellers, 2000; Martin et al., 1994).

\[
\log a_c = -\left( \frac{1}{2.303} \right) \left[ \frac{\beta' v_1}{f_2(f_2 + \beta' v_1)} \right]
\]

Equation 4-8

In this case, \(a_c\) is the shift factor used to describe the diluent concentration effect on the polymer modulus. It serves the same purpose as the shift factor \(a_T\) in Equation 4-7. An important feature of this equation is the parameter \(\beta'\). This parameter modifies the volume fraction of the added diluent, \(v_1\). Therefore, the total free volume of the polymer containing adsorbate vapor molecules is a combination of the free volume of the polymer and the free volume of the vapor. Only a portion of the volume fraction of the vapor is free volume, \(f_1v_1\) or \(\beta'v_1\), and influences the modulus (Grate & Zellers, 2000; Martin et al., 1994). The swelling induced effects of vapor sorption do affect the response of quartz resonators. Instead of measuring the modulus changes directly, the above equations represent an analysis of using swelling effects induced by vapor sorption in a polymer film to determine the corresponding viscoelastic changes.

4.2 Thermodynamic Properties Upon Sorption

4.2.1 Sorption Enthalpies
Enthalpy is a thermodynamic property defined by the internal energy, \( E \), pressure, \( P \), and volume, \( V \), of a system. The relationship is shown in Equation 4-9:

\[
H = E + PV \tag{Equation 4-9}
\]

The enthalpy is distinguished as a state function because the values of each of the properties defining it depend on the system’s state. The values of state functions depend only on the current state of the system and not on the prior history of how the state was achieved.

When speaking of thermodynamic properties, it is implied that the term is referring to classical or equilibrium thermodynamics. Equilibrium is defined simply as a macroscopic system that, when left alone, reaches a state in which the system does not change with time. A state in equilibrium can be defined by a number of properties such as volume, pressure and temperature (van Ekeren, 1998).

In the studies of protein and polymer films presented here, the vapor activity of water or an organic adsorbate in contact with the adsorbent film is incrementally changed and held for a period of time (ranging from 10 to 90 minutes in these studies). During this process a change from one equilibrium state to another occurs. Upon an increase in the vapor activity, there is a corresponding mass increase, measured via the frequency difference in the QCM frequency output, indicating vapor sorption by the adsorbate film. This mass increase is dependent on the vapor diffusion through the film of a certain thickness, density, and relaxational processes upon sorption. Equilibrium is attained when a steady state is achieved between the vapor being sorbed and desorbed at
the same rate in the film. In the QCM/HCC measurements, this is visible as the mass
trace levels off after the vapor concentration has been increased or decreased.
Simultaneously, upon sorption there is a corresponding heat effect, measured by the
voltages produced from the thermopiles in the QCM/HCC. Upon sorption a heat pulse
is generated. As thermal equilibrium is attained, the thermal signal stabilizes and settles
to a steady state baseline signal. The third measurement that is carefully watched with
the QCM/HCC is the voltage output corresponding to the motional resistance of the
film. The QCM and film oscillation is affected upon vapor sorption depending on the
viscoelasticity (vide infra) of the film. Upon sorption there is a corresponding increase
in the motional resistance of the QCM and film oscillation. Equilibrium of the motional
resistance is attained when a steady state signal is achieved and coincides with the
corresponding mass and thermal signals.

There is a noticeable difference between the polymer (Chapter 5) and protein
(Chapter 6) studies presented here. The polymer films do appear to reach equilibrium
upon vapor sorption whereas the protein films do not. The mass, thermal, and motional
resistance signals all reflect this difference. The protein films exhibit hysteresis: the
amount of vapor being sorbed into the film is not the same amount being desorbed for
the same change in vapor activity. The sorption of water by proteins is not fully
reversible within the time scale of the experiment. Also the sorption steps in the protein
films were longer in many cases than in the polymer films, however, the mass traces,
especially at the higher vapor activities, did not appear to level off indicating the need
for a much longer equilibration period. A discussion of the hysteresis effects and the
interpretation of the enthalpy for the proteins is given in Chapter 6.
Most sorption processes can be characterized as being exothermic. The sorption process is spontaneous; therefore the change in free energy is negative, \( \Delta G < 0 \). As the gaseous adsorbate adheres to the surface of the thin film adsorbent, the translational freedom of the adsorbate is reduced leading to a negative change in entropy, \( \Delta S \). This leads to the conclusion that the change in enthalpy, \( \Delta H \) must be negative (exothermic) for \( \Delta G = \Delta H - T \Delta S \) to be negative (Adamson & Gast, 1997). This appears to be true in the studies presented here. However, to assume that this holds true for every system can be misleading (Thomas, 1961). In fact, in physisorption, the entropy change is always negative because this sorption is essentially a condensation of the gas molecules. During this condensation, the adsorbate loses some degrees of freedom therefore, \( \Delta S < 0 \), while the entropy of the adsorbent film remains essentially unaltered. Chemisorption, on the other hand, involves not only the condensation but also the breaking and forming of chemical bonds. Since some chemical reactions are endothermic, it is conceivable that some of the surface reactions are also endothermic. The \( \Delta S \) of the gaseous adsorbate as stated above is negative, but in chemisorption there is a chance that the corresponding chemical reaction with the film increases the entropy of the film adsorbent leading to \( \Delta S > 0 \). Therefore, an endothermic chemisorption is possible if the \( T \Delta S \) term is numerically greater than the \( \Delta G \) term. It is important to account for the \( \Delta S \) effects not only of the gaseous adsorbate, but also of the chemical changes occurring on the surface of the adsorbent (Thomas, 1961).

In the experiments of polymer and protein films shown in Chapters 5 and 6, the sorption processes are exothermic and the corresponding desorption processes are endothermic. A discussion in the protein chapter states that hydration studies of
proteins involved the interaction of water with a various types of bonds. Some of the individual protein-water, water-water, and protein-protein interactions may actually be larger in magnitude than the reported hydration enthalpies, but the combination of these interactions cancel some of the individual contributions. The measured enthalpy becomes an average of these exothermic and endothermic events.

Gas adsorption, as measured using the QCM/HCC, can thermodynamically be characterized as integral adsorption. For a gaseous adsorbent, $X_2$, and a thin film adsorbate, $X_1$, this can be written as:

$$n_1 \cdot X_1 \text{ (adsorbent at T)} + n_2 \cdot X_2 \text{ (gaseous adsorbate at P,T)} = n_2 \cdot X_2 \text{ adsorbed on } n_1 \cdot X_1 \quad \text{Equation 4-10}$$

where $n_1 \cdot X_1$ are the moles of thin film adsorbent, $n_2 \cdot X_2$ are the moles of gaseous adsorbate, and $n_2 \cdot X_2$ are the moles of adsorbed gas.

For a process being carried out at constant volume, the integral heat of adsorption, $Q_i$, is equal to an energy change in the thin film adsorbent, $\Delta E_2$, for a mole of the gaseous adsorbate, $q_i = \Delta E_2$ (Adamson & Gast, 1997). The integral calorimetric heat, $q_i$, is:

$$q_i = \left( \frac{Q}{n_2} \right)_v \quad \text{Equation 4-11}$$

Specifically, for our heat conduction calorimeter, the heat evolved or absorbed in the thin film on the QCM is allowed to flow to or from an aluminum heat sink, which surrounds the QCM chamber. The heat flow is measured as it passes through the
thermopile walls placed under the QCM’s and in contact with the heat sink. At a steady state, the heat production rate, or thermal power, \( P \), is directly proportional to the thermopile potential, \( U \) as shown in Equation 4-12:

\[
P = \varepsilon U
\]

Equation 4-12

where \( \varepsilon \) is the calibration coefficient (W/v) and is discussed in Chapter 3. This is a form of the Tian equation, also discussed in Chapter 3 in the section on the thermal sensors, the thermopiles.

The measured heat quantity, \( dQ \), involved in the process during a time, \( dt \), is (Wadsö, 1995):

\[
dQ = \varepsilon U \, dt
\]

Equation 4-13

Integration of the measured heat quantity yields:

\[
Q = \varepsilon \int_{t_1}^{t_2} U \, dt
\]

Equation 4-14

This leads to the integral calorimetric heat for our instrumentation resembling the form of Equation 4-11:

\[
q_i = \frac{\varepsilon \int_{t_1}^{t_2} U \, dt}{n^2}
\]

Equation 4-15
where \( n_2 \) is the term for the moles of adsorbed molecules and \( q_i \) is reported in units of kJ/mol.

### 4.2.2 Sorption Isotherms

Solid-vapor interactions are often characterized and viewed by plotting a sorption isotherm. Data plotted as an isotherm consists of measuring the amount of vapor adsorbed by a given amount of adsorbate, usually measured in \( g_{\text{adsorbate}} / g_{\text{adsorbent}} \), as a function of the vapor pressure with temperature being held constant. The equilibrium vapor pressure is used to determine the vapor activity, \( a \), and is defined as:

\[
a_{\text{adsorbate}} = p/p^o
\]

**Equation 4-16**

where \( p^o \) is the vapor pressure in the standard state and \( p \) is the equilibrium pressure during the experiment. It is noted that the amount of vapor adsorbed (e.g. water in the case of proteins) at a fixed vapor activity and temperature generally cannot be equal to the amount of bound, condensed vapor (e.g. water), especially in the case of proteins, but it is hoped that a proportionality exists so that a relative scale of sorption could be established (Kuntz & Kauzmann, 1974). Using the QCM/HCC, the adsorbate activity is controlled by using two mass flow controllers and a gas bubbler. By changing the gas flow through the bubbler containing the desired adsorbate and then mixing this flow with determined amounts of the carrier gas, a calculated vapor activity, \( a \), is achieved. The mass uptake of adsorbate by a thin film (polymer and protein in this case), is measured as a function of vapor activity.
In an attempt to classify sorption isotherms and to fit the data to models, many theories have surfaced. Generally the theories fall into two categories: surface or solution models. Three types of isotherms will be discussed here for their relevance to our polymer and protein studies. Two of these isotherms, the Langmuir and BET, fall into the surface category, while the third, the D’Arcy and Watt isotherm is an example of a solution model.

To provide a little background, in 1916, Langmuir proposed a model for the adsorption process. His work focused on chemisorption processes but it can also be applied to physisorption. Chemisorption results in monolayer formation over the adsorbent being studied. Equilibrium is established between the gas phase and the partially formed monolayer. At a pressure, \( P \), and a fractional surface area coverage, \( \theta \), a dynamic equilibrium is reached where the rate of desorption of the adsorbed material equals the rate of condensation of the adsorbate (the gas phase molecules). In his theory, Langmuir suggested that the rate of desorption, is proportional to the fraction of surface covered, \( \theta \), and can be written as \( k_1 \theta \), where \( k_1 \) is a proportionality constant. Both the vapor pressure, \( P \), and the fraction of the surface not covered by the adsorbed molecules, \( 1-\theta \), are also proportional to the rate of adsorption. When the expressions for the rate of evaporation, \( k_1 \theta \), and for the rate of condensation, \( k_2 P(1-\theta) \), are equated, a relationship is seen between the equilibrium surface coverage and the gas pressure (Barrow, 1996):

\[
k_1 \theta = k_2 P(1-\theta) \tag{Equation 4-17}
\]
Upon rearrangement and introducing $b = k_1/k_2$, the isotherm is shown to equal:

$$\theta = \frac{bP}{1 + bP} \quad \text{Equation 4-18}$$

The Langmuir isotherm is often used to characterize chemisorption processes. At lower values of $P$, an initial steep rise of the isotherm is seen. When the value of $P$ is larger, $\theta$ is no longer simply proportional to the pressure, $P$ and the isotherm approaches a constant value of unity.

Because some adsorption processes for polymer and protein samples are the result of physisorption and undergo the formation of multilayer coverage of an adsorbent, the Langmuir isotherm does not adequately describe the physical events taking place. Isotherms that increase rather than flatten out suggest a secondary sorption stage. In 1938, S. Brunauer, P.H. Emmett, and E. Teller worked out an isotherm to describe this process. The isotherm describing this type of adsorption is named in their honor, the BET isotherm. The derivation of the BET isotherm follows the same form as the Langmuir in that there is a balancing of the forward and reverse rates. The heat of adsorption, $Q$, of the first layer may have a special value where in the succeeding layers the heat, $Q_v$ is the heat of condensation of the liquid adsorbate. The entire adsorption process can be described in two steps namely (1) the attachment of molecules to sites on the solid surface and (2) the ensuing attachment of molecules to sites already occupied by adsorbed molecules. A constant, $c$, is used to express the ratio of the strength of binding to the solid surface and to the absorbed molecules. The volume of adsorbed gas, $W$, is proportional to the volume of gas that would produce a monomolecular layer,
\( W_m \) and is expressed as \( W/W_m \). The expression for the BET isotherm is (Gregory, 1995; Kuntz & Kauzmann, 1974):

\[
\frac{W}{W_m} = \frac{Kx}{1 + Kx} + \frac{x}{1 - x} \quad \text{Equation 4-19}
\]

In the above equation, \( x \) is the ratio of the partial pressure of the gas and its saturation pressure, \( p/p^o \) and \( K \) is a proportionality constant. The first term on the right hand side of the equation resembles that of a Langmuir isotherm and accounts for the number of binding sites to accommodate a monolayer of adsorbate. The second term accounts for weakly binding multilayers. This additional term did help in fitting sorption data however other models evolved to try to describe sorption data.

In response to the surface models, a few solution models were explored such as the Flory, the Hailwood and Horrobin, the D’Arcy and Watt, and the Schwarz (Kuntz & Kauzmann, 1974). Some of these models closely resemble the BET isotherm and seemed to use this treatment as a springboard to add various terms to the equation in an attempt to explain and model the sorption process, especially proteins in this case. The D’Arcy and Watt isotherm will be discussed here because of its relevance to our hydration studies of the protein lysozyme. Several investigators have measured the hydration of lysozyme and have determined parameters for the D’Arcy and Watt isotherm (Careri et al., 1979; Lüscher-Mattli, 1986; Lüscher-Mattli & Rüegg, 1982). In Chapter 6 we present our lysozyme hydration data and show it in relation to this model. The D’Arcy and Watt isotherm is a five parameter model and is shown in Equation 4-20 (Gregory, 1995):
where $W$ is the uptake of adsorbate, $W_m$ and $K$ are the same constants as above and proportional to the energy of adsorption and the number of binding sites respectively, $x = p/p^0$, $C$ is a constant proportional to the number and affinity of weak binding sites and $D$ and $\gamma$ correspond to the number of and water affinity of the multilayer binding sites (Lüscher-Mattli & Rüegg, 1982). The first term resembles the Langmuir type isotherm and in this case accounts for the hydration of the stronger binding sites, the middle term is a linear term added to account for the adsorbate binding to weaker sites, and the last term resembling the BET isotherm is included to account for multilayer adsorbate formation (Rupley & Careri, 1991). Three distinct regions mark the isotherm. The first region spans from 0 to $\sim 0.1 \frac{g_{adsorbate}}{g_{adsorbent}}$ marking the hydration of the polar, ionizable side chains. From their lysozyme hydration studies, Careri, et al., state that $\sim 40$ molecules of water are accommodated per protein molecule. In the second region, from $\sim 0.1$ to $0.38 \frac{g_{adsorbate}}{g_{adsorbent}}$ marks the onset of the transition from monolayer to multilayer coverage. Binding occurs along the polypeptide backbone and among weaker, less polar groups. Careri states that the protein at this point accommodates $\sim 100$ water molecules per protein molecule. In the third region ranging from 0.38 to 1.0, water condensation takes place at the weak binding sites and the formation of multilayer water continues (Careri et al., 1979).

To help classify and categorize the various isotherms, in 1945, Brunauer characterized adsorption isotherms into five principal forms. These are shown in Figure 4-3:
Type I resembles a Langmuir isotherm characterizing a monolayer. Type II models a physisorption process with multilayer formation. This isotherm follows the BET isotherm described above. Type III is a rarer form and imitates a system where the heat of adsorption is \( \leq \) the heat of adsorbate liquefaction. Type IV and V level off before reaching the saturation pressure and may show hysteresis effects. These two are said to model capillary condensation phenomena (\textit{vide infra}) (Adamson & Gast, 1997).

Sorption isotherms will be presented for the polymer and protein films. The hydration of the lysozyme film resembles the D’Arcy and Watt isotherm shown above.
and is characterized as a sigmoidal Type II from Brunauer’s classification. The isotherms for the hydration of myoglobin start off in a similar manner but show a marked difference with a gap appearing at $a_w = 0.35 – 0.51$. A discussion will follow Chapter 6. The polymer sorption isotherms will be presented in Chapter 5. They represent data from films ranging in thickness from 0.75 to 8.5 µm and involved sorption measurements of water and ethanol. These sorption isotherms also resemble the D’Arcy and Watt isotherm but do not exhibit as sharp a knee in the initial sorption stages.

4.2.3 Partition Coefficients

When a vapor is sorbed into a thin film coated on a QCM, the QCM produces responses proportional to the partition coefficient, $K$. The partition coefficient gives a measure of the equilibrium distribution of vapor from the gas phase into the sorbent phase which leads to information regarding the interactions between the vapor and the sorbent. This quantification gives rise to a ratio of the concentration of the vapor in the sorbent phase, $C_s$, to the concentration of the vapor in the gas phase, $C_v$. This relationship is seen in Equation 4-21 (Grate, 2000; Grate *et al.*, 1995):

$$K = \frac{C_s}{C_v}$$

*Equation 4-21*

where $K$ is related to the standard Gibb’s free energy of solution with a gaseous solute, $\Delta G^o_s$ through the relationship:

$$\Delta G^o_s = -RT \ln K$$

*Equation 4-22*
with the standard states being the unit concentrations in the gas phase and the solution phase.

Because the QCM responds to molecules in the sorbent phase, it is a detector for $C_s$, and this relationship is commonly employed in exploring the QCM as a vapor-sensing device. Measured $C_s$ can be determined from experiments and compared with calculated $C_s$ value if the $K$ and $C_v$ values are known. This provides a means to test a sensor response and see its applicability as a simple mass-loading measurement.

A difficulty lies in the fact that $K$ values are seldom available. Most partition coefficient values for organic vapors into polymer films are measured at elevated temperatures ranging from $325 – 425$ K. Some researches have set out to determine $K$ values at $298$ K for the sorption of organic vapors by polymer films. Inverse gas phase chromatography (IGC) was used to make these measurements (Grate, 2000).

When using a QCM to measure $K$ values, the QCM response is related to the mass of vapor sorbed through **Equation 4-23**:

$$
\Delta f_v = \frac{n \Delta f_s C_s K}{\rho_s}
$$

**Equation 4-23**

The frequency shift of the sorbent phase, the film before vapor sorption, is denoted by $\Delta f_s$. This frequency shift provides a measure of the amount of sorbent polymer on the active QCM surface. The QCM response to the mass of adsorbed vapor is shown as the frequency shift, $\Delta f_v$. The density of the sorbent phase is $\rho_s$. For mass loaded QCM responses, the amplifying factor, $n$, is equal to 1. For thick films, swelling induced
modulus changes may occur which then increase the QCM response (vide supra). If this occurs, n is then equal to an amplifying factor (Grate, 2000).

The relationship in Equation 4-23 can be misleading. As a polymer adsorbs a vapor, the volume of the polymer film increases. In 1992, Grate, et al., modified the above to account for swelling induced modulus changes in a polymer film and its effect on the QCM frequency (Grate et al., 1992). This was again modified in 2000, to correct for the fact that the free volume in the polymer upon swelling has contributions from both the free volume of the polymer plus the free volume associated with the vapor (Grate & Zellers, 2000; Martin et al., 1994). In his treatment using surface acoustic wave devices, SAW, Grate shows that $\Delta f$, the total frequency shift, now accounts for vapor sorption due to both gravimetric and swelling effects in the polymer film. This is seen in Equation 4 – 24 (Grate & Zellers, 2000):

$$\Delta f_v = \left(\frac{\Delta f_v C_v K}{\rho_s}\right) + \beta' \left(\frac{C_v K}{\rho_L}\right) \left(\frac{\Delta f_s A_{SAW}}{\alpha}\right)$$ \hspace{1cm} \text{Equation 4-24}

where $\beta'$ is a plasticizing parameter which relates the diluent volume fraction to the free volume, $\rho_L$ is the density of the vapor as a liquid, $\rho_s$ is the density of the sorbent phase, $\alpha$ is the coefficient of thermal expansion of the polymer, and $A_{SAW}$ is the kHz change in frequency due to a $1^\circ$C change in temperature per kHz of coating on the resonator surface. The term $\Delta f_s A_{SAW}/\alpha$ therefore gives the frequency change that occurs due to a fractional volume increase in the polymer film and is modified by the parameter $\beta'$. 
In the polymer sorption studies presented here, the partition coefficient values were found by calculating the $C_s$ and $C_v$ values and analyzing the ratio of these. The concentration of the sorbent phase was found by **Equation 4-25**:

$$C_s = \frac{(f_r - f_i) \times 1.9793 \text{cm}^2 / 56.6 \text{Hz} \mu \text{g}^{-1} \text{cm}^2}{[(f_o - f_i) \times 1.9793 \text{cm}^2 / 56.6 \text{Hz} \mu \text{g}^{-1} \text{cm}^2] / \rho_f}$$  \hspace{1cm} \text{Equation 4-25}$$

where $f_o$ is the resonant frequency of the uncoated QCM, $f_r$ is the QCM resonant frequency with a dry sample film and $f_i$ is the QCM frequency at a point, $i$, indicating that the film has reached an equilibrium upon vapor sorption. The numerator determines the mass, $\mu g$, of water sorbed in the film and the calculation follows the Sauerbray relationship as discussed in Chapter 3. The denominator yields the volume of the adsorbent thin film phase. The first portion of the denominator is the Sauerbray relationship used to determine the mass of the dry film. Dividing this mass by the density of the film, $\rho_f$, gives the volume.

Likewise, the concentration in the vapor phase was calculated using **Equation 4-26**:

$$C_v = \frac{p}{RT} = \frac{n}{V}$$  \hspace{1cm} \text{Equation 4-26}$$

where $p$ is the vapor pressure, $R$ is the gas constant, and $T$ is the temperature. Ideal behavior is assumed for the vapor and using the ideal gas law, the concentration is found in mol/L. This is then converted to $\mu g/L$ so that upon comparison with $C_s$ the unitless quantity $K$ is found.
For a system in equilibrium, it would be expected that the value of K is fairly constant for a given polymer upon vapor sorption. In our presentation of K values for polymer sorption there is a change in K values upon increasing vapor sorption and in increasing vapor film thickness. The results show that polymer swelling does influence the determination of K and needs to be accounted for when reporting these values.

4.2.4. Diffusion Coefficients

Using the mass trace of the QCM/HCC, it is possible to get an idea of the rate of diffusion of the gas molecules adsorbing on a thin film. In our experiments, a thin (protein or polymer in these studies) film is exposed to incrementally increasing or decreasing changes in an adsorbate vapor activity. Initially this isolated system is not in equilibrium and a mass exchange occurs until the solid and gas phases reach thermodynamic equilibrium. The mobility of the adsorbate molecules through the adsorbent matrix is controlled by molecular diffusion. Some sorption experiments expose an adsorbent to a controlled vapor activity and measure the mass of the dry sample and then again the mass is measured once equilibrium at the new vapor activity has been achieved (Bull, 1944; Poole & Finney, 1986). This is effective in obtaining data for sorption isotherms but it does not provide information about the kinetics of the sorption process. When using the QCM/HCC for sorption studies, the sorption process is monitored through the transient stage of an adsorbate being introduced to the thin film adsorbent. In measuring this sorption process and change from one state of equilibrium to a new one, it is possible to determine the rate of diffusion in the adsorbent material.
It is interesting to view the adsorption process in light of the adsorption time. A molecule in the gas phase approaching the surface of a solid and not attracted to the surface, would stay in the area of the surface in the order of a molecular vibration time, or approximately $10^{-13}$ sec. The molecule would retain its original energy. A term called the accommodation coefficient is used to describe the energy in terms of the molecule’s temperature. In this previous example, the accommodation coefficient would be zero. In 1911, Knudsen defined the accommodation coefficient, $\alpha$, as:

$$\alpha = \frac{T_1 - T}{T_2 - T_1}$$  \hspace{1cm} \text{Equation 4-27}$$

where $T_1$ is the temperature of the gas molecules before they strike the surface, $T_2$ is the temperature of the surface, and $T_3$ is the temperature of the molecules that leave the surface (Adamson & Gast, 1997).

In the event that attractive forces are present, the average time of stay, $\tau$, of the molecule on the surface is:

$$\tau = \tau_o e^{Q/RT}$$  \hspace{1cm} \text{Equation 4-28}$$

where $\tau_o$ is approximately $10^{-12}$ to $10^{-13}$ seconds. $Q$ is the energy of adsorption or the interaction energy. When $\tau$ is on the order of several vibration periods, it can be assumed that adsorption has occurred. Physically this means that an equilibrium temperature has been achieved between the molecule and the surface. Desorption of the
molecule would occur in a direction that is independent of the molecule’s arrival (Adamson & Gast, 1997).

Diffusion in this case is a measure of the change in location of the adsorbate with time. The driving force of diffusion is a rate of change of concentration along the diffusion direction. By applying diffusion studies, it is possible to get a measure of the rate of adsorption of gas molecules interacting with the solid film interface. The flux, \( J \), denotes the rate of migration of a property. The flux can be understood as the quantity of a property (e.g. gas molecules), which passes through a given area, \( A \), in a certain time period. This value is divided by the area and the duration of the interval (Atkins, 1998). In transport properties, the flux of a gas is found to be proportional to the first derivative of the concentration. Fick’s first law of diffusion defines this proportionality of the flux of matter to the concentration and is expressed as:

\[
\text{Rate of diffusion} = J = -D \frac{\delta c}{\delta x} \tag{Equation 4-29}
\]

where \( \delta c \) is the concentration gradient, and \( \delta x \) is the diffusion direction. The proportionality constant, \( D \), is the diffusion coefficient and depends on the nature of the diffusing adsorbate and the receiving adsorbent in the studies shown here.

It is helpful to take this relationship one step further and describe the effect of diffusion on the concentration in a volume element. In this case, the net rate of the diffusing substance accumulating in a volume, \( A \delta x \), is calculated. This is performed by calculating the rate at which the substance enters the volume element at \( x \) and the rate with which it leaves at \( x+\delta x \). Fick’s second law defines this relationship as (Barrow, 1996):
\[
\frac{\delta c}{\delta t} = D \frac{\delta^2 c}{\delta x_2^2}
\]

Equation 4-30

where the quantity \(\delta c/\delta t\) is the rate of change of concentration with the expression showing the rate of change of the amount in the volume element.

Studies of the diffusion of a gaseous adsorbate in an adsorbent thin film have been the subject of many studies especially in the area of drying kinetics (Cairncross et al., 1995; Doumenc & Guerrier, 2001; Guerrier et al., 1998; Pinto, 1999). One clarifying point in many of the studies is the determination of whether the diffusion process follows Fickian behavior. In glassy polymers, many studies report that Fickian behavior is followed. It is noted that water transport in glassy polymers follows Fickian diffusional behavior. The small size of water molecules and their affinity for hydrophilic polymers contribute to the diffusion process being unaffected by the free volume distribution in the polymer matrix (Pinto, 1999). In the non-glassy region, however, polymer relaxation and the increase in free volume dominate the diffusion rates and mechanics. This is seen with sorption studies of small organic molecules with the diffusion process growing even more complex with large organic solvent molecules. When the rubbery to glassy transition is taken into account, non-Fickian behavior is seen and characterized by two regimes marked by large concentration gradients (Guerrier et al., 1998). In drying studies, the first regime is a fast step where the adsorbate concentration is high and the evaporation flux is close to values of the pure adsorbate. Upon evaporation, the polymer film shrinks and a second slower regime becomes apparent. The adsorbate concentration near the interface decreases resulting in a decline in the diffusion and evaporation rates.
In the studies presented here, the diffusion coefficients are determined for polymer and protein films following a treatment by Hernandez (Hernandez-Muñoz et al., 1999). The analysis uses an equation found by solving Fick’s differential equations according to the following assumptions: (1) the (polymer) film has a constant thickness, (2) the temperature and pressure are kept constant, (3) the sorption process is Fickian in other words, the adsorbate concentration is constant throughout the experiment. The rate of the mass uptake, \( r_m \), is given as:

\[
\frac{m^t_p - m^i_p}{m^\infty_p - m^i_p} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n + 1)^2 \pi^2} \times \exp\left(-\frac{D(2n + 1)^2 \pi^2 t}{l^2}\right)
\]

Equation 4-31

in the above equation, \( D \) is the diffusion coefficient, \( m^t_p \) is the mass of the film plus sorbate uptake at any time, \( t \). The superscripts, \( i \) and \( \infty \) denote the initial and final times and the film thickness is \( l \). Within a sorption rate, \( r_m \), range of 0.3 < \( r_m < 0.85 \), Equation 4-31 can be simplified to the first term of the summation. An error of < 0.1% is noted for this simplification (Hernandez-Muñoz et al., 1999). The equation thus reduces to:

\[
\frac{-1}{\pi^2} \ln\left(\frac{(m^\infty_p - m^i_p)\pi^2}{8(m^\infty_p - m^t_p)}\right) = \frac{D}{l^2} t
\]

Equation 4-32

This equation was used in our analysis where the left hand side was plotted versus the time, \( t \). The diffusion coefficient was calculated from the slope of the line. When the mass data is plotted according to this equation, three regions of the curve are distinguishable. In the first part, the data starts from the x-axis origin and is
characterized by the curve being convex. This data is in the kinetic region where $0 < r_m < 0.3$. The convex curvature is due to an inconsistency in the simplified equation in the limit of short time periods. The second region between $0.3 < r_m < 0.85$, is a straight line and can be used to determine the diffusion coefficient, $D$. The third region is noted when $r_m$ approaches 1. In this region, the numerator of the logarithmic term approaches 0. Therefore, the best region to use Equation 4-32 is in the second region where the rate is defined as $0.3 < r_m < 0.85$ (Hernandez-Muñoz et al., 1999). This equation was applied to our polymer studies and the results will be shown in Chapter 5. For the determination of the diffusion coefficient in the protein films, the equation was slightly modified to account for a linearly shifting mass trace baseline. The modified form of the equation is shown in Chapter 6.

4.3 Rheological Properties of Thin Films

4.3.1 Introduction

Because of the widespread application of thin polymeric films, the rheological properties are useful and necessary pieces of information in studying fundamental applications of polymer films. The term rheology pertains to the study of the deformation and flow of materials (Menard, 1999). A subcategory of rheology includes dynamic mechanical analysis (DMA) and is described as the application of an oscillatory force to a sample and the analyzing of the material’s response to the force (Menard, 1999). A simplification of the properties measured in DMA can be described as two-fold: (1) damping effects, where energy is lost as heat and (2) elasticity or the sample’s ability to recover from a deformation. In our studies utilizing the QCM/HCC, a thin film is subject to an oscillatory force causing a shear deformation. The oscillator
driver used in these experiments gives two output signals, the QCM oscillation frequency and a voltage, which can be used to calculate the motional resistance of the QCM oscillation. Because the film is in contact with the QCM it interacts mechanically with it influencing the resonant frequency and damping. These two parameters provide an insight into the rheological properties of a sample thin film.

The mechanical properties of thin polymer films have not been amenable to experimental measurements. Many of the dynamic mechanical testing techniques used for bulk viscoelastic materials cannot be applied to thin films because of difficulty in mounting the thin films (Brodt et al., 1995; Domack & Johannsmann, 1996; Vinci & Vlassak, 1996) (Ferry, 1980). Oftentimes, the mechanical properties are obtained by extrapolating information obtained from measurements on the behavior of thicker polymer samples. Error may result however, because the substrate and deposition techniques often influence the structure and dynamics. Using a quartz crystal microbalance, it is possible to obtain information regarding the mechanical properties of thin films (Johannsmann, 2001; Lee et al., 2002; Lucklum & Hauptmann, 2001; Martin et al., 2000).

A thin film undergoing oscillatory motion is subject to stress and an ensuing strain (vide infra). In this area, work has been done to characterize thin films in the low frequency range, (~ 10 Hz to kHz), however a sparse amount of work has been done in characterizing the mechanical properties of thin films in high frequency ranges (e.g. MHz) (vide supra).
4.3.2 Viscoelasticity

In order to define some of the behaviors of thin films undergoing oscillatory motion, it is helpful to look at some of the guiding principles, namely elasticity and viscoelasticity.

**Elasticity** is a mechanical property used to describe elastic solids. A solid is said to have elasticity when it is deformed by external forces and then returns to its original shape and dimension when the external force is removed. Hooke’s Law is used to explain the elastic properties of solids in terms of stress and strain. Stress, $\sigma$, is a quantity that is proportional to the force causing a deformation. Strain, $\gamma$, is a measure of the degree of the deformation. In general, Hooke’s Law states that stress is proportional to the strain with the proportionality constant depending on the material and on the nature of the deformation (Serway, 1982). The proportionality constant for a perfectly elastic solid is named the elastic modulus and is seen by the relationship:

\[
\text{elastic modulus} = \frac{\text{stress} (\sigma)}{\text{strain} (\gamma)} \quad \text{Equation 4-33}
\]

The type of deformation of interest in these studies is the shear deformation of a thin, solid film. Shear forces cause the deformation of a material by inducing slippage along a plane or planes parallel to the imposed stress. A shear deformation is a measure of the resistance to motion of the planes of a solid sliding past each other. The proportionality constant in this case is named the **shear modulus**, $G$ (Serway, 1982).

The type of material being studied often complicates the simple proportional relationship between stress and strain. Some thin films upon sorption of the adsorbate lose their rigid, solid characteristics. Instead, a system can exhibit a combination of
solid and liquid characteristics. **Viscoelastic** is the term used to describe these unique solids which exhibit both viscous and elastic behavior (Ferry, 1980). When deformed, viscoelastic materials are able to store some of the energy thus resembling elastic materials but one differing factor is that a portion of the energy is dissipated as heat. A viscoelastic material does not maintain a constant deformation under constant stress. Instead, the material creeps or in other words, continues to slowly deform with time. When this occurs, the material demands less stress to hold it; the stress relaxes. As for fluid behavior, an ideal liquid flowing under constant stress normally dissipates the energy input as heat. A viscoelastic material, on the other hand, stores some of the energy input and uses this for elastic recoil thus recovering part of its deformation when the stress is removed.

A perfectly elastic solid undergoing a sinusoidally oscillating stress, is characterized by the strain being in phase with the stress. Similarly, a perfectly viscous liquid is characterized by the strain being $90^\circ$ out of phase with the stress. Viscoelastic materials are characterized by the phase between the strain and stress lying somewhere in between these two degrees (Ferry, 1980). Linear viscoelastic behavior is noted when both the stress and the strain are infinitesimal and the time-dependent stress-strain relationship can be described by linear differential equations with constant coefficients. In this case, the ratio of stress to strain is not dependent on the magnitude of stress, but is solely a function of time or frequency. Polymers are a classical example of viscoelastic materials. The mechanical properties of polymers are dominated by viscoelastic behavior.
4.3.3 Complex Shear Modulus, G

When using a QCM, the thin film adhered to the surface oscillates in a sinusoidal, periodic fashion at a particular frequency, \( \nu \) (Hz) cycles/sec, or \( \omega (2\pi \nu) \), radians/sec. When the viscoelastic behavior is linear, the strain alternates sinusoidally but is out of phase with the stress. This can be seen in Figure 4-4 (Menard, 1999):

\[
\text{Force (dynamic)} \quad \text{Stress = FA} \\
\text{Time} \quad \text{Phase angle } = \delta
\]

**Figure 4-4.** A plot depicting a polymer sample subject to a sinusoidal oscillating stress. Within the sample material elastic limits, the sample response is a similar strain wave however it is shifted by an angle, \( \delta \) (Menard, 1999).

The resulting strain, \( \gamma \), in a periodic experiment is seen in the relationship:

\[
\gamma = \gamma_o \sin \omega t \quad \text{Equation 4-34}
\]

where \( \gamma \) is the strain at a time, \( t \), \( \gamma_o \) is the maximum strain, and \( \omega \) is the oscillation frequency as defined above. The phase shift between the applied stress and the resulting strain is the angle, \( \delta \). The elastic response at any time is then seen as:
\[ \gamma(t) = \gamma_o \sin(\omega t + \delta) \quad \text{Equation 4-35} \]

Following the treatment by Menard the above can also trigonometrically be written as (Menard, 1999):

\[ \gamma(t) = \gamma_o [\sin(\omega t)\cos\delta + \cos(\omega t)\sin\delta] \quad \text{Equation 4-36} \]

The above equation permits us to separate the in-phase and out-of phase components of the strain. The in-phase strain, \( \gamma' \) and the out-of-phase strain, \( \gamma'' \) are:

\[ \gamma' = \gamma_o \sin(\delta) \quad \text{Equation 4-37} \]

\[ \gamma'' = \gamma_o \cos(\delta) \quad \text{Equation 4-38} \]

Therefore the vector sum of these two components gives the overall complex strain of the sample:

\[ \gamma^* = \gamma' + i\gamma'' \quad \text{Equation 4-39} \]

In the QCM measurements, a film is subject to a shear strain defined as the shear modulus \( G \). The shear modulus as seen from above is a complex quantity and is given by (Aklonis & MacKnight, 1983):

\[ G = G' + iG'' \quad \text{Equation 4-40} \]
The in-phase quantity, $G'$, is the shear storage modulus and quantifies the storage of energy. The out-of-phase quantity, $G''$, is the shear loss modulus and defines the dissipation of energy. A common parameter that is measured which gives the ratio of the storage and loss components is the tangent of the phase angle, $\tan \delta$, where:

$$\tan \delta = \frac{G''}{G'}$$ \hspace{1cm} \text{Equation 4-41}

### 4.3.4 Complex Shear Modulus Dependence on Frequency and Sorption

The complex shear modulus is dependent on the probing frequency, the sample temperature and the amount of adsorbate added to a sample. For a material that is glassy/rigid at room temperature, $25^\circ$C, the shear storage modulus, $G'$, typically would have a values $\sim 10^8$ to $10^9$ Pa. As stated above, the frequency of the measurement does factor into the $T_g$ and shear modulus values. When a polymer with a segmental chain motion, $\tau$, is subject to a shear deformation on a much shorter time scale than $\tau$, the strain is accommodated elastically and the material is in the glassy state (Martin et al., 1994). In this glassy region, the shear loss modulus is less than the shear storage modulus, $G'' \ll G'$. For the same material, the shear loss modulus, $G''$, is typically a lower value at $25^\circ$C, $\sim 10^5$ to $10^6$ Pa.

If the sample is deformed on a time scale larger than the segmental chain motion, $\tau$, the strain is accommodated inelastically and the material is in a rubbery state. In this region, $G''$ is equivalent to or less than $G'$, $G'' \leq G'$. As a rule for probing at different angular frequencies, $\omega$, a material is glassy when $\omega \tau \gg 1$, and rubbery when $\omega \tau \ll 1$. The maxima in $G''$ occurs when $\omega \tau \sim 1$ (Martin et al., 1994).

The segmental chain motion is very sensitive and dependent on the temperature. By changing the temperature alone, a material can undergo a glass transition shifting from
elastic to viscoelastic behavior. In relation to the temperature and frequency, the glass transition is defined as (Lucklum & Hauptmann, 1997):

\[ \tau (T_g) = \frac{1}{\omega} \]

**Equation 4-42**

The glass transition of a material makes itself manifest through moduli measurements of a material, in this case the complex shear modulus, \( G \). For the above example of a glassy material at 25°C, as the temperature is increased, \( G' \) decreases in a curved fashion with a greater declining slope in the \( T_g \) range. The value of \( G' \) eventually levels off in the range of ~ 10⁶ Pa. Upon increasing temperature, \( G'' \) goes through a maximum in the \( T_g \) region, ~ 10⁷ Pa, and then decreases to about 10⁶ Pa. This behavior is shown schematically in **Figure 4-5** (Lucklum & Hauptmann, 1997).

---

**Figure 4-5.** The shear storage, \( G' \), and shear loss modulus, \( G'' \), as a function of temperature for a polyisobutylene (PIB) film on a 5 MHz QCM as measured by Lucklum, *et al.* (Lucklum & Hauptmann, 1997).
Similar sorption data and its effect on the shear modulus have been measured. Upon vapor sorption the same trend is seen in $G'$ and $G''$ (Katz & Ward, 1996).

Likewise, for a glassy polymer at 25°C with a shear storage, $G'$, and loss modulus, $G''$, similar to the example shown in Figure 4-5, as sorption occurs, the film becomes plasticized and may lose some of its glassy characteristics. At 25°C, the added diluent causes a decrease in the shear storage modulus, $G'$. There would be a corresponding influence on the shear loss modulus, $G''$. The effects of additional adsorbate can be envisioned as a left-ward shifting of the curves in Figure 4-5.

4.3.5 QCM Use in Measuring Rheological Properties

As mentioned above, several investigators have successfully used QCM technology to probe the rheological properties of thin films. The techniques usually involve impedance analyzer measurements (Hillman et al., 2001; Lucklum et al., 2001) and can vary in forcing the QCM to oscillate at different harmonics (Johannsmann, 2001) or in simply cutting the oscillator driver power and monitoring the decay of the acoustic wave propagating through the QCM and film (Rodahl et al., 1997). A QCM generates and detects acoustic waves that are propagating in the quartz resonator and in the contacting media. Using an impedance analyzer, the acoustic conditions of the QCM surface are monitored by measuring the electrical admittance, $Y_{el}$, or the electrical impedance, $Z_{el}$, $(1/Y_{el})$ spectrums around the QCM resonant frequency. As discussed in Chapter 3, these measurements can then lead to information on the complex shear modulus, $G$, and whether the QCM is operating in the gravimetric or non-gravimetric regime. It is not always practical to employ impedance analyzer measurements. To purchase an impedance analyzer, generally an excess of $35,000 is needed. Impedance
analyzer measurements are often time consuming and complex because several types of scans are employed such as frequency, conductance, admittance, suceptance and phase angle measurements. In an attempt to determine the damping effects of a film-coated QCM upon vapor sorption, we have explored two techniques to approximate the viscoelastic changes in a film upon vapor sorption. These include the “Δf-ΔR Approach” (Lucklum & Hauptmann, 2000a) and the “Fast Three-Step Method” (Behling et al., 1999) to approximate the damping effects and the $G'$ and $G''$ values.

4.3.5.1 Δf-ΔR Technique

As stated previously, using the Maxtek Phase Lock Oscillator to drive the QCM at its resonant frequency, we are able to obtain two parameters, the resonant frequency, $f_r$, of the QCM and the motional resistance, $R$, of the QCM oscillation. The mechanical vibration of the QCM is influenced by the acoustic properties at the surface of the quartz crystal. Because of the piezoelectric nature of quartz (vide supra), these changes in the mechanical vibrations are also reflected as changes in the QCM electrical behavior (Vig, 2002). It is this electrical behavior that is monitored and yields the two output parameters that we receive. Not only does the mass of a film on the QCM influence this electrical behavior but the shear modulus, $G$, of the coating also contributes to the QCM response. The QCM frequency measurements are used to determine the mass loading changes upon vapor sorption. When the shear storage modulus is high (~ 1 GPa), the film on the QCM is rigid and oscillates in unison with the QCM. The QCM frequency shift is due only to the thickness of the film in this region. Complexities arise when the shear storage modulus is small (~ 1 MPa) (Lucklum et al., 1999). In this case the material is soft or rubbery and upon oscillation,
the upper film surface lags behind the oscillation at the QCM-film interface. Using only the frequency measurement to determine the mass loading leads to incorrect mass values. When the film is in this rubbery region, it is necessary to obtain some measure of the damping effects on the QCM frequency. This is determined from the motional resistance output from QCM oscillator.

The resonant frequency, \( f_0 \), is a measure of the maximum of the in-phase admittance, the real part. The motional resistance, \( R \), is defined as the reciprocal of the in-phase admittance magnitude at \( f_0 \), as shown in Figure 3-7 (Lucklum & Hauptmann, 2000a). In the oscillator circuit, \( R \) is determined from the amplification needed to maintain a constant amplitude in the oscillation (Eichelbaum et al., 1999; Lee et al., 2002). When it is assumed that the quartz properties are unchanged, \( \Delta R \) is due solely to the acoustic energy dissipation in the film coating.

The acoustic load impedance, \( Z_L \), on a QCM has both imaginary and real components. The \( \Delta f \) and \( \Delta R \) measurements can be approximated to reflect these real and imaginary parts of \( Z_L \). The following approximations are employed for this (Behling et al., 1998):

\[
\frac{\Delta f}{f} = -\frac{\text{Im}(Z_L)}{\pi Z_{cq}} \quad \text{Equation 4-43}
\]

\[
\frac{\Delta R}{2\omega L} = \frac{\text{Re}(Z_L)}{\pi Z_{cq}} \quad \text{Equation 4-44}
\]
where $L$ is the motional inductance defined in Chapter 2 and Appendix C, $f$ is the bare QCM resonant frequency, and $Z_{eq}$ is the characteristic QCM impedance.

In the analysis of our polymer data, the change in both the resonant frequency and the motional resistance upon vapor sorption is plotted as $\Delta R/\Omega$ versus $\Delta f/\text{Hz}$. The dependence of these two terms on the shear modulus is used to estimate the viscoelastic effects in the polymer films upon vapor sorption.

**4.3.5.2 The Fast Three-Step Method**

When a thin film exhibits viscoelastic properties, the discrepancy between the oscillatory motion between the upper and lower surfaces of the film lead to a phase shift, $\varphi$, defined as (Lucklum & Hauptmann, 2000b):

$$\varphi = \omega \sqrt{\frac{\rho_f h_f}{G}}$$  \hspace{1cm} \text{Equation 4-45}

where $\omega$ is the angular frequency, $\rho_f$ the film density, $G$ the shear modulus, and $h_f$ the film height. The phase shift is therefore dependent on these properties of the film and how they change upon vapor sorption. Very small phase shifts, can be achieved with small film heights and/or a large shear modulus. In this situation, $\varphi$ offers a negligible contribution to the mass calculation from the QCM frequency. Upon vapor sorption however, the film density, height, and shear modulus changes. It is important to trace these contributions and monitor how they are affecting the QCM mass measurements.

The “Three Step Method” presented by Behling, et al., utilizes a set of approximations for $\varphi$ which in turn lead to some approximations in the equation for
calculating the acoustic load impedance, $Z_L$ (Behling et al., 1999). The goal then is to use this value of $Z_L$ to calculate the shear modulus, $G$. Each approximation of $\phi$ yields a limiting equation of $Z_L$. The process is described below with a few modifications, which we employed:

1. Impedance analysis is used to measure the electrical admittance, $Y_{el}$ at the resonant frequency. This value in turn is used to calculate the acoustic load impedance, $Z_L$. The equation for this is shown in **Equation 3-9**. Because we do not routinely utilize an impedance analyzer, it is necessary for us to make a first approximation for the value of $Z_L$. Dr. Ralf Lucklum generously provided us with an Excel spreadsheet that he uses to model the admittance and impedance spectra of film-coated QCMs. The spreadsheet, ZYSYN_QP.xls, allows for input parameters for the quartz constants, the film thickness and density. Dr. Allan Smith added calculations to determine $\Delta f$, $\Delta R$, and the ratio of the actual frequency shift with that predicted by the Sauerbray equation. By using this spreadsheet, it is possible to determine a first approximation for the real and imaginary components of $Z_L$ from the $\Delta f$ and $\Delta R$ values substituted into **Equations 4-43** and **4-44**.

2. A set of approximations is used for $\phi$ ranging from 0 to $\pi$. These approximations are meant to cover different possible ranges of $\phi$ and to allow for a rearrangement of the equation used to relate the acoustic load impedance, $Z_L$ and the shear modulus, $G$. The equation for $Z_L$ is given here and described more fully in Chapter 3:

$$Z_L = i \cdot M \cdot v$$  \hspace{1cm} **Equation 4-46**

where $M$ is the mass factor and $v$ is the acoustic factor. Each of these is defined as:
\[ M = \omega \rho f h_f \]  

**Equation 4-47**

\[ \nu = \frac{\tan \phi}{\phi} \]  

**Equation 4-48**

\( \phi \) is defined in **Equation 4-44**. Rearrangement of the equations in the limits of \( \phi \) then allow for a determination of first values for \( G' \) and \( G'' \). These equations are shown in **Appendix C**. It is noted that not all of the values for \( G' \) and \( G'' \) can simultaneously be valid. These values are then substituted into the exact equation to solve for \( Z_L \):

\[
Z_L = i \sqrt{\rho_f G} \tan \left( \omega h_f \sqrt{\frac{\rho_f}{G}} \right) 
\]

**Equation 4-49**

which then give a set of \( Z_L \) values representing the real and imaginary components.

3. The best of the solutions from step 2 are used in an iterative process in **Equation 4-50**:

\[
y(\phi) = \frac{\tan \phi}{\phi} - \frac{Z_L}{iM} = 0
\]

**Equation 4-50**

and solved for \( \phi \).

For our studies with the polymer films, portions of this Three-Step Method have been employed. Step 1 is used with the modifications in getting initial values for \( Z_L \) from the \( \Delta f \) and \( \Delta R \) data that we measure. In Step 2, we have used TK Solver 4.0 to input the initial values for the real, \( Z_L' \), and imaginary, \( Z_L'' \), parts of \( Z_L \) into the
modified equations. From this we are able to calculate values of $G'$ and $G''$. As stated above, not all of the approximations yield valid values for $G'$ and $G''$. We are able to recalculate values of $Z_L'$ and $Z_L''$ in the second part of Step 2. However, according to the theory this is for the purpose of comparing the value with the measured value. Our initial value of $Z_L$ is not directly measured and this portion of the step has not been frequently utilized. Step 3 has also not been performed. Our main interest was to secure good approximate values of $G'$ and $G''$ to (1) determine whether upon sorption our polymer films were in the glassy or rubbery region and (2) determine whether the QCM was operating in the gravimetric regime.

4.4 Conclusion

Studies involving the behavior of thin films upon sorption of a gas adsorbate are necessary because of the broad applications of thin films. Using the QCM/HCC, we are able to extract three parameters during vapor sorption of a thin film: mass changes, thermal changes, and motional resistance changes. As shown above, these three measured values lend themselves to a broad range of data analysis applications ranging from thermodynamic parameters to rheological properties. An appealing feature of the QCM/HCC is that measurements are made simultaneously and trace the whole sorption/desorption process as opposed to static techniques that measure the parameters before and after sorption has taken place. Applications of the QCM/HCC in measuring the sorption processes in polymer and protein films will be presented in the following two chapters. The data analysis will follow the techniques outlined above.
List of References


Chapter 5. Thermodynamic and Rheological Properties of a Thin Film of Tecoflex, a Cycloaliphatic Poly (ether urethane), Upon Solvent Vapor Sorption

5.1 Introduction to Studies of Polymer Thin Films

The study of polymer-solvent interactions has attracted much attention through the years in the applications of: drying of polymers and paint coatings (Ngui & Mallapragada, 1999; Saby-Dubreuil et al., 2001), microlithography (Price & Buley, 1991), biological sensors (Bunde et al., 1998; Muramatsu et al., 1987; Rodahl et al., 1997; Sakti et al., 1999; Smyth et al., 2001; van Noort et al., 2001), polyimide protective coatings for microelectronic packaging (Bluestein et al., 1999), and chemically sensitive and selective polymer coatings for organic vapor determination (Lucklum et al., 1991; Matsuura et al., 1997; Schierbaum et al., 1992; Schierbaum et al., 1995) (Grate et al., 1996; Grate & Wise, 2001). Sorption of vapors leads to insights into the chemical and physical properties of a polymer. The area is of interest to investigators developing polymer materials with a high degree of functionality (Ichikawa et al., 2001).

The uptake of an adsorbent vapor by a polymer thin film can induce a variety of complex relaxational effects such as free volume changes, swelling, plasticization, the onset of a glass transition, heat capacity changes, and modifications in the viscosity. Some of these effects have been discussed in Chapter 4. In the studies presented here, we are able to probe the mass and thermal changes upon vapor sorption of a thin film. In the sorption process the QCM subjects the thin film to an oscillatory shear stress allowing us to also probe the vapor sorption effects on the shear storage and loss moduli, $G'$ and $G''$. These effects were discussed in Chapter 4.
5.1.1 Calorimetry of Polymers

Differential scanning calorimetry, DSC, is widely used in the study of polymers. In this technique, the difference in heat flow rate, or power, between a sample and reference is monitored against time as the temperature is scanned within a fixed range (Haines et al., 1998). DSC results for polymers usually display the heat capacity, $C_p$, as a function of temperature. A discontinuity is seen in the $C_p$ value around the glass transition. Complexities arise because the glassy state is a metastable state, depending on thermodynamics and kinetics (the rate of cooling) and not a thermodynamically stable phase independent of kinetic parameters (van Ekeren, 1998).

In his review regarding the future direction of thermal analysis and calorimetry as applied to polymers, Mathot states that current research focus includes the need for “real-life” measurements. He states that during polymeric processing, it is common to have high cooling rates, high pressures, and high shear rates. These variables all influence the glass transition and crystallization of the polymer material (Mathot, 2001).

In the area of high cooling rate measurements, Mathot asserts the usefulness of a new form of calorimetry, high performance differential scanning calorimeter, HPer DSC. This type of DSC offers the capability of using controlled linear heating and cooling scan rates of several hundred degrees per minute. Two benefits of HPer DSC include (1) the ability to better mimic processing conditions in the laboratory and (2) the ability to suppress some time-dependent reorganization processes such as cold-crystallization, recrystallization, and annealing.

High pressure DSC (up to 550 MPa) has surfaced as an important tool to probe polymer responses to high pressure processing. Two processing techniques, injection
molding and extrusion, involve pressures up to a few hundred MPa. There is a deficiency in high pressure polymer studies leaving this as an open area to investigators.

Another future direction in the area of “real life” calorimetry includes the challenge of performing in-situ and real-time measurements. During the polymerization process it would be helpful to study the interaction between polymerization-crystallization-morphology. The author notes two possibilities for this: the use of scanning probe microscopy techniques or the utilization of combining several different techniques. With our QCM/HCC instrumentation at the present time we do not have the ability to scan temperature or increase the pressure of measurements, however, we are able to mimic in-situ situations of polymer sorption or drying.

Another emerging application and modification of DSC includes temperature modulated differential scanning calorimetry (TMDSC) (Ribeiro & Grolier, 1999). Using this method, the total differential heat flow is separated into two components: a reversing heat flow which is related to the heat capacity and a non-reversing heat flow which is related to the kinetics. By separating these components, it is possible to detect weak transitions, heat capacity changes in a quasi-isothermal mode, and to distinguish superimposable phenomena.

5.1.2 QCM Studies of Polymer Sorption

There exists a large magnitude of research involving QCMs to probe vapor sorption of polymer films. The studies range in their approach and methodology and include:

- using impedance analysis with QCM technology to probe the viscoelastic effects of polymer sorption (Bandey et al., 1999; Behling et al., 1998a; Hillman et al., 2001; Lucklum & Hauptmann, 2001; Martin et al., 2000; Muramatsu & Kimura, 1992)
- analyzing of impedance analysis measurements to study resonant frequency shifts and bandwidth measurements as the QCM oscillates at different harmonics (Johannsmann, 1999; Johannsmann, 2001)

- utilizing a “sandwich configuration” for thick films where a polymer film is coated with a second aluminum overlayer. The elastic properties of the overlayer are known and the overlayer enhances the shear stress of the polymer film (Wolff & Johannsmann, 2000).

- combining QCM technology with dielectric and thermopile measurements to monitor capacitance and temperature changes upon vapor sorption (Schierbaum et al., 1992; Zhou et al., 1996).

- analyzing the decay of the acoustic wave propagating through a thin film (Rodahl & Kasemo, 1996)

Many of the above-mentioned methodologies are focused toward polymer-coated QCM use as vapor sensors. Because of their sensitivity as mass detectors, thickness shear mode resonators, TSM, and surface acoustic wave devices, SAW, are commonly used to monitor volatile organic compounds (VOCs). These sensors are typically coated with a polymer film, which then acts as a chemically sensitive interface (Hierlemann et al., 2000).

Three features make polymers appealing for their use as sensors: rubbery polymers sorb vapors in a rapid and reversible manner, polymers can easily be put into the form of a thin film which adheres to surfaces, and varying the chemical structure of the polymer allows for vapor sorption selectivity (Grate et al., 1993). One of the demands of chemical sensors is that the adsorption process is reversible. To guarantee this usually an array of sensors are used. Each sensor is coated with a different organic polymer with each having partial selectivity to most VOCs. Mathematical methods are then used to analyze the data (Hierlemann et al., 2000; Lucklum et al., 1999; Lucklum et al., 1991).
5.2 Properties of Tecoflex®

Tecoflex® is a cycloaliphatic, poly(ether urethane), PEUT, synthesized and distributed through Thermedics Polymer Products (Woburn, MA) (2002). Introduced to the medical market in 1983, Tecoflex is used in many medical tubing applications e.g. for heart catheters. In 1985, Tecoflex became available to the optical industry as a medical grade polyurethane. Adding to this, in 1997, Tecoflex was introduced as a thermoplastic polyurethane interlayer film. This new application allowed for the lamination of glass and various plastics. This newer function of the polymer has proven useful for security reasons being used as security transparencies for banks, prisons, transportation areas and architectural areas. Interlayer films of this polymer are also used for the lamination of computer privacy screens, radio frequency interference filters, electromagnetic interference filters and computer touch screens.

Tecoflex is a random copolymer and its repeat units are shown in Figure 5-1.

Figure 5-1. The repeat units for the cycloaliphatic poly(ether urethane) Tecoflex.
The polymer has both a soft and hard $T_g$. The soft part (the short carbon chain) has a $T_g \sim -60^\circ C$, and a melting temperature, $T_m$, in the range of $155^\circ C – 190^\circ C$, while the hard portion (the cyclic portion) has a $T_g$ value of $\sim 40 – 50^\circ C$ and a $T_m \sim 310 – 375^\circ C$. The reported density is 1.11 g/ml

5.3 Tecoflex Sorption Studies Using the QCM/HCC – Past and Present

The coupling of QCM technology with heat conduction calorimetry has generated interesting results and provided a broader range of measurable parameters during the sorption process. Using the QCM/HCC able to measure:

(1) mass changes upon sorption processes

(2) corresponding heat effects

(3) motional resistance, a measure of damping that occurs in the polymer film as vapor sorption increases the film becomes plasticized and the viscoelastic nature become more evident, thus also affect the $T_g$

(4) applications of this data in determining sorption enthalpies, partition coefficients, diffusion coefficient

Dr. Hamid Shirazi a former colleague in our laboratory in his work to help develop and test the QCM/HCC studied the gas sorption of Tecoflex using six different solvents: toluene, chloroform, ethanol, acetone, n-hexane, and carbontetrachloride (Shirazi, 2000; Smith & Shirazi, 1999; Smith & Shirazi, 2000). At the time we did not have the capabilities to measure the output conductance voltage from the oscillator driver and therefore could not monitor the motional resistance changes upon vapor sorption by the tecoflex film. Three films that he studied were 0.70, 0.78, and 2.09 µm thick. For two
of these three films, 0.78 and 2.09 µm results for tecoflex sorption by ethanol will be compared with our results to add a variety of film thicknesses.

The studies presented here do not involve the variety of solvents used in the previous studies. Instead, a wider range of film thicknesses were used. The solvents were limited to ethanol and water. We wanted to:

(1) check the effect of the sorption processes on different thickness of polymer film.

(2) monitor the motional resistance upon sorption and compare different film thicknesses.

(3) evaluate the results in terms of sorption enthalpies, sorption isotherms, partition coefficients, and diffusion coefficients.

The work of four undergraduate researchers in our laboratory, Mr. Jason Riggs, Ms. Rebecca Mason, Betty Jacob and Anna Ayrapetova is gratefully acknowledged. Jason spent time in probing possible coatings to treat QCM surfaces to create hydrophilic and hydrophobic surfaces. He also spent time optimizing tecoflex solution concentrations to be used for drop coating. A film that he prepared, 4.71 µm thick, is included in the water and ethanol sorption studies. Becky spent time in our laboratory helping to make some additions to our instrumentation such as the new Nanovoltmeter preamplifier and she also worked with optimizing tecoflex solution concentrations. A film that she prepared by spin coating, 0.83 µm thick, is shown in the water vapor studies presented below. Betty and Anna worked with Hamid Shirazi and spent time exploring spin-coating techniques to prepare Tecoflex films to study the sorption of water and acetonitrile. Their investigations on a 1.1 µm film are included in the water vapor sorption studies.
In these studies Tecoflex films of varying thicknesses were exposed to controlled amounts of water and ethanol vapor. The mass and thermal signals were treated as stated in Chapter 3 and data was then analyzed as outlined in Chapter 4.

5.4 Experimental Procedure

Tecoflex (HP-60D) was purchased from Thermedics (Woburn, MA). The polymer is shipped as round pellets, \( \sim 2-3 \) mm diameter. Because the polymer is very hygroscopic before any solutions were made, the Tecoflex pellets were dried in an oven at \( \sim 55^\circ C \) so as not to induce any polymer melt.

Prior to coating, each 5 MHz QCM purchased from Maxtek, (P/N 149211-1, Model SC-501-1) was cleaned in Piranha solution, (one part 30% \( H_2O_2 \) in three parts 98% \( H_2SO_4 \)). Each QCM was immersed in the solution for \( \sim 2 \) min. and then rinsed vigorously with deionized water. The immersion in the Piranha solution and rinsing with water was repeated. Each QCM was then dried with a flow of \( N_2 \) gas. The QCM’s were then placed in the oven (\( \sim 60^\circ C \)) for further drying. Each QCM was mounted in the instrumental chamber and the resonant frequency was determined.

All but one of the films was prepared by spin coating. The spin coater (HRL Headway Research, Garland, TX) was generously at our disposal through the kindness of Dr. Yen Wei and his research group. Because many industries rely on the electronic, magnetic, and optical properties of thin, uniform films, spin-coating has become a well-studied and characterized process. In its simplest form, spin-coating can be characterized by four processes: (1) dispensing of enough coating solution onto the substrate, a QCM in this case, to wet the surface, (2) acceleration to the desired rotation speed and expulsion of excess fluid, (3) gradual fluid thinning resulting from spinning
at a constant rate, (4) evaporation of solvent as substrate continues spinning (Birnie, 2000). The viscous flow effects control the first part of the process while evaporation effects control the latter phases of the process.

Each of the Tecoflex coated QCM’s will be identified by the thickness of the film. For the **0.78** and **2.09 µm** films prepared by Hamid Shirazi and used as a comparison with our ethanol studies, the tecoflex solution was prepared by dissolving 20 mg/ml in warm chloroform (~ 40°C). These films were cast by spin coating at 3000 rpm. The tecoflex was added via a glass pipette and repeatedly dropped onto the QCM surface as it was rotating on the spin coater, until the desired thickness was achieved.

For the **0.83 µm** film prepared by Becky Mason, a 60 mg/ml solution of Tecoflex was prepared in chloroform. The goal was to prepare a viscous solution of Tecoflex. The increased concentration was used in an attempt to apply the Tecoflex only once before the spin coater was turned on and not in repeated succession as the spinning process was proceeding. For this process, the top of the QCM (the area of the QCM gold electrode) was covered with Tecoflex applied via a glass pipette. The spin coater was turned immediately on at 2700 rpm for one minute. A visibly thin uniform film was produced.

The **0.75 µm** film was created by using the same 60 mg/ml solution. The top gold surface was covered and the spin coater was set at 4500 rpm for 45 sec. To coat the **4.7 µm** film, Jason Riggs used the drop-coat method. The solution was dropped onto the QCM surface, allowed to spread naturally and the solvent evaporated off by placing the QCM in the oven for ~ 2 hours.
To achieve the 8.5 µm film, a more viscous solution of tecoflex/chloroform was used, 100 mg/ml. In this trial, a generous portion of Tecoflex was poured from a beaker onto the top QCM gold electrode surface. The spin coater was then started at 2000 rpm for one minute. To anneal/dry the tecoflex coated QCM was placed in the oven at 50°C for two hours.

Each of the tecoflex coated QCM’s was then placed in the QCM/HCC chamber and further dried with a flow of N₂ gas prior to each of the experiments.

A summary of the newly studied Tecoflex coated QCM’s is listed in Table 5-1. The initial parameters for H. Shirazi, B. Jacob, and A. Ayrapetova’s films are not included but their results are shown as a means of comparison. The mass was determined using the Sauerbray relationship (Chapter 3). The thickness of the film was found by utilizing the relationship between the film mass and density (1.11 g/ml) of Tecoflex.

Table 5-1. QCM data for coating of Tecoflex films used in the sorption studies.

<table>
<thead>
<tr>
<th>QCM #</th>
<th>Frequency before coating Hz</th>
<th>Frequency after coating Hz</th>
<th>Mass of Tecoflex film µg/cm²</th>
<th>Thickness of Tecoflex film µm</th>
<th>Method of coating</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>4991687.1</td>
<td>4986988.1</td>
<td>83</td>
<td>0.75</td>
<td>Spin</td>
</tr>
<tr>
<td>35</td>
<td>4991539.8</td>
<td>4986303.5</td>
<td>93</td>
<td>0.83</td>
<td>Spin</td>
</tr>
<tr>
<td>40</td>
<td>4997270.2</td>
<td>4967677.9</td>
<td>523</td>
<td>4.7</td>
<td>Drop</td>
</tr>
<tr>
<td>34</td>
<td>4994142.6</td>
<td>4940602.7</td>
<td>946</td>
<td>8.5</td>
<td>Spin</td>
</tr>
</tbody>
</table>

For the water sorption experiments, deionized water was placed in the glass, gas bubbler, the chamber was sealed and clamped and then placed in the constant temperature bath at 25°C.
A compilation of the films used and the experimental parameters for water sorption are shown in Table 5-2.

**Table 5-2.** Tecoflex – water sorption experiments studied with the QCM/HCC.

<table>
<thead>
<tr>
<th>File</th>
<th>Water Vapor Activity / $a_w$</th>
<th>Incremental Step for $a_w$</th>
<th>Time for each step min.</th>
<th>Film mass $\mu g/cm^2$</th>
<th>Film thickness $\mu m$</th>
<th>Motional resistance data available</th>
</tr>
</thead>
<tbody>
<tr>
<td>02-01-08-2</td>
<td>0 - 0.80</td>
<td>0.16</td>
<td>26</td>
<td>83</td>
<td>0.75</td>
<td>y</td>
</tr>
<tr>
<td>02-06-11-1*</td>
<td>0.08 - 0.84</td>
<td>0.15</td>
<td>35</td>
<td>83</td>
<td>0.75</td>
<td>y</td>
</tr>
<tr>
<td>01-08-07-1</td>
<td>0 - 0.80</td>
<td>0.16</td>
<td>18</td>
<td>93</td>
<td>0.83</td>
<td>n</td>
</tr>
<tr>
<td>99-10-26-2</td>
<td>0 - 0.80</td>
<td>0.16</td>
<td>26</td>
<td>119</td>
<td>1.1</td>
<td>n</td>
</tr>
<tr>
<td>01-11-20-2</td>
<td>0 - 0.80</td>
<td>0.16</td>
<td>26</td>
<td>523</td>
<td>4.7</td>
<td>y</td>
</tr>
<tr>
<td>01-09-11-1</td>
<td>0 – 0.80</td>
<td>0.16</td>
<td>70</td>
<td>946</td>
<td>8.5</td>
<td>n</td>
</tr>
<tr>
<td>01-09-27-1</td>
<td>0 - 0.80</td>
<td>0.16</td>
<td>70</td>
<td>946</td>
<td>8.5</td>
<td>y</td>
</tr>
<tr>
<td>01-12-20-3</td>
<td>0 - 0.80</td>
<td>0.16</td>
<td>70</td>
<td>946</td>
<td>8.5</td>
<td>y</td>
</tr>
</tbody>
</table>

* sorption at 40°C

The ethanol sorption experiments are summarized in Table 5-3. For the ethanol sorption experiments, dehydrated, 200 proof ethanol (Pharmco) was used.

**Table 5-3.** Tecoflex – ethanol sorption experiments studied with the QCM/HCC.

<table>
<thead>
<tr>
<th>File</th>
<th>EtOH Vapor Activity / $a_{EtOH}$</th>
<th>Incremental Step for $a_{EtOH}$</th>
<th>Time for each step min.</th>
<th>Film mass $\mu g/cm^2$</th>
<th>Film thickness $\mu m$</th>
<th>Motional resistance data available</th>
</tr>
</thead>
<tbody>
<tr>
<td>01-08-30-1</td>
<td>0.26 – 0.58</td>
<td>0.07</td>
<td>26</td>
<td>83</td>
<td>0.75</td>
<td>n</td>
</tr>
<tr>
<td>02-01-09-3</td>
<td>0 - 0.32</td>
<td>0.07</td>
<td>26</td>
<td>83</td>
<td>0.75</td>
<td>y</td>
</tr>
<tr>
<td>02-01-10-1</td>
<td>0.26 – 0.58</td>
<td>0.07</td>
<td>26</td>
<td>83</td>
<td>0.75</td>
<td>y</td>
</tr>
<tr>
<td>99-07-15-1</td>
<td>0.13 – 0.45</td>
<td>0.07</td>
<td>26</td>
<td>86</td>
<td>0.78</td>
<td>n</td>
</tr>
<tr>
<td>99-07-30-1</td>
<td>0.13 – 0.64</td>
<td>0.13</td>
<td>26</td>
<td>227</td>
<td>2.09</td>
<td>n</td>
</tr>
<tr>
<td>01-11-26-1</td>
<td>0.26 – 0.58</td>
<td>0.07</td>
<td>26</td>
<td>522</td>
<td>4.7</td>
<td>y</td>
</tr>
<tr>
<td>01-09-06-1</td>
<td>0.26 – 0.58</td>
<td>0.07</td>
<td>70</td>
<td>946</td>
<td>8.5</td>
<td>n</td>
</tr>
<tr>
<td>01-09-25-1</td>
<td>0.26 – 0.58</td>
<td>0.07</td>
<td>70</td>
<td>946</td>
<td>8.5</td>
<td>y</td>
</tr>
</tbody>
</table>
5.5 Results of Sorption Measurements

The initial data of the sorption experiments will be shown in each section. The data from the QCM/HCC includes the (1) applied solvent activity and when water is used as the solvent, an added feature is the (2) water vapor activity measured via the relative humidity meter, (3) the mass signal from the QCM frequency, (4) the thermal power from the voltage output of the thermopiles, (5) the corresponding heat effects found by integrating the thermal power using the software Origin, (Microcal, Northampton, MA), (6) the motional resistance of the film measured as an output voltage proportional to the conductance. Through a simple equation, the output conductance voltage from the phase lock oscillator is converted to the motional resistance (see Chapter 3). Because the addition of the phase lock oscillator occurred after some of the initial studies, this data is not available for all of the films. The films where this information is available are indicated in Tables 5-2 and 5-3. From the output conductance voltage, the power generated and dissipated in the QCM was also calculated and monitored.

5.5.1 Water Sorption by a Tecoflex film.

Five different films were used to study the water sorption of the polymer Tecoflex. All of the experimental runs were performed at 25°C except for the thinnest film, 0.75 µm, which was also tested at 40°C. The hydration ranges spanned from a water vapor activity, \( a_w \), of 0 to 0.80. The 40°C sorption study ranged in \( a_w \) from 0.08 to 0.84. Figures 5-2 to 5-8 show the results of these studies. The initial data for Anna and Betty’s, 1.1 µm film is not shown here but their results are included in the plots summarizing the data analysis such as the sorption enthalpies, isotherms, diffusion coefficients and partition coefficients.
Figure 5-2 displays results of water sorption of the thinnest polymer film. At the higher water vapor activities, $a_w$, the measured $a_w$ is less than the applied $a_w$. Two factors can account for this; the first is that the relative humidity meter is external to the water bath. It is possible that if there is a noticeable temperature difference between the $25^\circ C$ constant temperature bath and the ambient room temperature, the water vapor activity measured by the relative humidity meter will be slightly different. The second possibility for the discrepancy is that the meter the relative humidity meter is downstream from the QCM/HCC chamber and measures the relative humidity after the water vapor has interacted with the film. If the film was sorbing larger amounts of water, this would be reflected as less water vapor reaching the RH meter. At the lower $a_w$ the two values, applied and measured, appear to be the same. When looking at the mass trace, there does appear to be some hysteresis, the film took in more water than was desorbed. At the higher $a_w$, from the slope of the mass trace, it appears that more time could have been allotted for the film to reach equilibrium with the water vapor. With the film taking in water, swelling and free volume increases would be expected causing slow structural relaxation processes in the polymer thus allowing the film to adsorb more water vapor at the higher water vapor activities. This is evidenced by the mass change seen in the steps. At the lower $a_w$, the film sorbs $\sim 0.2 - 0.3 \ \mu g$ of water, where at the higher $a_w$, the film consistently sorbs $\sim 0.5 - 0.7 \ \mu g$ of water. The four cycles appear to be very repeatable. The corresponding thermal signal is shown along with the integrated heat. The motional resistance changes are considered to be small. A bare QCM has a motional resistance of $\sim 12$ to $13$ ohms. This polymer thin film-coated QCM exhibits a motional resistance of $\sim 14.5$ ohms. The motional resistance changes
upon sorption are defined incrementally and correspond with the $a_w$ changes. These changes span only 1 ohm for the sorption processes. The thermal power generated in the QCM was also calculated and plotted to show its change upon vapor sorption. The total power changes by only 0.05 µW through the process indicating that any relaxational effects are minimal as recorded by the motional resistance. The film appears to be in the glassy state throughout the sorption process. Upon vapor sorption, the $T_g$ would be lowered from the normal starting values of -60 °C and 40-50°C for the soft and hard parts respectively. However, because the QCM measurements are at 5 MHz instead of approximately 10 Hz, the starting value for the $T_g$ is much higher. When applying the general rule of a 6-7°C increase in the $T_g$ for every decade of Hz increase (as outlined in Chapter 4) in the probing frequency, the starting $T_g$ values become, ~ -30°C for the soft part and ~ 70 to 80°C for the hard part. Upon vapor sorption at 25°C the hard part of the polymer may still be in its glassy state.

The same 0.75 µm film is shown in Figure 5-3 however this experiment was performed at 40°C. Because of the higher temperature, the water vapor activity could not be measured by the relative humidity meter. At the higher temperature, the film did adsorb more water in each incremental step than the trial at 25°C. The film does not display the same hysteresis effects seen in the mass trace of Figure 5-2. The amount of water sorbed at each step does appear to level off indicating that equilibrium has been reached. There are a few anomalies present in the mass trace such as the second step of the second full cycle, upon sorption and desorption, there is an over shoot in the mass signal and then a leveling off. In the third full sorption cycle, this sharp mass increase appears at the fourth step of the sorption process. The generated thermal signals are
shown and the corresponding integrated heat. The baseline of the thermal power trace is slightly noisier than that measured at 25°C. The motional resistance was noisier than the measurements at 25°C, by a factor of 10. Therefore, the data depicted in the graphs has been smoothed to show the incremental changes upon sorption. The thermal power dissipated in the QCM is negligible and spans 0.3 µW. The erratic behavior can be seen in the thermal power in the QCM. At 5 MHz even though the $T_g$ can be predicted to be $\sim 70 - 80^\circ$C for the hard part of the polymer, the increased measuring temperature and the vapor sorption both lower the initial value of the $T_g$.

The experiment on the 0.83 µm film shown in Figure 5-4 was done before we had the capability of measuring the motional resistance. The mass trace shows the same pattern as the 0.75 µm film at 25°C shown in Figure 5-2. At the higher $a_w$, the mass changes occur in larger steps than seen in the lower $a_w$ steps. There does not appear to be any evidence of hysteresis. The thermal power trace reveals a slight overshoot in the baseline after each sorption step and then a returning to the baseline. It is not clear as to why this overshoot in the baseline occurred. One possible explanation would be that more time is needed for the sorption step to reach an equilibrium state. However, this is not reflected in the mass trace; each sorption step appears to reach equilibrium. The behavior seems to be isolated to the thermal signal. Another possibility includes transfer of the thermopile voltage. If there was a noticeable difference between the constant temperature bath and the outside preamplifier the Seebeck effect may play a part in causing a voltage potential between the warmer and colder ends of the cables. The effect is small and not visible in the mass trace. The integrated heat is then shown for each sorption step.
**Figure 5-5** depicts the 4.7 µm film that was deposited on the QCM by drop coating. The mass trace follows the same pattern as shown in the previous two films. Motional resistance data was available for the studies done on this run. The starting base motional resistance for this film increased from ~ 12 ohms for a bare QCM to ~ 76 ohms for this film coated QCM. The motional resistance data closely mimics the mass changes and reveals incremental changes in the film structural properties upon vapor sorption. Because the low powered phase lock oscillator (PLO) was used, the power generated in the QCM changed by less than 0.8 µW.

**Figures 5-6, 5-7 and 5-8** are the results of the 8.5 µm film with the results in 5-8 studied four months after those shown in 5-6 and 5-7. **Figure 5-6** are the results of the first studies done on the 8.5 µm film. The applied and measured $a_w$ values closely match each other except at the highest $a_w$ values. The measured $a_w$ at this point is slightly less than the applied. The film may be able to hold more water at this point releasing less water to the activity meter. From the mass trace, it appears that equilibrium is attained with each sorption step. The corresponding thermal power and integrated heat traces are also shown.

The study in **Figure 5-7** shows the same film undergoing water sorption with the motional resistance data now available. The applied and measured water vapor activities are closely matched in the first sorption cycle, however there are some differences in the second sorption cycle. The measured $a_w$ is larger than the applied $a_w$. This could result from less water being sorbed in the film or that the relative humidity meter could have been at a slightly higher temperature since it was outside of the constant temperature bath. The mass trace does not reveal that less water was sorbed by
the film, leading to the conclusion that the relative humidity meter was at a slightly higher temperature. It is interesting to note that the motional resistance of the dry film coated QCM in this case is ~ 175 ohms. The overall change in the motional resistance from the first sorption step to the last is ~ 75 ohms whereas for the 4.7 µm film the overall motional resistance changes spanned ~ 35 ohms. The low powered PLO was used and the power generated in the QCM changed by less than 0.5 µW.

Figure 5-8 depicts the same film studied four months later. Only one sorption cycle was performed, however the time scale for the sorption was the same as the previous run. The mass and thermal traces closely resemble the previous two runs. The starting motional resistance for the film is ~ 7-8 ohms less. This may be due to the fact that the film was slightly drier in this run and also that slow relaxational changes in the polymer over time led to greater ordering of the chains.
Figure 5-2. Tecoflex/water sorption, 0.75 µm film at 25°C. a) applied and measured $a_w$, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance, f) thermal power generated in crystal.
Figure 5-3. Tecoflex/water sorption, \(0.75 \, \mu\text{m}\) film, at \(40^\circ\text{C}\). a) applied \(a_w\), b) mass trace, c) thermal power, d) integrated heat, e) motional resistance, f) thermal power generated in QCM.
**Figure 5-4.** Tecoflex/water sorption, 0.83 µm film, at 25°C.  
**a)** applied aw,  
**b)** mass trace,  
**c)** thermal power,  
**d)** integrated heat.
Figure 5-5. Tecoflex/water sorption, 4.7 µm film at 25°C. a) applied $a_w$, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance, f) thermal power generated in the QCM.
Figure 5-6. Tecoflex/water sorption, 8.5 µm film, at 25°C. a) applied and measured $a_w$, b) mass trace, c) thermal power, d) integrated heat.
Figure 5-7. Tecoflex/water sorption, 8.5 µm film, at 25°C. a) applied and measured $a_w$, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance, f) thermal power generated in QCM.
Figure 5-8. Tecoflex/water sorption, 8.5 µm film, at 25°C. a) applied and measured $a_{w}$, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance, f) thermal power generated in QCM.
5.5.2 Ethanol Sorption by a Tecoflex Film.

The results of three different films, 0.75, 4.7, 8.5 µm, being exposed to ethanol vapor will be shown here. Two films studied by Hamid Shirazi, 0.78 and 2.09 µm, were also exposed to ethanol vapor; the results of these two films will be shown in the analysis of the sorption isotherms, hydration enthalpies, diffusion coefficients and partition coefficients. The applied vapor activity differed for some of the experimental runs. The first three data sets for the 0.75 µm film are shown in Figures 5-9, 5-10, and 5-11. Figure 5-9 displays data for the film being exposed to an ethanol vapor activity, $a_{\text{EtOH}}$, range from 0.26 to 0.58. The data reveal the simultaneous mass and thermal changes corresponding to the vapor activity changes. Motional resistance data was not available for this experimental run.

Figure 5-10 depicts the 0.75 µm film exposed to $a_{\text{EtOH}}$ ranging from 0 to 0.32. The motional resistance data was noisy and the graph shows data that has been smoothed to better see the incremental changes. When comparing this experimental run with that of water sorption shown in Figure 5-2, the film adsorbs more than five times more ethanol than water for the same vapor activities.

The results shown in Figure 5-11 are for ethanol sorption in the same activity range as those shown in Figure 5-9, $a_{\text{EtOH}} = 0.26 – 0.58$. These results show the same film studied four months apart. The overall magnitude and pattern of the mass changes appear to be very repeatable. The mass trace in Figure 5-11 shows slight hysteresis at the higher vapor activities. The thermal power trace when compared for each run appears to be repeatable. The motional resistance was noisy, however, it can be seen that the motional resistance closely corresponds to the sorption events. The effect of the
higher ethanol vapor concentrations does not noticeably affect the motional resistance when this is compared with the results in Figure 5-10, the same film studied at lower vapor activities.

**Figure 5-12** reveals data for the 4.7 µm film. This film was studied at the same ethanol vapor concentrations as the 0.75 µm film shown in Figures 5-9 and 5-11, \( a_{e\text{OH}} = 0.26 – 0.58 \). As expected, the thicker film is able to sorb greater amounts of ethanol almost ten times the amount. The mass trace for the 4.7 µm film reveals no hysteresis and is repeatable for the three full cycles shown. The thermal power shows a very stable baseline with repeatable peaks corresponding to the sorption steps. The starting motional resistance value is higher in this ethanol study than that shown for the same film studied with water, shown in Figure 5-5, ~ 75 ohms for the water versus ~ 175 ohms for the ethanol. Previous to the data shown in the figures, the film was exposed to a half cycle of the vapor, from 0.58 to 0.26 for the water in Figure 5-5 and ethanol in Figure 5-12. Water and ethanol are present in the film at the start of the studies. The ethanol appears to have a greater effect on the structural relaxation processes of the Tecoflex film. The motional resistance for this 4.7 µm film undergoing ethanol sorption does not show the same magnitude of increase at the higher activities when compared to the mass increase. The water sorption for the same film differs because the magnitude of the motional resistance change is similar to the mass changes.

**Figures 5-13** and 5-14 depict the ethanol sorption data for the 8.5 µm film exposed to \( a_{e\text{OH}} = 0.26 – 0.58 \). The two files represent data taken two weeks apart. The motional resistance data was not available for the sorption processes shown in **Figure 5-13**. The amount of ethanol sorbed in the two trials is very similar. In comparing this
data with the water sorption shown in Figures 5-7 and 5-8 for the same film, the film is able sorb almost 4 times as much ethanol than water. The magnitude of the thermal signals in Figures 5-13 and 5-14 are similar, however, in Figure 5-13, the thermal power trace shows incremental stepwise changes in the baseline. The Maxtek low powered PLO was used for the data shown in Figure 5-14. From Figure 5-14 it can be seen that the power generated in the crystal was small in magnitude ~ 0.6 µW. The starting motional resistance, ~250 ohms, is larger in magnitude than seen in any of the other films. At the highest sorption peaks, the motional resistance is noisier than at the lower vapor activities. The motional resistance is calculated from the conductance output voltage. There is an inverse relationship between the two. When the conductance voltage is smaller, the signal to noise ratio decreases and this is reflected in the motional resistance calculation and plot.

When comparing the water and ethanol sorption studies, the motional resistance data is more pronounced in the ethanol studies and especially so in the thicker films, e.g. 4.7 µm and 8.5 µm. The motional resistance changes are sensitive to any deviations of QCM acting as a gravimetric tool. In general, an increase in the motional resistance indicates increased viscoelastic contributions from the polymer material. Researchers note that it is not possible to pinpoint a maximum allowed resistance before the QCM is not operating in the gravimetric regime. A maximum allowed resistance would depend on the loss factor, κ, defined as the ratio of the shear loss modulus to the shear storage modulus, $G''/G'$ (Lucklum et al., 2000).

Following the data for the ethanol sorption, the thermopile calibration results are shown. The calibration procedure and equations are included in Chapter 3.
Figure 5-9. Tecoflex/ethanol sorption, 0.75 µm film, at 25°C. a) applied EtOH vapor activity, b) mass trace, c) thermal power, d) integrated heat.
Figure 5-10. Tecoflex/ethanol sorption, 0.75 µm film, at 25°C. a) applied EtOH vapor activity, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance, f) thermal power generated in the QCM.
Figure 5-11. Tecoflex/ethanol sorption, 0.75 µm film, at 25°C. a) applied EtOH vapor activity, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance, f) thermal power generated in QCM.
Figure 5-12. Tecoflex/ethanol sorption, 4.7 µm film, at 25°C. a) applied EtOH vapor activity, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance, f) thermal power generated in QCM.
Figure 5-13. Tecoflex/ethanol sorption, 8.5 µm film, at 25°C. a) applied EtOH vapor activity, b) mass trace, c) thermal power, d) integrated heat.
Figure 5-14. Tecoflex/ethanol sorption, 8.5 µm film, at 25°C. a) applied EtOH vapor activity, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance, f) thermal power generated in QCM.
5.5.3 Calibration of Thermopiles.

As discussed in Chapter 3, using the Maxtek PLO, it is possible to use the known power generated in the QCM as a means of electrically calibrating the thermopiles. Results for three of the films are shown in Table 5-4. Calibrations were performed both before and after each experimental run. The deviation between the pre- and post-calibrations is minimal, with the thickest film, 8.5 µm, displaying the largest deviation.

Table 5-4. Calibration coefficients for the thermopiles used in measuring the heat effects caused by the sorption of a thin film of Tecoflex.

<table>
<thead>
<tr>
<th>File</th>
<th>Film thickness</th>
<th>ε</th>
<th>Experimental condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>02-01-09-1</td>
<td>0.75</td>
<td>3.2285</td>
<td>pre-run</td>
</tr>
<tr>
<td>02-01-11-1</td>
<td>0.75</td>
<td>3.2634</td>
<td>post-run</td>
</tr>
<tr>
<td>01-11-27-1</td>
<td>4.7</td>
<td>3.0322</td>
<td>pre-run</td>
</tr>
<tr>
<td>01-11-29-1</td>
<td>4.7</td>
<td>3.0030</td>
<td>post-run</td>
</tr>
<tr>
<td>01-12-20-1</td>
<td>8.5</td>
<td>2.9720</td>
<td>pre-run</td>
</tr>
<tr>
<td>01-12-21-1</td>
<td>8.5</td>
<td>3.1280</td>
<td>post-run</td>
</tr>
</tbody>
</table>

The calibrations were performed using the regular powered PLO with the film-coated QCM. From the conductance output voltage, the power generated in the crystal was calculated. The output voltage proportional to the thermal signal was recorded and the steady-state voltage was used to determine the calibration coefficient, ε. Examples of the plots are shown in Figure 5-15. In (a), the thermopile output voltage, proportional to the thermal power, is plotted. When the PLO is connected, heat is generated in the crystal and upon disconnecting the PLO, an endothermic signal is seen indicating a loss of heat being generated in the crystal. In (b), the steady state output
conductance voltage is recorded. This is used to calculate the generated thermal power in the crystal.

![Figure 5-15. Calibration of the thermopiles. (a) The voltage proportional to the thermal power generated in the 0.75 µm polymer film coated QCM. Exothermic signals are in the downward direction. (b) The output conductance voltage from the phase lock oscillator.](image)

5.6 Analysis and Discussion of Sorption Processes

The water and ethanol sorption of the Tecoflex films were analyzed for their thermodynamic and rheological properties. The steady state mass and thermal traces both indicate that upon sorption and desorption, equilibrium was reached thus making the data amenable to a thermodynamic analysis.

5.6.1 Sorption Enthalpies

Sorption enthalpies were found by taking the quotient of the integrated heat in kilojoules and the moles of water or ethanol sorbed in the film. A description of this thermodynamic parameter and the equations used in the calculations are found in Chapter 4.
Figure 5-16 displays the summary of results for five different Tecoflex films undergoing water sorption. The 0.75 µm film was the only sample done at 40°C and is shown here for comparison. The larger magnitude of the $\Delta_{\text{sorption}}H$ at 40°C is expected when compared to the values measured at 25°C, however, the $\Delta_{\text{vaporization}}H$ of water at 40°C is ~ -44.44 kJ/mol and the measured values are larger than this. A calibration of the thermopiles was not done at 40°C and this could be the source of the larger enthalpy value. The $\Delta_{\text{vaporization}}H$ of water at 25°C is –43.98 kJ/mol (Lide, 1997). For a physisorption process, the $\Delta_{\text{sorption}}H$ would be similar in magnitude to the $\Delta_{\text{vaporization}}H$, a discussion of this is included in Chapter 4. The magnitude of the enthalpy values shown here are close to –44 kJ/mol leading to the conclusion that the Tecoflex sorption can be categorized as physisorption. At the lower water vapor activities, there is a broader distribution among the enthalpy values. Studies of polymer drying are useful in describing polymer-vapor interactions at low vapor activities. The removal of a solvent from a polymer film is a complex process depending on the molecular structure of the gaseous adsorbate, the drying conditions, and whether the amorphous polymer is in a glassy or rubbery state. As the water vapor activity increases, the enthalpy values in all of the films approach ~ 44 kJ/mol. The 1.1 µm film is consistently lower in magnitude while the 4.7 µm film is consistently higher in magnitude except at the highest vapor activities.

Figure 5-17 is a plot summarizing the $\Delta_{\text{sorption}}H$ values for ethanol sorption in the Tecoflex films. The $\Delta_{\text{vaporization}}H$ for ethanol is -42.32 kJ/mol (Lide, 1997). The $\Delta_{\text{sorption}}H$ values for ethanol appear to focus around this value, which classifies the sorption process as physisorption. Unlike the plot depicting the water sorption
enthalpies, the ethanol sorption displays a broader distribution of values at the higher vapor activities. Due to the much larger motional resistance changes in the ethanol sorbed films, the film appears to be undergoing structural changes upon ethanol sorption. It is noted that only two films were studied at the lower vapor activities. Most of the experimental runs were conducted at $a_{\text{EtOH}}$ values between 0.26 and 0.58, leading to the greater number of data points in this range. The thickest film, 8.5 µm is consistently lower in magnitude upon sorption and desorption. The 4.7 µm film is the only sample that does not show a linear pattern. For both sorption and desorption, the enthalpy values increase in magnitude at the second and third highest activities and then decrease at the highest activity. This was the only film coated by drop coating; however, further studies would be needed to draw a conclusion regarding the coating method and vapor sorption. Polymers are characterized as being metastable; because of this many structural changes are possible as a function of time and temperature (Mathot, 2001). It would seem possible that coating methods might also contribute to some of the measured differences.
Figure 5-16. Enthalpy of sorption of water in the Tecoflex films.

Figure 5-17. Enthalpy of sorption of ethanol in the Tecoflex films.
5.6.2 Sorption Isotherms

The sorption isotherms for the water and ethanol experiments are shown in Figures 5-18 and 5-19 respectively. For both plots, the mass of water or ethanol sorbed in the film is plotted versus the vapor activity.

The water sorption isotherm in Figure 5-18 shows continuity among the different films and the amount of water being sorbed. All films show an almost linear dependence at the lower vapor activities with a slight curvature appearing at the higher vapor activities. The 0.83 µm and 1.1 µm films sorbed slightly less water from the mid-point of the vapor activities to the highest value, while the 4.7 µm film sorbed slightly more water at the higher water vapor activities.

For the ethanol sorption isotherm shown in Figure 5-19, there is a broader distribution in the sorption values. Approximately 4 times the amount of ethanol was sorbed into the Tecoflex films compared to the water sorption. Ethanol in this case appears to be a good plasticizer of ethanol based on the amount sorbed into the films and the greater motional resistance values seen in the initial data. An adsorbate that is a good plasticizer also creates more free volume in a film and likewise causes increased swelling. Generally, the individual isotherms for each film show a similar linear behavior at the low vapor activities but begin to show curvature at even lower vapor activities than seen in the water sorption isotherm. The 4.7 µm film behaved curiously by sorbing almost 50% more ethanol than the other films. Again, it is not clear whether the coating method may have influenced the internal structure of the film causing great relaxation effects upon sorption.
In both water sorption and ethanol sorption, the thicker films have a greater curvature in the isotherm plots at the higher vapor activities.

**Figure 5-18.** Sorption isotherm for water sorption in Tecoflex films.

**Figure 5-19.** Sorption isotherm for ethanol sorption in Tecoflex films.
Polymer swelling upon vapor sorption is characterized in sorption isotherms by an extended region of gradually increasing slope. The slope then turns more steeply concave upward above a vapor activity of 0.7 (Riven et al., 2001). The water sorption isotherm in Figure 5-18 when compared to the ethanol sorption isotherm in Figure 5-19 does not have the same characteristic swelling features. When a least squares third-order polynomial fit was performed on the two isotherms, the slope of the water isotherm was 1.32 whereas the slope of the ethanol isotherm was 1.09. The slope of the ethanol isotherm then starts to curve more markedly concave upward upon sorption. The sorption steps were not taken to activities of 0.7 for the ethanol studies. However, the water studies go beyond activities of 0.7 yet do not exhibit the same curvature.

The characterizing of vapor sorption in polymeric materials has been the subject of many studies (Okuzaki et al., 1999; Riven et al., 2001; Russell & Weinkauf, 2001). Vapor sorption in glassy polymers is often described as consisting of three distinct mechanisms. The lower vapor activity ranges constitute two of the mechanisms and are described using the Dual-Mode Model while the rubbery and glassy polymers in high vapor activity ranges are characterized by the Flory-Huggins isotherm model.

The Dual-Mode Model used in the lower vapor activity range is a combination of Henry’s Law to explain sorption in the low solubility limit and a Langmuir sorption component that is described as “hole-filling” or the sorption of the excess free volume associated with the glassy state (Russell & Weinkauf, 2001). A general form of the Dual-Mode isotherm is shown in Equation 5-1 (Okuzaki et al., 1999):

\[
C = C_D + C_H = k_D p_i + \frac{C_n b p_i}{1 + b p_i}
\]

Equation 5-1

where \( C \) is the total sorbed vapor concentration (g g\(^{-1}\)) defined as the weight ratio of the
sorbed vapor and dry polymer film. $C_D$ is the linear term describing the Henry’s Law sorption at low vapor activities while $C_H$ is the Langmuir type sorption. The constants include the Henry’s Law solubility constant, $k_D$, the Langmuir capacity constant which is a concentration term is $C'_H$, the Langmuir affinity constant is $b$ and has units of pressure, and $p_i$ is the vapor partial pressure (Okuzaki et al., 1999).

The positive curvature in the isotherm at higher vapor activities is characteristic of non-ideal behavior and is best pictured using the Flory-Huggins model. The model is given in Equation 5-2 (Russell & Weinkauf, 2001):

$$\ln \left( \frac{p_i}{p^o} \right) = \ln v_s + (1 - v_s) + \chi (1 - v_s)^2$$

Equation 5-2

where $p^o$ is the saturated vapor pressure, $v_s$ is the volume fraction of sorbed vapor in the polymer film, and $\chi$ is the Flory interaction parameter defining the polymer-adsorbate interaction energy (Russell & Weinkauf, 2001).

### 5.6.3 Partition Coefficients

The background theory and equations used to determine the partition coefficients are shown in Chapter 4. The partition coefficient, $K$, is a unitless quantity that reflects equilibrium between the adsorbate gas molecules and adsorbent thin film. $K$ is the proportionality constant between the concentration of vapor molecules sorbed in the film, $C_s$, and the concentration of vapor molecules in the vapor phase, $C_v$. At a fixed temperature, $K$ should be constant for a given gaseous adsorbate and thin film adsorbent.

*Figures 5-20 and 5-21* display the results for water and ethanol sorption in Tecoflex films of varying thicknesses. As can be seen, the $K$ values are not constant for the water sorption nor for the ethanol sorption. The partition coefficient results for water
sorption in Figure 5-20 show much less scatter in the $K$ values than do the ethanol results in Figure 5-21.

**Figure 5-20.** Partition coefficients for water sorption in Tecoflex films. Film thicknesses are listed in the legend.

**Figure 5-21.** Partition coefficient for ethanol sorption in Tecoflex films.
For the water sorption results, the 4.7 µm film has a higher K value than the other films at each sorption step. Likewise the 0.83 µm and 1.1 µm films have consistently lower K values. Of the three studies done on the 8.5 µm film, the first experiment, shown as the open tilted triangles, are slightly higher than the other two experimental runs. The next 8.5 µm study was done two weeks after the initial experiment and the third study was performed 4 months later. In the first 8.5 µm run, two possibilities may have contributed to the higher K value: the film was newly made and although dried, there may have been trapped solvent molecules that had not yet diffused out. The second possibility is that according to an engineer at Thermedics, the manufacturers of Tecoflex, the polymer properties will change slightly after the polymer has had a chance to settle (Walder, 2002). The 0.75 µm film falls in line with the 8.5 µm film results. Based on the other results, 0.75 µm would be expected to have values near the 0.83 µm and 1.1 µm films. The K values for each of the films increases upon increasing water vapor activity. This indicates that the film is able to sorb more water at the higher vapor activities as also noted in the mass traces for each film shown above.

The K values for ethanol sorption show a broader distribution. The 0.78 µm film is nearly constant across the vapor activity ranges. The 4.7 µm film displays slightly different behavior from the other films. At the lower ethanol vapor activities, the K values for the films flatten out and upon increasing vapor activity, the values begin to show a linearly increasing dependence. The 4.7 µm film shows a stronger linear relationship even at its lowest measured vapor activity. The thickest film, 8.5 µm, exhibits the greatest increase in K values at the higher $a_{\text{EtOH}}$ values. With the exception
of the 0.78 µm film, the K values are in the same general range as those seen in the water sorption. At the higher $a_{\text{EtOH}}$ values however, a steeper increase is seen in K.

A general conclusion can be drawn that at the higher vapor activities for both water and ethanol, the polymer undergoes relaxational effects, free volume increases, and increased sorption is possible. It is noted that when a polymer is in the rubbery region, the rate of analyte sorption increases and K values are much higher (Lucklum et al., 2000). Although it is more complex to measure the gravimetric response of rubbery polymers, they are more sensitive to changes in material properties making them better suited for chemical sensing.

As discussed in Chapter 4, researchers have investigated correction factors to account for polymer swelling and partition coefficient calculations (Grate et al., 1998; Grate & Zellers, 2000; Martin et al., 1994). Because the concentration of the vapor in the polymer film, $C_s$, is describe in terms of mass per volume, any changes in the polymer volume are likewise going to affect the concentration term. When using QCMs, as a polymer swells, the shear modulus, $G$, decreases. This modulus change causes an amplification of the gravimetric response of the QCM. Grate, et al., used independent methods to determine the partition coefficients of four polymer/adsorbate pairs and then compared these with results from surface acoustic wave sensors, SAW, and thickness shear mode resonators, TSM (Grate et al., 1998). The K values calculated from SAW and TSM devices varied in comparison with the independently measured K values. The polymer $T_g$ and viscoelastic properties determined how closely the K values matched each other. Grate, et al. developed interesting conclusions worth
noting here. From their SAW results they drew two conclusions (Grate et al., 1998; Grate & Zellers, 2000):

1. For acoustically thin films, the viscoelastic contribution to the partition coefficient depends on the polymer being used and its initial shear modulus, $G$. The shear modulus need not be high indicative of the polymer being in the glassy state. In fact, for an acoustically thin film, if the initial shear modulus, $G$, is low, the sensor still behaves primarily in the gravimetric regime. Any decrease in the modulus from an initially low value will not produce significant frequency shifts.

2. For an acoustically thin film, the swelling-induced modulus effect has a noticeable influence when the initial modulus value is high, indicating that the polymer is in the glassy state. Any decreases in the modulus from an initial high value have an effect on the frequency shift of the SAW device.

In their comparison of TSM and SAW devices, the SAW devices were more sensitive to shear modulus changes for some of the polymers studied.

For our Tecoflex partition coefficient a future project may include determining $K$ from an independent method so as to also pursue a correction factor to account for the polymer swelling.

In designing chemical sensors, fundamental data regarding the polymer/analyte interactions is paramount. Sorption enthalpies and entropies are not always readily available. As seen above, using the QCM/HCC it is possible to directly measure sorption enthalpies. For other investigators who do not have this technology readily available, an indirect method for determining the sorption enthalpies must be used. An
example of this is shown in the work of Hierlemann, et al. (Hierlemann et al., 2000). The group used QCMs coated with polymer films ranging in thickness from 0.44 to 0.67 µm. They used six different polymer films and studied the sorption of an extensive range of organic adsorbates (19 plus water). Partition coefficients were calculated from the QCM gravimetric measurements. The group then studied the sorption effects at different temperatures and again calculated the partition coefficients. From the temperature dependence of the K values, the van’t Hoff equation was used to determine the overall sorption enthalpy, $\Delta_{\text{sum}H}$. This relationship is shown in Equation 5-3.

\[
\ln K = -\frac{\Delta_{\text{sum}H}}{RT} + \frac{\Delta_{\text{sum}S}}{R} \tag{Equation 5-3}
\]

where K is the measured partition coefficient, T is the temperature, R is the gas constant, $\Delta_{\text{sum}H}$ is the overall sorption enthalpy, and $\Delta_{\text{sum}S}$ is the overall sorption entropy. The researchers then plotted $\ln K$ versus $1/T$ to determine the $\Delta_{\text{sum}H}$ from the tangent slope $\delta \ln K / \delta (1/T)$ while $\Delta_{\text{sum}S}$ was determined from the intercept. Conceptually, the $\Delta_{\text{sum}H}$ value is seen as the additive contributions from the enthalpy of vaporization, $\Delta_{\text{vap}H}$, and the enthalpy of the polymer-adsorbate mixing, $\Delta_{\text{mix}H}$, which is a measure of the intermolecular interaction energies (Hierlemann et al., 2000).

Although thermodynamically the above relationships are valid, investigators would need to ensure that the K values from the TSM resonators were valid and that the TSM was operating in the gravimetric regime. From the K values shown in Figures 5-20 and 5-21, swelling effects occur in film coatings of varying thicknesses and in different
adsorbate vapors. The QCM/HCC offers a method to directly measure the enthalpy of sorption as opposed to indirect methods, which rely on many experiments done over a range of temperatures.

5.6.4 Diffusion Coefficients

Diffusion coefficients were also measured for the water and ethanol sorption of the Tecoflex films. As stated above, the theory and equations can be found in Chapter 4. Diffusion coefficients give an indication of the rate of the sorption processes. These processes are usually characterized as being Fickian, adhering to Fick’s Laws of diffusion, or non-Fickian, showing deviations from the diffusion laws. Figures 5-22 and 5-23 depict the diffusion coefficients, D, for water and ethanol in the Tecoflex films.

For the D values for water sorption shown in Figure 5-22, the diffusion coefficients appear to center about two distinct lines. The thinner films generally have lower D values while the thicker films have greater D values. The diffusion coefficients for water center around $\sim 4-5 \times 10^{-12} \text{ cm}^2\text{s}^{-1}$ for the 0.75 µm and 1.1 µm films while the 4.7 µm and 8.5 µm films have values $\sim 4-5\times10^{-11} \text{ cm}^2\text{s}^{-1}$. Diffusion of water in the thicker films is an order of magnitude faster than that found in the thinner films. The 0.83 µm film follows a different trend from the other films. Within the middle water vapor activity ranges, the diffusion coefficient for this 0.83 µm film increases but then decreases at the higher vapor activities. The 4.7 µm film closely matches the D values for other 8.5 µm film. In some of the previous analyses such as the sorption enthalpy plots and the isotherms, this 4.7 µm film did not always follow the general trend of the other films. The D values for the 8.5 µm film closely match each other given that the experiments were performed four months apart. Ageing did not seem to affect the film
within this four month time span. The 4.7 µm and 8.5 µm films show a consistent and repeatable increase in the diffusion coefficient at the highest water vapor activity.

The diffusion coefficients for ethanol sorption are shown in Figure 5-23. The measured D values follow the same trend seen for the water sorption where D for the thinner films is lower than in the thicker films. The 0.75 µm film centers about $D \sim 1 \times 10^{-12}$ cm$^2$s$^{-1}$, while the 4.7 µm and 8.5 µm films center about $D \sim 1 \times 10^{-10}$ cm$^2$s$^{-1}$. A difference of almost two orders of magnitude exists between the thinner and thicker films. For the 0.78 µm film, only one averaged data point was available, $D \sim 3 \times 10^{-11}$ cm$^2$s$^{-1}$. This D value falls in the lower region of the thicker films. More scatter among the D values is evident for the thicker films undergoing ethanol sorption than seen for the same films sorbing water. Taking into account the motional resistance data for the thicker films, it is expected that greater swelling and relaxation effects need to be accounted for in the thicker films. Experiments were not performed taking the thicker films to the lower ethanol vapor ranges. This would be an interesting future project.

When a sorption process is Fickian, the rate of sorption is proportional to the concentration of the adsorbate entering or leaving the film. The diffusion process should be independent of the thickness of the film (Riven et al., 2001). According to these criteria, both the water and ethanol sorption processes in Figures 5-22 and 5-23 reveal that diffusion in the Tecoflex films is non-Fickian. Rivin et al. comment that non-Fickian behavior results from diffusion coupled with relaxational processes (Riven et al., 2001). The relaxational processes include two components: temperature transients and physical relaxation. Physical relaxation results from swelling effects and
increased free volume within the polymer film. The slight temperature transients occur when a process is non-Fickian.

When sorption occurs, an increase is temperature is seen due to the heat of condensation of the vapor. This is shown in the exothermic peaks in the thermal power traces for each of the experiments. When this occurs, the vapor activity is lowered in the region of the slightly higher film temperature. This effect momentarily decreases the sorption rate. Similarly, when desorption occurs, the film undergoes a temperature decrease as seen in the endothermic peaks shown in the experimental thermal power traces. The lower film temperature relative to the vapor has the effect of increasing the vapor activity thus slowing the desorption rate. The decrease in film temperature upon desorption equals the increase in film temperature upon sorption. Because of the low activation energy for diffusion, the temperature variations are noted to have little effect on D. Relaxational processes however bring about slow volume increases which cause an increase in sorption ability. Equilibrium is not attained as readily and slight temperature transients result (Riven et al., 2001).

In Figure 5-22 for the water sorption, the thicker films display an increase of D at the higher vapor activities. This effect is present in Figure 5-23 for the ethanol sorption however, the effect is less pronounced. The ethanol sorption experiments need to be carried out at higher vapor concentrations to confirm this effect.
Figure 5-22. Diffusion coefficients for Tecoflex/water sorption. Results are plotted as a function of water vapor activity and film thickness.

Figure 5-23. Diffusion coefficients for Tecoflex/ethanol sorption. Results are plotted as a function of ethanol vapor activity and film thickness.
5.6.5 Attempts at Isolating the Shear Modulus, G

The water and ethanol sorption studies of Tecoflex films varying in film thickness raise some interesting questions. In the above studies, it was assumed that the QCM was operating in the gravimetric regime and therefore acting as a microbalance. The sorption isotherms, partition coefficients and diffusion coefficients all indicate that some swelling took place in the polymer films upon vapor sorption. This effect is most evident when comparing the thicker films to the thinner films and when comparing the water sorption studies to the ethanol sorption studies.

The response of the QCM is dependent not only on the film mass but also on the shear modulus, G. As stated in Chapter 4, a polymer film in the glassy state has a high shear modulus ~ 1 GPa. In this case the film resonates synchronously with the QCM and the QCM acts as a microbalance. As a polymer film swells, its viscoelastic properties influence its behavior and the upper film surface lags in its oscillation when compared to the QCM/film interface. In this case, the shear modulus often decreases to ~ 1 MPa indicating that the material has gotten rubbery (Lucklum et al., 1998). The shear parameters of a film are difficult to determine. The real and imaginary parts of the acoustic load impedance, $Z_L$, can be determined from impedance analyzer measurements as shown in Equation 3-9 or an approximation can be made from the change in the QCM resonant frequency and motional resistance data as shown in Equations 3-10 and 3-11. Four unknown values contribute to the difficulty in determining the shear parameters namely, the film density, film thickness, and the real and imaginary contributions of the shear modulus, G.
In our sorption studies of the polymer Tecoflex, we wanted to determine the viscoelastic effects upon the QCM measurements. The motional resistance changes, ΔR, reflect an energy dissipation in the film coating but it is not easy to determine the magnitude of these effects on the shear modulus and if a deviation is occurring from the mass sensing region of the QCM. In our attempts to gain further insight, four avenues were pursued:

1. DSC measurements of Tecoflex to confirm the T_g of the polymer.
2. Cold temperature frequency measurements to accurately determine the film thickness.
3. Plots of Δf-ΔR to gain insight into the magnitude of the modulus changes.
4. Analysis involving a “Three-Step” approximation method for determining the shear modulus, G.

Each of these is discussed briefly in the following sections.

5.6.5.1 Differential Scanning Calorimetry Results

Differential scanning calorimetry as explained above is a well-established technique in determining the glass transition of substances. As stated in Chapter 4 however, the glass transition itself is a complex phenomenon yet is often used to characterize the state of a polymer. To confirm the stated values for the T_g of Tecoflex, ~ -60°C for the soft part and ~ 40-50°C for the hard part, we were able to use DSC measurements to independently measure the T_g of the polymer. The DSC measurements on the Tecoflex samples were run through the generosity of Dr. Andrew McGhie of the Laboratory for Research on the Structure of Matter (LRSM) at the University of Pennsylvania. Two instruments were used: a TA Instruments, 2920 DSC and a TA Instruments, SDT 2960 DTA-TGA, Differential Thermal Analysis-Thermogravimetric Analysis, for the
gravimetric weighing of the Tecoflex samples. The results are shown in the figures below.

![DSC scans of an 8.5 mg half pellet of Tecoflex.](image)

**Figure 5-24.** DSC scans of an 8.5 mg half pellet of Tecoflex. The y-axis is the heat flow in Watts/gram and the x-axis is the temperature in °C. The scans were performed at a rate of 3°C/min. a) Two symmetric endotherms appeared on this first scan possibly caused by the evaporation of water. b) A second scan repeated after the first scan cooled. Broad endotherms are noted at ~ 80°C and 160 °C. c) A third scan of the same pellet after purged in a He environment for 12 hours. A T_g appears to occur at ~ 83.2°C. d) A fourth scan of the Tecoflex after cooling from the previous run. A T_g appears at ~ 86.5 °C.
Figure 5-24 depicts four scans of a Tecoflex pellet as shipped from the manufacturers but then cut in half. Each scan was run at 3°C/min. and the plots are shown as the heat rate, W/g, versus the temperature, °C. This 8.5 mg sample was stored in a dessicator for two days prior to the first scan shown in (a). However, Tecoflex is known to be hygroscopic and was exposed to the ambient atmosphere in the cutting and preparation process. After this scan, the Tecoflex sample was taken out to observe any effects upon heating. The sample was clear but appeared to slightly flow during the heating process. The two endothermic peaks both ~ 5.2 J/g were believed to be water evaporating from the polymer sample.

In (b) the sample was covered and not scanned again until two days after the initial scan shown in (a). In this scan, a broad endotherm appears ~ 80°C and another appears at ~ 160°C. Because these appear to be inconclusive another scan was performed. In (c) the same sample is scanned but previous to this the sample was purged in a helium atmosphere for 12 hours. The heat flow versus temperature plot reveals a more pronounced endotherm which was interpreted as a T_g ~ 83.2°C. The sample was allowed to cool and another scan was run. The scan shown in (d) is very similar to the previous run. An endotherm at ~ 86.5°C was interpreted to be a T_g.

We were not able to scan below 25°C and therefore could not measure the T_g of the soft part of the polymer. From these results, we were able to confirm what was stated to us from the manufacturers, that the T_g is difficult to pinpoint. The T_g measurements depend on the prior history of the polymer, annealing, and settling time. In fact, for the hard portion of Tecoflex, the T_g can range anywhere from ~ 30°C to 120°C. It is noted that the manufacturers record different T_g values within the first weeks of the polymer
being processed and that it takes a month before the reported $T_g$ value of ~ 50°C to 60°C is measured (Walder, 2002). Overall, these independent DSC measurements confirmed for us that our Tecoflex films were above their $T_g$ for the studies done at 25°C.

### 5.6.5.2 Cold Temperature Measurements

The film height measurements reported for the Tecoflex films used in these studies were calculated using the mass of the dry film coating the QCM. From the reported Tecoflex density (1.11 g/ml) and the mass of the dry film, the volume of the sample was determined. The relationship between the volume and the QCM measured area (1.9793 cm$^2$), was then used to determine the film height. The films were determined to be dry by observing the mass trace. When no slope was visible in the mass trace and when the QCM frequency reached a stable reading of ± 0.1 Hz, the film was considered dry.

Some investigators use independent measurements such as ellipsometry or profilometry to determine film thickness. Lucklum, et al., report using cold temperature QCM frequency measurements to determine the film thickness (Behling et al., 1998b) (Lucklum et al., 1998; Lucklum & Hauptmann, 1997). In their studies, QCMs were coated with polyisobutylene (PIB). PIB is noted to be a rubbery material at room temperature with a $T_g$ ~ -68°C. The absolute frequency shift of a dry PIB film coated QCM was measured at –50°C. The film is considered to be stiffer at this temperature therefore yielding a more accurate frequency shift due to the mass of the film with negligible contributions from the viscoelastic effects. The following equation is given as an accurate approximation to determine the film height (Behling et al., 1998b; Lucklum & Hauptmann, 1997):
\[
\begin{align*}
    h_r &= \left[ \frac{2}{\tan\left(\frac{\alpha_q f_r}{2}\right)} - \frac{4K_q^2}{\alpha_q f_r} \right] \frac{Z_{eq}}{2\pi f_r \rho_f} \\
    \text{Equation 5-4}
\end{align*}
\]

where \(h_r\) is the height of the film, \(\alpha_q\) is the wave phase shift in the quartz and is further defined in **Appendix C**. The series resonant frequency of the quartz with a zero phase shift is given as \(f_r\), \(K_q\) is the electromechanical coupling factor for a lossless quartz, \(\rho_f\) is the film density, and \(Z_{eq}\) is the specific acoustic impedance of the quartz crystal.

To confirm our film thickness calculations, this cold film frequency measurement method was employed. The 8.5 µm film was placed in the Maxtek QCM holder. This was then mounted in an airtight dessicator containing \(\text{P}_2\text{O}_5\) as a dessicant. The regular powered Maxtek PLO was used as the oscillator driver, a multimeter was used to monitor the output conductance voltage and the HP 53513A frequency counter was used to monitor the QCM frequency. The dessicator unit was placed in an insulated box containing a reservoir of dry ice. This created an ambient temperature of \(-78.4^\circ\text{C}\) for the frequency measurements of the 8.5 µm film (1997). This temperature was chosen for two reasons: dry ice was readily available and it would produce a temperature lower than the \(T_g\) value for the soft portion of the Tecoflex unit, \(T_g \sim -60^\circ\text{C}\). At this colder temperature, it was believed that the film would be in a rigid state and no viscoelastic effects would then contribute to the QCM frequency shift. The results of these measurements are shown in **Table 5-5**.
Table 5-5. QCM frequency and conductance voltage measurements of 8.5 µm Tecoflex film at –78.4°C

<table>
<thead>
<tr>
<th>Time of measurement</th>
<th>Resonant frequency (Hz)</th>
<th>Conductance voltage (volts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room temperature</td>
<td>4942297.8</td>
<td>0.452</td>
</tr>
<tr>
<td>In cold, 5 min.</td>
<td>4942326.4</td>
<td>0.461</td>
</tr>
<tr>
<td>In cold, 2 hours</td>
<td>4942935.3</td>
<td>0.611</td>
</tr>
<tr>
<td>Δcold measurements (2 hr. – 5 min.)</td>
<td>608.9</td>
<td>0.15</td>
</tr>
</tbody>
</table>

When recording the frequency measurements, the QCM frequency never stabilized and after two hours there was a continual frequency increase of ~ +10 Hz every five minutes. The conductance voltage did not change as dramatically. Because a stable frequency was never reached at the cold temperature, these values were not used to calculate the film height.

After corresponding with Dr. Ralf Lucklum of Otto-von-Guericke-University, Magdeburg, Germany, some insights were given as to the continual frequency increase at the cold temperature. He suggested that the thermal expansion coefficient of the Tecoflex is probably much different from that of the quartz. This difference can cause a mechanical stress with the oscillating QCM. He noted that when employing the cold temperature measurements, a cold temperature should be chosen that is still above the polymer T_g. When the T_g is lower than the measuring temperature, the film is able to relieve any induced stresses. A cold temperature below the T_g introduces the possibility of stress, bending of the QCM and frequency changes. He noted that the quartz itself does not see a significant increase in the equivalent resistance measurements due to this effect (Lucklum, 2002). This was observed in our measurements and is shown as the...
small overall change in the conductance voltage. Another trial was not performed and this is discussed in the future applications in Chapter 7.

5.6.5.3 Analysis of $\Delta f$ – $\Delta R$ Plots

Two measurable parameters from an oscillating QCM include the change in resonant frequency, $\Delta f$, and the change in the motional resistance, $\Delta R$. As shown in Chapter 3, Equations 3-10 and 3-11, the real part of the acoustic load impedance, $Z_L'$, and the imaginary part, $Z_L''$, can be derived from $\Delta R$ and $\Delta f$ respectively. Lucklum, et al., acknowledge that the $\Delta f$ and $\Delta R$ responses are uniquely linked to the shear modulus, $G$ (Lucklum & Hauptmann, 2000). Although the shear modulus itself is difficult to calculate the $\Delta f$ and $\Delta R$ measurements can be used to estimate the viscoelastic contributions to the shear modulus. Analyzing $\Delta f$ and $\Delta R$ data cannot be solely used to determine the shear parameters. In their modeling of the $\Delta f$ and $\Delta R$ values in interpreting the viscoelastic contributions, Lucklum shows that several combinations of $G'$, $G''$, and film thickness, $h_f$, can all result in the same $\Delta f$, $\Delta R$ values (Lucklum & Hauptmann, 2000). A few plots of $\Delta R$ versus $\Delta f$ are shown in Figure 5-25 to compare the results from different film thicknesses as well as the effects of different adsorbates, water and ethanol in this case.

In Figure 5-25, the film thicknesses are compared as well as the different adsorbates. The 0.75 $\mu$m is depicted in (a) and (b) showing the values for the water and ethanol sorption respectively. The ethanol sorption produces the great change in the motional resistance. For both studies, the motional resistance does not noticeable start to increase until what would be the third sorption step. Results for the 4.7 $\mu$m film are shown for the water and ethanol sorption in (c) and (d). The incremental $\Delta R$ changes are almost
ten times greater in the ethanol sorption than seen for the water sorption. The initial concave curvature is slightly different for the water when compared to the convex curvature of the initial ethanol sorption steps. Overall the 4.7 µm plot shows an S form where the water plot appears more linear. Data for the 8.5 µm film is shown in (e) for the water sorption and (f) for the ethanol sorption. In (e) the results depict the 8.5 µm film studied four months apart. There is little change in the results during this time period. The ethanol sorption shows slightly more curvature.
Figure 5-25. ∆R versus ∆f plots for Tecoflex sorption. a) water sorption, 0.75 µm film, b) ethanol sorption, 0.75 µm film, c) water sorption, 4.7 µm film, d) ethanol sorption, 4.7 µm film, e) water sorption, 8.5 µm film, f) ethanol sorption, 8.5 µm film.
The Δf and ΔR values are useful because they can be used as inputs in models to determine G values. Such a model is the Excel spreadsheet given to us by Dr. Ralf Lucklum, ZYSYN_QP.xls. Although more than one set of G values are possible, the modeling does help to narrow the possibilities. The spreadsheet has not been routinely employed in our calculations yet but it is foreseen to be a valuable tool in using the Δf and ΔR information to extract G’ and G”.

5.6.5.4 Employing the “Three-Step Method” for Calculating the Shear Moduli

In their attempt to investigate shear modulus calculations for film coated QCMs Behling, et al. devised a “Three Step Method (Behling et al., 1999).” This method was discussed in Chapter 3 and shown to rely on a family of approximations used to simplify the “tan ϕ” term and likewise, the equations for calculating G as shown in Equations 3-12, 3-14, and 3-16. The method relies on the measured electrical impedance, which is then used to calculate the acoustic load impedance, ZL. This value is then used in the simplified equations to determined first approximation values for G. The final step involves an iterative process to obtain the best value of G.

The equations used for the modification were adapted and incorporated into a mathematical problem solving software, TK Solver 4.0 (UTS Software). The Three-Step Model original equations and adapted equations used in the TK Solver rule sheet are shown in Appendix D.

Because we do not routinely use an impedance analyzer, the real and imaginary parts of the acoustic load impedance, ZL’ and ZL”, were determined from the approximations shown in Equations 3-10 and 3-11. The actual frequency and motional resistance data are thus used to make the first approximations. When using these ZL’ and ZL” values
to solve for the shear storage and loss moduli, $G'$ and $G''$, the software calculates values for each of the approximations. Using the TK Solver model, it is possible to obtain some values for $G'$ and $G''$ however, not all of the equations can be solved simultaneously leaving some solutions discarded. Behling, et al. note a word of caution when obtaining shear modulus values. To obtain unique solutions for the shear parameters, the exact knowledge of the film thickness and density must be known. Errors may arise when these values are not exactly known because multiple possible combinations exist for the film height, $h_f$, and $G'$ and $G''$ (Behling et al., 1997). From these shear modulus values, work still needs to be done on the TK Solver model to use the iterative process in finding the best values for $G'$ and $G''$. It may then be possible to utilize the spreadsheet generously sent to us from Dr. Ralf Lucklum, ZYSYN_QP.xls, to model some values of the shear modulus and use these to compare with the TK Solver calculations.

5.7 Conclusion

QCM/HCC studies of vapor sorption in polymer films provides a wealth of information to characterize the sorption thermodynamics, kinetics, and rheological processes. Comparisons were made with regards to the adsorbate vapor and the film thicknesses in these sorption studies of Tecoflex films. Two different adsorbate vapors were used: water and ethanol. For each adsorbate, films ranging in five different thicknesses were studied. Some general conclusions can be drawn from the sorption studies presented here:

1. The Tecoflex films sorb almost four times more ethanol than water vapor when comparing grams adsorbed.
2. For both water and ethanol sorption, the thicker films have higher diffusion rates than the thinner films. For water the rate as determined from the diffusion coefficient, $D$, is one order of magnitude greater while for ethanol two orders of magnitude separate the thicker and thinner films.

3. Upon sorption the motional resistance changes, $\Delta R$, are more pronounced in the thicker films than in the thinner films. When comparing ethanol and water sorption, the motional resistance changes are greater for ethanol sorption leading to the conclusion that ethanol is a better plasticizer for Tecoflex, causing swelling and free volume increases.

4. The Tecoflex/water sorption enthalpies showed some scatter among the different film thickness at the lower vapor activities. At the higher vapor activities the $\Delta_{\text{sorption}}H$ values converged at $\sim 44$ kJ/mol, the value for $\Delta_{\text{vaporization}}H$ of water.

5. The Tecoflex/ethanol sorption enthalpies displayed some scatter at the higher vapor activities. Greater relaxation effects occur at the higher vapor activities as evidenced by the $\Delta R$ measurements and may contribute to some of the deviation in values. The thicker films generally had $\Delta_{\text{sorption}}H$ values lower than the expected value for the $\Delta_{\text{vaporization}}H$ of ethanol, $-42.3$ kJ/mol (Lide, 1997).

6. The partition coefficient values increased with increasing film thickness for both water sorption and ethanol sorption. The variations in the $K$ values appear to be the result of swelling and increased free volume effects in the polymer films. These effects lead to an overall increase of the polymer volume thus affecting the concentration calculations.
7. The water sorption isotherm displaying grams of water as a function of water vapor activity, shows an almost linear dependence with a slight increased curvature at the higher water vapor activities.

8. The ethanol sorption isotherm shows a linear dependence of grams of ethanol versus the vapor activity at the lower activity values. The dependence takes on an increased curved dependence at the midpoint of the water vapor activities. To see the effect fully, the measurements will need to be carried to higher vapor concentrations.

9. When employing the electrical calibration method for the thermopiles, for three different films, the pre- and post-calibrations varied by only ~ 0.03 W/v verifying that the procedure is very repeatable and unchanged after the film has undergone sorption cycles.

10. Diffusion in Tecoflex films by both water and ethanol appears to be non-Fickian as evidenced by the difference in values when comparing the thinner films and the thicker films. The smaller diffusion coefficient values for the thinner films reflect the polymer relaxation effects hindering the diffusion process as the adsorbate penetrates the film. The larger diffusion coefficient values for the thicker films reflect the diffusion of water not being as hindered by relaxation effects. Water diffusion from the film would first leave from the surface and upper portions of the film. Because the film is not being dried, water buried in the lower portions of the film do not have to diffuse out. The water at the surface of the thicker films is less hindered by the relaxational effects.

11. From the DSC measurements of Tecoflex, the sorption studies conducted here appear to be below the $T_g$ and indicate that the polymer is in the glassy state at the start of the studies.
12. The measured $\Delta f$ and $\Delta R$ values give some insight into the $Z_L$ real and imaginary values. The actual calculation of the shear modulus from these values is not straightforward however the $\Delta f$ and $\Delta R$ values can be used to estimate $Z_L$ and viscoelastic contributions to the change in shear modulus.
List of References


Chapter 6. Hydration Studies of Protein Films

6.1 Introduction

Water in itself is an interesting molecule because of its unique characteristics such as its strong hydrogen bonding with other water molecules as well as with polar molecules. The large cohesive energy among water molecules gives rise to its high boiling point, its strong surface tension, and its unwillingness to dissolve nonpolar, hydrophobic solutes (Eisenhaber & Argos, 1996). The combination of studying water-protein interactions provides even more interesting and unusual data. The influence of water on protein structure, folding, function, and energetics is a topic that has seen considerable research interest and has been spotlighted in several reviews (Bellissent-Funel, 1999; Gregory, 1995b; Kuntz & Kauzmann, 1974; Rupley & Careri, 1991). Many functionally important motions in proteins are suppressed when a protein is dry, and the fast, structural relaxation and conformational processes do not resume until hydration occurs. Water has long been seen as a natural solvent and it is noted that most interactions between macromolecules in a biological system occur in an aqueous environment (Wennerström, 2000). However, many unanswered questions linger in regards to protein-water interactions such as, the effect hydration water has on unfolding and folding, the magnitude of the effects, the influence on intramolecular interactions. All of these topics suffer from a lack of actual experimental data. The incomplete information has led to many conjectures and discussions on protein hydration which have swayed in their interpretations through the years (Makhatadze & Privalov, 1993).
Proteins are unique in the fact that they are soluble yet retain a compact structure in water. Many proteins in an aqueous environment fold into a globular conformation with nonpolar groups gathering in the interior of the macromolecule while the polar groups remain exposed at the surfaces. By utilizing this type of conformation, the protein adopts a minimum free energy due to the reduced interactions between the nonpolar groups and water molecules. The large variation in chemical composition and the complex surface of proteins determine the nature of its interactions. The polar groups interact with water molecules through electrostatic interaction, namely, hydrogen bonding (Norde, 2000). It can be misleading, however, to presume that all of the protein apolar surfaces are buried in the protein center. The apolar residues do tend to reside in the interior of the molecule, however the solvent accessible surface of the protein may still consist of a considerable amount of apolar sites. In some cases this may even be up to 40 – 50% of the surface (Norde, 2000). When the apolar sites are somewhat evenly distributed over the surface of the macromolecule, the protein is water-soluble and nonaggregating whereas prominent apolar patches on the surface lead to protein aggregation.

6.1.1 Techniques Used to Study Protein Hydration

In spite of the variety of techniques with which the dependence of protein dynamics and protein hydration has been investigated, the role of water in biological macromolecules especially at the molecular level still remains unclear (Poole & Finney, 1986). Studies of protein hydration can be classified as to whether the technique recorded time averaged or dynamic properties and whether the protein sample was in a solid or solution state (Careri, 1999). A summary of the methods used to study protein
hydration include: quartz crystal microbalance gravimetric techniques (Gascoyne & Pethig, 1977), Mössbauer spectroscopy, microwave absorption (Parak, 1986), X-ray diffraction, IR, UV-VIS spectroscopy, Circular dichroism, NMR, NMRD (nuclear magnetic relaxation dispersion) (Denisov et al., 1996), ESR, dielectric relaxation (Bone, 1996; Pethig, 1992), sorption thermodynamics, gravimetric methods such as a dessicator storage (Poole & Finney, 1986), viscosity measurements, as well as molecular dynamic and Monte Carlo simulations (Karplus, 2000; Petukhov et al., 1999; Peyard, 2001; Tarek & Tobias, 1999).

Although molecular dynamic simulations have opened a whole new aspect of chemistry and provide valuable insight into probing chemical behaviors and activities, it is noted that when comparing experimental and simulated hydration sites in proteins, problems may arise. Two reasons for possible problems include: (1) simulated sites represent the best reproduction of solvent distribution and oftentimes do not comply with the actual steric restrictions. (2) no crystal packing effects are included in the simulations.

Several authors have reviewed hydration methods (Bellissent-Funel, 2000; Careri, 1999; Mattos, 2002; Phillips & Pettitt, 1995; Sterpone et al., 2001). Each of these methods used to study the hydration of proteins provides a piece of the total picture. It is noted that oftentimes, when results from different methods are compared, they contradict each other (Phillips & Pettitt, 1995). Protein hydration is often reported in the form of a sorption isotherm where $h$, the $g_{\text{water}}/g_{\text{protein}}$, is plotted versus the water vapor activity, $a_w$. When measuring water adsorption and desorption, proteins exhibit hysteresis with the adsorption curve usually lying below the desorption curve. When
comparing a series of protein samples, it is recommended that the samples are identically prepared, wet or dry, and that the sample is rehydrated or dehydrated in the same direction (Poole & Finney, 1986). At low humidities, conformational states of proteins may become locked or frozen in. The conformation state varies not only with the degree of hydration but also on the rate of hydration or dehydration (Lüscher-Mattli & Rüegg, 1982). The diversity of measuring processes provides a somewhat variety in results. The environment of the protein as it was being studied contributes greatly to the types of results. These factors need to be considered before comparing results from other studies. Because of this, there is a need for further studies concerning protein hydration to help clarify these pieces of the picture.

### 6.1.2 Protein Hydration Characteristics

Within specific hydration ranges, \( h, \frac{\text{g}_{\text{water}}}{\text{g}_{\text{protein}}} \), biological macromolecules tend to undergo hydration in certain defined increments (Careri, 1999; Reinisch et al., 1989; Yang & Rupley, 1979). Based on his studies of the protein lysozyme, Careri, et al. have summarized the hydration effects of proteins based on hydration ranges (Careri et al., 1979). A summary of their table is provided below.
Table 6-1. A summary of the hydration processes in globular proteins. The summary is provided by Careri and based on his work with the protein lysozyme (Careri, 1999; Rupley & Careri, 1991).

<table>
<thead>
<tr>
<th>Hydration Level $h_{\text{water} / \text{protein}}$</th>
<th>Structure</th>
<th>Dynamics</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 0.07</td>
<td>Using 1 Å level of comparison, low hydration structure of protein same as that at full hydration. Water interacts primarily with charged groups (ca 2 water/group). At 0.07: surface water undergoes transition from dispersed to clustered state: associated with completion of charged group hydration.</td>
<td>Low water mobility. Protein motions frozen. Enzymatic activity negligible.</td>
</tr>
<tr>
<td>0.07 – 0.15</td>
<td>Water interacts mainly with polar protein surface groups (ca 1 water/polar site). Water clusters centered on charged and polar sites. Fluctuation of size and arrangement of clusters.</td>
<td>Protein exhibits internal motion (H exchange), increase from 1/1000 at 0.04 to full solution rate at 0.15.</td>
</tr>
<tr>
<td>0.15 – 0.25</td>
<td>At 0.15: surface water establishes long-range connectivity in a 2-dimensional percolative phase transition. Protein surface spanned by network of H-bonded water; network characterized by fluctuating and random connectivity, amount of connections increasing with hydration level.</td>
<td>At 0.15: Dielectric measurements reveal long-range proton movements along percolative networks.</td>
</tr>
<tr>
<td>0.25 – 0.38</td>
<td>At 0.25: beginning of water condensation onto weakly interacting unfilled patches of protein surface.</td>
<td>In lysozyme, increase in enzymatic activity and motion of noncovalently bound ligand. Water motion strongly increases with increased hydration. Considerable mobility of hydration water and small free-energy difference from bulk solvent, term “bound water” seems inappropriate. Enzymatic activity of lysozyme 1/10 of its solution value.</td>
</tr>
<tr>
<td>0.38</td>
<td>Monolayer of water covering the surface. Interaction with charges and polar surface groups selects locally ordered arrangements of hydration water. Fluctuation between various instantaneous arrangements, as in liquid water. Diffraction studies show: for most proteins, clusters or threads of H-bonded water; some proteins however, extensive H-bonded networks.</td>
<td>Full internal motions of proteins.</td>
</tr>
</tbody>
</table>
In her hydration studies of proteins, Lüscher-Mattli extensively studied \( \alpha \)-chymotrypsin in order to review and confirm hydration models and studies performed on other globular proteins (Lüscher-Mattli & Rüegg, 1982). From the sorption-desorption scanning experiments, two major hydration ranges were noted which coincide with the ones presented above: (1) low-humidity range \((h < 0.1)\), strong hydration sites are favored and occupied. The dry biopolymer undergoes pronounced structural rearrangements. (2) mid-to-higher humidity range, \((0.1 < h < 0.3-0.5)\) structural rearrangements are mostly complete; the solid protein is present in its solution conformational state.

It is noted that discussion as to conformations and hydration states has gone through pendulum swings as to whether or not conformation changes occur upon dehydration at low water activity levels. Recent reviews suggest that there is no clear-cut answer to this question and that answers depend on the extent of hydration and the protein being studied (Gregory, 1995a).

6.1.3 Practical Applications of Protein Hydration Studies

Protein-water interactions are important in a variety of practical applications. One area in which the interaction of water with proteins provides critical information is food systems. Many foods are comprised of proteins and polysaccharides and their interaction with water regulates their structure and properties. In the last decade, the research of water interacting with food proteins has been closely associated with polymer studies. This has led to a new experimental and interpretive approach known as “food polymer science (Slade & Levine, 1998).” Water, interacting with proteins,
functions as a plasticizer thus lowering the glass transition ($T_g$) and simultaneously affecting the thermomechanical properties, and the physical and chemical stability of foods (Matveev et al., 2000; Slade & Levine, 1998). The dependence of the $T_g$ on the water content is important in food development and in processes such as drying, mixing, freezing and storage (Matveev et al., 2000). It is noted that water acting as a plasticizer “drops the $T_g$ of most biological materials from about 200°C (for anhydrous polymers, e.g. starch, gluten, and gelatin) to about -10°C or so (under physiological conditions of water content), without which they would be glassy in their native, in vivo state (Slade & Levine, 1995).” A broad distribution in relaxation times upon protein hydration adds even more evidence of proteins exhibiting glass transition behavior (Green et al., 1994; Teeter et al., 2001).

Another practical application of studying hydration effects in proteins involves the process of heat-treating foods. Heat treatment is a common step in food processing and in producing protein concentrates. Heat treatment, however, often is followed by the unwanted and undesirable effect of protein denaturation. The presence of water affects the protein during heat treatment (Hägerdal & Martens, 1976). Protein hydration water has been studied in conjunction with heat treatment of myoglobin to determine protein stability and its effects on food processing. Studies have been performed by equilibrating a protein at a fixed water content. Differential scanning calorimetry (DSC), scans were then done to evaluate the protein stability as the temperature was scanned. Results from these measurements aid in protecting proteins from environments where irreversible denaturation would occur.
Great interest surrounds the binding of nitric oxide (NO) with the heme group in myoglobin. This formation of nitrosylmyoglobin (MbFe$^{\text{II}}$NO) in meat products is important in understanding mechanisms of processes occurring in muscle tissue and how to apply this in protecting foods from oxidative reduction. Because part of the mechanism of binding NO involves the removal of water from the heme pocket, hydration effects play a part in this research (Møller & Skibsted, 2002).

Low temperature experiments have been performed to better understand hydration effects of proteins in extreme temperatures. Much research interest has focused on understanding supercooling and its effect on protein function. It is noted that protein hydration water at $0.4 \text{ g}_{\text{water}}/\text{g}_{\text{protein}}$ does not crystallize at low temperatures (Sartor et al., 1995; Sartor & Johari, 1997; Sartor et al., 1994). Studying these effects can lead to understanding how some organisms are able to survive at subzero temperatures (Doster et al., 1986). Other low-temperature experiments have been performed to understand protein stability. These studies have shown that just as proteins denature upon heating, proteins may also undergo cold-denaturation upon cooling (Griko & Privalov, 1992; Privalov et al., 1986).

Hydration of proteins also plays a factor in protein-protein interactions and protein interfacial interactions. This aspect has been the focus of attention by groups researching soil and food science as well as biotechnology and biomedical applications (Norde, 2000). Hydration of proteins affects the protein conformation as well as the solvent accessible surface area (ASA). Several researchers note that the further work is needed in understanding protein hydration and that this topic is just as daunting as trying to understand protein folding (Finney, 1999; Janin, 1999).
6.1.4 Calorimetric Studies of Proteins

With the growing capabilities to prepare and purify biological molecules and the increased sensitivity of calorimeters, the field of thermodynamically characterizing biological macromolecules has emerged. Previous to the current growth of biocalorimetric data, the thermodynamics of biological macromolecules relied on model dependent, van’t Hoff analyses of equilibrium data (Plum, 1995). Direct calorimetric data has been steadily replacing these indirect and sometimes inaccurate model analyses (Sturtevant & Liu, 1995; Sturtevant, 1997). Studying protein energetics has aided in developing insight into their biological functions and reactions. Calorimetry provides several methods to directly measure heat effects of different processes and in this case, hydration of proteins. In the past, calorimetry was used to study the energetics of sharp transitions. The trend in research today however, is to explore complex interactions and functions. This has pushed the development of very sensitive microcalorimeters that use reasonable solution concentrations. This in turn has opened new avenues for calorimetric exploration. New aspects of calorimetry include nanocalorimetry in which the title reflects the extreme sensitivity (Privalov et al., 1995). In a recent review of using microcalorimetry to study biological macromolecules, Privalov notes that three areas of interest have emerged (Privalov & Privalov, 2000): (1) heat involved in complicated changes of macromolecules and their complexes under varying conditions, (2) specifying the state of macromolecules and determining absolute values for thermodynamic parameters, (3) utilizing the thermodynamic values to determine and confirm structural information. Although a host of calorimetric methods exist (e.g. combustion calorimetry, heat conduction calorimetry, scanning calorimetry, batch, flow
and titration calorimeters), two widely employed methods in studying proteins include differential scanning calorimetry (DSC) and isothermal titration calorimetry (ITC). These methods will be briefly discussed as to their contributions in studying protein thermodynamics. A third newer instrumentation, an isothermal sorption calorimeter will be discussed to compare its relevance to our QCM/HCC analysis of protein hydration.

6.1.4.1 Differential Scanning Calorimetry (DSC)

DSC measurements have been used to provide a wealth of information regarding thermally induced order-disorder transitions (DiLorenzo et al., 1999). DSC measurements provide heat capacity, $C_p$, data of the two stable macroscopic states of proteins, the native and denatured states (Makhatadze & Privalov, 1995). The excess $C_p$ of a protein/buffer solution is measured as a function of temperature. The transition enthalpy, $\Delta H$, is determined from the integration of the $C_p$ versus T curve. Analyzing this temperature dependence of the enthalpy leads to a full thermodynamic characterization of a biological macromolecule in its native states and the intermediate states leading to its unfolding (Creighton, 1993). Measurements can then be done varying the pH, ionic strength, and use of denaturants.

Recent advances in DSC designs (DSC microcalorimeters) have increased the experimental values measured for $C_p$ of biological macromolecules (Privalov et al., 1995; Privalov, 1997). Because protein solutions are very dilute, $C_p$ measurements of the solute molecules were often difficult to distinguish from the overpowering background solvent heat capacity. DSC measurements on proteins are performed on sample sizes of < 1ml and concentrations ranging from 0.1 mg/ml to 10 mg/ml. A
protein solution undergoes a temperature scan with the usual heating rates being between 0.1 and 2 K/min. The energy needed to increase the temperature of the sample is observed (Makhatadze & Privalov, 1995; Rösgen & Hinz, 1999). To account for any buffer effects, a temperature scan is performed on the sample and separately on the dialysis buffer solution. The heat capacity, \( C_p \), calculations rely on the heat capacity difference between these solutions. This value, the apparent heat capacity, \( \Delta C_{p,app} \), is used as the foundation for the \( C_p \) calculations. The effects of the buffer solution are accounted for so that the measured energy is from the protein:

\[
\Delta C_{p,app} = c_{p,buffer} \times m_{buffer} - c_{p,protein} \times m_{protein} \tag{Equation 6-1}
\]

the mass of the buffer and protein are denoted by \( m_{buffer} \) and \( m_{protein} \). The heat capacity can then be solved by utilizing the specific volume, \( v = V/m \). The protein heat capacity is shown by (Privalov et al., 1995; Rösgen & Hinz, 1999):

\[
C_{p,protein} = C_{p,buffer} \times \frac{v_{protein}}{v_{buffer}} - \frac{\Delta C_{p,app}}{m_{protein}} \tag{Equation 6-2}
\]

When using low buffer concentrations, the heat capacity of the buffer can be approximated as the heat capacity of water at room temperature. If this approximation does not hold for a particular experiment and exact data are required, an added measurement can be made of the water against the buffer. Thus, the heat capacity difference between the buffer and protein would be known.
When a thermal folding – unfolding two state transition occurs, the $C_p$ values can be found for the native, $C_p^N$ and unfolded, $C_p^U$ states (Makhatadze & Privalov, 1994; Taylor et al., 1999).

\[ \Delta U^C_p(T) = C_p^U(T) - C_p^N(T) \]  \hspace{1cm} \text{Equation 6-3}

Integration of the area under the $C_p$ curves yields the calorimetric enthalpies, $\Delta H$.

The DSC data of $\Delta U^C_p$ then allows for the determination of the enthalpy and entropy difference between the native and unfolded states at any other temperature by using \textbf{Equation 6-4} for the enthalpy:

\[ \Delta N^C_H(T) = \Delta N^C_H(T_i) + \int_{T_i}^{T} \Delta U^C_p dT \]  \hspace{1cm} \text{Equation 6-4}

\textbf{Equation 6-5} is used for the entropy difference:

\[ \Delta N^C_S(T) = \Delta N^C_H(T_i) / T_i + \int_{T_i}^{T} \Delta U^C_p d \ln T \]  \hspace{1cm} \text{Equation 6-5}

And likewise, the change in the Gibbs free energy can be calculated by utilizing \textbf{Equation 6-6}:

\[ \Delta N^C_G(T) = \Delta N^C_H(T) - T \Delta N^C_S(T) \]  \hspace{1cm} \text{Equation 6-6}
Several researchers have published a plethora of work in using DSC to study proteins. On major contribution by Makhatadze and Privalov is the tabulation of the heat capacities of the peptide units and the side chains of the amino acid residues (Makhatadze & Privalov, 1996). From these measured heat capacity values, it is possible to calculate a first approximation of partial heat capacity for an unfolded protein in an aqueous solution. Summing the partial heat capacities of the individual amino acid residues leads to the total partial heat capacity (Makhatadze & Privalov, 1995). DSC studies have constituted one of the main pillars of thermodynamic data of bio-macromolecules in the past two decades.

### 6.1.4.2 Isothermal Titration Calorimetry (ITC)

In the past fifteen years an enormous amount of bio-calorimetric data has been determined using isothermal titration calorimeters (ITC). ITC has developed into one of the most widely used instruments in determining binding constants of protein ligand interactions (e.g. protons, salts, drugs, enzymes, lipids, nucleic acids) (Doyle, 1997). Isothermal titration calorimeters were first built by S. J. Gill at Colorado University and I. Wadsö at Lund University (Privalov & Privalov, 2000). In 1990, John Brandts introduced a new titration calorimeter design which increased the sensitivity of measurements at least 10 to 40 times that of previous instruments while reducing the time to produce a protein-ligand binding curve by a factor of at least 20 (Fisher, 1995; Wiseman, 1989). MicroCal Corp. (Northampton, MA) markets a commercial version of this design bearing the name, OMEGA. The principal operation of ITC involves small aliquots of one reactant being added, via an injection syringe (typically 25 – 250
μL), and then mixed in a solution containing the second reactant. The reactant is added in a stepwise manner with the number of injections, the volume per injection, and the time between injections determined by the researcher. For each stepwise injection, the resulting heat flux is measured and integrated to determine the enthalpy. Analyzing the titration curve leads to information concerning the number of binding sites, n, the enthalpy of binding, ΔH, the equilibrium binding constant, K, and from that the free energy of binding, ΔG.

In a typical binding experiment, a binding model is used to determine the heat involved in the process. For example, in a 1:1 stoichiometry of the binding of a ligand [L] to a protein [P] the relationship is (Fisher, 1995; Wiseman, 1989):

\[
P + L \leftrightarrow PL
\]  

**Equation 6-7**

The bound ligand concentration [PL], is:

\[
[PL] = [P] \cdot \frac{K[L]}{1 + K[L]}
\]  

**Equation 6-8**

where the total protein concentration \([P]_t = [P] + [PL]\), and K is the binding constant. In ITC the heat, q, absorbed or evolved in a protein-ligand binding is proportional to the above concentration of bound ligand [PL] where (Fisher, 1995):

\[
q = V \Delta H \cdot \frac{K[L]}{1 + K[L]}
\]  

**Equation 6-9**
Two methods of data analysis can then be employed. The first involves making initial estimates of $K$ and $\Delta H$ and fitting Equation 6-9 in a plot of $q$ versus $[L]_t$ data. An iterative process is then employed to refine constant values of $K$ and $\Delta H$. A second method of data analysis is to plot the derivative of the heat, $q$, with respect to $[L]_t$, $dq/d[L]_t$. This provides a plot of $\Delta H$ values for each injection. The curve is then fitted to determine $K$.

Although an enormous amount of ITC data abounds and has been instrumental in studying protein ligand interactions, the technique differs from our studies of slow hydration processes of proteins. The protein samples used in ITC are in solution, where ours are in a polymeric, glassy-solid state. The samples are stirred and this causes a slight Joule heating. Although it is possible to add small increments of water (~1-2µL), this is still a much larger amount than the lowest water vapor activity measurements that we can achieve. Because ITC is so widely used, it is instructive to note its contributions in the understanding of protein energetics.

### 6.1.4.3 Sorption Calorimetry

The isothermal sorption microcalorimeter is a relatively new calorimetric instrument that is akin to the QCM/HCC in the fact that the solvent vapor activity is varied. The vapor then interacts with a solid/crystalline sample and the corresponding thermal signal upon sorption is measured. The sorption calorimeter directly measures the partial molar enthalpy change when water evaporates from the liquid phase and recondenses to the sorbent phase (Wadsö & Markova, 2000). The design of the instrument consists of a twin setup comprised of a sample and reference side. Each side
is composed of two vessels situated one above the other. The lower vessel measures the sample being studied and the upper vessel contains the solvent (in this case water) that will be sorbed. At the start of an experiment, the upper chamber is injected with the solvent to be sorbed. The rate of vapor interaction with the solid sample is dependent on the diffusion of the vapor phase as it travels through a tube to the connecting sample vessel. Unique to this instrument is that the thermal signals are measured simultaneously and individually from both the vaporization and sorption vessels. From these calorimetric results it is possible to measure the rate of vaporization from the vapor source, the vapor activity in the sorption chamber and the differential enthalpy of sorption (Markova et al., 2000). The water flow, \( q_m \), from the vapor source to the sample vessel is found by:

\[
q_m = \frac{P_v}{\Delta_{vap} H M_W}
\]

**Equation 6-10**

where \( q_m \) is measured in g/s, \( P_v \) is the measured thermal power of vaporization, \( \Delta_{vap} H \) is the enthalpy of vaporization of water, and \( M_W \) is the molecular weight of water. The water gained by the sample is denoted as \( c_w \) and found by evaluating the following equation:

\[
c_w = \int (q_m dt) / m_o
\]

**Equation 6-11**

where \( m_o \) is the starting mass of the sample. And from this the differential enthalpy of sorption, \( \Delta_{sorp} H \), in relation to the steady state of the liquid water is:
\[ \Delta_{\text{ sorp}} H = \left( P_s - P_v \right) / q_m M_w \]

Equation 6-12

where \( P_s \) is the thermal power of sorption, and \( P_v \) is the thermal power of vaporization.

It is noted that sorption calorimeters as that described above have many potential applications in studying protein-water interactions and has been used in studying water sorption of pharmaceutical materials (Markova et al., 2001).

6.1.5 New Prospects in Measuring Protein Hydration Using the QCM/HCC

The QCM/HCC provides a unique calorimetric method of studying protein hydration. Other methods of hydrating samples utilize measurements before and after the hydration process (Poole & Finney, 1986). The QCM/HCC is used to record measurements during the hydration process. As the sample is kept in an isothermal environment, the water vapor activity in contact with the sample, a protein film, is varied at well-defined intervals, thus allowing for the rate of hydration to be measured. In their comprehensive review of protein hydration, Kuntz and Kauzmann report in their discussion on calorimetric measurements of proteins that there exists “few reports of water-protein systems in which the water content is the major variable (Kuntz & Kauzmann, 1974).” Since this review in 1974, the same statement could well be made today. Using the QCM/HCC, simultaneous measurements are made on the heat evolved during the hydration processes, the mass of water being sorbed, as well as measurements regarding the softening of the protein film indicative of the protein undergoing a transition from a dry structure to a semi-fluid hydrated state. Investigators state that large amplitude fluctuations of proteins occur on a time scale of \( 10^{-7} \)s. The QCM operates with a vibrational period of \( 2 \times 10^{-7} \)s (Reinisch et al., 1989).
Because of this time resolution, the QCM can capture these large structural fluctuations and measure these as a change in the motional resistance of the protein film.

In comparison to the above mentioned isothermal sorption calorimeter, both instruments measure the mass uptake of water as well as the thermal activity involved with this process. As an added feature, the QCM/HCC provides flexibility in scanning both the hydration and dehydration. And, structural properties of the film are also analyzed during the course of the hydration process. Sorption enthalpies and isotherms as a function of water vapor activity are analyzed from these direct calorimetric measurements.

6.1.6 Studies Involving Protein Films

The physical state of protein films has not been extensively characterized or studied with little being reported in the literature (Poole & Finney, 1986). A few reported studies on protein films include: (1) a QCM study (Reinisch et al., 1989) measuring the mass and damping effects upon hydration, (2) a Raman resonance (Feng & Tahcikawa, 2001) study determining binding of water to the heme group, (3) a circular dichroism (CD), far-UV, and IR spectroscopic analysis of protein and peptide films (Safar et al., 1993) and (4) gravimetric and IR studies of lysozyme films (Careri et al., 1979).

Reinisch, et al., coated a QCM by allowing myoglobin dissolved in 0.1M potassium phosphate buffer to dry on the surface of the QCM. Using a light microscope and a scanning electron microscope, they reported that the surface of the dry film appeared cracked and wrinkled at a spatial resolution of 0.1 mm.

The studies done by Feng, et al. using spectroscopic methods, report that the native structure of myoglobin is maintained upon film formation, however the surface
characteristics of the film were not probed. Results from this study will be discussed in the myoglobin section.

Safir, et al., used transmission electron microscopy (TEM) to deduce that proteins form thin films on glass and mica with an amorphous, noncrystalline appearance. They then used circular dichroism (CD) spectroscopy to analyze three aspect of protein films, namely to determine (Safar et al., 1993): (1) the solid state secondary structure and compare solution and aggregate conformations, (2) the stability of secondary structure components in transition from solution to aggregate and in thin films, (3) solvent and temperature effects on the secondary structure of the protein in the solid state. Their results for the protein myoglobin showed that the CD spectra for a myoglobin solution and a solid-state film indicated that the $\alpha$-helical patterns were basically the same with a slight decrease of $\alpha$-helical content in the film. Upon heating the film to $132^\circ \text{C}$, the $\alpha$-helical content dropped while the $\beta$-sheet conformation dominated. The secondary structure of the film appeared to undergo an irreversible temperature induced $\alpha$ to $\beta$ transition. The investigators were able to convert the $\beta$-structure myoglobin film to the stable $\alpha$-helical conformation at room temperature using formic acid (Safar et al., 1993).

Careri, et al., cast films of lysozyme from an aqueous solution onto IR windows. The hydration was studied gravimetrically and through IR spectroscopy. They do not report any physical description to characterize the film (Careri et al., 1979).

**6.1.7 Outline of Protein Studies Using the QCM/HCC**

The QCM/HCC has been used to study the hydration of two proteins, lysozyme and myoglobin. Thin films (1 – 3 $\mu$m) of each protein were exposed to controlled step-wise
changes in water vapor activity while under isothermal conditions. The water vapor activity ranged between 0 and 0.80 for the films. All of the studies were performed at 25°C with the exception of one myoglobin film studied at 37°C. Sorption enthalpies and isotherms were determined from the measurements. In addition, the myoglobin films were also evaluated as to the changes in the film motional resistance upon vapor sorption and analyzed as to the effects of the buffer solution used.

6.2 Continued Analysis of Lysozyme Hydration

6.2.1 Review of Previous Studies Using the QCM/HCC

Protein function is dependent on water. Because of its robustness and its relative abundance and ease of preparation, the protein lysozyme has been the subject of extensive hydration studies. Yang and Rupley noted that the enzymatic activity of lysozyme changes as a function of hydration (Yang & Rupley, 1979). Lysozyme is shown to be inactive at low hydrations with its activity beginning at approximately 0.2 g water / g protein. Their studies included measuring the rotational relaxation rate of lysozyme using an electron spin resonance (ESR) probe. The relaxation rate followed the same dependence on hydration as did the enzymatic activity.

These studies on the hydration of lysozyme were our initial work in utilizing the QCM/HCC for protein studies. Dr. Hamid Shirazi, a former graduate student in our laboratory, was instrumental in the work on preparing the films and guiding the experimental process and data analysis. The lysozyme film studied was 250µg and 1µm and no buffer was used in preparing the solution and casting the films. The experimental results, data analysis and discussion have been reported (Shirazi, 2000; Smith et al., 2002).
The studies can be summarized in viewing the sorption isotherm and plot of the heat generated per mole of water sorbed by the lysozyme film. **Figure 6-1** depicts the sorption isotherm. The triangular markers depict our hydration measurements. The other lines on the plot stem from parameters for lysozyme used in fitting a sorption isotherm utilizing the D’Arcy Watt equation (Gregory, 1995a; Rupley & Careri, 1991). The dashed line marks the hydration of the polar and more strongly bound sites, dash-dot line denotes the hydration of the weaker binding sites, the dotted line represents the formation of a multilayer of water, and the solid line constitutes the summation of these contributions. Our experimental data closely follows the data used to construct this lysozyme isotherm.

**Figure 6-2** features the heat generated per mole of water, $\Delta Q/\Delta n$, in the lysozyme film. The values are plotted versus the water vapor activity. The results follow a trend seen in the literature (Bone, 1996; Hinz, 1986; Lüscher-Mattli & Rüegg, 1982). At the lower $a_w$ values, the $\Delta Q/\Delta n$ values are larger in magnitude explained by the fact that in this region binding of water is occurring among the polar sites on the protein. In the higher $a_w$ region, the hydration water is binding to the weaker sites and participating in a multilayer of water formation, thus the lower in magnitude $\Delta Q/\Delta n$ values.

One additional piece of information that we have since analyzed is the rate of hydration of the lysozyme film. From this information the diffusion coefficients were tabulated. A discussion of this follows in the next sections.
Figure 6-1. A sorption isotherm for the hydration of a 1µm film of lysozyme. The experimental data is depicted by the triangular markers. The various lines are from previous literature defined parameters using the D’Arcy Watt isotherm for lysozyme hydration.
Figure 6-2. The heat generated per mole of water ($\Delta Q/\Delta n$) for the hydration of a 1µm lysozyme film. The values at the lower $a_w$ are larger in magnitude reflecting the hydration of the more polar, strongly bound sites while the values at the higher $a_w$ approach the $\Delta_{vap}H$ for water $\sim 44$ kJ/mol, reflecting the formation of multilayer water sites.
6.2.2. Kinetic Analysis of Lysozyme Hydration

Studying the diffusion of water in a protein film can be compared to the extensive studies that have been done on solvent diffusion in polymers. This area has seen considerable research because of its applications to polymer films such as the drying of paints and varnishes (Guerrier et al., 1998), diffusion of inks and dyes (Pinto et al., 1999), drug delivery and medical applications (Waggoner et al., 1993), and diffusion through building materials such as wood (Wadsö, 1994a). Diffusion coefficients are sought after values especially for their applications to industries involved in coating technologies and food processing and packaging. In these areas it is vital to have good control over the drying rate and solvent diffusion. A variety of models have been proposed to determine diffusion coefficients. Because most industrial applications involve blends of polymers and copolymers, their properties as mixtures are not well characterized. Models have served in characterizing some systems (Doumenc & Guerrier, 2001; Mézin et al., 1996).

Although protein-protein and protein-water interactions involve a wider variety of electrostatic associations and bonds than what is seen in a single type of polymer, polymers are closely related to protein chains and can serve as prototypes in studying diffusion coefficients.

6.2.2.1 Model of Diffusion

As a first approximation, many times the solvent diffusion is assumed to follow Fick’s Law, where diffusion is proportional to the concentration gradient. The studies of diffusion in the protein films shown here can be categorized as following the transient-sorption method (Wadsö, 1994a). This method involves measuring the mass.
change that occurs as the water vapor activity over the sample is changed. The weight change curve can be plotted versus the square root of time to evaluate whether the process obeys Fick’s Law of diffusion (Crank, 1975). A linear behavior of the initial slope confirms an adherence to Fick’s Law.

The lysozyme data was first analyzed according to Hernandez-Munoz (Hernandez-Munoz et al., 1999). The equation is based on adherence to Fick’s differential equations and is shown in **Equation 6-13**:

\[
\frac{-1}{\pi^2} \ln \left( \frac{D^2}{8m^\infty - m^i} \right) = \frac{D}{l^2} t
\]

**Equation 6-13**

where D is the diffusion coefficient, m is the mass of the film and sorbed vapor, the subscript p, refers to a polymer film, the superscripts refer to: t, the sorbed vapor at any time, \( \infty \), the final time and i, the initial time. The thickness of the film is \( l \) and in units of cm. Three assumptions are made in employing this equation: (1) the film thickness is constant, (2) pressure is constant, and (3) temperature is constant.

The sorption process in the lysozyme film occurred over long time spans and hysteresis was noticed upon desorption. Because of this, the mass baseline was not flat and there was a noticeable linear increase and decrease in the baseline. The treatment followed in Hernandez was modified to account for the small shift in baseline. The graphing software, Origin 6.0 (MicroCal, MA) was used to perform a non-linear least squares fit to the mass versus time data. A four parameter equation was used and the derivation of this is included in Chapter 4. The fitting equation was of the form:
\[ m_t = p_1 + p_2 \cdot e^{-p_3 \cdot x} + p_4 \cdot x \] \hspace{1cm} \text{Equation 6-14}

where \( m_t \) is the mass at any time, \( x \) is the time and the third parameter, \( p_1 \) is the mass at infinity, \( p_3 \) is the rate constant \((s^{-1})\) and \( \pi \) is the term used to define the diffusion coefficient \( D \) where \( p_3 \) is equal to \((\pi^2 D)/l^2\). The fourth parameter, \( p_4 \), accounts for the linearly increasing or decreasing baseline. The Origin software employs a Levenberg-Marquardt iteration where the fitter computes a variance-covariance matrix using values from the previous iteration. A sample of the experimental curves and the fit curves will be shown in the results.

6.2.2.2 Analysis of Experimental Results

The lysozyme film was exposed to water activity stepwise changes of 0.03. For each of the experimental runs, the equilibration time at each water vapor activity step was one hour. Because of the noticeable hysteresis effects, the experimental run in the lowest activity range (0 – 0.09) was repeated allowing for a five-hour equilibration period. The diffusion coefficients were evaluated for each sorption and desorption step.

For the lowest hydration steps, 0 – 0.03 water vapor activity, \( a_w \), the water sorption and desorption steps are shown in Figure 6-3.
Figure 6-3. Mass vs. time traces for a 1 µm lysozyme film shown as the solid black line. The dotted line in each is the simulated curve for a first approximation of the parameters, p₁ through p₄. The dashed line is the actual fit of the data produced from Origin. 

- **a)** sorption from a_{w} = 0 – 0.03, 1 hr. equilibration,
- **b)** sorption, a_{w} = 0 – 0.03, 5 hr. equilibration,
- **c)** desorption, a_{w} = 0.03 – 0, 1 hr. equilibration,
- **d)** desorption, a_{w} = 0.03–0, 5 hr. equilibration.
In Figure 6-3, (a) and (b) both depict the mass versus time traces upon a stepwise increase in \( a_w \), from 0 – 0.03. The solid line is the experimental data, the dotted line is the simulated curve with first approximations for the four parameters, the dashed line is the fit curve yielding the results for the four parameters. The lysozyme film is the same in both cases however the equilibration time was increased from one hour in (a) to five hours in (b). When comparing the sorption plots with the one and five hours of equilibration, the \( p_3 \) values, which define the rate of hydration, differ by an order of magnitude (e.g. \( 3 \times 10^{-3} \) versus \( 3 \times 10^{-4} \)) with the five hour step claiming the much slower hydration rate. The same trend is found on the desorption plots where (c) is the trace for a one hour equilibration, (d) for the five hour equilibration period. The fourth parameter, \( p_4 \), is a measure of the increase or decrease of the mass trace baseline. At equilibrium, the mass trace would level off and almost no change would be seen in the baseline. When comparing the sorption and desorption traces of one and five hour intervals, the baseline in b) and d), the five hour equilibration periods are almost two orders of magnitude smaller. Clearly it is seen that the hydration processes in the one-hour steps did not reach full equilibration. The slight changes even in the baseline for the five-hour periods suggested that a much longer time scale is needed to achieve full equilibration.

Figure 6-4 presents a view of the higher hydration range. The mass versus time curves are shown for the same lysozyme film with hydration being measured at \( a_w = 0.86 – 0.90 \).
Figure 6-4. Mass versus time traces for lysozyme film at $a_w = 0.86 - 0.90$. a) Water sorption in the lysozyme film, 1 hr. equilibration. b) Water desorption of lysozyme film, 1 hr. equilibration.

The rate values, $p_3$, are of the same order of magnitude, however the slope of the baseline, $p_4$, is an order of magnitude larger than the values seen at the lower $a_w$. The mass traces approach a leveling off but appear to need much more time to reach equilibrium with the water vapor interacting with the film. At this higher water vapor activity, it would be expected that the protein’s polar sites have been hydrated. Slow structural changes may be occurring to allow for other sites to interact with the water. At this level a multi-molecular network of water is also forming.

From the values obtained for $p_3$, the diffusion coefficients, $D$, were calculated and plotted. Figure 6-5 depicts the diffusion coefficient for each of the lysozyme studies.
Figure 6-5. Diffusion coefficients, $D$, for a 1 µm lysozyme film measured over a range of water vapor activities, 0 – 0.90. a) values measured after 1 hr. equilibration periods with the set $a_w$. b) values measured after a 5 hr. equilibration period at low $a_w$.

In Figure 6-5, values for $D$ were found for both the water sorption and desorption of the lysozyme film. The values for the one-hour equilibration periods, (a), center around $3 \times 10^{-12}$ cm$^2$s$^{-1}$, while values for the five hour equilibration period, (5), are an order of magnitude smaller and center around $3 \times 10^{-13}$ cm$^2$s$^{-1}$.

At equilibrium and if the diffusion follows Fick’s law, the diffusion coefficient, $D$, is a constant and is proportional to the net rate of flow of water vapor (in this case) across a plane of fixed area, and to the rate of change of the molar concentration. If the temperature, pressure and the area of diffusion are the same, $D$ should also remain the same and not be dependent on the time. From above, however, the measured $D$ does
appear to depend on time as evidenced by the very slow approach to equilibrium, thus indicating non-Fickian behavior for water sorption of the protein film. This behavior has been cited for the water sorption of wood and collagen (Pineri et al., 1978; Wadsö, 1994b). When comparing protein dehydration to polymer/solvent drying research, for polymers, it is found that the diffusion coefficient can decrease by several orders of magnitude when the solvent concentration decreases. The polymer layer is characterized by large concentration gradients (Guerrier et al., 1998). The gradients are categorized as two successive steps: (1) a fast regime where the solvent concentration is high allowing for the flux of solvent to the surface interface, (2) a slow regime where the solvent concentration has decreased near the interface upper layer. Because of relaxation processes in polymers, plasticization effects and glass/rubber transitions, the diffusion of a solvent becomes concentration dependent in these cases. These complications point towards non-Fickian diffusion and appear to be seen in our protein analysis.

6.3 Hydration of Myoglobin

6.3.1 Structure and Function of Myoglobin

Kendrew, et al., first elucidated the structure of Myoglobin (Mb) more than 40 years ago (Kendrew et al., 1960). His work revealed that Mb is a relatively small globular protein, 17.8 kDalton, with 153 amino acid residues folded into 8 α-helices. The polypeptide chain upon folding provides a nonpolar pocket which both houses and stabilizes an electroactive iron porphyrin ring.
Most often, the reported function of myoglobin consists of the storage of oxygen (O₂, g) in the muscles and to assist in delivering O₂ to the mitochondria (Parak & Nienhaus, 2002). This is achieved through the reversible binding of O₂ to the central ferrous (Fe II) atom of this heme molecule, thus forming oxymyoglobin (MbFe²⁺O₂). This process occurs at 1 atm and 36⁰C in humans with a dissociation constant (K_D) ~ 1 µM (Brunori, 2000). When a ligand binds to Mb, researchers have outlined several reaction steps (1) the ligand moving into the heme pocket, (2) a water molecule being displaced, (3) the Fe atom moving in-plane to form a hexacoordinate complex, (4) the formation and stabilization of the Fe-ligand bond (Møller & Skibsted, 2002). In vivo,
Fe in the heme group of myoglobin remains in the ferrous state (FeII) when the protein is both oxygenated and deoxygenated. However, in vitro, FeII gradually oxidizes to the ferric state (FeIII) and H₂O replaces O₂ as the iron ligand. The molecule no longer is able to bind O₂. This oxidized form of myoglobin is known as either methmyoglobin or even more commonly as metmyoglobin (metMb). The “met” form does not accumulate in vivo due to the presence of an enzyme system which reduces the ferric to the ferrous state (Phillips & Richards, 1981).

Myoglobin, however, does not bind O₂ exclusively, the ferrous state of Mb also binds carbon monoxide (CO) and nitric oxide (NO) with even higher affinities thus forming carbonmonoxymyoglobin (MbFe⁺⁺CO) and nitrosylmyoglobin (MbFe⁺⁺NO). Researchers have even suggested that another function of Mb is the scavenging of NO, which is a powerful respiration inhibitor (Parak & Nienhaus, 2002). Interestingly, NO binds more strongly to Mb than O₂ but a critical difference is that the NO does not replace the O₂ in a ligand exchange reaction. Instead, the NO is oxidized by the coordinated O₂ when it enters the heme cavity (Møller & Skibsted, 2002). The NO binding to the oxymyoglobin is catalyzed and effectively oxidized in what would otherwise be a slow process.

Although the molecule is packed tightly, there are four internal cavities that play a role in the protein’s function (Parak & Nienhaus, 2002). A curious phenomenon of myoglobin is that the x-ray structure shows no channel wide enough for the above-mentioned diatomic molecules to enter or leave the heme pocket. A static representation of myoglobin therefore is not the most accurate picture and the protein is better described dynamically including the opening and closing of these channels (Parak
Figure 6-7 depicts the coordination plane of the heme pocket. It is characterized by the FeII tetracoordinated in the center. The Histidine residues at the 5<sup>th</sup> and 6<sup>th</sup> coordination sites are also shown.

![Figure 6-7](image)

**Figure 6-7.** Structure of the heme group in myoglobin. In the center of the heme rests the tetracoordinated, divalent (Fe<sup>2+</sup>) iron. Because the heme is buried in the myoglobin, there is a high specificity for binding of diatomic gases, namely O<sub>2</sub>. The fifth coordination position is occupied by the proximal Histidine (His 93). The distal Histidine (His 64) at the 6<sup>th</sup> coordination position serves to obstruct access to select gases to the heme (Stryer, 1981).

**6.3.1.1 Equilibrium Configurations**

A functional protein actually exists in more than one state. The heme environment of myoglobin has been the subject of much research and experimentation. From the above diagram, two Histidine residues reside at the 5<sup>th</sup> and 6<sup>th</sup> coordination positions of the Fe in the heme group. The 5<sup>th</sup> coordination position of the Fe joins the porphyrin ring to the protein polypeptide chain through the N on the imidazole ring of Histidine.
93, His93. The second Histidine, His64, resides in the vicinity of the 6th coordination position of the Fe. This distal heme acts as a barrier and obstructs access to the 6th coordination position so that only O2 and a few other small molecules could bind to the Fe. The distal heme demands that a small molecule binding to the 6th site of the Fe be positioned at a determined angle, one that happens to be ideal for O2.

Normally functioning Mb fluctuates between two states, deoxy- (deoxyMb) and oxymyoglobin (oxyMb). These two states are marked by having different conformations and different properties. Within these two states of functioning Mb, there exist a number of conformation substates (CS). Conformational substates have the same general structure but may differ in the fine details. Although they perform the same functions, the rates of performance may differ (Frauenfelder & Gratton, 1986). The two states of Mb, deoxyMb and oxyMb, exemplify these differences in states. In oxymyoglobin, the ligand is bound to the heme iron with the heme being in a planar coordination. The d-electrons of the iron become paired and are characterized as being in a low-spin (S=0) singlet configuration. DeoxyMb differs in structure because the iron has moved out of the heme plane by \( a_o \sim 0.45 \, \text{Å} \) and is a high-spin (S=2) quintet state (Champion, 1992; Feng & Tahcikawa, 2001; Frauenfelder & Gratton, 1986).

Researchers note that two distinct types of motions occur in proteins namely: equilibrium fluctuations (EF) and functionally important motions (FIMS) (Frauenfelder & Gratton, 1986). A FIM describes the motion of a protein in changing from one state to another while an EF is marked by a protein in a particular state moving from one substate to another without changing its state, namely an equilibrium configuration (Yang & Rupley, 1979). Therefore, a macromolecule such as oxymyoglobin may exist
in a large number of conformational substates. Barriers between CS are generally in the order of 100 kJ/mol. The complexity does not end here. Investigators note evidence of CS being further divided into conformation sub-substates (CS$^2$) separated by energy barriers of 10 – 50 kJ/mol and these are further divided into sub-sub-sub-states (CS$^3$) with barriers between these substates in the order of a few kJ/mol (Frauenfelder & McMahon, 2001). Myoglobin has been shown to exhibit these three tiers of CS with CS$^1$ being shown to exist from measurements involving the time dependence of the CO and O$_2$ molecules binding to the heme proteins and involves possibly the rearrangement of the protein molecule as well as the hydration layer. Evidence for the CS$^2$ in myoglobin was shown through NMR and Mössbauer studies and would include motions of larger units in a protein such as helices. CS$^3$ states were shown to exist from studies performed using specific heat and dielectric relaxation at temperatures down to 0.2 K and include motions of a few atoms or small groups (Frauenfelder & McMahon, 2001).

In the case of Mb having a hierarchy of conformational substates, there would also exist a hierarchy of motions visible while the macromolecule is in equilibrium or during its function. Mb exhibiting three CS would also exhibit three types of equilibrium fluctuations (EF) within a particular state, EF1, EF2, EF3. To describe an EF however, a distribution of relaxation times is required. A distribution of times implies that the barriers between the substates span a range of energies.

Because of the hierarchy of states and motions, similarities have been drawn between proteins and amorphous solids and glasses. Investigators note that the theories of glass transition may apply to protein motions (Frauenfelder & Gratton, 1986; Slade & Levine, 1995).
6.3.1.2 Hydration Studies of Myoglobin Films

Myoglobin thin films have been studied using Resonance Raman (RR) Spectroscopy (Feng & Tachikawa, 2001). In their work, Feng and Tachikawa dissolved metMb in a 0.1M phosphate buffer, pH 7.4, and cast metMb films on several substrates such as a quartz plate, gold, silver, platinum and a GC electrode. They sought to determine if metMb remained in its native state when cast as a film and they studied the electron transfer properties of metMb under different conditions. Their spectroscopic data revealed that the metMb remained in its native state when cast as a film, however, the transition from the solution state to a drier film did cause a conformational change. The iron of myoglobin in an aqueous solution is in its high spin state (S=2), while in the film, iron was shown to be in its low spin state (S=0). Once the metMb film was redissolved in a buffer solution, the Raman spectra were restored and were indicative of aqueous metMb with Fe in the high spin state. Their studies, which included FT-IR and UV-VIS spectroscopy, as well as, cyclic voltammetry, revealed that water bound to the Fe in the heme evaporates from the pocket as the metMb film is formed. In solution, the distal histidine (His64) helps to bind the weakly coordinated water to the heme. Upon drying, the water is able to escape and a slight restructuring takes place. The N on the imidazole ring of the His64 residue moves in closer contact with the central Fe and changes it to a low spin state. This is depicted in Figure 6-8.
When the imidazole side chain occupies the 6\textsuperscript{th} coordination site of the Fe, the Fe is pulled into the heme plane and the porphyrin ring is flat. This was supported by electrochemical experiments, which measured an increase in the heterogeneous electron transfer rate between the protein and the electrode.

The hydration of myoglobin has been the study of previous QCM measurements (Reinisch et al., 1989). Reinisch, et al., studied a myoglobin film dried on the surface of a QCM and separately studied a 0.1M potassium phosphate buffer film, pH 7.0 dried on a QCM. They sought to confirm measurements by other methods that protein hydration occurs in discrete steps. Two properties were recorded upon hydration, the change in resonant frequency, $f_o$, of the film coated QCM and the change in the vibrational amplitude, $A$. As noted in Chapter 4, the change in $f_o$ is proportional to a change in the film mass upon hydration. The vibrational amplitude is dependent on the motional resistance, $R$, and is a measure of the damping of the film and the dissipative
effects upon hydration. The vibrational amplitude is shown by **Equation 6-15** (Reinisch et al., 1989):

\[
A(\omega_o) = \left( \frac{1}{M \omega_o} \right) \frac{F_o}{R}
\]

Equation 6-15

where \(\omega_o\) is the angular frequency (\(\omega_o = 2\pi f_o\)), which is the series resonant frequency of the oscillating quartz and M is the total effective mass of the oscillator. The constant amplitude of the driving force is \(F_o\), and is periodic with \(\omega_o\) where:

\[
F(t) = F_o \cos(\omega_o t)
\]

Equation 6-16

From their studies on a myoglobin/buffer film versus a buffer film, the investigators noted that the myoglobin/buffer system damps the QCM to a greater degree than the buffer film alone. Upon hydration of the myoglobin film, the vibrational amplitude was plotted versus the QCM frequency shift. From this plot, the amplitude decreases in two defined steps and the hydration of a protein film does appear to occur in discrete steps.

### 6.3.2 Experimental

The myoglobin samples were obtained through the generosity of Dr. Anthony Addison. The myoglobin was originally purchased from Sigma (M-1882), as lyophilized, essentially salt-free, type III with the iron being in the ferric state (FeIII). This oxidized form of myoglobin is referred to as metmyoglobin (metMb). The
molecular weight reported by Sigma is 18,800 Daltons which corrects for 4.8% H$_2$O when the myoglobin was prepared.

A 0.5M sodium phosphate buffer solution of pH 7 was prepared using anhydrous monosodium phosphate (NaH$_2$PO$_4$), Sigma, Reagent grade, Lot 95F-0128 and trisodium phosphate dodecahydrate, tribasic (Na$_3$PO$_4$ ·12H$_2$O), MCB Matheson, Coleman, and Bell, Reagent grade. For one liter of sodium phosphate buffer solution (PBS), 4.2689 g of the monobasic phosphate was combined with 5.4829 g of the tribasic phosphate. These were dissolved in 1 L of degassed, deionized water. Subsequent buffer was prepared in the same manner. For the dialysis process, 0.05M sodium phosphate buffer was prepared by diluting 100 ml of the above 0.5M solution in 1 liter of degassed, deionized water.

The starting concentration of the myoglobin was aimed to be about 4 mg/ml, which was achieved by dissolving 200 mg of myoglobin in 50 ml of PBS. This concentration was based on a laboratory used in the inorganic chemistry curriculum at Drexel University. The solution was prepared in an Erlenmeyer flask and stirred gently but continuously so as not to cause any denaturation made visible by the appearance of foam on the surface of the solution.

After three hours of stirring, the solution was filtered to capture any undissolved particulates of myoglobin. Upon weighing the undissolved particulates on filter paper and subtracting for the mass of filter paper, the concentration was approximated to be 2.3 mg/ml. Approximately 27 ml of myoglobin solution was transferred into dialysis tubing. The remaining myoglobin solution was stored in silanized vials kindly provided by Dr. Kevin Owens from his laboratory.
The dialysis tubing (Spectra Por 3, Part No. 132720, Lot # 22623) used had a molecular weight cut-off (MWCO) of 3,500, a flat width of 18 mm, and a diameter of 11.5 mm. The volume/length ratio of the tubing was 1.0 ml/cm. Preparation of the dialysis tubing included cutting a length of the tubing (e.g. 35 cm) and boiling this in deionized water for approximately 15 min. The tubing was then allowed to cool, then rinsed, and placed in fresh deionized water to be boiled again. The process was repeated 3 times. Extra cut/boiled pieces of dialysis tubing were stored in a beaker of deionized water at 4°C.

Two separate samples of myoglobin were dialyzed. The first was an experimental trial to determine a minimal concentration of buffer for the dialysis. The first trial consisted of three dialysis steps.

Approximately 27ml of myoglobin solution was transferred to the dialysis tubing. The bottom end of the tubing was tied and approximately 4 cm was left as extra at the top to provide an air bubble for the tubing to float. The top was then sealed with a dialysis clamp. According to Spectra Por, the dialysate volume should be ~ 100 times the sample volume.

For this first dialysis trial, the myoglobin filled dialysis tubing was placed in a large graduated cylinder with 1.5 L of 0.05M sodium phosphate buffer solution. A magnetic stir bar was used to gently stir the solutions for 12 hours. The dialysis tubing was then placed in a large graduated cylinder with 1.5 L deionized water. After 4-5 hours, the myoglobin dialyzing in the deionized water precipitated as a suspension in the tubing. The tubing was placed in a large graduated cylinder of 1.5L of deionized water once again. In this third dialysate change, the myoglobin precipitated out of the solution.
This was an example of “salting in.” When too few ions are present in solution the buffer salts no longer shield the protein-protein ionic charges. The attractive forces between individual protein molecules become dominant causing aggregation and precipitation (Voet et al., 1999). This solution was then discarded and another trial was started.

For the second trial of dialysis, 16 ml of the original myoglobin solution in 0.5M sodium phosphate buffer solution was placed in the dialysis tubing. This was then placed in a large graduated cylinder with 1.6 L of 0.05M sodium phosphate buffer solution. The myoglobin in the tubing was gently stirred for 4.5 hours. No further dialysis was done on this myoglobin sample.

The dialyzed myoglobin solution was used to prepare thin films on the QCM’s (Maxtek, 5 MHz, polished, P/N 149211-1, Model SC-501-1). Three different films were used for the myoglobin analysis. The first was coated using an airbrush (Badger, Franklin Park, IL, USA, Model 200). The ensuing two films were coated using an oscillating capillary nebulizer (OCN) vide infra (Perez et al., 1999).

Prior to coating, each QCM was cleaned with Piranha solution (1:3, 30% H₂O₂ : 98% H₂SO₄). The QCM’s were placed in the piranha solution for 1 minute and then rinsed vigorously with deionized water. The process was repeated a second time. The QCM’s were then dried using a continuous flow of N₂ gas.

Two of the QCM’s were then coated with a thin film of poly-L-lysine. This coating was applied to make the surface of the QCM hydrophilic. Because of no apparent change in facilitating the coating, the poly-L-lysine was not applied to the third QCM.
The first myoglobin film was coated on QCM #19 using the airbrush. The basic operation of an airbrush is that it contains a solvent reservoir, which is drawn upon by a mild flow (60 psi) of an inert carrier gas (e.g. N\textsubscript{2}). The solvent is then sprayed through a nozzle onto the QCM surface. The airbrush was held about 20 cm from the QCM surface and slowly and repeatedly moved across the QCM surface. After a thin build up of substrate, a dry gas flow was applied to help the substrate dry. Continual applications were then repeated until the desired film thickness was achieved. The airbrush appeared to coat the QCMs too thickly on some trial runs. The myoglobin solution on the QCM quickly became thick and viscous. It was difficult to get a visibly uniform coating using the airbrush. The film on QCM #19 appeared to be the most uniform and was used in the first experimental run.

The other two films were prepared using the oscillating capillary nebulizer (OCN). Jason Riggs, a senior undergraduate researcher in our laboratory, did the background work of ordering the components, assembling, and testing the OCN for the coating of thin films on QCM’s. The OCN is a device that allows a solvent to be sprayed as a very fine, uniform mist. The solution to be nebulized is contained in a gas tight syringe (for this study, Hamilton, #81020, 100 µl). The syringe is then mounted on a syringe pump (Harvard Apparatus, Model 22) and connected to the OCN via a luer adapter fitting (Upchurch, PEEK quick connect, 10.32 to female). PEEK tubing (Upchurch, #1536, 1/16” o.d., 0.007” i.d.) is connected to the other end of the luer lock. Through this PEEK tubing, a fused silica capillary (Polymicro, #200026, 80 mm, 50 µm i.d., 150 o.d.) is inserted. This capillary is inserted and protrudes through a tee fitting (Upchurch, #0.429, 1/16” ss, 0.040” thru hole)). The other end of the tee is mounted
with PEEK tubing (Upchurch, #1532, 1/16”o.d., 0.02”i.d.). The 50 µm i.d. fused silica capillary is friction mounted into a larger fused silica capillary (Polymicro, #200026, 30mm, 250 µm i.d., 350 µm o.d.) and both of these are inserted into the larger PEEK tubing (0.02” i.d.). Stainless steel tubing (1/16” o.d., 0.040” i.d.) is fit into the top of the tee fitting. This stainless steel tubing is connected to a cylinder of helium gas. As the helium is introduced into the larger capillary, the inner capillary is forced to oscillate. As a result of the oscillation, the solution is then nebulized at the capillary tip (Perez et al., 1999).

![Diagram of the oscillating capillary nebulizer, OCN.](image)

**Figure 6-9.** The oscillating capillary nebulizer, OCN, used to coat thin films on the surface of QCM’s (Perez et al., 1999).

When first trying to coat QCM’s with the myoglobin solution, the protein seemed to clog the OCN. Instead of a fine mist, the OCN sputtered the nebulized myoglobin solution. Dr. Kevin Owens suggested silanizing the glass parts involved with the OCN so as to avoid any protein adhering to the glass surfaces. This was performed using siliconizing fluid (dimethyldichlorosilane, PAR #9356). The syringe barrel and glass
capillaries were first aspirated with 1:1 HNO$_3$ and then rinsed with deionized water. Methanol was then aspirated through the glass parts. A stream of dry nitrogen gas was employed to dry the glass surfaces. The glass surfaces were then exposed to the siliconizing fluid. Excess fluid was removed and the glass syringe bore and capillaries were cured in an oven at 65°C for 10 hours.

The OCN was then reassembled and the dialyzed protein solution was drawn into the gas tight syringe. The syringe pump was set to inject 2 µL/min, but this rate was varied to both 5 µL/min and 10 µL min during the coating process. The helium flow was regulated at 30 psi. Because the outer (250 µm i.d.) glass capillary is friction mounted, some trials were required to ensure that the capillary would not discharge from the PEEK tubing. It was found that turning the syringe pump on first helps in achieving this.

Of the two films coated with the OCN, the first QCM was held in a Drexel-built QCM mount as it was coated. Using this holder, the whole surface of the QCM was covered, a total area of 5.067 cm$^2$. The second was placed in the Maxtek QCM holder (Model CHT-100) the QCM frequency was monitored as the film was coated. The frequency was displayed on a Tektronix frequency counter. A total area of 2.849 cm$^2$ was coated on the QCM surface.

Data for the three, coated QCM’s is presented in Table 6-2. Mass measurements were taken on a Mettler (H542) balance.
Table 6-2. QCM data before and after coating with myoglobin/buffer solution.

<table>
<thead>
<tr>
<th>QCM #</th>
<th>19</th>
<th>12</th>
<th>31</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass before coating</td>
<td>mg</td>
<td>440.57</td>
<td>441.88</td>
</tr>
<tr>
<td>Mass after poly-L-lysine</td>
<td>mg</td>
<td>440.79</td>
<td>442.17</td>
</tr>
<tr>
<td>Mass after myoglobin film applied (dried)</td>
<td>mg</td>
<td>441.86</td>
<td>443.76</td>
</tr>
<tr>
<td>Mass of myoglobin film from Mettler</td>
<td>µg</td>
<td>1070</td>
<td>1590</td>
</tr>
<tr>
<td>Mass</td>
<td>µg/cm²</td>
<td>211</td>
<td>314</td>
</tr>
<tr>
<td>Frequency before coating</td>
<td>Hz</td>
<td>4979235.7</td>
<td>4995240.6</td>
</tr>
<tr>
<td>Frequency after poly-L-lysine</td>
<td>Hz</td>
<td>4979207.5</td>
<td>4995193.7</td>
</tr>
<tr>
<td>Frequency after myoglobin</td>
<td>Hz</td>
<td>4972814.2</td>
<td>4954387.9</td>
</tr>
<tr>
<td>Mass of myoglobin film from QCM</td>
<td>µg/cm²</td>
<td>223</td>
<td>* 721</td>
</tr>
<tr>
<td>Motional resistance before coating</td>
<td>Ω</td>
<td>9.349</td>
<td>15.129</td>
</tr>
<tr>
<td>Motional resistance after poly-L-lysine</td>
<td>Ω</td>
<td>11.523</td>
<td>15.444</td>
</tr>
<tr>
<td>Motional resistance after myoglobin (dry)</td>
<td>Ω</td>
<td>12.179</td>
<td>*98.912</td>
</tr>
</tbody>
</table>

The discrepancy between the Mettler mass measurement and the mass determined from the QCM frequency shift of 12 µg for QCM #19 and 69 µg for QCM #31 can be due to several factors. The Mettler was used by several lab members and may have been rezeroed in the days between the measurements. The film on QCM #19 was not visibly as uniform as the films on the other two reported QCM’s and may have caused the slight divergence in masses. The film on QCM #31 was visibly uniform. Within the QCM/HCC chamber, the top part of the chamber is clamped on top of the QCM. It has been noticed that clamping and reclamping may cause an average deviation of 50 Hz in the frequency measurements. The parameters of the QCM indicate that there is a 56.6 Hz shift per µg per cm². The clamping effect may have contributed to the discrepancy. QCM #12 was not fully dried while in the QCM and the frequency reflects this.
For the three films discussed in these experimental runs, the first was analyzed at 37°C, films two and three were analyzed at 25°C. For each experimental run, the sample film (myoglobin-buffer coated QCM) was placed on the left side of the QCM/HCC. A clean QCM was placed on the right side to be used as a reference QCM.

**6.3.2.1 Myoglobin/Buffer Film Coated with Airbrush Studied at 37°C**

The film on QCM # 19 upon completion of coating was stored in a dessicator with phosphorus pentoxide ($\text{P}_2\text{O}_5$) at room temperature for 24 hours. The sample QCM was then placed in the QCM/HCC and allowed to equilibrate for a few hours until a stable thermal baseline was achieved and no detectable mass change was noticed (noted by a stabilization in frequency, $\pm 0.5\text{Hz}$). Four experimental runs were performed on this film. The conditions for each of the four experiments are listed in Table 6-3.

**Table 6-3.** Experimental conditions for airbrush coated myoglobin film on QCM studied at 37°C.

<table>
<thead>
<tr>
<th>Experimental File</th>
<th>Water vapor activity ($a_w$) p/p₀</th>
<th>Incremental step for $a_w$</th>
<th>Time for each step min</th>
<th>Film condition previous to run $a_w$</th>
<th>Time / hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>02-06-27-1</td>
<td>0.00 – 0.15</td>
<td>0.03</td>
<td>66</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>02-06-28-1</td>
<td>0.15 – 0.30</td>
<td>0.03</td>
<td>80</td>
<td>0.27</td>
<td>7</td>
</tr>
<tr>
<td>02-06-29-1</td>
<td>0.30 – 0.44</td>
<td>0.03</td>
<td>88</td>
<td>0.30</td>
<td>3</td>
</tr>
<tr>
<td>02-06-30-1</td>
<td>0.30 – 0.44</td>
<td>0.03</td>
<td>88</td>
<td>0.30</td>
<td>3</td>
</tr>
</tbody>
</table>

For each of the experiments, to achieve the correct water vapor activity, the water vapor concentration was measured in parts per million (ppm). The step-wise change of 1860 ppm corresponded to a 0.03 change in water vapor activity. Results for each of the four experimental runs are listed in the results and discussion.
6.3.2.2 Myoglobin/Buffer Film, (314 µg/cm², 2.3 µm), Coated with OCN, Studied at 25°C

A second film of myoglobin was made on QCM #12 using the OCN. The film appeared very uniform, thin and homogeneous. Instead of storing the film in a dessicator of drying agent, the film was stored in a dessicator containing a saturated NaCl solution, which maintained a constant water vapor activity environment of 0.75. The experimental boundaries for each run on this film are listed in Table 6-4.

Table 6-4. Experimental parameters for QCM #12, myoglobin film studied at 25°C

<table>
<thead>
<tr>
<th>Experimental File</th>
<th>Water vapor activity (a_w)</th>
<th>Incremental step for a_w</th>
<th>Time for each step</th>
<th>Film condition previous to run</th>
<th>Film condition previous to run</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p/p_o</td>
<td></td>
<td></td>
<td>a_w</td>
<td>Time / hr</td>
</tr>
<tr>
<td>02-07-08-2</td>
<td>0.67 – 0.76</td>
<td>0.03</td>
<td>88</td>
<td>0.75</td>
<td>3</td>
</tr>
<tr>
<td>02-07-09-1</td>
<td>0.51 – 0.67</td>
<td>0.03</td>
<td>88</td>
<td>0.64</td>
<td>3</td>
</tr>
<tr>
<td>02-07-10-1</td>
<td>0.35 – 0.51</td>
<td>0.03</td>
<td>88</td>
<td>0.51</td>
<td>3</td>
</tr>
<tr>
<td>02-07-11-1</td>
<td>0.35</td>
<td>0.03</td>
<td>calibration</td>
<td>0.35</td>
<td>3</td>
</tr>
<tr>
<td>02-07-11-2</td>
<td>0.35 – 0.51</td>
<td>0.03</td>
<td>88</td>
<td>0.35</td>
<td>3</td>
</tr>
<tr>
<td>02-07-12-1</td>
<td>0.51 – 0.67</td>
<td>0.03</td>
<td>88</td>
<td>0.51</td>
<td>3</td>
</tr>
<tr>
<td>02-07-15-2</td>
<td>0.67 – 0.76</td>
<td>0.03</td>
<td>88</td>
<td>0.67</td>
<td>3</td>
</tr>
</tbody>
</table>

Unlike the previous experiment of a myoglobin film at 37°C which was dried and the hydration was measured from low water vapor activities, this film was preconditioned in a higher water vapor environment. The measurements were made at the upper end of the water vapor activities with the measurements proceeding with decreasing activities. Originally, the experiment was slated to test the film at a higher water activity up to 0.80. The film lost oscillation at this hydration level. The experiment was restarted with the higher limit of the water activity being 0.76.
6.3.2.3 Myoglobin/Buffer Film, (231 µg/cm², 1.7 µm), Coated with OCN, Studied at 25°C

A third myoglobin film was made on QCM #31 again using the OCN as the coating method and a thin, uniform, homogeneous film was produced. After coating, the QCM covered film was placed in a dessicator with a saturated NaCl solution so as to maintain a constant water vapor activity of 0.75. Just as in the previous trials, this film was first measured at the higher water vapor activities followed by experimental runs of decreasing water vapor activity. The experimental limits are listed in Table 6-5 for each of the tests done on this film.

Table 6-5. Experimental parameters for QCM #31, myoglobin film studied at 25°C.

<table>
<thead>
<tr>
<th>Experimental File</th>
<th>Water vapor activity (a_w) p/p₀</th>
<th>Incremental step for a_w</th>
<th>Time for each step min</th>
<th>Film condition previous to run a_w</th>
<th>Time/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>02-07-25-2</td>
<td>0.67 – 0.76</td>
<td>0.03</td>
<td>88</td>
<td>0.75</td>
<td>15</td>
</tr>
<tr>
<td>02-07-26-2</td>
<td>0.76 – 0.86</td>
<td>0.03</td>
<td>88</td>
<td>0.73</td>
<td>2</td>
</tr>
<tr>
<td>02-07-28-2</td>
<td>0.67 – 0.76</td>
<td>0.03</td>
<td>88</td>
<td>0.76</td>
<td>22</td>
</tr>
<tr>
<td>02-07-29-2</td>
<td>0.51 – 0.67</td>
<td>0.03</td>
<td>88</td>
<td>0.64</td>
<td>5</td>
</tr>
<tr>
<td>02-07-30-2</td>
<td>0.35 – 0.51</td>
<td>0.03</td>
<td>88</td>
<td>0.48</td>
<td>3</td>
</tr>
<tr>
<td>02-07-31-2</td>
<td>0.16 – 0.35</td>
<td>0.03</td>
<td>88</td>
<td>0.32</td>
<td>5</td>
</tr>
<tr>
<td>02-08-02-1</td>
<td>0 – 0.16</td>
<td>0.03</td>
<td>88</td>
<td>0.16</td>
<td>16</td>
</tr>
<tr>
<td>02-08-03-2</td>
<td>0 – 0.16</td>
<td>0.03</td>
<td>99</td>
<td>0.16</td>
<td>2</td>
</tr>
<tr>
<td>02-08-04-2</td>
<td>0.16 – 0.35</td>
<td>0.03</td>
<td>88</td>
<td>0.16</td>
<td>4</td>
</tr>
<tr>
<td>02-08-05-2</td>
<td>0.35 – 0.51</td>
<td>0.03</td>
<td>88</td>
<td>0.35</td>
<td>3</td>
</tr>
<tr>
<td>02-08-06-2</td>
<td>0.51 – 0.67</td>
<td>0.03</td>
<td>88</td>
<td>0.51</td>
<td>3</td>
</tr>
<tr>
<td>02-08-07-1</td>
<td>0.51</td>
<td>0.03</td>
<td>calibration</td>
<td>0.51</td>
<td>2</td>
</tr>
<tr>
<td>02-08-08-2</td>
<td>0.51 – 0.76</td>
<td>0.03</td>
<td>0.4</td>
<td>0.51</td>
<td>12</td>
</tr>
<tr>
<td>02-08-09-2</td>
<td>0.76 – 0.86</td>
<td>0.03</td>
<td>88</td>
<td>0.76</td>
<td>3</td>
</tr>
<tr>
<td>02-08-16-2</td>
<td>0.35 – 0.65</td>
<td>0.016</td>
<td>88</td>
<td>0.32</td>
<td>35</td>
</tr>
</tbody>
</table>
6.3.2.4 Sodium Phosphate Buffer Film Coated with OCN, Studied at 25°C

The OCN was used to coat a film of 0.05M sodium phosphate buffer. The coating conditions used with the OCN were the same as those used for the myoglobin solutions. The initial setup measurements are shown in Table 6-6.

**Table 6-6.** Parameters of initial coating of sodium phosphate buffer solution.

<table>
<thead>
<tr>
<th>QCM #</th>
<th>Mass before coating (mg)</th>
<th>Mass after PBS film applied (dried, mg)</th>
<th>Mass of PBS film from Mettler (µg/cm²)</th>
<th>Frequency before coating (Hz)</th>
<th>Frequency after PBS (Hz)</th>
<th>Mass of PBS film from QCM (µg/cm²)</th>
<th>Motional resistance before coating (Ω)</th>
<th>Motional resistance after PBS (dry, Ω)</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>441.33</td>
<td>771.75</td>
<td>65.3</td>
<td>4991605.7</td>
<td>4989486.4</td>
<td>74.1</td>
<td>9.349</td>
<td>16.092</td>
</tr>
</tbody>
</table>

Two experimental runs were performed on this buffer film at 25°C. The conditions for these are listed in Table 6-7.

**Table 6-7.** Experimental parameters for sodium phosphate buffer film hydration experiments.

<table>
<thead>
<tr>
<th>Experimental File</th>
<th>Water vapor activity ((a_w)) (p/p_o)</th>
<th>Incremental step for (a_w)</th>
<th>Time for each step (min)</th>
<th>Film condition previous to run (a_w)</th>
<th>Time/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>02-08-30-3</td>
<td>0.35 – 0.48</td>
<td>0.015</td>
<td>60</td>
<td>0.35</td>
<td>12 hr.</td>
</tr>
<tr>
<td>02-09-12-1</td>
<td>0 – 0.50</td>
<td>0.025</td>
<td>varied 10 to 120</td>
<td>0</td>
<td>10 days</td>
</tr>
</tbody>
</table>
After coating the PBS film, the QCM was stored in a dessicator with P$_2$O$_5$ for two hours at room temperature. Before the first experimental run, the QCM was placed in the calorimeter and allowed to equilibrate for several hours at a water vapor activity of 0.35. The original intention was to study the film over a water vapor activity range of 0.35 – 0.65, to cover the range where the interesting behavior was seen in the myoglobin film. However, upon starting the run, the film lost oscillation at a water vapor activity of 0.60. After manually setting the water vapor activity it was determined that the highest water vapor activity that the film would oscillate was 0.48. For the first run, this became the range that was studied.

In the second trial, the film was allowed to dry for a few days. The stepwise changes in the water vapor activity varied and were manually changed incrementally when the frequency change was <2 Hz. Because the stepwise changes varied, the hydration process was viewed to a water vapor activity of 0.50. This provided some interesting data to compare with the myoglobin/buffer film.

6.3.3 Results and Discussion

Each of the experimental runs are shown below within their respective heading. The data was analyzed according to the methods discussed in Chapter 3. To determine the film thickness, the density of myoglobin was used. The specific volume of myoglobin has been measured as 0.741 cm$^3$/g, which leads to a density of 1.349 g/cm$^3$ (Chalikian et al., 1996; Hinz, 1986).

Because a buffer solution is needed to get myoglobin into solution, some of the buffer remains in the film. By making a film of buffer solution, an attempt has been made to separate the effects of the buffer solution undergoing hydration and
dehydration. UV-VIS spectra of the original myoglobin/buffer solution and of the redissolved film confirm the presence of aquametmyoglobin.

6.3.3.1 Myoglobin Film Coated with Airbrush Studied at 37°C

The mass, thermal, and motional resistance traces were measured for the myoglobin/buffer film and these are shown in Figures 6-10 through 6-13. Figure 6-10 depicts the results in the low water vapor activity, $a_w$, range. With each increase and decrease in $a_w$, a defined mass change occurs along with a simultaneous thermal signal, and a corresponding change in the motional resistance. Exothermic events are denoted by the downward going peaks while endothermic events are marked by the upward going peaks. The integrated thermal signal then yields information as to the heat effects per mass of water gained or lost and allows us to calculate the sorption enthalpies. The motional resistance change for this film at low $a_w$, is minimal. From a plot of the motional resistance versus time, (e), it can be seen that the change is $<0.2 \, \Omega$. The myoglobin/buffer film is behaving as a rigid film in this region and causes no power dissipation in the crystal at this point. A slight decrease following by an immediate increase in the mass occurs at 57,000 sec and lasts for $\sim 290$ sec. It is unclear why this occurs, however some slight structural changes may have caused the transient signal marking a mass loss of 0.4 µg and an ensuing 0.2 µg mass increase.

Figure 6-11 shows the next stepwise changes in $a_w$, 0.15 – 0.30. The thermal, mass and motional resistance traces follow the same pattern of increasing and decreasing upon corresponding changes with the $a_w$. Upon desorption, however, the motional resistance begins to show an erratic behavior with fluctuations ranging $\sim 40 – 50 \, \Omega$. The power generated in the crystal from the low-powered Maxtek PLO driving the
QCM oscillation is calculated to be an average of 4.5 µW in the mid portion of the graph (approximately 25,000 to 47,000 sec). The erratic motional resistance changes account for < 1.5 µW of power being dissipated in the crystal. This is shown in (f).

The time scale shown in (f) reflects the conditioning stepwise changes (0 to 23,000 sec) in a_w previous to the first full sorption/desorption cycle. During the desorption steps, the erratic behavior appears in the motional resistance. This measurement provides a window to view the properties of the myoglobin film. It appears that upon drying within this range, some structural changes are occurring in the film as well as perhaps small fissures and cracks.

Figures 6-12 and 6-13 track the mass, thermal and motional resistance changes that occur upon a_w changes of 0.30 – 0.44 each. Because of the irregular mass and motional resistance changes, it was not possible to increase the measurements beyond a_w = 0.44, the film lost oscillation beyond this point. The deviations in the mass changes reveal that the hydration steps were not at equilibrium. Two factors appear to contribute to the irregular mass changes: (1) the increased water sorption processes are causing larger structural changes in the film, (2) the higher measurement temperature, 37°C, may increase the effects of sorption induced structural changes. Drawing a similarity between the protein film and polymer behavior, at higher temperatures, polymers become looser noted by an increase in vibrational and rotational motions leading to the onset of a glass transition. It is interesting to note the similarities and repeatability between the two files represented in Figures 6-12 and 6-13. With the changes in mass upon the sorption events, the integrated heat and motional resistance both closely match each event and appear to correspond very well. These measurements were instructive in
watching how closely the mass, thermal power, and motional resistance events are linked. In Figure 6-12 (e), the relationship between the motional resistance and the thermal power generated in the crystal is shown. The QCM oscillator generates a known amount of heat in the QCM. As the film becomes less rigid, more of that heat is dissipated as can be seen in the endothermic step-wise changes. The changes in motional resistance are minimal in this case and vary by ~ 4 Ω. These motional resistance changes account for a small heat dissipation, < 0.2 µW. Because of the irregular events, sorption enthalpies and isotherms were not evaluated for these experiments at 37°C, and it was decided to perform the next set of experiments at 25°C so as to explore a wider range of hydration.
Figure 6-10. Myoglobin/buffer hydration/dehydration, 37°C, $a_w = 0 – 0.15$. a) applied water vapor activity, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance.
Figure 6-11. Myoglobin/buffer hydration/dehydration, 37°C, $a_w = 0.15 – 0.30$. a) water vapor activity, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance, f) thermal power generated in the QCM.
Figure 6-12. Myoglobin/buffer hydration/dehydration, 37°C, $a_w = 0.30 - 0.44$. 

**a)** water vapor activity, 

**b)** mass trace, 

**c)** thermal signal, 

**d)** integrated heat, 

**e)** motional resistance, 

**f)** power dissipated in crystal
Figure 6-13. Myoglobin/buffer hydration/dehydration, 37°C, $a_w = 0.30 - 0.44$. a) water vapor activity, b) mass trace, c) thermal signal, d) integrated heat, e) motional resistance
6.3.3.2 Myoglobin Film (314 µg/cm², 2.3 µm) Coated with OCN, Studied at 25°C

The first studies provided us with information as to the sensitivity of our instrumentation and validated that our motional resistance measurements closely follow the film behavior upon sorption and desorption of a solvent. From these initial studies, we decided to conduct the further studies at 25°C to get a broader range of hydration effects. The film at 37°C appeared to be lossy when looking at the mass and motional resistance data. By lowering the temperature, the film would stay in a more rigid state so that higher water vapor activities could be measured. Also, the previous film was dried in a dessicator before the experiments and further dried with a constant stream of N₂(g) at 40 cc/min in the QCM/HCC. Because the low hydration ranges appear to have some structural effects on the previous film, this new set of experiments was started at higher water vapor activities. This film coated using the OCN appeared very uniform and thin, whereas the film coated with the air brush (in the previous section) had noticeably slightly thicker coating spots than others. After coating the QCM, the film was placed in a dessicator with a constant aw = 0.75 until the start of the experiments.

Figure 6-14 depicts the results from the first experimental run performed at aw = 0.67 – 0.76. The mass, thermal power, and motional resistance measurements all reflect the controlled stepwise changes in the aw. The two hydration/dehydration cycles appear to be very repeatable. Pronounced hysteresis is observed in the mass trace denoted by the amount of water leaving the myoglobin/buffer film being less than the amount being sorbed into the film. This is also reflected in the measured water vapor activity, (f). The aw is measured downstream from the QCM/HCC chamber, after the water vapor has interacted with the film. When the same amount of water is not being sorbed into
the film, the measured $a_w$ will be higher in magnitude than the originally applied $a_w$. Likewise, when more water is being held in the film, the measured $a_w$ is reflected as being lower in magnitude than the applied water vapor activity.

Figure 6-15 shows graphs of the mass, thermal and motional resistance changes upon corresponding changes in the applied $a_w$. All appear to show the expected behavior however, the motional resistance changes raised some questions. Instead of the expected increasing step-wise changes followed by the decreasing ones, the motional resistance first decreases upon sorption, then mid-way through the increasing step, the motional resistance also begins to increase. On the descending steps, the motional resistance decreased as expected but then mid-way through the step, the measurements begin to increase. The effects are small and account for $\sim 0.2 – 0.3 \, \mu W$.

Figures 6-16 and 6-17 show data from the myoglobin/buffer film being exposed to $a_w$ changes between 0.35 and 0.51. The prior film conditions were different for each run. Figure 6-16 shows data when the film was held at a $a_w = 0.51$ for three hours prior to the experiment. Figure 6-17 depicts the data after the film was held at $a_w = 0.35$ for three hours prior to the experiment. In Figure 6-16, the mass trace did not appear to start off at equilibrium. The initial mass of the hydration cycle appeared to have needed more time because the film was still desorbing water. The motional resistance was carefully tracked during the hydration cycle. In contrast to this, Figure 6-17, depicts a mass trace, which appears to have started in a more stable state of equilibrium. The mass traces in both figures reveal hysteresis effects. The motional resistance in this case yielded only an erratic signal whose amplitude was $30 \, \Omega$. It appears that holding the film at the lower humidity caused some slight structural effects/changes in the film.
Instead of possibly damaging the myoglobin/buffer sample, it was decided to bring the film back to a higher \( a_w \) and observe the effects on the motional resistance.

**Figure 6-18** shows data from the myoglobin/buffer film brought back to a \( a_w = 0.51 \) – 0.67. This experimental run was conducted with the same \( a_w \) conditions as those shown in **Figure 6-15**. There are some marked differences between the data for these two runs. The mass trace in **Figure 6-18** at \( a_w \approx 0.60 \) begins to show a much larger increase in the amount of water uptake. At this point, there is a mass change of \( \approx 25 \mu g \) versus an \( \approx 15 \mu g \) mass change for the same corresponding change in \( a_w \). A pronounced hysteresis is seen in (b) of **Figure 6-18**. The motional resistance did not register signals and was off-scale for a portion of the experiment. The last portion (~ at 40,000 sec) the resistance did come back on scale and registered expected step-wise signals. The measured water vapor activity records some of the film sorption effects. The irregular step changes reflect more water being held in the film and less actually reaching the relative humidity meter. The film appeared to be going through more dramatic structural changes than seen in the previous experimental runs.

Two other experiments were started with this film in which the film was going to be exposed to \( a_w = 0.67 \) – 0.76. The film lost oscillation, however the thermal signals were recorded fine. Because it was unclear whether or not taking the film to a lower \( a_w \) had caused damage to the protein/buffer film, it was decided to remove this QCM and cast a new film to be studied.
Figure 6-14. Myoglobin-buffer hydration/dehydration, 25°C, $a_w = 0.67 - 0.76$. a) water vapor activity, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance, f) water vapor activity as measured by the relative humidity meter.
Figure 6-15. Myoglobin/buffer hydration/dehydration, 25°C, $a_w = 0.51 – 0.67$. a) applied water vapor activity, b) mass trace, c) thermal signal, d) integrated heat, e) motional resistance, f) measured water vapor activity.
Figure 6-16. Myoglobin/buffer hydration/dehydration, 25°C, $a_w = 0.35 - 0.51$.  a) measured water vapor activity, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance, f) thermal power dissipated in QCM.
Figure 6-17. Myoglobin/buffer hydration/dehydration, 25°C, $a_w = 0.35 - 0.51$. a) applied water vapor activity, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance, f) measured water vapor activity.
Figure 6-18. Myoglobin/buffer hydration/dehydration, 25°C, $a_w = 0.51 - 0.67$. a) applied water vapor activity, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance with inset of magnified portion, f) measured water vapor activity.
Figure 6-18 shows the data for the calibration of the left thermopile. The calibration procedure is explained in Chapter 3. The Maxtek, low powered PLO was used to calibrate the left, sample side thermopile. The results are shown in the table above and in Figure 6-19.

**Figure 6-19.** Calibration of left thermopile using the low powered Maxtek Phase Lock Oscillator, with myoglobin-buffer film on QCM #12, aw = 0.35. **a)** steady-state thermal signal measured from thermopiles, **b)** steady-state conductance measured from the PLO.

**Table 6-8** Summary of left thermopile calibration, myoglobin-buffer film on QCM #12, held at aw = 0.35.

<table>
<thead>
<tr>
<th>Left Thermopile Calibration Using Low Powered PLO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Δ thermal signal</td>
</tr>
<tr>
<td>Average Δ conductance signal</td>
</tr>
<tr>
<td>Power generated in QCM</td>
</tr>
<tr>
<td>Calibration coefficient, ε</td>
</tr>
</tbody>
</table>
**Figure 6-20** displays the heat effects on the film that were generated per mole of water, $\Delta Q/\Delta n$, as the film was hydrated/dehydrated. The results are not being titled as $\Delta_{\text{hydration}} H$ values because the system did not appear to be at equilibrium in many of the sorption steps as evidenced by the pronounced hysteresis. A more detailed discussion of this analysis follows in the next section. The negative, exothermic values denote the sorption processes and the positive, endothermic values indicate the results of the desorption processes. Hydration of the film began at $a_w = 0.67 - 0.76$ and then followed in decreasing steps to $a_w = 0.35$. These results are shown as the shaded squares. The outlined squares indicate the $\Delta Q/\Delta n$ as the film was rehydrated and include values up to $a_w = 0.67$. Several attempts were made to hydrate the film to $a_w = 0.76$, however the film lost oscillation. This often indicates significant structural changes in the film properties.

The hydration followed the same pattern as seen in the lysozyme film, with the values at the lower $a_w$ being larger in magnitude and then decreasing to approach $\sim 44$ kJ/mol at the higher $a_w$. The value of 27 kJ/mol at $a_w = 0.64$ in the desorption cycle diverges from the other values. When referring to **Figure 6-18**, this hydration cycle was not at equilibrium thus resulting in this value. The process appears to have needed a much longer time for equilibrium to be attained. This is also the point immediately before the film lost oscillation.

It is interesting to note the large $\Delta Q/\Delta n$ values at $a_w = 0.38, 0.48, 0.51$, with results being $-1158, -154$ and $122$ kJ/mol, respectively. These sizeable values are only seen the first time the film is hydrated. The second time the film is hydrated, the magnitude of the values can be compared to those seen for lysozyme in **Figure 6-2**.
Figure 6-20. The heat generated per mole of water, $\Delta Q/\Delta n$, in the protein/buffer film. The inset provides a closer view of the regions $\sim 45$ kJ/mol in magnitude. The hydration started at $a_w = 0.67 – 0.76$ decreasing to $a_w = 0.35$. These values are shown as the solid black squares. The outlined squares are the data starting at $a_w = 0.35$ and increasing to $a_w = 0.67$. The film lost oscillation after this point.
6.3.3.3 Myoglobin/Buffer Film, (231 µg/cm², 1.7 µm), Coated with OCN, Studied at 25°C

The above studies at 25°C provided some interesting insights into protein hydration. The anomaly in the hydration of the myoglobin-buffer film at a water vapor activity of 0.35 to 0.67 was puzzling. To test whether this was an artifact of the film or some unusual process, another myoglobin-buffer film was prepared. Using the OCN, a thin, visibly uniform film was cast onto the QCM. The previous myoglobin-buffer coated QCM was driven using the low powered Maxtek PLO. Because the hydration effects appeared to put more demand on the oscillator driver, it was decided that the regular powered Maxtek PLO would be used as the oscillator driver for this set of studies. Using this, we were also able to explore higher water activity ranges. This more extensive set of studies included a_w ranging from 0 to 0.86 and included starting the film at higher a_w and decreasing the a_w in step-wise changes to 0. Following this, the film was then systematically brought back to the higher water vapor activities.

Figure 6-21 shows the data received with the film being exposed to a_w = 0.67 – 0.76. Because the mass, thermal and motional resistance traces were stable, it was decided to try this film at higher a_w. Results from a_w = 0.76 – 0.86 are displayed in Figure 6-22. Although the mass and thermal signals reflect the step-wise changes, the motional resistance began showing erratic signals. The erratic motional resistance signals continued through the experimental runs until the film reached a_w = 0.45 as seen in Figure 6-23. It is interesting to note, that at this particular run with a_w = 0.35 – 0.51, the mass and thermal signals do not exhibit an equilibrium being reached. Prior to the run, the film was held at the second highest a_w = 0.48 for that particular run. It appears
that being held at that $a_w$, the film had a chance to take up much more water than was noted in the previous experiment. A question arose as to whether at that activity, the film had gone through some structural changes and was able to take on more water. The desorption steps needed much longer intervals at this point. The step-wise changes were consistent throughout the experiments and averaged 88 min. for each step.

The following experimental runs shown in Figures 6-26 and 6-27 show the film being brought to a $a_w = 0$. For the first half of the hydration cycle in Figure 6-26, the motional resistance was still erratic. The film then recovered a stable state and the regular patterned motional resistance could be recorded.

Figures 6-27 and 6-28 both show the film at $a_w = 0 - 0.16$. The graphs in Figure 6-28 show the data when the step-wise change was increased by 12 minutes. This did not have a noticeable effect on the experiment. Once again, in these figures, the motional resistance is erratic at the lowest hydrations and on the desorbing steps.

Figures 6-29 and 6-30 which show the film behavior at hydration levels $a_w = 0.16 - 0.35$ and $a_w = 0.35 - 0.51$. The mass and thermal signals follow the expected patterns, however, once again, the motional resistance signal, upon desorption, becomes erratic.

The results shown in Figures 6-25 and 6-30 are interesting to compare. Both show data taken at $a_w = 0.35 - 0.51$. As mentioned above, the data in Figure 6-25 was held at $a_w = 0.48$ for three hours prior to the study. This data followed measurements previously taken at higher water vapor activities. The data in Figure 6-30 show the film behavior after it was held at $a_w = 0.35$. An obvious difference exists between the mass and thermal traces of these two experiments. It does appear that the direction of
hydration of the protein/buffer film does have an influence on the amount of water sorbed into the film and the rate of hydration.

Two other files show this marked different behavior, which appears to be dependent on the prior history of the protein/buffer film. Figures 6-24 and 6-31, both depict results from $a_w$ changes ranging from 0.51 – 0.67. The data in Figure 6-14 was held at $a_w = 0.64$ for three hours prior to the experiments. The graphs in Figure 6-31 show data after the film was held at $a_w = 0.51$ for three hours prior to the experiment. Although both files show erratic motional resistance behavior, the film that was held at the lower $a_w$ as seen in (e) of Figure 6-31 goes off scale and does not register a sensible signal at several time intervals during the run.

Figures 6-32 and 6-33 show the film behavior as it is brought back to the higher water vapor activity ranges. Again, in (e) of Figure 6-32, the erratic motional resistance behavior is seen upon desorption of the second cycle. In Figure 6-33, the motional resistance for the first full cycle is erratic in its signal however for the second full hydration cycle, the signal reveals the ordered step-wise changes. It appears that a conditioning of the film has taken place and that it was more reception of the hydration processes after being exposed to the same water vapor activity ranges immediately following one full hydration cycle.

Further comparisons follow the presentation of the initial data.
Figure 6-21. Myoglobin/buffer hydration/dehydration, 25°C, $a_w = 0.67 - 0.76$. a) measured water vapor activity, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance.
Figure 6-22. Myoglobin/buffer hydration/dehydration, 25°C, $a_w = 0.76 - 0.86$. a) measured water vapor activity, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance.
Figure 6-23. Myoglobin/buffer hydration/dehydration, 25°C, $a_w = 0.67 \rightarrow 0.76$. a) measured water vapor activity, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance.
Figure 6-24. Myoglobin/buffer hydration/dehydration, 25°C, $a_w = 0.51 - 0.67$. a) measured water vapor activity, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance.
Figure 6-25. Myoglobin/buffer hydration/dehydration, 25°C, $a_w = 0.35 - 0.51$. a) measured water vapor activity, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance.
Figure 6-26. Myoglobin/buffer hydration/dehydration, 25°C, \( a_w = 0.16 - 0.35 \). a) measured water vapor activity, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance.
Figure 6-27. Myoglobin/buffer hydration/dehydration, 25°C, $a_w = 0 - 0.16$, a) measured water vapor activity, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance.
Figure 6-28. Myoglobin/buffer hydration/dehydration, 25°C, $a_w = 0 – 0.16$. a) measured water vapor activity, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance.
Figure 6-29. Myoglobin/buffer hydration/dehydration, 25°C, $a_w = 0.16 - 0.35$. a) measured water vapor activity, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance.
Figure 6-30. Myoglobin/buffer hydration/dehydration, 25°C, $a_w = 0.35 - 0.51$. a) measured water vapor activity, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance.
Figure 6-31. Myoglobin/buffer hydration/dehydration, 25°C, $a_w = 0.51 - 0.67$. a) measured water vapor activity, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance.
Figure 6-32. Myoglobin/buffer hydration/dehydration, 25°C, $a_w = 0.67 - 0.76$. a) measured water vapor activity, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance.
Figure 6-33. Myoglobin/buffer hydration/dehydration, 25°C, $a_w = 0.76 - 0.86$. a) measured water vapor activity, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance.
The total mass uptake of water was compared for this set of experimental data. This is displayed in Figure 6-34. The dashed line at \( a_w = 0.67 - 0.76 \), marks the first experimental run. The next trial is shown by the solid line at \( a_w = 0.76 - 0.86 \). The following experiments are represented by the solid lines and follow in decreasing order. At \( a_w = 0 - 0.16 \), the trend was changed and the film was gradually brought back to the higher water vapor activities. These trials are noted by the dotted lines on the graph.

For each of the experimental runs, it is noted that the film absorbed more water when being brought from the higher water vapor activities than from those trials where the film had previously been at a lower water vapor activity. The most obvious example of this is the trial done at \( a_w = 0.35 - 0.51 \), represented by (4) on the total mass graph. Figure 6-35 shows a closer view of this occurring in a different file. The dashed line shows data from the myoglobin/buffer hydration where the film was held at \( a_w = 0.64 \) prior to the run and the solid line shows the hydration results when the film was held at \( a_w = 0.51 \) prior to the test. There is \( \sim 20 \mu g \) difference in the amount of water taken up by the film. For the data represented by the solid line, it appears that if given a much longer time scale, the film may once again approach the same masses. The desorption steps reveal a more pronounced hysteresis in the film when the film was held at a lower water vapor activity prior to the run. By the end of the run, the gap between the mass differences lessens to about \( 15 \mu g \). Figure 6-36 displays the mass changes (solid line) upon water sorption with the measured water vapor activity (dashed line) superimposed on this. The measured \( a_w \) is not the same as that applied because of inconsistencies in the hydration of the film as discussed above.
Figure 6-34. Summary of mass change data for myoglobin/buffer hydration/dehydration. The dashed line at $a_w = 0.67 - 0.76$ marks the first run. The following experiments proceeded from $a_w = 0.76 - 0.86$ (solid line) down to $a_w = 0 - 0.16$. The film was then brought back from $a_w = 0$ to $a_w = 0.86$ in the experiments noted by the dotted lines.
Figure 6-35. Comparison of myoglobin/buffer hydration/dehydration, $a_w = 0.51 - 0.67$. Film condition prior to the experimental runs different for each. The dashed line is the data after the film was held at an $a_w = 0.64$. The solid line is data from the film being held at $a_w = 0.51$.

Figure 6-36. Comparison of mass and $a_w$ of myoglobin/buffer film at $a_w = 0.51 - 0.67$. Prior film history is different in each experimental run. a) the film was held at $a_w = 0.64$ for 3 hours prior to the run, b) the film was held at $a_w = 0.51$ for 3 hours prior to the run.
Water sorption isotherms of proteins typically take a sigmoidal shape and are usually classified as sigmoidal Type II isotherms (Kuntz & Kauzmann, 1974). These isotherms are distinguished by four characteristic features: (1) at \( a_w \sim 0 \) to 0.05 the water bound to the protein increase linearly and rapidly, (2) at \( a_w \sim 0.1 – 0.3 \) a distinct knee appears believed to mark the hydration of the polar sites, (3) at \( a_w \sim 0.3 – 0.9 \) a steady increase in water is noted however it is slower than the first sections. This marks the onset of hydration of the weaker binding sites, (4) at \( a_w > 0.9 \) the hydration process increases much more rapidly and marks the onset of multilayers of water.

Using the data revealing the mass of water sorbed and desorbed in the myoglobin/buffer film, the mass percent was calculated and tabulated as \( g_{water}/g_{film} \). The sorption isotherm, Figure 6-37, shows appropriate increasing and decreasing values except for the experimental runs between \( a_w = 0.30 \) and 0.55. This region reveals an anomaly in the expected hydration behavior. When the film was taken from the higher water vapor activities, the film responded by sorbing/desorbing water in much larger increments than seen in the runs previous to this. The film resumed the “expected” behavior at water vapor activities < 0.30. When the film was being brought back to the higher hydration levels, water sorption between \( a_w = 0.35 – 0.51 \), followed the pattern of the previous runs, however there appeared a large gap where this experiment ended and the next run began. In their comprehensive review of protein hydration, Kuntz and Kauzmann suggest that an abrupt break in a water sorption isotherm at constant pressure may indicate a protein/water phase change from a dry solid state to a solution state (Kuntz & Kauzmann, 1974). The authors note that such behavior had not been seen in
protein/water systems and that instead of a solid/solution state, the protein may better be described as a glass/solution state.

Initially our results proved to be interesting and possibly indicated that we may have captured this phase change that had not previously been measured. To confirm our conjectures, we decided to test the film in the critical range of $a_w = 0.30 - 0.60$. These results can be seen in Figure 6-38. The $a_w$ step changes were decreased almost in half from 0.03 to 0.016 with each step being held for $\sim 88$ min. At $\sim a_w = 0.52$, very large thermal signals are seen along with much larger increases in the mass gain. This trend continues until the film is brought to an $a_w = 0.64$. At this point, the film is brought back down in water vapor activity. The regular step-wise mass and thermal decreasing signals occur along with the regular step-wise changes in motional resistance until the film reaches an $a_w \sim 0.50$. At this point the film resumes its dehydration with much larger incremental steps. The motional resistance does not show any repeatable signals at this point however, even though the signals are somewhat erratic, they are not large in magnitude. These large dehydration effects are seen until the film is brought to $\sim a_w = 0.43$. Once again the film proceeds with the dehydration but the effects are much smaller in magnitude.

The data from this experiment was inserted into the isotherm data and the results are shown in Figure 6-39. The closer hydration steps provided information to now link the sorption and desorption steps with the previous series of hydration steps. Hysteresis effects are evident in the data for both isotherms as was also seen in the mass traces above, e.g. Figures 6-33 to 6-35. Hysteresis is defined as an absence of reversibility where a reversible process is defined as a system being at equilibrium throughout the
whole process being studied and is never more than infinitesimally removed from a state of equilibrium. Rupley and Careri, in their comprehensive review of protein hydration, remark that the literature states, but makes a poor case, for the statement that the impression is given that small samples or thin films display less hysteresis than larger samples. From the measurements in this study, it can be stated that pronounced hysteresis does occur in thin films.

The hysteresis effects in the sorption isotherms of Figures 6-37 and 6-39 strongly resemble an effect known as capillary condensation. This type of hysteresis loop effect is often seen in gas adsorption to a porous surface (Lyklema, 2000). From their Young’s modulus investigations, Morozov, et al., observe that a protein crystal surface is not flat and actually is a system of microcapillaries of extensive curvature. Upon dehydration, these capillaries may deform as evidenced from protein shrinkage upon dehydration. Once deformed, the centers that once bound water may now be able to interact with each other thus forming new bonds.

Several proposals have been issued in trying to explain hysteresis effects including (Rupley & Careri, 1991): (1) capillary condensation in interstices of the solid material, (2) metastable states brought about by phase changes within the adsorbate, (3) protein conformational changes brought about by low hydration and reverse more slowly than the rate of water sorption, (4) a limited number of nucleation sites (charged sites) for condensation water upon sorption, however upon desorption, the nucleation would not be a limiting factor.

One factor that has not been discussed and will be treated with following the thermodynamic interpretation is the effect of the presence of the buffer in the film.
Figure 6-37. Water sorption isotherm for myoglobin/buffer film. The solid triangular data markers show the results for the first experiments starting at the higher $a_w$ and then progressing in controlled steps to $a_w = 0$. The film was then rehydrated and brought systematically back to the higher water vapor activities.
Figure 6-38. Myoglobin/buffer hydration/dehydration, 25°C, $a_w = 0.35 - 0.64$, 0.016 $a_w$ stepwise change. a) measured water vapor activity, b) mass trace, c) thermal power, d) integrated heat, e) motional resistance with an inset showing a portion where the regular step-wise change in the motional resistance of the film was recovered.
Figure 6-39. Sorption isotherm including data from experimental run $a_w$ changed in 0.016 steps from $a_w = 0.35 – 0.64$, each step held for ~90 min. The triangular shapes with the line drawn through mark the data for this. The other triangular markers, shaded and unshaded, show data for the previous trials.
The $\Delta_{\text{hydration}}H$ determined from the QCM/HCC experiments with proteins, as with most protein hydration measurements, is not only the amount of heat involved in the hydration process, but it constitutes a summation of several processes. Because the $\Delta_{\text{hydration}}H$ is a summation of individual interactions, it is not possible to determine the individual contributions of specific amino acid residues such as hydrogen bonding, salt-bridge interactions, hydrophobic bonding, and proton ionization (Fisher, 1995). Each individual residue’s interaction with water can produce either positive or negative enthalpic contributions. The resulting $\Delta_{\text{hydration}}H$ value may actually be smaller in magnitude than some of the specific individual interactions.

With the QCM/HCC, we are able to report $\Delta_{\text{sorption}}H$ values for many films that we have studied. However, to report values for a state function such as $\Delta H$, it is implied that the sorption process is reversible. Hysteresis, as stated above, is the absence of full reversibility. Because of the presence of hysteresis when evaluating protein samples, it is generally believed that the equilibrium state is not defined well enough for a thermodynamic analysis. Some authors argue that a high reproducibility of hysteresis in an isotherm does make the data amenable to a thermodynamic interpretation (Rupley & Careri, 1991). In spite of this argument, instead of reporting $\Delta_{\text{hydration}}H$ values, the data obtained here will be reported as heat/mole values, $\Delta Q/\Delta n$.

Another complication in the thermodynamic treatment of the sorption data exists from contributions stemming from conformation changes and mechanical deformations. Morozov, et al. studied the elastic properties and sorption isotherms of lysozyme crystals. In fact several investigators have noted that the measured values for protein hydration cannot be assigned only to the protein-water interactions. They concluded
that conformational changes and swelling effects also contribute to the thermodynamic interpretation (Kuntz & Kauzmann, 1974; Morozov et al., 1988). In fact Morozov, et al., propose an equation to evaluate all of the possible contributions to the total free energy of hydration, $\Delta G_o$ (Morozov et al., 1988):

$$\Delta G_o = \Delta G_h + \Delta G_\sigma + \Delta G_a + \Delta G_w - \Delta G_s + \Delta G_c$$  \hspace{1cm} \text{Equation 6-17}

From Morozov’s treatment, the parameters in the above equation include: $\Delta G_h$ – the dehydration energies of separate groups in the protein chain, $\Delta G_\sigma$ – the contributions from the changes in the area of the water-vapor-protein interface, $\Delta G_w$ – the energy of uniform extension of water in capillaries under the action of capillary forces, $\Delta G_a$ – the energy released during the formation of new bonds between the dehydrated centers of water binding, $\Delta G_s$ – the energy needed to concentrate the intracrystalline salt solution, for solid samples containing salts, and $\Delta G_c$ – the energy spent for salt crystallization, again for solid samples containing salts. Some authors agree that the above effects possibly do contribute to the thermodynamic analysis, however they disagree as to the magnitude of the effects (Rupley & Careri, 1991).

From our motional resistance data, we do see systematic as well as erratic thermal contributions made from the film being hydrated or dehydrated. The thermal effects due to changes in motional resistance however are generally $< 2 \mu W$ in the data shown above.
Figure 6-40 and Table 6-9 show the data from the calibration of the left, sample side, thermopile, with the protein/buffer film being held at $a_w = 0.51$. The calibration coefficient, $\varepsilon$, was determined to be 3.368 W/v.

Figure 6-40. Calibration of left thermopile using the regular powered Maxtek Phase Lock Oscillator, with myoglobin/buffer film on QCM #31, $a_w = 0.51$. a) thermal signal from QCM/HCC, b) conductance voltage from Maxtek PLO.

Table 6-9. Summary of left thermopile calibration, myoglobin/buffer film on QCM #31, held at $a_w = 0.51$.

<table>
<thead>
<tr>
<th>Left Thermopile Calibration Using Regular Power PLO</th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Average $\Delta$ thermal signal</td>
<td>5.799E-5</td>
<td>volts</td>
</tr>
<tr>
<td>Average $\Delta$ conductance signal</td>
<td>2.5001</td>
<td>volts</td>
</tr>
<tr>
<td>Power generated in QCM</td>
<td>1.95E-4</td>
<td>Watts</td>
</tr>
<tr>
<td>Calibration coefficient, $\varepsilon$</td>
<td>3.368</td>
<td>W/v</td>
</tr>
</tbody>
</table>
Figure 6-41 displays the $\Delta Q/\Delta n$ values for all of the original myoglobin/buffer hydration experimental runs with this film. The series of measurements began at the higher water vapor activities ($a_w = 0.76 - 0.86$) and continued in a progression of experiments to a water vapor activity of 0. These measurements starting at the high $a_w$ and decreasing to 0 are indicated by the shaded in squares. The next series of experiments then proceeded from $a_w = 0$ and progressed to the higher humidities. These sets of experiments are denoted by the outline squares. The same pattern holds true for Figure 6-42, except in this graph the data for the experimental run at $a_w = 0.35 - 0.51$ is omitted so that the heat/mole effects at the other hydration ranges are more evident.

In general, the hydration and dehydration processes follow a similar trend, with the exception of the anomalous behavior at $a_w = 0.35 - 0.51$ in Figure 6-41, where at lower $a_w$ values, the $\Delta Q/\Delta n$ values are large in magnitude, e.g. $\sim 80$ kJ/mol. This is most often interpreted to reflect the hydration of the more strongly polar sites of the protein. With increasing water vapor activity, the values roughly linearly decrease in magnitude to $\sim 45$ kJ/mol. At this point, multilayers of water are generally believed to be forming in the hydration process and the $\Delta Q/\Delta n$ values reflect the $\Delta_{vaporization}^H$ value of water, $\sim 44$ kJ/mol. The values at $a_w = 0.38$ and 0.41 corresponding to $-1362$ kJ/mol and $-255$ kJ/mol, especially caught our eye and again questions were raised as to whether this could be indicative of a phase change or crystallization in the protein or an effect of the buffer. The data in Figure 6-43 proved to be puzzling. This displays the $\Delta Q/\Delta n$ data for the smaller $a_w$ changes (0.016) in the region of 0.35 – 0.51. Although the magnitude of the $\Delta Q/\Delta n$ values are large, $\sim 80+$ kJ/mol, they do not come near some of the values attained in the first experiment in this region, $\Delta Q/\Delta n \sim -1362$, and $-255$ kJ/mol. When
compared with Figure 6-20, the $\Delta Q/\Delta n$ data for the previous film displayed the same intriguing results at $a_w$ = 0.38, 0.48, and 0.51. The $\Delta Q/\Delta n$ values reach magnitudes of ~ -1158 kJ/mol, 159 kJ/mol and -121 kJ/mol respectively.

Figure 6-41. Heat per mole of water, $\Delta Q/\Delta n$, generated for water sorption in myoglobin/buffer film, 25°C, $a_w$ = 0 to 0.86. All experimental runs included except for trial with shorter $a_w$ increments of 0.016. The experiments started at the higher $a_w$ ranges and proceeded to 0. These are marked as shaded in squares. The experiments starting at $a_w$ = 0 and increasing in $a_w$ are marked by the outlined squares.
Figure 6-42. Heat generated per mole of water, $\Delta Q/\Delta n$, of water sorption in myoglobin/buffer film. Experimental run for $a_w = 0.35 – 0.51$, descending, omitted as well as the experimental results from run with $a_w$ step-wise changes of 0.016. The experiments started at the higher $a_w$ ranges and proceeded to 0. These are marked as shaded in squares. The experiments starting at $a_w = 0$ and increasing in $a_w$ are marked by the outlined squares.
Figure 6-43. Heat generated in myoglobin/ buffer film per mole of water, $\Delta Q/\Delta n$, for $a_w$ interval = 0.016, 90 min. per step.
When comparing the data in Figure 6-41 and 6-43, ΔQ/Δn values of the same magnitude were expected as a possible result. Although the values in Figure 6-41, representing the much smaller aw step-wise changes, 0.016, are relatively large in magnitude, ~ 80 kJ/mol, the same region ~ aw = 0.45 – 0.51 does not have the very large ΔQ/Δn values. One possibility may be that the film was conditioned during the first trial shown in Figure 6-41. Whatever event produced that amount of heat may not have been an easily reversible process. This led us to probe not only the hydration effects on the protein but also to explore the hydration effects on the buffer present in the film.

Because of the presence of the sodium phosphate buffer in the film, the system is more accurately defined as a three-component system, protein-buffer-water. When water vapor is brought into contact with the protein-buffer film, the water may interact with a variety of protein sites as well as with the buffer. This leads to the concept of preferential or selective solvation. According to their review, Kuntz and Kauzmann define selective or preferential hydration of a macromolecule as occurring when, “the concentration of water in the vicinity of the macromolecule is higher than the average water concentration in the solution (Kuntz & Kauzmann, 1974).” Preferential hydration is symbolized by Γ and is given by the equation:

$$\Gamma_i = \left( \frac{M_i}{M_3} \right) \left( \frac{\delta m_i}{\delta m_3} \right)_{aw}$$  \hspace{1cm} \text{Equation 6-18}$$

where $\Gamma_i$ is the preferential solvation of the macromolecule by the $i^{th}$ component usually reported in units of grams i per gram of macromolecule. $M_i$ and $m_i$ are the molecular weight and molality of i respectively. $M_3$ and $m_3$ are the same values for the macromolecule(Kuntz & Kauzmann, 1974). The review cites data from Bull and
Breese depicting a graph of \( g_{\text{water}}/g_{\text{protein}} \) versus increasing buffer-salt concentration in moles\( \text{salt}/g_{\text{protein}} \times 10^4 \). The graph shows that with increasing buffer-salt concentrations, the preferential hydration of the macromolecule decreases even though the water uptake of the sample increases.

Another equation to interpret the preferential hydration, \( \Gamma_w \), is shown in Equation 6-19 (Kuntz & Kauzmann, 1974):

\[
\Gamma_w = W - 1000n_2/m_2
\]

where the total uptake of water at a set water vapor activity is \( W \), \( n_2 \) denotes the moles of salt per gram of macromolecule, and \( m_2 \) is the molality of the salt in the two-component salt-water system.

From the results shown in the review it is determined that the presence of salts have a very large effect on protein hydration. For example, the presence of LiCl causes a dehydrating effect in proteins, while the presence of Na\(_2\)SO\(_4\) increases the protein hydration. It is stated that the competitive binding of water and salt to the protein can have several effects: (1) the salt displacing water from the protein hydration shell, (2) ion hydration causing new water to be brought into the immediate area of the protein, (3) altering of the protein conformation, (4) altering of the bulk water structure (Kuntz & Kauzmann, 1974). The authors state the need for studies to be done by other techniques to confirm past results and to add new data in the area of salt binding studies. Investigators have probed the effects of buffer concentration in regards to protein heat capacity and effects on folding-unfolding as well as effects on glass
transition temperatures but not necessarily in regards to hydration effects (Inoue & Ishikawa, 2000; Privalov, 1997). A hydration study of a sodium phosphate buffer follows this discussion.

Before proceeding, the hydration of myoglobin/buffer film also provided data used to determine the diffusion coefficients, D. As noted above in the discussion of diffusion coefficients as determined from the hydration of a lysozyme film, the sorption processes in proteins may not adhere to Fickian behavior. This was noticed in the lysozyme film when different hydration times were compared. Different hydration times for the aw step-wise changes were not utilized so these comparisons cannot be made with the myoglobin/buffer film. Also as stated above, many of the sorption steps had not reached full equilibration and this may affect the value of D. When comparing the lysozyme and the myoglobin/buffer films, the values of the diffusion coefficients are similar. The values for the lysozyme center around 4E-12 cm²s⁻¹, while the myoglobin/buffer values average about 8-9E-12 cm²s⁻¹. Data for the myoglobin/buffer system are shown in Figures 6-44 and 6-45.
### Figure 6-44

Fitting of mass vs. time traces for myoglobin/buffer film. Data is shown as the solid black line. The dotted line is the simulated curve for the first approximation of the parameters. The dashed line is the actual fit of the data produced from the software, Origin. **a)** sorption at $a_w = 0.42 - 0.45$, **b)** desorption at $a_w = 0.45 - 0.42$.

### Figure 6-45

Diffusion coefficients, $D$, for 1.7 µm myoglobin/buffer film measured over a range of $a_w = 0 - 0.86$. The equilibration for each step was ~ 90 min.
6.3.3.4 Sodium Phosphate Buffer Film Coated with OCN, Studied at 25°C

The interesting behavior of the myoglobin/buffer film in the two film-coated QCM’s studied at 25°C at water vapor activities of 0.35 to 0.55, led to interesting discussions regarding three possibilities: (1) myoglobin crystallization, (2) phase change behavior of the myoglobin, (3) hydrate formation occurring with the sodium phosphate buffer. In an attempt to separate the effects of the buffer hydration and the protein hydration, a mole ratio of each in the film was calculated. This was determined by verifying the concentration of the myoglobin dialyzed solution and the concentration of myoglobin in the film. The concentration of the myoglobin in each was determined by UV-Vis spectrophotometry. For this measurement a Perkin-Elmer Lambda 2 UV-Vis spectrophotometer was used. The instrument was interfaced to a PC via the software UV Winlab (version 2.70). These results are seen in Figure 6-46.

![Figure 6-46. The UV-Visible spectrum of the myoglobin stock solution dialyzed with 0.05M sodium phosphate buffer and the myoglobin film redissolved in solution.](image-url)
The characteristic peaks in the visible spectrum of the α-band at 635 nm, the β-band at 505 nm, and the Soret band at 409.5 nm, along with the UV peak at 280 nm all confirm that the stock and redissolved film solutions are aquametmyoglobin. Although the myoglobin concentrations from the stock solution and from the film differ, the spectra confirms that the myoglobin in the film was in its native state and of the same form as in the stock solution. Using a molar absorptivity, \( \varepsilon \) of 157 mM\(^{-1}\)cm\(^{-1} \) at 409.5 nm (Rothgeb & Gurd, 1978), the concentration of myoglobin in the film was determined to be 1.1 \( \mu M \) while that of the dialyzed stock solution was 4.8 \( \mu M \). The concentration of the film, redissolved in buffer solution, was not used for the subsequent calculations because there was a noticeable residue of protein/buffer adhering to the QCM after rinsing to redissolve the film. Using a Mettler balance, it was determined that 120 \( \mu g \) of protein/buffer was still on the QCM surface and did not dissolve in the solution, thus the concentration calculation was reduced. The concentration of buffer and of protein in the stock solution were then used to calculate the mass of buffer and protein in the film. The buffer:protein mass ratio was determined to be 100:1.

This buffer:protein ratio confirmed that the buffer was in excess. A film of just sodium phosphate buffer was made to see the effects of the hydration of the buffer and to compare measurements with the myoglobin/buffer film. Figures 6-47 and 6-48 show these results.

In Figure 6-47, results are shown for the hydration of the sodium phosphate buffer film. The \( a_w \) was incrementally changed in 0.015 steps between the range of 0.352 and 0.480. The original experimental setup included a hydration range extended to an \( a_w \) of
0.640. The film lost oscillation in the pre-conditioning steps and it was determined that an $a_w = 0.480$ was the best upper range to use. The mass change upon hydration revealed some curious results. The lower $a_w$ ranges demonstrated the expected step-wise increase and decrease of mass upon the hydration/dehydration processes. At $a_w = 0.464$ and 0.480 however, the mass change, determined from the QCM frequency shift, decreased upon water sorption. In the desorbing steps from $a_w = 0.48$ to 0.45, the mass signal actually increased upon desorption. Because the mass measurement is directly linked to the QCM frequency, the oscillation frequency leads to a direct link with the film properties (vide supra). The motional resistance was then analyzed to find any clues leading to patterns in the film behavior. The motional resistance as shown in (d) was erratic and in fact off-scale so no clear signal was measured.

The thermal trace in (c) closely followed the changes in the mass as evidenced by the peaks recorded upon sorption/desorption. Another anomaly occurred with the thermal baseline becoming more endothermic in incremental steps. For the hydration events, an exothermic shift would be expected from the heat being generated. The same holds true for the desorption steps. The baseline becomes incrementally more exothermic upon the loss of water, an endothermic event. When exploring this question, it was realized that the change in thermal power generated in the crystal by the phase lock oscillator itself was much more noticeable than had previously been seen. As the film was taking in more water, the power generated in the QCM from the PLO was actually decreasing thus shifting the observed thermal power signal. The dissipation can be accounted for by using the original output conductance voltage and solving for the power dissipated in the crystal. In this particular experiment, the motional resistance data proved to be
useless therefore voiding the ability to cancel out this effect. A second experiment was then discussed.

The second trial with the same sodium phosphate buffer film is shown in Figure 6-48. The applied and measured water vapor activities are depicted in (a). The film was allowed to dry for ten days under a stream of dry nitrogen at 25°C. The film was then held at a water vapor activity of 0.0. Instead of using the automated step-wise changes in the LabView VI, this run was operated manually. Stepwise $a_w$ changes of 0.025 were used and the time intervals varied from 10 to 120 min. The $a_w$ was increased or decreased when the hydration event appeared to reach equilibrium, verified by a negligible change in the QCM oscillation frequency, < 2 Hz. The data displayed shows a hydration range of $a_w = 0.400$ to 0.525, where at this point (~91,500 sec) the film-coated QCM lost its oscillation frequency. The drying of the film and gradually increasing the hydration from $a_w = 0$ did have the effect that the QCM did not lose oscillation as early in the hydration steps as the previous run. The $a_w$ was decreased to 0.500 and oscillation was regained. The remainder of the data is shown to $a_w = 0.17$.

The mass changes upon hydration are depicted in (b). Once again, the mass changes corresponded with the hydration up to $a_w = 0.450$, changing when the $a_w$ reached 0.475 and 0.500. At this point, the curious decreases in mass were seen until the QCM lost oscillation at $a_w = 0.525$. In the dehydration processes the same phenomena occurred where upon desorption, the mass change recorded an increase of mass at $a_w = 0.500$ and 0.475. These two values closely correspond the region in the first run where this phenomena occurred, $a_w = 0.464$ and 0.480. From this it appears that a critical
hydration range is $a_w = 0.450$ and 0.464 because it is within this step that the unusual mass change begins to occur.

The thermal trace is outlined in (c) where interestingly, once again the thermal baseline becomes incrementally more endothermic upon the exothermic hydration processes. Likewise, for the endothermic dehydration steps, the baseline becomes incrementally more exothermic. What differs in this run from the first is that the motional resistance did register incremental signals corresponding to the hydration/dehydration events unlike the erratic signals seen in the first run. The motional resistance is shown in (d). From the equations provided by Maxtek, the motional resistance is converted to its original conductance output voltage. This voltage is then converted to the power generated in the crystal. As stated above, as the film took on more water, the motional resistance of the film changed in such a way as to decrease the thermal power generated in the QCM. Thus an endothermic baseline shift was observed upon sorption. The same holds true for the desorption steps: as water left the film, the PLO was able to increasingly drive the QCM at its resonant frequency utilizing its full power thereby shown as the exothermic incremental steps in the baseline.

The heat dissipation was accounted for and subtracted from the thermal baseline giving the thermal trace seen in (e). The peaks were then integrated yielding the heat, $\Delta Q$, produced by the hydration/dehydration events.
Figure 6-47. Hydration/dehydration of sodium phosphate buffer film, 25°C, aw = 0.35 – 0.48. a) measured water vapor activity, b) mass trace, c) thermal power trace, d) motional resistance with an inset displaying a closer view of a specified time period. No regular pattern of motional resistance changes was seen.
Figure 6-48. Sodium phosphate buffer film, 25°C, $a_w = 0.40$ to 0.53 to 0.23. 

- **a)** applied and measured water vapor activity,
- **b)** mass change,
- **c)** total thermal power generated,
- **d)** motional resistance,
- **e)** thermal power generated in the film, power dissipated in QCM subtracted out,
- **f)** integrated heat.
A tabulation of the hydration results and the heat per mole, \( \Delta Q/\Delta n \), is displayed in Figure 6-49. The overall magnitude of the values are larger than those seen in the myoglobin /buffer and lysozyme films. In the myoglobin/buffer and lysozyme samples shown above, as the \( a_w \) increases, the \( \Delta Q/\Delta n \) approached \( \sim 44 \) kJ/mol. This is evidenced in Figures 6-2, 6-20, and 6-42 where at \( a_w = 0.50 \) for lysozyme and \( a_w =0.60 \) for the myoglobin system, the values of \( \Delta Q/\Delta n \) approach \( \sim 44 \) kJ/mol. For the sodium phosphate buffer film, the \( \Delta Q/\Delta n \) values in Figure 6-49 do not approach this value until reaching a lower \( a_w, \sim 0.22 \).

One puzzling difference can be seen in Figure 6-43 where the \( \Delta Q/\Delta n \) results are plotted for the myoglobin/buffer run that measured the smaller \( a_w \) steps at long intervals. In this case, the \( \Delta Q/\Delta n \) values actually increase in magnitude at lower hydration steps. It is difficult to interpret the results, however, based on the discussions above, with the longer equilibrium period at a particular \( a_w \), there is an increased opportunity for structural fluctuations to occur in the protein. As water leaves the film, some sites on the protein may now preferentially bind with the salt thus producing excess exothermic signals. The increase in the endothermic magnitudes at lower \( a_w \) is just as puzzling. It is known that upon dehydration, phosphates release water to form P-O-P linkages (de Jager & Prinsloo, 2001; Ghule et al., 2001). Some studies show this as an effect that occurs upon heating.

Upon hydration two values are of special interest before the QCM frequency became irregular: these include the heats generated at \( a_w = 0.424 \) and 0.450, with values of \(-527.6 \) and \(-2830.6 \) kJ/mol. Although large values were seen in the protein hydration, the value at \( a_w = 0.450 \) is easily twice as large as that seen in the three-component
systems above. Likewise, upon desorption these large values are seen, e.g. $a_w = 0.450$, 0.424, and 0.400 each having values of 534 kJ/mol, 1186 kJ/mol, and 388 kJ/mol. These results led to discussions involving the possibility of hydrate formation among the sodium phosphate molecules.

![Graph showing heat generated per mole of water, $\Delta Q/\Delta n$, of sodium phosphate buffer film upon hydration at 25°C. The $a_w$ was change in increments of 0.025. The graph shows data of the film being hydration at a start of 0.425 $a_w$. The film lost oscillation at 0.525 $a_w$. The dehydration steps are then presented. These data points are shown by the shaded squares. The asterisks denote data points where the film oscillation actually increased upon sorption. The validity of these data points are suspect.](file:02-09-1)

**Figure 6-49.** Heat generated per mole of water, $\Delta Q/\Delta n$, of sodium phosphate buffer film upon hydration at 25°C. The $a_w$ was change in increments of 0.025. The graph shows data of the film being hydration at a start of 0.425 $a_w$. The film lost oscillation at 0.525 $a_w$. The dehydration steps are then presented. These data points are shown by the shaded squares. The asterisks denote data points where the film oscillation actually increased upon sorption. The validity of these data points are suspect.
The hydrolysis of phosphates is of interest to investigators studying possible materials for heat storage, agricultural aspects, food additives, and detergents to name a few applications (Van Wazer, 1961). The sodium phosphate buffer, cast on the QCM as a film, may have formed crystal hydrates during the water sorption process. A general equation exhibiting the formation of crystal hydrates is of the form (Burylev et al., 1995):

$$X(s) + nH_2O(g) = X \cdot nH_2O(s) \quad \text{Equation 6-20}$$

where $X$ is the compound, which in our case for the sodium phosphate buffer is of the form, $H_wNa_xP_yO_z$. Burylev, et al. utilized a modified approach to calculate the standard enthalpy for the formation of a salt hydrate shown in Equation 6-21:

$$\Delta_f^oH(X \cdot nH_2O),s, 298K = \Delta_f^oH(X),s, 298K - \Delta_f^oH(H_2O),s \quad \text{Equation 6-21}$$

In their work, the group calculated the standard enthalpies of formation of various hydrous sodium salts and formulated a comprehensive table of $\Delta_fH$ values (Burylev et al., 1995). They then compared these calculated values with measured values for the hydrated salt formations. They note that the maximum deviation between the calculated and measured values is 2% whereas most of the deviations are < 1%. A portion of their table concerning the sodium phosphates is shown in Table 6-10.
Table 6-10. Experimental and calculated standard enthalpies of formation of hydrous sodium salts, kJ/mol (Burylev et al., 1995).

<table>
<thead>
<tr>
<th>Compound</th>
<th>-Δ_fH° (X, s, 298K)</th>
<th>-Δ_fH° (XnH2O, s, 298K) experiment</th>
<th>-Δ_fH° (XnH2O, s, 298K) calculated</th>
<th>δ, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na₃PO₄</td>
<td>1916.9±1.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na₃PO₄·12H₂O</td>
<td></td>
<td>5480.3±6.3</td>
<td>5480.9</td>
<td>0.01</td>
</tr>
<tr>
<td>Na₃PO₄·8H₂O</td>
<td></td>
<td></td>
<td>4292.9</td>
<td></td>
</tr>
<tr>
<td>Na₃PO₄·6H₂O</td>
<td></td>
<td></td>
<td>3698.9</td>
<td></td>
</tr>
<tr>
<td>Na₃PO₄·0.5H₂O</td>
<td></td>
<td></td>
<td>2065.4</td>
<td></td>
</tr>
<tr>
<td>Na₄P₂O₇</td>
<td>3166.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na₄P₂O₇·10H₂O</td>
<td>6125.4</td>
<td>6136.4</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Na₂H₂PO₃·2.5H₂O</td>
<td>1928.0</td>
<td>1930.3</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>NaHPO₄</td>
<td>1536.8±1.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaHPO₄·H₂O</td>
<td>1830.9</td>
<td>1833.8</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>1747.57±1.26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na₂HPO₄·12H₂O</td>
<td>5297.66±1.26</td>
<td>5311.57</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>Na₂HPO₄·7H₂O</td>
<td>3821.46±1.26</td>
<td>3826.57</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Na₂HPO₄·2H₂O</td>
<td>2346.05±1.26</td>
<td>2341.57</td>
<td>-0.19</td>
<td></td>
</tr>
<tr>
<td>Na₂H₂P₂O₇</td>
<td>2763.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na₂H₂P₂O₇·6H₂O</td>
<td>4527.9</td>
<td>4545.6</td>
<td>0.39</td>
<td></td>
</tr>
</tbody>
</table>

From our three-fold analysis using (1) the Δ_rH values, (2) stable water vapor activities for salt hydrate pairs (vide infra), and (3) solubility curve information of the Na₂O-P₂O₅-H₂O system (vide infra), four of the above salt hydrates were possible matches with the sorption studies presented here. The four salts and their enthalpies of reaction for the salt hydrates include: (1) Na₄P₂O₇·10H₂O, Δ_rH = -540 kJ, (2) Na₂HPO₄·12H₂O, Δ_rH = -648 kJ, (3) Na₂HPO₄·7H₂O, Δ_rH = -381 kJ, (4) Na₂HPO₄·2H₂O, Δ_rH = -115 kJ. For each of these, the reaction enthalpy per mole of water ~ -50 kJ/mol. The ΔQ/Δn experimental values particularly for (1) the buffer film, a_w = 0.45, -
2831 kJ/mol, and \( a_w = 0.42 \), 1186 kJ/mol, (2) the myoglobin/buffer film QCM #12, \( a_w = 0.38 \), -1158 kJ/mol, (3) myoglobin/buffer film QCM #31, \( a_w = 0.38 \), -1362 kJ/mol, all are much larger in magnitude than the –50 kJ/mol calculated value. The system appears to be complicated by buffer-buffer, protein-buffer, and protein-protein interactions. Integration of the thermal signals was also complicated and dependent on the baseline assignment.

Salt hydrate pairs have been studied for their use as water buffers in organic reactions such as enzyme catalysis (Halling, 1994; Halling, 1992). The salt hydrates have been shown to maintain very stable water activity levels essential to these types of reactions. Assuming ideal behavior, a mixture of two salt hydrate forms of the same salt will be in equilibrium at a characteristic water vapor pressure. For example at a certain \( a_w \) the salt pair of \( \text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O} \) may be in equilibrium with \( \text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O} \) (often written in the shorthand notation of \( \text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O} \)) and this equilibrium extends until enough water has been added to convert the salt sample entirely to \( \text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O} \). At this point the water vapor activity increases and a new salt hydrate appears, in this case \( \text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O} \) and this pair is then in equilibrium. Halling has summarized some of the water vapor activities at which certain pairs of salt hydrates are in equilibrium. From his data and data gathered he fit \( a_w \) versus \( 1/T \) (temperature) data and listed characteristic constants for the salt hydrates. He used the equation relating the temperature dependence, \( T \), of the vapor pressures, \( P \), of the salt hydrate pairs and the pure water:

\[
\log P = A - B/T \quad \text{Equation 6-22}
\]
where $A$ and $B$ are constants for a given salt hydrate pair. In terms of $a_w$, the equation holds true for the form:

$$\log a_w = A - \frac{B}{T} \quad \text{Equation 6-23}$$

If the constants for a salt hydrate pair are known, the equilibrium can be calculated at a certain temperature. Some of Halling’s findings that relate to our sodium phosphate system are reported in Table 6-11 (Halling, 1992).

**Table 6-11.** Properties of salt hydrate pairs for $a_w$ control.

<table>
<thead>
<tr>
<th>Salt pair</th>
<th>Water capacity mmol g(^{-1})</th>
<th>Max T °C</th>
<th>Constant A</th>
<th>Constant B</th>
<th>Data T °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Na}_2\text{HPO}_4\cdot 12/7$</td>
<td>14.0</td>
<td>35</td>
<td>1.619</td>
<td>512.3</td>
<td>15-30</td>
</tr>
<tr>
<td>$\text{Na}_2\text{HPO}_4\cdot 7/2$</td>
<td>18.6</td>
<td>48</td>
<td>1.370</td>
<td>472.4</td>
<td>15-25</td>
</tr>
<tr>
<td>$\text{Na}_4\text{P}_2\text{O}_7\cdot 10/0$</td>
<td>22</td>
<td>80</td>
<td>1.458</td>
<td>527.0</td>
<td>35-80</td>
</tr>
<tr>
<td>$\text{Na}_2\text{HPO}_4\cdot 2/0$</td>
<td>11.2</td>
<td>95</td>
<td>1.328</td>
<td>630.8</td>
<td>20-30</td>
</tr>
</tbody>
</table>

The water capacity is calculated as the water given up when 1 g of the higher hydrate is completely converted to the lower hydrate. The maximum temperature refers to the temperature above which the higher hydrate is not known to be stable. The constants are the values that were found by fitting $a_w$ values to Equation 6-23 and the temperature range of the experimental vapor pressure is given in the last column.

From these above values Halling has provided tables of water activity values at different temperatures for a good number of salt hydrate pairs. Some of his findings at 25°C for the system we studied, $H_w\text{Na}_x\text{P}_y\text{O}_z$, are provided in Table 6-12 (Zacharis et al., 1997).
**Table 6-12.** Water activity values for selected salt hydrate pairs of the form $H_wNa_xP_yO_z \cdot H_2O$ at 25°C (Halling, 1992).

<table>
<thead>
<tr>
<th>Salt Pair</th>
<th>Equilibrium $a_w$ at 25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$_2$HPO$_4$ · 12/7</td>
<td>0.80</td>
</tr>
<tr>
<td>Na$_2$HPO$_4$ · 7/2</td>
<td>0.61</td>
</tr>
<tr>
<td>Na$_4$P$_2$O$_7$ · 10/0</td>
<td>0.49</td>
</tr>
<tr>
<td>Na$_2$HPO$_4$ · 2/0</td>
<td>0.163</td>
</tr>
</tbody>
</table>

When comparing these values to our data, the Na$_2$HPO$_4$ · 7/2 and the Na$_4$P$_2$O$_7$ · 10/0 salt pairs show equilibrium $a_w$ values closest to the areas where our experimental values showed large $\Delta Q/\Delta n$ values, namely at $a_w = 0.450$ and 0.424 for the buffer film and 0.38 for the myoglobin/buffer films. For the myoglobin films, it may be possible that the salt hydrate pair of Na$_2$HPO$_4$ · 2/0 is in equilibrium but due to the presence of the myoglobin, the equilibrium $a_w$ value is increased from the 0.163 value in the table.

To further check and describe our data, phase equilibria data was used as a means of determining which salt hydrates may be present in our buffer film. Because of the interest in sodium phosphates as heat storage material, the Na$_2$O-P$_2$O$_5$-H$_2$O system has been studied at a variety of temperatures (Belton & Ajami, 1973; Broadbent et al., 1977; Morey, 1953; Van Wazer, 1958; Watanabe et al., 1994). When making our sodium phosphate buffer film, it was cast from a 0.05 M solution containing both anhydrous monosodium phosphate, NaH$_2$PO$_4$, and trisodium phosphate dodecahydrate, Na$_3$H$_2$PO$_4$ · 12H$_2$O. In solution the mixture is best characterized as a Na$_2$O-P$_2$O$_5$-H$_2$O system. The sodium phosphates are known to dissolve or precipitate incongruently thus yielding a different Na$_2$O/P$_2$O$_5$ ratio in solution from that of the solid phase. If higher
temperatures (>100°C) are used to precipitate sodium phosphates from solution, generally it is the anhydrous or lower hydrates that are formed whereas at lower temperatures it is the higher hydrates that are formed. Since our measurements are at 25°C, it can be expected that upon hydration/dehydration, the higher hydrates are formed. An extensive review was done by Wendrow and Kobe on the Na₂O – P₂O₅ – H₂O system (Wendrow & Kobe, 1952). In commenting on the system at 25°C, they reveal that the system is far from simple and in the phase diagram shown below as many as twelve different solid phases exist along with ten three-phase areas at this temperature. From their results they plotted the weight percent of Na₂O versus the weight percent of P₂O₅ and have shown the phase diagram for this system at different temperatures. Figure 6-50 is a portion of their reported phase diagram shown for 25°C (Van Wazer, 1958). The dashed lines mark the mole ratios of Na/P for a particular composition. Therefore, all compositions of a given Na/P ratio must lie on the same straight line. The area beneath the solubility curve denotes the single-phase, homogeneous solution. As some of the sodium orthophosphate is precipitated out of solution, the precipitate is given by the particular solid phase that falls along the mole ratio composition line. Two-phase regions existing between the solubility curve and the equilibrium solid phase are shown by the tie lines. Three phase regions in the diagram are numbered.
Figure 6-50. A phase diagram of the Na$_2$O – H$_2$O – P$_2$O$_5$, sodium orthophosphate system at 25°C. The squares mark crystalline solids. The two-phase areas are covered by tie lines and the three-phase areas are numbered (Van Wazer, 1958).
In attempting to see where our sodium phosphate buffer solution fit within this solubility curve the weight percent of Na₂O and P₂O₅ needed to be found. Dr. Allan Smith devised an Excel spreadsheet to calculate the weight percents of each depending on the composition of sodium phosphate used. These weight percents were then applied to the actual amounts and compositions that we used in making the buffer solution. From this we were able to extract the mole ratio of Na/P. The general balanced equation used in the spreadsheet relating the elemental composition of the inorganic hydrate to its composition of Na₂O, P₂O₅ and H₂O is:

\[ H_w Na_x P_y O_z \cdot nH_2O \rightarrow 0.5yP_2O_5 + 0.5xNa_2O + ((2n+w)/2)H_2O \]  \hspace{1cm} \text{Equation 6-24}

where the mole balance for the elemental oxygen is given by a relationship with the coefficients:

\[ z = (5Y + x + w)/2 \]  \hspace{1cm} \text{Equation 6-25}

A picture of the spreadsheet used in the calculations is shown in Figure 6-51. Although it is stated that trisodium phosphate dodecahydrate (Na₃PO₄ · 12H₂O) was used to prepare our buffer solution, it is noted that there is no alkali-free dodecahydrate of trisodium phosphate. This composition of trisodium phosphate with 12 molecules of water always contains some free alkali. The composition is better shown as 4(Na₃PO₄·12H₂O) · NaOH (Wendrow & Kobe, 1952). For the calculations shown in the spreadsheet, it is this composition that is used for the trisodium phosphate. From our calculations we determined a Na/P ratio of 1.64.
A molar mass and composition calculation for use with Wendrow and Kobe

<table>
<thead>
<tr>
<th>Element</th>
<th>At. Mass</th>
<th># Atoms</th>
<th>Partial Mass</th>
<th>Wt%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>22.98977</td>
<td>1</td>
<td>22.9896</td>
<td>19.16%</td>
</tr>
<tr>
<td>H</td>
<td>1.00794</td>
<td>2</td>
<td>2.0159</td>
<td>1.68%</td>
</tr>
<tr>
<td>P</td>
<td>30.973761</td>
<td>1</td>
<td>30.9738</td>
<td>25.82%</td>
</tr>
<tr>
<td>O</td>
<td>15.9994</td>
<td>4</td>
<td>63.9976</td>
<td>53.34%</td>
</tr>
</tbody>
</table>

Formula weight of the anhydrous salt: 119.9770 100.00%

Moles water in the hydrate: 0

H: 1.00794 0 0.0000 0.00%
O: 15.9994 0 0.0000 0.00%

Mass of the water: 0.0000 0.00%

Formula weight of the hydrate: 119.9770 100.00%

Weight percent Na2O: 25.83%
Weight percent P2O5: 36.13%
Weight percent water: 38.05%

Figure 6-51. A depiction of the Excel spreadsheet used to determine the mole ratio of Na/P for comparison of our experimental sodium-orthophosphate solution with phase diagram values.

From the weight percents from our calculations, 25.8% for Na2O and 36.1% for P2O5, these allowed us to fix a point on the phase diagram. A line was drawn from this point through the origin. This line marks the 1.6:1 mole ratio of Na/P. Upon precipitation, the first hydrate encountered along this line is Na2HPO4 • 12H2O and further drying yields the hydrate Na2HPO4 • 8H2O. However, if Na2HPO4 • 12H2O is the first hydrate encountered, our ΔQ/Δn values are smaller than the calculated value of ΔH° = -5311.57 kJ/mol for this hydrate. Due to the complex nature of the phase diagram of
the Na₂O – P₂O₅ – H₂O system, and the possible formation of a variety of hydrates, it
does seem difficult to merge the data analyses from the three approaches given here: (1)
ΔfH⁰ values, (2) a_w values where two hydrates are in equilibrium and (3) phase diagram
results. It would seem that the phase diagram results most closely incorporates our
actual buffer preparation and data.

6.4 Conclusions

In conclusion, the QCM/HCC has been shown to provide sensitive and
simultaneous data upon the hydration of two different protein films, lysozyme and
myoglobin. Measureable quantities include: (1) water sorption isotherms, (2) water
sorption enthalpies, (3) viscoelastic changes upon hydration, and (4) water diffusion
coefficients in proteins. An appealing characteristic of the instrumentation is that
different proteins are amenable to this type of study because only a small sub-milligram
samples are needed.

Several observations of the protein hydration process can be made:

(1) The direction of hydration does make a difference in the amount of water the film
will absorb/desorb. If the film was started from a higher a_w it generally took up more
water than experiments done in the same a_w range but started from a lower a_w.

(2) The hydration process has components with long time scales (~ hours). Further
hydration studies should allow for longer equilibration steps in the
hydration/dehydration scans.

(3) The motional resistance measurements give a glimpse into the structural properties
of the film and how the film structure changes upon water sorption/ desorption. The
desorption processes appear to cause structural changes in the film as evidenced by the sometimes erratic motional resistance readings.

(4) In some of the hydration processes where two full cycles were performed, the motional resistance seemed to resume normal step-wise signals upon the second cycle, perhaps indicative of the film being conditioned.

(5) The presence of the sodium phosphate buffer in the myoglobin film has a pronounced effect on the hydration measurements. It appears that upon the film’s first hydration, the phosphate buffer salts interact strongly with the water and some of the buffer sites are preferentially hydrated, thus forming hydrates of the sodium phosphate buffer. Large changes in the hydration state of the buffer are observed over small variations in water activity. Upon the second hydration cycle, these large quantities of heat generated per mole, Q/n, are not observed.

(6) A hydration study of the sodium phosphate buffer yielded large $\Delta Q/\Delta n$ values for $a_w \sim 0.45 – 0.51$ also confirming that the large values seen in the myoglobin/buffer film were effects of the buffer being hydrated.
List of References


Chapter 7. QCM/HCC Future Developments

The studies presented here are part of a dynamic process. As with any research, the current work in a field provides a foothold so that continuing investigations could occur. New researchers provide different outlooks and visions of certain projects with the ability carry the projects further along. In the studies discussed above, there are a few interesting avenues for further investigations. A brief description is given of possible future aspects regarding the instrumental development and experimental applications of the studies presented here.

7.1 Continuing Instrumental Developments

The QCM/HCC has proven to be a versatile, sensitive instrument capable of a variety of applications (Shirazi, 2000; Smith et al., 1998; Smith & Shirazi, 2000; Smith et al., 2002). A unique aspect of the instrument is the ability to study the thermodynamic and rheological properties of thin films as a function of vapor activity. Dr. Allan Smith is currently engaged in pursuing a second-generation model of the QCM/HCC. As with any instrumental technique, researchers try to enhance the performance, improve the ease of use, and incorporate new capabilities to further increase an instrument’s contributions to scientific studies. The following is a listing of some general enhancements, which we have gathered from our experience in using the instrument and our discussions on the technique.

1. The LabView Virtual Instruments (VI’s) written by Dr. Hamid Shirazi have contributed to the ease and automation of controlling the instruments and collecting the data involved in the QCM/HCC measurements. Dr. Allan Smith has already pursued improving the LabView VI’s, which control the user-defined experimental parameters,
data acquisition and control of the individual instruments comprising the whole. Since the experiments presented here, the VI has been modified to include a greater flexibility in changing the time-scale of individual sorption steps within a sorption cycle. Within one full sorption cycle, each individual sorption step may be assigned a unique length of time, where previous to this, each step was assigned one fixed length of time at the start of the experiment. Another added feature is that the user can modify the data-sampling rate. This feature will allow for longer sorption studies while helping to cut down on the data file size for experiments running a few days. Previously, the VI had two modes of operation, a manual setting and an automated setting. Different variations of these modes are now available. The user can choose from manual settings on the VI, as well as, two automated settings involving ramp options for the sorption steps. The input and output parameters for the vapor concentrations have been changed from units of ppm to activity. Included in the VI is an electronic log where the user can document input experimental parameters that will be stored as a text file.

2. The constant temperature water bath provides a very stable, isothermal environment. Although the water bath is very stable, it is not always convenient to perform experiments at different temperatures. Time is required to change the bath temperature and attain thermal equilibrium. The water bath also demands that some time be taken for periodically changing the water and cleaning the inside. Another method of a stable temperature controlled environment would increase the flexibility in use of the QCM/HCC.

3. From discussions on current calorimeters, a temperature scanning capability would increase the usefulness of the QCM/HCC. One aspect this would enhance is the area of
drying studies because mass changes and rheological properties would simultaneously be monitored. When scanning temperature, it is possible to determine the $T_g$ from a change in heat capacity. As a dual means of probing this relaxational effect, the $T_g$ could also be monitored by the QCM’s motional resistance output data.

4. With our current instrumentation, cables from the QCM electrodes to the frequency counter are placed through a stainless steel tube to carry them away from the water bath and the calorimeter to a rack housing the frequency counter. It may be helpful to further investigate a re-routing of the cables. A few times after changing a film and reconnecting the instrument, feedback from the QCM oscillations would occur causing both channels of the frequency counter to read the same QCM. Pressing the reset button on the PLO often rectified this; however, better-shielded and shorter cables may help to reduce this effect.

5. Wiring from the thermopile to the preamplifier follows the same route as the QCM cables. For increased sensitivity and accuracy two aspects could be looked at: shortening the length of the cables shorter and maintaining the cables at the same temperature as the experiment housed in the constant temperature bath. Because of the Seebeck effect, a temperature difference causes a proportional change in the Seebeck voltage. The magnitude of the voltages leaving the QCM/HCC instrument is very small. Large temperature differences between the calorimeter chamber and the preamplifier housed outside the constant temperature bath would have an effect on the magnitude of these voltages. Insulation and a constant temperature environment for the cables and preamplifier would reduce the influence of ambient air conditioning or heating on the voltage readings.
6. Another improvement would be a relative humidity or vapor meter that could be housed in the water bath so as to ensure that the measurement temperature is the same as the actual experimental temperature.

7. It may be helpful to further investigate the possibility of automating the thermal baseline assignment before integration. This is a time consuming part of the data analysis and sometimes dependent on the person analyzing the data. However, when talking to the software engineers from Origin (Microcal), they agree that a human defined baseline is more accurate than a software defined baseline.

8. Having a stable, repeatable calibration method has added to the clarity of our results. This electrical calibration closely matches the heat flow of our regular experiments. One interesting approach to the calibration method may be to investigate a possible test reaction for sorption processes that can be used as a chemical calibration.

7.2 The 2-Drop Isothermal Heat Conduction Calorimeter

Using the 2-Drop Calorimeter in the physical chemistry laboratories has provided students with an introduction to calorimetry and exposure to some types of chemical processes that may be studied. Further investigations to enhance the calorimeter’s use include:

1. Pursuing biochemical titration experiments to be used in the undergraduate physical chemistry laboratories such as protein-ligand binding, or reactions involving some pharmaceutical based aspects.

2. Continuing the use of the home-built 2-Drop model because student’s gain exposure to the inner workings of the calorimeter and practice hands-on experience with calibration techniques.
3. Varying concentrations of BaCl$_2$ and 18-crown-6 ether to develop a working experiment with the 2-Drop Calorimeter.

4. Having students continue to learn the art of reading and interpreting a power vs. time plot.

5. Having students devise and sketch a calorimeter giving them a list of parameters of which to be mindful.

6. Performing the evaporation or titration experiments at different temperatures so students could see the temperature effects.

7. Modifying the water sorption of molecular sieves (zeolites) experiment. We took an initial look at possibly setting up a sorption experiment based on the technique used by Lars Wadsö and Natalia Markova (Wadsö & Markova, 2000). Tubing or a glass pathway would connect the reference and sample sides. Water would be placed in the reference chamber and zeolites would be placed in the sample side. The two sides would be isolated until ready to begin the study. At this point, the pathway between the reference and sample chambers would be unblocked and water vapor would be free to flow through the tubing to the sample cell. The voltage output of the thermopiles associated with the sample and reference sides would be read individually. The reference side would record the heat due to the vaporization of the water while the sample side would record the heat due to the Zeolite sorption of water. The excess heat between the two sides would give the heat due to the adsorption of the water molecules. Further investigation is needed in constructing the pathway between the sample and reference sides.
7.3 Polymer Sorption Studies

Because of the magnitude of polymer sorption studies and the broad techniques used in research to analyze polymers, there are a host of other methods in which to compare and analyze our results. A few possible methods are given below.

1. An interesting approach would be to compare the experimental isotherms with theoretical models, including the Dual-Mode and Flory-Huggins solution models (Okuzaki et al., 1999; Riven et al., 2001; Russell & Weinkauf, 2001). This would involve investigating the parameters and constants for a particular polymer material being fit to these equations.

2. The QCM/HCC could be used to explore the sorption effect on other polymers. A wealth of studies have been done on polyisobutylene (PIB), $T_g \sim -76^\circ$C, poly(methyl methacrylate) (PMMA), $T_g \sim 106^\circ$C and polyethylene (PE), $T_g \sim -20, -130^\circ$C (Behling et al., 1998; Domack & Johannsmann, 1996; Ferry et al., 1953; Fitzgerald et al., 1953; Moore & Wanke, 2001; Plazek & Ngai, 1996; Price & Buley, 1991; Russell & Weinkauf, 2001; Wolff et al., 1997). It would be interesting to compare QCM/HCC sorption results on these polymers with results from other methods.

3. Based on the models proposed by Grate, et al. (Grate et al., 1995; Grate & Zellers, 2000) further exploration of our studies could include determining a correction factor for polymer swelling. Some possibilities include independently measuring the thermal expansion coefficient for the polymer thus giving insight into the free volume. Another avenue is to explore the volume occupied by the adsorbate molecule and estimate the effects in the polymer adsorbent molecule. A correction factor could then be used to modify partition coefficient results.
4. Using the phase lock oscillator, to monitor the frequency and motional resistance of film-coated QCM’s upon vapor sorption, provides an insight into the shear modulus of the film. Additional information, such as the film density and film height as they change upon vapor sorption, is needed to accurately calculate the shear modulus, $G$. It would be helpful to explore an independent method of obtaining the film thickness. However, it would take some engineering to devise an in-situ method that would not disturb the calorimetric measurements.

5. By using different film thicknesses, it may be possible to use the equations for $G'$ and $G''$ in order to set up simultaneous equations to determine the four unknowns in the equations, the film density, film thickness, shear storage and loss moduli.

6. Another method that could be further continued is to pursue the cold temperature frequency measurements of a thin film. It may be beneficial to design an insulated chamber with a device for variable temperature control depending on the polymer being studied.

7. An ongoing aspect would be to explore the theoretical models and their possible incorporation with our experimental data. Time may be well invested in developing a better working computer model for the Three-Step Method. In the calculations, the equations need to loop back three times to different parts, time would be needed to correctly write this in a mathematical software. Dr. Ralf Lucklum’s Excel spreadsheet, ZYSYN_QP offers many potential applications to our studies. It can be used as a means of obtaining a first approximation for acoustic load impedance, $Z_L$, and how this changes upon polymer swelling due to vapor sorption.
7.4 Protein Hydration Studies

Protein hydration studies are of value in a number of fields as stated in Chapter 6. To expand and help improve the direct calorimetric measurements provided by the QCM/HCC, the following suggestions might be considered.

7.4.1 Lysozyme

1. Lysozyme studies can be continued by creating films of varying thicknesses. When the QCM/HCC lysozyme studies were done, we did not have the capability of measuring the motional resistance. It would be interesting to compare the motional resistance data with that of myoglobin. Because no extra buffer is needed to get lysozyme in solution, the sorption effects would reflect the actual protein-water hydration and not a complex three-component system.

2. The sorption studies would be enhanced by allowing for longer time intervals for each sorption step to monitor hysteresis effects.

3. The films of varying thicknesses and studied with longer sorption steps could then be compared with results from previous QCM/HCC studies as well as studies from other sources such as Bone, Lüscher-Mattli and Careri (Careri et al., 1979; Lüscher-Mattli, 1986).

4. As done in the myoglobin studies, an intriguing approach would be to vary the starting water vapor activity in the sorption studies. It was noted with the myoglobin/buffer film that the starting water vapor activity does have an effect on the amount of water uptake within a defined water vapor activity range.
7.4.2 Myoglobin

Analyzing the myoglobin proved to be challenging because of the presence of the buffer in the film. However, the presence of the buffer does more closely mimic the state of a protein in-vivo. A few helpful approaches for further studies of the myoglobin-buffer-water system and listed below.

1. When dialyzing the myoglobin-buffer solution, work could be continued in determining the minimum concentration of buffer needed before the protein precipitates out of solution. This would decrease the presence of the buffer in the myoglobin-buffer film.

2. A greater understanding could be gained by further probing of the complex, three-component protein-buffer-water system. A more in-depth thermodynamic analysis may ensue based on previous investigations of three component complex systems (Kuntz & Kauzmann, 1974).

3. An unexplored area with this instrument would be to probe the effects of myoglobin sorption of nitrous oxide (NO) and anesthetic gases. Several investigators have probed the binding of NO to myoglobin and used DSC measurements to explore the interaction of anesthetic gases with myoglobin (Møller & Skibsted, 2002; Tanner, 1998; Tanner, 2001). The QCM/HCC offers an avenue to directly and simultaneously measure the mass, thermal, and motional resistance changes upon an adsorbate vapor binding to the protein film.

7.4.3 Other Proteins/Aspects of Protein Studies

1. Future QCM/HCC applications could involve continuing the study of hydration effects on other proteins. Relatively few direct calorimetric studies have been
performed on protein hydration. An interesting next step may be to expand the types of proteins we have studied (Smith et al., 2002).

2. Further experimental work could be continued probing the hydrate formation of buffer salts. Few experimental results are found in the literature.

3. Hydration studies of one type of secondary structure, such as a peptide that is solely $\alpha$-helical, would give information on the hydration of specific portions of proteins. This would be similar to the approach in Privalov, et al. work utilizing DSC studies to determine the heat capacities of individual amino acids (Privalov et al., 1995; Scholtz & Baldwin, 1995).

In working with the QCM/HCC, many intriguing research projects and possibilities have emerged. In the words of Sir Humphrey Davy, “Nothing tends so much to the advancement of knowledge as the application of a new instrument. The native intellectual powers of men in different times are not so much the causes of the different success of their labours, as the peculiar nature of the means and artificial resources in their possession (Hager, 1995).”
List of References


Appendix A. 2-Drop Calorimeter Student Handout

CHEMISTRY 356-358, Physical Chemistry Laboratory

The Cutting Edge of Experimental Thermodynamics: Isothermal Heat Conduction Calorimetry

A physical chemistry laboratory experiment under development at Drexel University, with the cooperation of scientists and engineers at Dow Chemical Company and Lund University, Lund, Sweden.

Allan L. Smith, Hamid Shirazi, Sr. Rose Mulligan

Objective: To explore the use of isothermal heat conduction calorimetry as a means of measuring a variety of heat changes such as determining the enthalpies of vaporization and reaction, evaluating the radiant power of a light source, measuring the heat of water adsorption of zeolites and identifying the heat of metabolism from small insects.

Introduction: Calorimetry is the measurement of heat. All chemical reactions and processes and all biological processes, which are ultimately chemically based, are accompanied by the generation or absorption of heat. Thermodynamics, the science underlying the interpretation of calorimetric measurements, is extremely well understood and allows for the determination of many useful chemical properties of substances.

Types of Calorimetry

Because of the central importance of energy changes in chemical reactions, calorimetry is usually a part of any experimental chemistry curriculum. Most general chemistry texts and physical chemistry lab manuals mention one type of calorimeter, the bomb
Calorimeter. By measuring the heat change in reactions in which the standard enthalpies of formation are known for all but one of the reactants or products, the unknown enthalpy of formation can be determined. Bomb calorimeters are used for determining enthalpies of combustion of organic compounds but are limited in the type of heat changes that could be measured.

Calorimetry as practiced in both academic and industrial research labs, however, is much more diverse. In current literature, forty-four researchers in thermodynamics identifying important areas for development in the 21st century hardly mention combustion calorimetry, but describe dozens of applications of heat conduction calorimeters (Letcher, 1999). Common types of calorimetry are:

**Solution calorimetry:** a method of measuring the total heat evolved in a chemical process in solution, the process being carried out inside an adiabatic container such as a dewar flask.

With the proper kind of solution calorimeter, one can measure the heat evolved or consumed in fast reactions carried out when one reactant is incrementally added to another. Such experiments are called *thermal titrations*, and they have been widely used in biochemical systems to determine both enthalpy change and binding constant for the formation of enzyme-substrate complexes.

**Differential scanning calorimetry (DSC):** a method of comparing the heat capacities and heat generation or absorption within a sample and a reference as the temperature of both is raised at a constant rate.

**Thermal gravimetric analysis (TGA):** the loss of mass of a sample is continuously monitored as the temperature is raised at a constant rate. Although
not strictly a calorimetric measurement, thermal gravimetric analysis is often combined with differential scanning calorimetry; the acronym is **DSC/TGA**.

If the molar enthalpy change for a reaction is already known, measuring the rate of heat evolution in slow reactions determines the time dependence of the extent of the reaction and thus the rate of reaction. With calorimeters of high sensitivity it is possible to measure the heat given off by an unconnected dry cell battery as it slowly loses its chemical energy, an explosive as it slowly decomposes on the shelf, or even the heat generated by an exercising insect or a germinating seed.

**Comparison of Adiabatic and Heat Conduction Calorimetry**

Both the bomb calorimeter and the solution calorimeter are examples of **adiabatic calorimetry**. When a chemical process occurs in an adiabatically isolated system of known heat capacity, $C$, the temperature change, $\Delta T$, is measured and the heat change is calculated from the equation:

$$Q = C\Delta T$$  \hspace{1cm} \text{Equation A-1}

In **heat conduction calorimetry**, the reaction vessel is isolated adiabatically from its surroundings except for contact with a heat flow sensor, which in turn is connected to a large heat sink such as a constant temperature bath. The heat flow sensor generates an output voltage proportional to the flow of heat, $dQ/dt$, through the sensor from the reaction vessel to the heat sink. This heat flow sensor signal is recorded as a function of time, and the total heat generated in the process is obtained by **integrating the heat flow signal over the duration of the experiment**:
Heat Flow Sensors – Thermocouple Plates (TCP)

The key to making heat conduction calorimetry practical is the availability of sensitive and relatively inexpensive heat flow sensors. Ingemar Wadsö (Wadsö, 1997) has pioneered the use of thermopiles as heat flow sensors. They are commercially available in the form of thermoelectric heat pump (Melcor, 1995) devices that operate on the inverse Peltier effect. The Peltier effect is responsible for the generation of a thermocouple voltage signal. Two dissimilar conducting materials (such as copper and constantan wire), connected at two points of differing temperature, generate a voltage difference proportional to the temperature difference. In the inverse Peltier effect, a flow of current through two dissimilar conductors causes a temperature difference to develop across the two connection points of the dissimilar materials. In a thermoelectric heat pump, when a voltage is applied to a thin array of dissimilar conducting pairs of materials, heat is pumped from one side of the array to the other side. In the commercial devices, the dissimilar materials are small rectangular pieces of n- and p-doped BiTe semiconductors. Thermoelectric heat pumps are used, for example, in cooling the integrated circuits used in computers. Figure A-1 depicts the structure of the type of thermocouples used (Tellurex, 2002). The two-drop calorimeter was constructed using TCP, CP1.4-71-045L from Melcor (Trenton).

\[ Q = \int \frac{dQ}{dt} \ dt \]  

Equation A -2
Figure A-1. Thermocouple Plate (TCP) (Tellurex, 2002)

One TCP is comprised of a large number of thermocouples connected in series electrically to give a high output voltage. They are connected in parallel thermally to give a high ratio of output voltage with a temperature difference.

The Working Equations for a Heat Conduction Calorimeter

The Tian equation is employed to calculate the thermal power from the measured signal. The output voltage, $U$ (V), from the heat flow sensors recorded as a function of time may be converted into the heat flow rate, $P$ (W), by multiplication with the calibration coefficient, $\varepsilon$ (W/V). If we are interested in the kinetics of rapidly changing processes the Tian equation takes into account the heat capacity and the rate of temperature change of the reaction vessel (Bäckman et al., 1994).
\[ \frac{dQ}{dt} = P = \varepsilon \left( U + \tau \frac{dU}{dt} \right) \quad \text{Equation A-3} \]

Here \( \tau \), the time constant, of the calorimeter is given by

\[ \tau = \frac{C}{k} \quad \text{Equation A-4} \]

Where \( C \) (J/K), is the heat capacity of the sample and its holder cup and \( k \) (W/K), is the heat conductance of the TCP. The calibration coefficient, \( \varepsilon \) (W/V), takes into account the thermal conductance of each thermocouple divided by the material constant of the thermocouple(Bäckman et al., 1994). The calibration coefficient is usually found from electrical calibrations, as discussed below. Using Equation A-3, when the signal, \( U(t) \), is numerically differentiated to give, \( \frac{dU}{dt} \), the actual thermal power produced in the sample from the measured voltage may be calculated.

When the thermal power changes slowly, at steady state conditions, \( \frac{dU}{dt} = 0 \). The Tian equation maybe reduced to:

\[ P = \varepsilon * U \quad \text{Equation A-5} \]

For the experiments described here, the reduced form of the Tian equation, Equation A-5, may be used.

**Design and Construction of a Simple Heat Conduction 2-Drop Calorimeter**

Thomas C. Hofelich, a chemist in the Analytical Sciences Laboratory at Dow Chemical Company, has developed a sensitive and inexpensive heat conduction calorimeter, which he has used extensively at Dow for measuring heat production when small quantities of reagents are mixed(Hofelich et al., 1994). Hofelich calls his device “The
2-Drop Calorimeter,” because the heat evolved when one drop of reactant A is added to one drop of reactant B can be measured.

Dr. Lars Wadsö, an engineer at the Division of Building Materials, Lund University, Sweden, has also developed a similar, inexpensive, isothermal heat conduction calorimeter (Wadsö, 1998). Experimental applications he has developed include:

- **Polymer science:** Curing reaction of a standard epoxy
- **Food science:** Microbiological growth in food
- **Material science:** Steel corrosion
- **Coatings technology:** Oxidation of linseed oils
- **Biotechnology:** Heat production in waste compost

Commercial versions of the type of calorimeter described here have recently been introduced. Typical industrial uses include the determination of the heat evolved upon mixing materials of both known and unknown origin (hazardous evaluation), and the study of the effect of concrete additives on the hydration of cement paste (Hofelich et al., 1997; Wadsö, 1998).

In this experiment, a 2-drop calorimeter constructed at Drexel from Hofelich’s and Wadsö's plans will be used (Hofelich, 1997). The sample chamber of the 2-drop calorimeter is a small glass vial of 2 cm³ volume. Inside the insulated box housing the calorimeter, the sample vial rests in an aluminum cup in good thermal contact with a Melcor thermopile resting on a large aluminum block functioning as a heat sink. There are two identical thermopile – aluminum cup – sample vial combinations, one serving as the **reference** and one serving as the **sample chamber**. **Figure A-2** is a schematic picture (Wadsö, 1999) of an isothermal heat conduction calorimeter.
Figure A-2. Isothermal Heat Conduction 2-Drop Calorimeter

Calibration of the instrument

Thermal calibration of the 2-drop calorimeter is achieved by applying a known voltage, \( V \), across a resistor of resistance, \( R \), attached to the side of the aluminum cup where:

\[
V = I \times R \quad \text{Equation A-6}
\]

\[
P = I \times V \quad \text{Equation A-7}
\]

thereby leading to:

\[
P = \frac{V^2}{R} \quad \text{Equation A-8}
\]
The thermal power generated in the resistor is:

\[ \frac{dQ}{dt} = P = \frac{V^2}{R} \quad (W) \quad \text{Equation A-9} \]

When a steady state output voltage is obtained, the calibration coefficient may be found from

\[ \varepsilon = \frac{P}{U} \quad (W/V) \quad \text{Equation A-10} \]

where \( U \) is the total measured voltage output. Typical values of \( \varepsilon \), for commercially available thermopiles are 2.4 – 2.5 W/V. Figure A-3 depicts a typical steady state output voltage used during the calibration.

![Figure A-3. Calibration thermal signal for 2-Drop Calorimeter](image)

**Enthalpy of Vaporization of Organic Solvents**

The 2-drop calorimeter provides an easy method for determining the enthalpies of vaporization of volatile organic solvents. As only one drop of the solvent is released into the aluminum cup, the thermopile reads the endothermic event as the solvent evaporates and the thermal signal is gathered. By measuring the mass of 20 drops of
solvent and using the solvent’s molecular weight calculating the mass of one drop, we can compute the molar enthalpy of vaporization of the solvent.

The calibration coefficient from the calibration is used to convert the voltage ($U$) to a thermal signal ($W$). Integration of the area under each thermal signal curve, $U$, gives the heat signal, $Q$, in Joules. Using the measured heats and the amount of solvent in each drop, the molar enthalpy of vaporization is found:

$$\Delta_{vap}H = \frac{\varepsilon \int U dt}{n} \quad \text{Equation A-11}$$

Here, $n$, is the moles of solvent.

**Laser Power Meter**

The TCP used in the 2-drop calorimeter are sensitive enough to be used as a laser power meter. In this experiment, the radiant power of a light source, a helium-neon laser, is measured. The 5 mW laser will be directed on the top surface of the sample aluminum cup blackened with graphite. After leaving the laser on for a few minutes, a steady-state output voltage will be reached. The output voltage, $U$, multiplied by the calibration coefficient, $\varepsilon$, gives the thermal power (W). The percentage of light absorbed and reflected can then be found

$$\left\{\text{Power (measured) / Power (radiated)}\right\} \times 100 = \% \text{ absorbed} \quad \text{Equation A-12}$$
Thermal Titration of Tris(hydroxymethyl)aminomethane, (THAM), C(CH$_2$OH)$_3$NH$_2$, with 3M HCl

Using heat conduction calorimeters, thermal titrations carried out when one reactant is incrementally added to another enable the measurement of the heat evolved in fast reactions. This method has been widely used in biochemical systems to determine both the enthalpy change and the binding constant for the formation of enzyme-substrate complexes. Performing a thermal titration in this calorimeter requires only microliters of the titrating solution. In this experiment, a small sample of reagent A, either solution or solid, is placed in the vial, and the titrating reagent B is held in a 1000 µl syringe mounted above the vial. Single drops of B are added to the vial, and the thermal signal generated from each addition is recorded.

As the first drops of titrant are delivered, a large exothermic signal is seen. Two or three drops at a time are delivered after the signal returns to the baseline. Releasing the drops of titrant is repeated until the expected equivalent molar volume is achieved indicating that the endpoint is reached. The titration proceeds according to:

\[
\text{H}^+ (\text{aq}) + \text{Cl}^- (\text{aq}) + \text{C(CH$_2$OH)$_3$NH}_2 (\text{aq}) \rightarrow \text{C(CH$_2$OH)$_3$NH}_3^+ (\text{aq}) + \text{Cl}^- (\text{aq})
\]

Equation A-13

The volume of HCl required to neutralize all of the THAM is used to determine the enthalpy of reaction for this titration. At any intermediate point in the titration, the ratio of the added volume to the total volume of HCl equals the ratio of the added number of drops to the total number of drops.

The number of moles, \(n_i\), in each drop of titrant is, \(n_i (\text{HCl}) = V_i \text{HCl} \ast M_{\text{HCl}}\), where \(V_i\), is the volume of titrant used and \(M\), is the molarity of the titrant. Each peak,
corresponding to the \( i \) \(^{th} \) drop of the titrant, is integrated to give the heat, \( Q_i \) (J), Equation A-2. The total heat in all peaks before the endpoint, \( \Sigma_i Q_i \), divided by the moles of titrant added before the endpoint, \( \Sigma_i n_i \), gives the enthalpy of the process.

\[
\Delta_r H = \frac{\Sigma_i Q_i}{\Sigma_i n_i}
\]  
Equation A –14

Another method of calculating the enthalpy of reaction is to employ the moles of THAM used. Because of difficulty in seeing the syringe gradients, we will be using Equation A-15, the moles of THAM for our calculations.

\[
\Delta_r H = \frac{\Sigma_i Q_i}{\Sigma_i n_{\text{THAM}}}
\]  
Equation A-15

Using the literature value (Eatough et al., 1974) for the enthalpies of formation of all reactants and products, Hess’s Law is used to calculate the \( \Delta_r H \). We assumed no concentration dependence for the \( \Delta_r H \) (s).

**Insect Metabolism**

Isothermal calorimeters have been explored as a means of measuring the heat of metabolism of small insects. The rates of heat exchange between small insects and their environments are believed to be controlled by several factors: radiative heat gain, convective heat loss, metabolism, and evaporation. These properties vary with the size, shape, orientation, and surface properties of the insect (Casey, 1988).

Isothermal microcalorimeters offer a non-damaging route to monitor the metabolism of insects. To simplify the multiple sources of possible heat exchange, the heat measured from an insect scurrying in the vial is taken as the heat of metabolism. The heat
measured is the sum of the heats from all of the insect processes that takes place in the calorimeter.

The integration of the peaks, Equation A-2, corresponding to the activity events, gives the heat, $Q$, in Joules. If we assumed that the heat evolved from the bug is all the heat of metabolism, it is interesting to try to calculate how much sugar is needed to generate the same amount of heat. An approximate value can be found by using the “burning” of glucose in oxygen as a model of metabolism.

$$C_6H_{12}O_6(s) + 6O_2(g) \rightarrow 6CO_2(g) + 6H_2O(l) \quad \text{Equation A-16}$$

From the $\Delta_f H$ of the products and reactants, the $\Delta_r H$ can be found from Hess’s Law. We can use this to determine the amount of glucose that would produce this amount of heat.

$$\text{mass}_{\text{glucose}}(g) = \left( \sum_i Q_i \times 180 \text{ g/mole}_{\text{glucose}} \right) / \Delta_r H_{\text{m. glucose}} \quad \text{Equation A-17}$$

**Heat of Water Adsorption on Zeolites**

Zeolites, or "molecular sieves" have a porous structure. The internal surface area of these pores is quite large (several hundred square meters per gram of zeolite). Zeolites used as molecular sieves usually come as spherical pellets with diameters of ca. 1-2 mm. When even a few milligrams of dry zeolites are exposed to water vapor, large exothermic heat flows are observed. Carefully keeping the zeolites in a dry environment is essential prior to the experiment or during the prep time. In this lab period, the exothermic event will not reach completion. We will therefore only use a ten-minute period of the water adsorption event to calculate the $\Delta_{\text{absorption}}H$ of the...
zeolites. The enthalpy of the adsorption process for this time period and mass of
zeolites is found:

$$\Delta_{\text{adsorption}}H = \frac{\varepsilon \int Udt}{\text{mass}_{\text{zeolites}}}$$

Equation A-18

which then yields the $\Delta H$ in Joules/gram.

**Procedure**

**Data Acquisition Setup:**

1. Put the top part of the insulating box on the calorimeter. This will allow the
   instrument to reach thermal equilibrium while setting up the computer interface
   and preparing for data acquisition.

2. Make the necessary connections from the calorimeter leads to the A/D computer
   interface terminal board. A single-ended setup is used. One lead is connected to
   pin 1 and the other to pin 18. The polarity (-/+ is arbitrary. If it is reversed, the
   magnitude of the signal remains the same, but the sign of the signal will change.

3. On the computer, open the **Agilent VEE Pro 6** software. There should be a
   shortcut on the desktop, if not, the software can be opened through Programs
   and Agilent VEE.

4. Close the “Tip of the Day”. Open a new file. In the folder VEE Programs, open
   **two_drop**. You will see the following control panel open:

![VEE control panel](image)

Figure A-4. VEE control panel for data acquisition of 2-Drop Calorimeter experiments.
5. On the A/D config icon, double click in the “Configure” box. The channel should read 0, the gain is 8, and the sample rate is 1 Hz. Click on the “Hardware” tab on the right hand side. Make sure the “channel type” is “single-ended.”

6. In the “Get Data Panel,” the channel should read 0, and points are 1.

7. Right click on a plain light blue part of the “strip chart.” A submenu will appear for the “y plot.” Select the “properties” option. Go to the “scales” tab. (Do not use the auto scale option because the graph constant updates, it will be difficult to see any changes unless you are following the “AlphaNumeric” display.)

8. Change the X Name to “Time (s)” and the Y Name to “Volts”. For the X, or Time axis the maximum should be 100 and the minimum should be 0. For the Y, or Volts axis, change the scale to 5m for the maximum (meaning 5 mV) and -5m for the minimum. (N.B. Your data is still recorded in volts, this is just scaling or magnifying the axis of the chart so that we will be able to see the signal.) The Y scale may be changed for any of the experiments.

9. It is crucial to save the data to a file for later data analysis. In the light blue section of the “To File” look for the raised light blue box, next to the words “To file:” Double click in there and at the bottom type in a name for your file. It will put the correct file extension, you just need to type in a name for the particular part of the experiment (e.g. calibration). You may want to further distinguish the file with your initials or the date. This will save the data in the file “VEE Programs.” Afterwards, you can move the file to your own folder under “My Documents.”

10. You can start the data collection in one of two ways – click on the green “start” button or click on the ► on the toolbar at the top.

11. Start the data collection to record the thermal baseline. You want to collect about 5 minutes of a stable baseline before starting the calibration.

12. In between each experiment, be sure to change the file name according the experiment being performed.

**Calibration of the Calorimeter**

1. While data for the baseline is being recorded, use a voltmeter to measure the voltage of the battery that you will use as your potential source.

2. Also measure the resistance of the resistor attached to the side of the sample aluminum cup and record its value. A1 kΩ chip-resistor is used as the heating element. However the thin copper wire leads on the resistor also impose some resistance. Measuring the resistance using an ohmmeter will account for this
The resistor is used only for the calibration and does not play a part in any of the other experiments.

3. Close the electrical circuit (turn on the switch, if one available) by connecting the battery to the leads from the calorimeter. After a few seconds (depending on the time constant) you will see a decrease (or increase depending on the polarity of the leads in the terminal board) in the signal indicating an exothermic process from the heating of the sample cup. Since the thermopiles are heat flux sensors, the signal will decrease in magnitude and eventually reach a steady state and level off. After a few minutes (e.g. 5 min.) disconnect the battery and the signal will return to the base line. Wait a short while to establish a baseline after the heating event. Repeat the calibration two more times and then stop logging data by clicking on the stop program ( ■ ) on the toolbar.

Experiment 1: Enthalpies of Vaporization

1. Weigh a clean glass vial and a lid. Fill a syringe with the organic solvent. Release 20 drops in a glass vial, cover to prevent any evaporation and find the mass. After finding the mass of 20 drops, divide to find the mass of one drop. Use the molecular weight to determine the amount of moles in one drop.

2. Fill a 1 ml syringe to the 0.5 ml mark with the organic solvent. Mount the syringe on the sliding top and align the syringe exactly above the aluminum sample cup (the one you calibrated that had the resistor attached to the side). You may lower the syringe a few mm into the cup, but the syringe should not touch the sides or the bottom of the aluminum cup.

3. Tighten all screws and put the insulating box on the calorimeter so that the syringe extends through the hole in the top part of the insulating box. Allow for thermal equilibrium to be reached.

4. Prepare for data acquisition in the same manner as described in the calibration procedure. Remember to change “To file” name. Start logs and keep recording the signal for a few minutes(e.g. 5min). This will provide for a baseline before the actual event.

5. Start pushing down the plunger on the syringe very slowly until you observe a change in the signal. This will indicate that a drop has disengaged from the syringe tip and has fallen into the aluminum cup. It is difficult to see the actual event. An endothermic signal will be observed until all of the liquid is vaporized. The signal will eventually return to the baseline.

6. Record a few minutes of baseline before starting the next drop. Repeat the measurement twice more after which time you can "stop" the data acquisition.
Experiment 2: Laser Power Meter

1. Use a ring stand to secure the He-Ne laser above one of the aluminum cups, the one that has a blackened graphite mark on the bottom.

2. Turn the laser on to get the system set-up. Remember to change the file name. Once you are ready to start the data acquisition, leave the laser on but cover over the opening so that no light is directed into the calorimeter.

3. Start the data acquisition and allow the system to reach thermal equilibrium and a baseline to be established (about 5 min). Remove the covering over the opening and allow for a steady-state thermal signal to be reached (about 8-10 min.).

4. Cover over the calorimeter top so that the light is not reaching the inside of the calorimeter. Allow for a baseline to be established (about 5 min). Repeat the above twice more.

Experiment 3: Thermal Titration of a Saturated Solution of Tris (hydroxymethyl) aminomethane (THAM), C(CH₂OH)₃NH₂, with 3M Hydrochloric Acid

1. In a vial prepare 10-12 mg of dried C(CH₂OH)₃NH₂ (THAM) in a saturated solution, and position the vial in the sample aluminum cup.

2. Fill a 1 ml syringe with the titrant, 3M HCl. Secure the syringe in the sliding top in the usual manner. Lower the tip of the syringe into the vial. Tighten all screws and put the insulating box on the calorimeter.

3. Allow the system to reach thermal equilibrium. Start logs and record the signal for a few minutes. Deliver one drop of the titrant. This will produce an exothermic signal.

4. Wait for the signal to return to the baseline and deliver another drop. Repeat this until the endpoint of the titration is reached. Once the endpoint is passed small peaks are still observed and may be due to the heat of dilution of the HCl.

Experiment 4: Insect Metabolism

1. Remove the insulating top of the calorimeter. Gently place a small size insect into the sample aluminum cup. A small glass vial or watch glass may be used as a lid to keep the insect in the aluminum cup.

2. Put the insulating box on the calorimeter and allow the system to reach thermal equilibrium before you begin recording the data. Sometimes, it is a challenging task to keep the insect awake. Since the environment inside the calorimeter is
cold and dark, the insect may fall asleep. Find a way to wake up the insect (use of chemicals and other torture methods are not allowed).

3. Once the log is started, the recorded thermal signal will show a baseline metabolism of the insect and peaks corresponding to activity events.

4. Record the thermal signal for about 15 min. to accumulate thermal signals from the base metabolism and activity peaks.

**Experiment 5: Heat of Water Adsorption on Zeolites**

1. Place a few of these spheres (5-8 or more) in a special 2-drop calorimeter vial and put the vial in an oven for a day or longer. This drying procedure should drive off all water molecules.

2. Remove the zeolites from the oven immediately covering the container with a piece of aluminum foil. It is essential to have a tight seal so that no water is absorbed prematurely.

3. Measure the mass of the vial, containing the dry spheres, and sealed with Al foil. Place the vial containing the zeolites on a heat sink and leave it there until the vial reaches room temperature. To speed up the cooling you may use some ice, but keep in mind the zeolites should be at and not below room temperature when they are placed in the aluminum cup.

4. Mount a glass tube in the sliding top. Align the tube above the sample aluminum cup. Lower the glass tube into the aluminum cup. Make sure the tube does not touch the bottom or the sides of the aluminum cup.

5. Place a small beaker of water on the heat sink before putting the insulating box on the calorimeter. Wait a while to ensure thermal equilibrium as well as a water saturated atmosphere inside the insulating box.

6. Start logs and wait a few minutes before removing the Al foil on the vial. Remove the Al foil and immediately, using a paper funnel, slide the zeolites through the glass tube into the aluminum cup. Raise the glass tube carefully without moving it sideways. If you leave the glass tube in place, it may obstruct the flow of water vapors into the aluminum cup.

7. Place the aluminum foil back on the empty vial and measure its mass one more time. Use this and the previous mass measurement to obtain the mass of the dry zeolite spheres.

**Data Analysis**

1. **Preparation of Data.**
a. Open a file (e.g. calibration) in Excel. The format is an ACSII file. We want the default options so when the dialogue box comes up, you can click on finished.

b. Column A is the recorded voltage signal. Click on A to highlight it and then right clicking. Insert a column. The voltage data moves to column B.

c. In column A insert the time, 1 second intervals.

d. Repeat for each of the files that will be using when you work on that data.

2. Calibration. Open the data in GRAMS 32. There should be a shortcut on the desktop, if not, go to Programs - Galatica – GRAMS 32. Follow the additional instructions about opening the file in GRAMS. Use the “Applications” and “Integration” functions. For the calibration, the height (depth) information will be shown on the right hand side. Determine the calibration coefficient, ε, in W/V, using Eqn. 10. For your lab write-up prepare a graph from the calibration by plotting the thermal signal (V) vs. time (s). The graphs for the lab report can be finished on your own. It is more crucial to get the GRAMS information while in the lab period. In a data table, show the battery voltage, the resistor’s resistance, and the three height values (volts), their average and standard deviation.

3. Enthalpy of Vaporization. Determine the mass and then the moles of the organic solvent used in each drop. Open the data in Excel, insert a time column and again follow the instructions for opening the file in GRAMS. Integrate and record the area under each curve to get the total heat, Q, in Joules. Show the area for each peak in a table (v*s). Take the average, to get the mean value for one drop. Using the sensitivity factor, ε (W/V), from the calibration experiment convert the thermal signal (U_vols) to thermal power (W). This integrated signal now multiplied by ε is the heat of the evaporation (Q). Use, Q / mol, to determine the Δ_vapH. Finally, compare the enthalpy to the literature value (e.g. from the CRC online). In the lab report, include a graph of the volts versus time (or power versus time).

4. Heat of Laser Light Absorption. Open the data in Excel and follow the attached procedure for opening the data in GRAMS. Integrate and record the height (volts) (depth) of each heating event. Take and average to get the mean light absorbed in the aluminum cup. Show the height of each peak in a table. Using the sensitivity factor, ε, from the calibration experiment, once again, convert the average thermal signal to thermal power (W). Compare this with the stated power output from the laser. Calculate the percent absorbed in the aluminum cup and the remaining amount that was reflected.

5. Thermal Titration. Determine the moles of THAM used in the titration. Open the data in Excel and follow the attached procedure for opening the data in GRAMS. Integrate and record the area of each exothermic peak. Show these values in a table (v*s) and add the values to get the total thermal signal from the reaction. Convert the
thermal signal (V) to thermal power (W) by using $\varepsilon$. These integrated values are now the heat of the reaction (Q) Determine the $\Delta H$ of the reaction. Compare this experimental value with a literature value for the reaction. You may have to use Hess’s Law and the heats of formation to calculate a literature value for the heat of reaction. In your report show a plot of the thermal signal versus the time as you prepared in the preceding parts.

6. Insect Metabolism. Follow the above procedure for opening the data in Excel and then in GRAMS to integrate the peaks. Because the thermal signals are much smaller in magnitude, work with a small portion of the data, one that has a substantial peak(s). Record the areas under the peaks chosen. These are due to heat given off by the insect’s metabolism. Show these values in a table (v*s) and sum them. Use $\varepsilon$ to convert the values to power (W). These integrated values multiplied by $\varepsilon$ are now the heat given off by the insect (Q). Use Hess’s Law to calculate the $\Delta H$ of glucose ($C_6H_{12}O_6$) combustion. Using the calculated $\Delta H$ and the heat measured from the peaks, find the mass equivalent that would be used to evolve the experimental amount of heat, $Q$. Convert to this value of glucose to milligrams.

7. Heat of Water Adsorption on Zeolites. Follow the above procedure for opening the data in Excel and then in GRAMS. Convert the thermal signal (V) to thermal power (W) and graph the data. Integrate to get the total heat, $Q$. Use the mass of zeolites used to find the moles. Determine the $\Delta_{\text{adsorption}} H$ for the selected time period.

Questions and Further Thoughts

1. When analyzing the vaporization graphs, because the volume of organic solvent released in one drop is highly reproducible, the area under the peaks should be the same. However, you may notice that the peaks have broadened. In analyzing the environment of the calorimeter, what may be some reasons accounting for this peak broadening?

A large percentage of the $\Delta_{\text{vap}} H$ is used in breaking the intramolecular bonds. What fraction of the $\Delta_{\text{vap}} H$ of the organic solvent is spent on expanding the gas vapor? (Assume ideal behavior.)

$$
\Delta_{\text{vap}} H = \Delta_{\text{vap}} U + \Delta_{\text{vap}} (PV) \quad \text{where} \quad \text{volume liquid} << \text{volume vapor}
$$

2. The experimental value of the $\Delta_{\text{vap}} H$ of each of the organic solvents is compared with the literature value and assumes that the experimental value is measured at room temperature, 25°C. Many times however, the lab temperature fluctuates according to the outside temperature. If the temperature in the lab is 18°C, how will this affect the evaporation process. What steps should be included in the calculation to account for this temperature change? Calculate the $\Delta_{\text{vap}} H$ of one of the organic solvents at 18°C. What is the percent difference?
3. The time constant, $\tau$, is the thermal response time of the calorimeter. The Tian equation takes into account the heat content of the sample and holder cup:

$$P = \varepsilon (U + \tau \frac{dU}{dt})$$

Because the reactions performed in these experiments are slower reactions, the time constant is considered negligible and we calculated the heat flow rate as:

$$P = \varepsilon \times U$$

If we had taken the time constant into account, how would this have changed the results of the integrated peaks? Why does the time constant need to be accounted for in a fast reaction taking only milliseconds?

4. If you were constructing a calorimeter for long-term studies on insects, what factors would have to be taken into consideration?
List of References


### Appendix B. Nomenclature and Abbreviations Used

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$</td>
<td>Wave phase shift in quartz</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>Transition region in glassy materials, gradual main chain relaxation</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>Linear coefficients of thermal expansion, (length/(length * °C))</td>
</tr>
<tr>
<td>$\alpha_0$</td>
<td>Coefficient of expansion of an ideal material</td>
</tr>
<tr>
<td>$\alpha_G$</td>
<td>Coefficient of expansion of a glass</td>
</tr>
<tr>
<td>$a_{adsorbate}$</td>
<td>Vapor activity of gaseous adsorbate</td>
</tr>
<tr>
<td>$a_c$</td>
<td>Alternating current</td>
</tr>
<tr>
<td>$a_c$</td>
<td>Shift factor used to describe the diluent concentration effect on the polymer modulus</td>
</tr>
<tr>
<td>$a_t$</td>
<td>Temperature shift factor for WLF equation</td>
</tr>
<tr>
<td>$a_w$</td>
<td>Water vapor activity</td>
</tr>
<tr>
<td>$A$</td>
<td>Area</td>
</tr>
<tr>
<td>$\AA$</td>
<td>Angstrom</td>
</tr>
<tr>
<td>AFM</td>
<td>Atomic force microscope</td>
</tr>
<tr>
<td>$A_{SAW}$</td>
<td>kHz change in frequency due to a 1°C change per kHz of coating on resonator surface</td>
</tr>
<tr>
<td>AT</td>
<td>Temperature compensated cut of quartz, 35°15' off of Y axis</td>
</tr>
<tr>
<td>$\beta$</td>
<td>Transition region in glassy materials, relaxation of side groups</td>
</tr>
<tr>
<td>$\beta'$</td>
<td>Plasticizing parameter relating the diluent volume fraction to the free volume</td>
</tr>
<tr>
<td>B</td>
<td>Constant in WLF equation, close to unity</td>
</tr>
<tr>
<td>BT</td>
<td>Temperature compensated cut of quartz</td>
</tr>
<tr>
<td>BET</td>
<td>Sorption isotherm model by S. Brunauer, P.H. Emmett, and E. Teller</td>
</tr>
<tr>
<td>BVD</td>
<td>Butterworth Van Dyke equivalent electrical circuit</td>
</tr>
<tr>
<td>$\delta_c$</td>
<td>Concentration gradient</td>
</tr>
<tr>
<td>$c_m$</td>
<td>Elasticity</td>
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<td>$c_q$</td>
<td>Complex shear modulus of quartz</td>
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<tr>
<td>$c_q^0$</td>
<td>Storage shear modulus of quartz</td>
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<tr>
<td>C</td>
<td>Sensitivity constant for a 5 MHz QCM, 56.6 Hz µg⁻¹cm²</td>
</tr>
<tr>
<td>C</td>
<td>Capacitance</td>
</tr>
<tr>
<td>C</td>
<td>D'Arcy and Watt constant proportional to the number and affinity of weak binding sites</td>
</tr>
<tr>
<td>$C_p$</td>
<td>Heat capacity</td>
</tr>
<tr>
<td>$C_0$</td>
<td>Shunt capacitance or static capacitance, gold electrodes of the QCM, wires and clamping</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>C1</td>
<td>Capacitance of the resonating QCM</td>
</tr>
<tr>
<td>C1</td>
<td>Universal constant for the WLF equation</td>
</tr>
<tr>
<td>C2</td>
<td>Capacitance of the added mass to the QCM</td>
</tr>
<tr>
<td>C2</td>
<td>Universal constant for the WLF equation</td>
</tr>
<tr>
<td>CS</td>
<td>Conformation substate</td>
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<tr>
<td>C_s</td>
<td>Concentration of analyte in the sorbent phase, thin film</td>
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<tr>
<td>C_v</td>
<td>Concentration of analyte in the vapor phase</td>
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<tr>
<td>δ</td>
<td>Transition region of glassy materials, characterized by local motions</td>
</tr>
<tr>
<td>δ</td>
<td>Angle of phase shift between the applied stress and the resulting strain</td>
</tr>
<tr>
<td>δ</td>
<td>Acoustic wave decay length</td>
</tr>
<tr>
<td>tan δ</td>
<td>Tangent of the phase angle, ratio of the storage and loss components</td>
</tr>
<tr>
<td>D</td>
<td>Diffusion coefficient</td>
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<tr>
<td>D</td>
<td>D'Arcy and Watt constant proportional number of multilayer binding sites</td>
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<td>DAQ</td>
<td>Data acquisition board</td>
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<tr>
<td>dc</td>
<td>Direct current</td>
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<tr>
<td>dq/dt</td>
<td>Heat flux</td>
</tr>
<tr>
<td>DMA</td>
<td>Dynamic mechanical analysis</td>
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<tr>
<td>DSC</td>
<td>Differential scanning calorimetry</td>
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<tr>
<td>DTA</td>
<td>Differential thermal analysis</td>
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<tr>
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<td>Piezoelectric constant of quartz</td>
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<td>Seebeck coefficient</td>
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<td>Permittivity of quartz</td>
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<td>Seebeck voltage</td>
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<td>dE_s</td>
<td>Change in Seebeck voltage</td>
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<td>ΔE_2</td>
<td>Energy change in the thin film adsorbent</td>
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<td>f</td>
<td>Oscillation frequency</td>
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<td>f</td>
<td>Fractional free volume</td>
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<tr>
<td>f_h</td>
<td>Fraction of heat flow that does not pass through the thermopile</td>
</tr>
<tr>
<td>f_g</td>
<td>Fractional free volume at T_g</td>
</tr>
</tbody>
</table>
\( f_o \) Series resonant oscillation frequency
\( f_2 \) Polymer free volume
\( f_r \) Resonant frequency
\( \Delta f_s \) Frequency shift of the sorbent phase before vapor sorption
\( \Delta f_v \) Frequency shift of the sorbent phase due to vapor sorption
\( F \) Force
\( \text{FIMS} \) Functionally Important Motions
\( \gamma \) Strain in an elastic deformation
\( \gamma \) Transitions characterized by bending and stretching motions
\( \gamma \) D'Arcy and Watt constant proportional to water affinity of the multilayer binding sites
\( \gamma_0 \) Maximum strain in an elastic deformation
\( \gamma' \) Elastic deformation in-phase strain
\( \gamma'' \) Elastic deformation out-of-phase strain
\( \gamma^* \) Elastic deformation complex strain
\( \Delta G \) Gibbs free energy
\( \Delta_{\text{mixing}} G \) Free energy of mixing
\( \Delta_{\text{sorption}} G \) Free energy of sorption
\( G \) Conductance
\( G \) Complex shear modulus (of a thin film)
\( G' \) Storage shear modulus
\( G'' \) Loss shear modulus
\( G_c \) Thermal conductance per thermocouple plate
\( G_c \) Crystal conductance
\( GC \) Gas chromatography
\( \text{GPIB} \) General purpose interphase board
\( G_q \) Complex shear modulus of quartz
\( G_s \) Source conductance
\( G_t \) Total conductance
\( \eta \) Viscosity
\( \eta_f \) Viscosity of the thin film
\( \eta_q \) Viscosity of quartz
\( h \) g H₂O/ g lysozyme
\( h \) g adsorbate/ g adsorbent
\( h_f \) Thickness of the thin film
\( h_q \)  Thickness of quartz
\( h'p \)  Constant in the D'Arcy Watt sorption isotherm
\( H \)  Enthalpy
\( \Delta H \)  Enthalpy change
\( \Delta_{\text{adsorption}}H \)  Enthalpy of adsorption
\( \Delta_{\text{condensation}}H \)  Enthalpy of condensation
\( \Delta_{\text{crystallization}}H \)  Enthalpy of crystallization
\( \Delta_{\text{dehydration}}H \)  Enthalpy of dehydration
\( \Delta_{\text{denaturation}}H \)  Enthalpy of denaturation
\( \Delta_{\text{fusion}}H \)  Enthalpy of fusion
\( \Delta_{\text{hydration}}H \)  Enthalpy of hydration
\( \Delta_{\text{mixing}}H \)  Enthalpy of mixing
\( \Delta_{\text{reaction}}H \)  Enthalpy of reaction
\( \Delta_{\text{soption}}H \)  Enthalpy of sorption
\( \Delta_{\text{vaporization}}H \)  Enthalpy of vaporization
\( \text{HCC} \)  Heat conduction calorimeter
\( \text{HEW} \)  Hen egg white
\( \text{HPLC} \)  High performance liquid chromatography
\( I \)  Current amplitude
\( i \)  Square root of -1
\( \text{i.d.} \)  Internal diameter
\( \text{IGC} \)  Inverse gas chromatography
\( \varphi \)  Acoustic wave phase shift
\( j \)  Square root of -1
\( J \)  Current density across the quartz of the QCM
\( \text{J} \)  Flux
\( \kappa \)  Electromechanical coupling coefficient
\( k \)  Thermal conductivity
\( k_1 \)  Proportionality constant used for rate of evaporation in Langmuir isotherm
\( k_2 \)  Proportionality constant used for rate of condensation in Langmuir isotherm
\( k_a \)  Rate constant for adsorption
\( k_d \)  Rate constant for desorption
\( k_q \)  Wave vector for shear wave in quartz
\( K \)  Partition coefficient/ equilibrium constant
\( K^2 \) Quartz electromagnetic coupling coefficient
\( K_{q}^{o2} \) Electromechanical coupling factor for lossless quartz
\( K_{q}^2 \) Electromechanical coupling factor for lossy quartz
\( K_c \) Equilibrium constant
\( K_D \) Dissociation constant
\( K_{eq} \) Equilibrium constant
\( \lambda_q \) Wavelength of the propagating acoustic wave in QCM
\( l \) and \( l_f \) Thickness of the thin film
\( \Delta l_q \) Change in the thickness of the resonating quartz
\( l_q \) Thickness of resonating quartz
\( L \) Inductance
\( L_1 \) Inductance of the resonating QCM
\( L_2 \) Inductance of the added mass to QCM
\( LCR \) Simple circuit consisting of an inductor, capacitor, and resistor
\( LEM \) Lumped element model
\( LF \) Low frequency
\( \Delta m \) Change in the mass
\( m \) Mass
\( M \) Mass factor in determining \( Z_L \)
\( Mb \) myoglobin
\( metMb \) metmyoglobin
\( MFC \) Mass flow controller
\( m_{p,\infty} \) Mass of the film and the sorbed solvent vapor at time infinity
\( m_{p}^i \) Initial mass of the film and the sorbed solvent vapor
\( m_p^t \) Mass of the film and the sorbed solvent vapor at time \( t \)
\( \Delta M_q \) Change in the mass of resonating quartz
\( M_q \) Mass of resonating quartz
\( \nu \) Frequency
\( n \) Number of moles
\( n \) Number of the overtone frequency
\( n_a \) Gas molecules adsorbed per gram of solid
\( n_q \) The ratio of the overtone frequency over the quartz resonant frequency
\( n_1^s \) Moles of thin film adsorbent
\( n_2^g \) Moles of gaseous adsorbate
N  Odd integer for the resonator harmonic number
NMR Nuclear magnetic resonance
OCN Oscillating capillary nebulizer
o.d. Outer diameter
p Instantaneous power
p_i Partial pressure
p/p^0 Vapor activity
p^0 Saturation vapor pressure
P Pressure
P Heat flux, thermal power
PBS Phosphate buffer solution
P_{crystal} Power generate in quartz crystal
PDMS Polydimethylsiloxane
PIB Polyisobutylene
PLO Phase lock oscillator
PVA Polyvinylalcohol
θ Fraction of monolayer, fraction of surface coverage
q Charge
q_i Integral calorimetric heat
Q Quality factor
Q Heat
Q_i Integral heat of adsorption
QCM Quartz crystal microbalance
ρ_f Density of thin film
ρ_L Density of vapor when in liquid phase
ρ_q Density of quartz
ρ_s Density of sorbent phase
ρ Electrical resistivity
r Acoustic wave reflectance coefficient
r Dissipation factor
rf Radio frequency
r_m Rate of mass uptake
rpm Rotations per minutes
R Resistance
R Motional Resistance
R Ideal gas law constant
\(\Delta R\)  
Change in motional resistnace

RH  
Relative humidity

R1  
Resistance of the resonating QCM

R2  
Resistance of the added mass to the QCM

\(\sigma\)  
stress in an elastic deformation

\(\text{sub-} T_g\)  
Sub-glass transition, \(\beta, \delta, \gamma\) relaxations

\(\Delta S\)  
Entropy

\(\Delta_{\text{mixing}} S\)  
Entropy of mixing

\(\Delta_{\text{adsorption}} S\)  
Entropy of sorption

\(\Delta_{\text{vaporization}} S\)  
Entropy of vaporization

S  
Sensitivity constant of a thermopile

SAW  
Surface acoustic wave device

\(S_{\text{exp}}\)  
Experimental thermopile sensitivity

\(S_{\text{id}}\)  
Ideal thermopile sensitivity

\(\tau\)  
Time constant in Tian equation

\(\tau\)  
Average time of stay of vapor molecule on the film surface

t  
Time

T  
Temperature

TA  
Thermal analysis

\(T_c\)  
Critical temperature

TCP  
Thermocouple Plate

\(T_d\)  
Temperature of denaturation

\(T_g\)  
Glass transition temperature

TG  
Thermogravimetry

TGA  
Thermogravimetric analysis

TLM  
Transmission line model

\(T_m\)  
Temperature of melt

TSM  
Thickness shear mode resonantor

U  
Voltage

v  
Acoustic factor in determining \(Z_L\)

\(v_1\)  
Volume fraction of diluent

V  
Voltage

\(V\)  
Volume

\(V^o\)  
Specific volume at absolute zero

\(V^o_L\)  
Specific volume extrapolated from the liquid state to absolute zero

\(V^o_G\)  
Specific volume extrapolated from the glassy state to absolute zero
\( V_f \)  Free volume  
\( VI \)  Virtual instrument  
\( V_q \)  Speed of the propagating acoustic wave in QCM  
\( V_s \)  Volume of the adsorbent polymer phase  
\( V_t \)  Specific volume (cc/g)  
\( V_v \)  Volume of the adsorbate liquid vapor  
\( VCO \)  Voltage controlled oscillator  
\( \omega \)  Angular frequency = 2\( \pi \nu \)  
\( W \)  Uptake of adsorbate in D'Arcy and Watt isotherm  
\( WLF \)  Williams-Landel-Ferry equation  
\( W_m \)  Proportionality constant proportional to the energy of adsorption  
\( x \)  Displacement  
\( \delta x \)  Diffusion direction  
\( X_1 \)  Thin film adsorbate  
\( X_2 \)  Gaseous adsorbent  
\( X_C \)  Capacitive reactance  
\( X_f \)  Reactance of the thin film  
\( X_L \)  Inductive reactance  
\( Y \)  Admittance  
\( Y_{EL} \)  Electrical admittance  
\( z \)  Ratio of acoustic impedance in quartz over that in the thin film  
\( Z \)  Thermoelectric material property, figure of merit  
\( Z_{AB} \)  Complex electrical input impedance  
\( Z_{eq} \)  Acoustic impedance of QCM  
\( Z_L \)  Complex acoustic impedance due to the mass loading  
\( Z_{Lp}, Z_{L'} \)  Real part of acoustic load impedance  
\( Z_{Lpp}, Z_{L''} \)  Imaginary part of acoustic load impedance  
\( Z_m \)  Total motional impedance in an equivalent electrical circuit  
\( Z_q \)  Acoustic impedance of quartz  
\( Z_1 \)  Motional impedance of the unperturbed quartz crystal  
\( Z_2 \)  Complex motional impedance created by an acoustically thick film
Appendix C. Quartz Constants

(Lucklum et al., 1997; Lucklum & Hauptmann, 1997; Lucklum & Hauptmann, 2000)

\[ \rho_q = 2.651 \times 10^3 \text{ kg m}^{-3} \] density
\[ \varepsilon_q = 3.982 \times 10^{-11} \text{ A}^2\text{s}^4\text{kg}^{-1}\text{m}^{-3} \] permittivity
\[ e_q = 9.53 \times 10^{-2} \text{ A s m}^{-2} \] piezoelectric constant
\[ \eta_q = 3.5 \times 10^{-4} \text{ kg m}^{-1}\text{s}^{-1} \] viscosity
\[ c_q = 2.947 \times 10^{10} \text{ N m}^{-2} \] piezoelectric stiffened elastic constant
\[ v_q = 3347 \text{ m s}^{-1} \] shear sound velocity
\[ K_q^{02} = \frac{eq^2}{(\varepsilon_q c_q)} \] electromechanical coupling factor for lossless quartz
\[ K_q^2 = \frac{e_q^2}{(\varepsilon_q (c_q + j\omega \eta_q))} \] electromechanical coupling factor for lossy quartz
\[ L_q = \frac{(\rho_q h_q^3)}{(8A e_q^2)} \] motional inductance of quartz crystal
\[ C_0 = \varepsilon_q(A/h_q) \] static quartz capacitance
\[ \omega = 2\pi f \] angular frequency
\[ \alpha = \omega(h_q/v_q) \] wave phase shift in quartz
\[ Z_q = \rho_q v_q = \sqrt{\rho_q c_q} \] specific quartz impedance

List of References


Appendix D. “Fast Three Step Method” Equations Used in TK Solver Model

Adapted equations as entered in TK Solver 4.0, File 3_Step
Original Equations in (Behling et al., 1999; Lucklum & Hauptmann, 2001)

<table>
<thead>
<tr>
<th>Rule</th>
</tr>
</thead>
<tbody>
<tr>
<td>;Fast Three-Step Method for Shear Moduli Calculation from Quartz Crystal Resonator Measurements</td>
</tr>
<tr>
<td>;Carsten Behling, Ralf Lucklum, Peter Hauptmann, IEEE 46(6), Nov. 1999, 1431-1438</td>
</tr>
<tr>
<td>Lq=(q<em>hq^3)/(8</em>Aq*eq) ; motional inductance of quartz crystal</td>
</tr>
<tr>
<td>Zq=sqrt(ρq*cq) ; specific quartz impedance</td>
</tr>
<tr>
<td>ω=2*pi()*f</td>
</tr>
<tr>
<td>ZLpp=(f/f)<em>-1</em>pi()*Zq</td>
</tr>
<tr>
<td>ZLp=(R/(2<em>ω</em>Lq))*(pi()^*Zq)</td>
</tr>
<tr>
<td>;M=ω<em>p</em>hf ; mass factor</td>
</tr>
<tr>
<td>;Using the ZLp,ZLpp from above to get first approx. for G',G&quot; values for approximations</td>
</tr>
<tr>
<td>;(G0p,G0pp)=power((,0,-1)<em>((1/M^2,0)+(1/ZLp,0))</em>(1/3<em>M^3,0)</em>(M,ZLpp)</td>
</tr>
<tr>
<td>;(G1p,G1pp)=power((,0,-1)<em>power((-2</em>M,-ZLpp),2)/power((1-pi()^2,0),2))</td>
</tr>
<tr>
<td>;(G2p,G2pp)=power((,0,-1)<em>(-pi()^2/8</em>ZLp^2,0)+(0,M<em>ZLpp)+(pi()/4</em>ZLp,0)<em>csqrt(pi()^2/4</em>ZLp^2,-4<em>M</em>ZLpp))</td>
</tr>
<tr>
<td>;(G2bp,G2bpp)=power((,0,-1)<em>((-2</em>M,-ZLpp),2)/power((1+3/2*pi(),0),2))</td>
</tr>
<tr>
<td>;(G3p,G3pp)=power((,0,-1)<em>power((-2</em>M,-ZLpp),2)/power((1+3/2*pi(),0),2))</td>
</tr>
<tr>
<td>;(G4p,G4pp)=power((,0,-1)*power((-M,-ZLpp),2)/power((pi(),0),2))</td>
</tr>
<tr>
<td>;Using the G',G&quot; values to recalculate the ZL values</td>
</tr>
<tr>
<td>;(sqG0p,sqG0pp)=csqrt(0./G0pp)</td>
</tr>
<tr>
<td>;(ZL0p,ZL0pp)=csqrt(ρ<em>G0p,ρ</em>G0pp)<em>ctan(0,ω</em>hf*sqG0pp)</td>
</tr>
<tr>
<td>; (sqG1p,sqG1pp)=csqrt(0./G1pp)</td>
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<tr>
<td>;(ZL1p,ZL1pp)=csqrt(ρ<em>G1p,ρ</em>G1pp)<em>ctan(0,ω</em>hf*sqG1pp)</td>
</tr>
<tr>
<td>; (sqG2p,sqG2pp)=csqrt(0./G2pp)</td>
</tr>
<tr>
<td>;(ZL2p,ZL2pp)=csqrt(ρ<em>G2p,ρ</em>G2pp)<em>ctan(0,ω</em>hf*sqG2pp)</td>
</tr>
</tbody>
</table>
### Rules (continued)

\[ (\text{sqG2bp, sqG2bpp}) = \sqrt{0, /G2bpp} \]
\[ (ZL2bp, ZL2bpp) = \sqrt{\rho * G2bp, \rho * G2bpp} * \text{ctan}(0, \omega * h * \text{sqG2bpp}) \]

\[ (\text{sqG3p, sqG3pp}) = \sqrt{0, /G3pp} \]
\[ (ZL3p, ZL3pp) = \sqrt{\rho * G3p, \rho * G3pp} * \text{ctan}(0, \omega * h * \text{sqG3pp}) \]

\[ (\text{sqG4p, sqG4pp}) = \sqrt{0, /G4pp} \]
\[ (ZL4p, ZL4pp) = \sqrt{\rho * G4p, \rho * G4pp} * \text{ctan}(0, \omega * h * \text{sqG4pp}) \]

- **Iteration process, solving for**

<table>
<thead>
<tr>
<th>Equation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>((0,0) = (ZL0p * \phi 0,0) - \text{ctan}(0, M * ((1/3) * (\phi 0^3))))</td>
<td>(\phi \sim 0)</td>
</tr>
<tr>
<td>((0,0) = (ZL1p * \phi 1,0) - \text{ctan}(0, M * ((1 - (\pi/2) + (2 * 1))))</td>
<td>(\phi \sim \pi/4)</td>
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<tr>
<td>((0,0) = (ZL2p * \phi 2,0) - \text{ctan}(0, M * ((\pi/2) - \phi 2)))</td>
<td>(\phi \sim \pi/2)</td>
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<td>((0,0) = (ZL2bp * \phi 2b,0) - \text{ctan}(0, M * ((8 * \phi 2b((\pi/2) - (4 * \phi 2b^2))))</td>
<td>(\phi \sim \pi/2)</td>
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<tr>
<td>((0,0) = (ZL3p * \phi 3,0) - \text{ctan}(0, M * ((-1 - ((3/2)) * \pi) + (2 * \phi 3))))</td>
<td>(\phi \sim 3/4\pi)</td>
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<td>((0,0) = (ZL4p * \phi 4,0) - \text{ctan}(0, M * (-\pi) + \phi 4)))</td>
<td>(\phi \sim \pi)</td>
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List of References


VITA

Sister Rose B. Mulligan I.H.M.

Place and Date of Birth

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<td>57th Annual Calorimetry Conference</td>
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“Applications of the Quartz Crystal Microbalance/Heat Conduction Calorimeter: Measuring the Thermodynamic and Mechanical Hydration Effects on a Myoglobin Thin Film.”

Smith, A. L.; Mulligan, R.