A MICROMACHINED THERMAL SENSOR FOR BIOCHEMICAL SENSING
AND POLYMER CHARACTERIZATION

A Thesis in
Electrical Engineering
by
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ABSTRACT

This thesis presents a micromachined calorimetric sensor for the measurement of the molar enthalpy change of enzymatic reactions and their characterization using high resolution thermal conductivity measurements. A polysilicon heater and a microthermopile sensor have been fabricated on a 2 µm thick, freestanding nitride-oxide-nitride membrane and integrated with glass or polymer microfluidic channels. The p-type polysilicon/gold microthermopiles have a responsivity of 1 V/W, temperature sensitivity of ~4.7 mV/K, and a time constant of less than 100ms.

Measurements of the heat of reaction from enzymatic catalysis of glucose, hydrogen peroxide and urea, were performed using glucose oxidase, catalase, and urease respectively in continuous flow configuration using the integrated microfluidic channel. A sensitivity of 53.5µV/M for glucose, 26.5µV/M for hydrogen peroxide and 17µV/M for urea was obtained. Detection limit for glucose measurement in the continuous flow mode is ~ 2 mM (30 pmole).

AC calorimetric measurements were performed by introducing a periodic heat signal using the heater and detecting the frequency dependent thermal signal response in the presence of various fluids and polymers. Thermal conductivity of different fluids and five typical polymers used in microfabrication was measured using this device. The results of the predicted thermal conductivity are compared to those available in literature for polyimide (~ 2%) and SU-8® (< 10%) polymers.

The device has also been used for monitoring real-time biochemical reactions as the thermal properties change with time due to the changing composition of the test fluid
from reactants to products. Real-time enzymatic reaction for (glucose, urea) and antigen-antibody binding reaction (BSA, human fibrinogen) have been observed. This is the first demonstration of monitoring biochemical reactions in real-time using this technique.

In summary, a versatile thermal sensor for the study of thermal characteristics of various biochemical samples and reactions has been designed. The integrated microfluidic channel allow for continuous flow testing of various samples. In addition to the determination of the thermal properties of various polymers and biomaterials, the sensor platform can be easily implemented as a thermal fluid flow sensor and a hydrogen sensor.
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Chapter 1

Introduction

A micromachined calorimeter for biochemical sensing and thermal characterization of polymers is presented in this thesis. Chapter 1 will begin with a brief description of the motivation and the goal of this thesis. This is followed by a review of biosensors including the principles of operation, figures of merit, and a brief review of the current developments. Basic concepts of bioenergetics, thermodynamics, and heat transport in biological samples are discussed in section 1.3. Section 1.4 presents a detailed description of calorimetric biosensors and biological reaction kinetics in the context of thermal sensing. The classification, history and previous work on calorimetric biosensors are introduced. In section 1.5, a review for the state of the art of large and micro-scale calorimeters is given. Finally, the chapter ends with a plan of the work to be presented in this thesis.

1.1 Motivation and Aim

Evolution, absorption, and transport of heat energy in biological systems are the very fundamental basis of life. All living things need energy to do work, such as maintain cell structure, function, grow and reproduce. Since energy cannot be created or destroyed;
cells typically capture, store and then use energy to sustain the basic metabolic activities. The ability to convert different forms of energy evolved early in life, and different cells show remarkably similar systems. Recent developments in micromachining technologies have made it possible to create miniaturized sensors capable of studying such fundamental properties. The aim of this thesis is to design, fabricate and demonstrate fundamental thermal measurements on biological molecules including the study of the evolution of heat in exothermic biochemical reactions and the measurement of the thermal properties of biological molecules and biological fluids. The micromachined thermopile based thermal sensor integrated with microfluidic reaction test-chamber allows for sampling of ~15 nl of sample volumes and real-time measurement of biochemical reactions.

1.2 Biosensor

Biosensor is a device, which responds to the presence of a particular analyte in a selective way through a biochemical reaction and can be used for qualitative or quantitative determination of the analyte. It incorporates a biological recognition element connected to a transducer (Fig. 1.1).
1.2.1 Recognition elements

Recognition elements are the key components for responding to the target analyte without interferences from others and to integrate the biological component with the selected transduction method. Depending upon the working principle, recognition elements can be divided into two categories—catalysis type and binding type. For the catalysis type, the biomolecule catalyses a change in a physicochemical parameter, while the binding type relies upon a strong binding of the analyte to the biomolecule [1]. There are four major biomolecules used as recognition elements which are listed in Table 1.1.

Figure 1.1: Schematic representation of biosensor construction.
1.2.2 Transducer Technologies

In this section we will briefly review the most important biosensing techniques. Excellent reviews of biochemical sensing principles, and their practical implementations as sensor systems are available [2-4].

1.2.2.1 Electrochemical Transducers

i. Potentiometric: sensors measure the potential difference between a reference electrode and the transduction electrode. Potential variation is due to the selective accumulation of the charged species on the sensor surface. The potential is proportional to the logarithm of the analyte concentration.

<table>
<thead>
<tr>
<th>Type</th>
<th>Biomolecule</th>
<th>Analyte</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalysis</td>
<td>Enzyme (Tissue, micro-organisms)</td>
<td>Substrate</td>
<td>Highly selective, catalytic activity, fairly fast acting, most commonly used, deactivation after relatively short period of time.</td>
</tr>
<tr>
<td>Binding</td>
<td>Antigen</td>
<td>Antibody</td>
<td>Highly selective and sensitive, powerful binding, no catalytic effect</td>
</tr>
<tr>
<td></td>
<td>Receptor Proteins</td>
<td>Hormones, Neurotransmitters</td>
<td>Have an affinity for a range of structurally related compounds.</td>
</tr>
<tr>
<td></td>
<td>Nucleic Acids</td>
<td>Nucleic Acids</td>
<td>Specific base-pairing</td>
</tr>
</tbody>
</table>

Table 1.1: Four major biomolecules used as recognition elements and their characterizations.
ii. Amperometric: sensors measure the Faraday current flow due to oxidation (reduction) of the biological system or of a species involved in the reaction of the analyte with the biomolecule. The current magnitude at a certain voltage is directly proportional to the analyte concentration. Most commercial biosensors are amperometric.

iii. Conductometric: Sensors measure the change in the electrical conductivity of the solution during reactions.

Field-effect transistor (FET) based biosensors mainly use potentiometric technique, but could also use amperometric or conductometric techniques.

1.2.2.2 Photometric Transducers

The techniques include:

i. Absorption spectroscopy measures ultraviolet or visible absorbance at a certain wavelength. The measured absorbance is linearly proportional to the analyte concentration.

ii. Fluorescence spectroscopy measures re-emitted radiation due to excited species decay. The change of wavelength between re-emitted radiation and original excitation gives advantages in analysis.

iii. Luminescence spectroscopy measures light emission without heat during biochemical reactions.

iv. Reflection spectroscopy measures the material adsorbed on an optical surface.
v. Light scattering spectroscopy measures particles size, size distribution and flowing in a medium by analyzing the scattered light intensity fluctuation as a function of time.

vi. Surface Plasmon Resonance (SPR) technique monitors the refractive index of the evanescent field layer, which will change as the analyte-receptor binding interaction occurs on the surface.

1.2.2.3 Piezoelectric Transducers

i. Gravimetric: These devices measure the mass changes occurring on the surface of the piezoelectric materials (crystals) during biochemical reactions. Typically, the piezoelectric crystals are configured as precision oscillators. The biomolecules are made to tightly bind to the surface such that the oscillation frequency shift of the piezoelectric crystal is proportional to the mass change. For quartz crystals, this relationship can be written as:

\[
\Delta f = -2.3 \times 10^6 f^2 \frac{\Delta m}{A_s}
\]

where \( \Delta f \) is the change in resonant frequency of the coated crystal, \( f \) is the resonant frequency of the unperturbed crystal, \( A_s \) is the area and \( \Delta m \) is the deposited mass. Since quartz is typically used in these sensors, the device is also known as a quartz crystal microbalance (QCM).

ii. Surface acoustic wave (SAW): The system uses changes in the propagation properties of surface acoustic waves caused due to the presence of biomolecular films. Surface acoustic waves of different kinds such as Rayleigh waves, Lamb
waves, etc can be used for such measurements [5]. Surface acoustic waves are typically generated using a radio frequency source in the 100-500 MHz range applied to the surface of a piezoelectric crystal using an interdigitated electrode configuration. The propagation properties of the waves are detected by a second set of electrodes. Since the waves only penetrate into the crystal to a depth of about one wavelength, they interact only with the surface coating of analyte species. Lithium niobate (LiNbO$_3$) is the most commonly used crystal for SAW device.

1.2.2.4 Thermal Transducers

i. Calorimetric: The devices measure heat production or absorption involved in biochemical processes. The heat produced is typically related to the amount of the substance analyzed.

ii. Thermal Property devices: Change of thermal properties such as specific heat, thermal conductivity and thermal diffusivity are measured during bioreaction.

The advantages and disadvantages of above transduction methods are listed in Table 1.2.
1.2.3 Biosensor Performance Specifications

Since biochemical sensors operate with many different detection techniques and are available in many different configurations, it is important to be able to specify their performance capabilities in a universal set of metrics. Any given sensor may be specified using several or all of the metrics listed below. However, there exists no sensor that can achieve high performance specifications on all of the listed metrics. In fact, current efforts in biochemical sensing technologies are focused on improving many of these parameters.

<table>
<thead>
<tr>
<th>Transduction Principle</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Electrochemical</strong></td>
<td>Simple, Low cost</td>
<td>Potentiometric: noise sensitive, Amperometric: mass transport properties are critical, Electrode fouling is a major issue for both these sensors</td>
</tr>
<tr>
<td><strong>Optical</strong></td>
<td>In-vivo application, no electrical interference, flexibility, small size, Multi-species detection, High sensitivity</td>
<td>Optical properties required for reaction media</td>
</tr>
<tr>
<td><strong>Piezoelectric</strong></td>
<td>No label, no additional chemicals.</td>
<td>Non-specific binding may cause error, SAW: not perform well in liquids due to large damping.</td>
</tr>
<tr>
<td><strong>Thermal</strong></td>
<td>General detection principle, possibility for continuous-flow operation, simple procedures</td>
<td>High cost, slow response</td>
</tr>
</tbody>
</table>

Table 1.2: Comparison of different transduction principles.
i. Sensitivity: The amount of change in a sensor’s output in response to a change in
the sensor’s input over the sensor’s entire range.

Sensitivity range/linear range/detection limits: it is important to know what
concentration range is detectable and what section of this range has linear
response. The lower level of this range is normally taken as the detection limit.

ii. Accuracy: The degree of correctness with which a measuring system yields the
“true value” of a measured quantity.

iii. Resolution: The smallest increment of change in the measured value that can be
determined from the instrument’s readout scale.

iv. Precision: The difference between the instrument’s reported values during
repeated measurements of the same quantity. Typically determined by statistical
analysis of repeated measurements.

v. Selectivity: The ability of a sensor to measure only one input parameter. Most
sensors will respond mainly to one analyte, with a limited response to other
similar analytes. This behavior is principally a function of the selective
component.

vi. Reversibility: One of the important features of biochemical sensors is its
capability to be used repeatedly. Reversibility relates to the ability of the sensor to
revert back to the unperturbed specifications when the input is completely
removed. Achieving high reversibility is a major challenge in biochemical
sensors.

vii. Response time: Biosensors output typically requires a certain time to stabilize,
which among many other factors includes the time for the biochemical reactions
to attain equilibrium rate. The required time is known as response time, which can vary from a few seconds to a few minutes.

viii. Recovery time: the time required between two measurements and is related to the reversibility of the biochemical reactions described earlier.

ix. Working lifetime: the time after which the response has declined by a given percentage (i.e. 5%) during continuous use.

1.2.4 Miniaturization of Biochemical Sensors

The advantages of microbiosensor over the conventional ones are: first, small sample quantity is required which is important since bio-analytes are very expensive or may not be easy to obtain in large quantities; second, their small size allows for implantation of the microbiosensor for in-vivo measurements; third, mass production via batch fabrication techniques makes the biosensors inexpensive to be disposable and their fabrication in array format possible; and fourth, the possibility of integration with on-chip electronics.

Miniaturization allows the sampling of small fluid volumes (~1nl - 1µl). But the sample volume required is fundamentally dictated by the desired analyte concentration. Fig. 1.2 [6] shows that biological chemicals associated with clinical chemistry assays (between $10^{14}$ and $10^{20}$ copies/ml) and immunoassays (between $10^7$ and $10^{18}$ copies/ml) might be readily assayed with very small sample volumes, in the range between picoliters and microliters. Unfortunately, numerous chemicals (and organisms) are routinely present at much lower concentrations, from less than 100 to $10^7$ copies/ml. To ensure at least one target molecule per sample volume, any test system must operate above
diagonal line in Fig. 1.2. These low-concentration samples include most sources of DNA. And the minimum sample volume required for accurate DNA assays is 100 µl. Otherwise preconcentration is needed.

Figure 1.2: Sample volume: scaling of concentrations and volumes [6].

Most biochemical analyte samples are complex mixtures of many different molecules. For example, the human blood not only has glucose in it, but several hundreds of other proteins, sugars, salts and cells. While biocatalysts and proteins (antibodies) are highly selective molecules, they still respond to a small range of closely related
molecules, i.e., it is nearly impossible to functionalize a sensor to have an infinite selectivity. The advent of microsensor systems has now made it possible to achieve high selectivity through the use of complex algorithms uniquely interpreting the data acquired through an array of sensors functionalized using a range of biomolecules with different sensitivities to the analyte molecule.

Reversibility is another traditional area of weakness in the performance of biochemical sensors. It mainly arises from the fact that reversing biochemical reactions is not always 100% efficient and therefore leads to a constant drift in the performance of the sensors. Microsensor systems are able to address this limitation by having real-time, on-chip calibration capabilities or alternatively through use and throw sensor modules. Using silicon-processing techniques to mass-produce miniaturized biosensors will dramatically reduce the cost per device. The cost per device can be expressed as [7]:

$$\text{Biosensor Cost} = \frac{\text{Design Cost}}{(# \text{ of Wafers}) \times (# \text{ of Sensors/Wafer})} + \frac{\text{Wafer Production Cost}}{# \text{ of Sensors/Wafer}} + \text{Assembly \\& Testing Cost per sensor}$$

For example, if the design cost is $200,000; wafer production cost is $500; each wafer contains 500 chips; assembly and testing cost per chip is $0.5. The cost per chip is $41.5 for 10 wafers being produced. The cost per chip drops to $1.9 for 1000 wafers being produced. This illustrates the necessary of mass production in order to achieve low cost. If the device is cheap enough to be disposable, the time-consuming recovery process is not needed. This will reduce the expensive lab hour and make bioanalysis more affable. Since new processes and techniques for micromachining and micropatterning of new non-silicon thin films are being constantly developed, biosensors using novel non-silicon sensors can also be batch fabricated.
Finally, miniaturization of biochemical sensors through the use of microfabrication techniques has made it possible to configure sensors with high sensitivity specifications which could not be achieved at the macroscale. The work described in this thesis on thermal biosensors is an example of such an improvement in the sensitivity.

1.2.5 Biosensors Applications

Biosensors have been used in many fields, including clinical diagnostics, process monitoring and environmental control. Clinical diagnostic applications claim close to 92% of the biosensor market, which was about $180B in year 2001 [8]. Measurements of blood, gases, ions and key metabolites are highly valuable for diagnostics under critical metabolic circumstances. Glucose, urea, lactate, sodium, potassium and calcium are some of the routinely needed assays for diagnostic work. The ideal biosensor might be an implanted sensor integrated with a microprocessor controlled drug delivery system for continuous monitoring of a metabolite. Such a device would be attractive for the treatment of chronic illnesses.

Biosensors also find widespread use for processes control in food and beverage industries. They can perform off-line or on-line real time monitoring on a range of reactants and products, such as sugars, yeasts, alcohols and carbon dioxide. Typically, their use helps to increase yields, improve automation and reduce reliance on human judgment. Biosensors also used to measure potential pollution analytes in air, water, soils and other environmental situations.
Ideally, biosensor should be inexpensive, rapid, automated, capable of sensing multiple analytes, highly selective, accurate, and sensitive. At present though, most biosensors fall short of these desired performance specifications and thus their use has been limited to specific problems where the necessary requirements are met. Most biosensors are unable to measure analyte concentration in the $10^{-9}$M range or below, which is often the requirement in the measurements of hormones and other serum components [2]. For on-line monitoring, implanted biosensors must additionally overcome the drawbacks arising from the “harsh conditions” such as the formation of biofilm to which some of them may be subjected. In these circumstances the availability, stability and preparation of the suitable receptor are also limiting factors.

The work described in this thesis relates to microfabricated thermal sensors for biochemical sensing and investigations. In the next section, detailed introduction of the principle of operation and the state of the art of thermal biosensors is reviewed.

### 1.3 Biological Thermodynamics and Heat transport

#### 1.3.1 Bioenergetics, Thermodynamics, and Calorimetry

Metabolism is the sum of all chemical activities in an organism, which can be divided into two broad classes:

i. Anabolism is the process in which larger more complex molecules are synthesized from simpler substances. Example of such a process is the synthesis of proteins from amino acids.
ii. Catabolism is the process in which larger molecules broken down into smaller ones. Example of such a process is the breakdown of starch molecules into glucose. In general, Catabolic processes release energy that drives anabolic processes.

Thermodynamics is the study of energy and its transformations. Entropy ($S_0$) measures system disorder/randomness. Enthalpy ($H$) is total potential energy of a system. Enthalpy is also thought of as the heat content of system. Free energy ($G_0$) is the amount of energy available to do work in a system, and is related to the entropy (disorder) and enthalpy (heat) as follows [9]:

$$G_0 = H - TS_0$$  \[1.3\]

In a typical chemical reaction occurring at constant temperature, the change in the free-energy can be related as:

$$
\Delta G_0 = \Delta H - T\Delta S_0
$$  \[1.4\]

where $T$ is temperature in Kelvin. Exergonic reactions release energy and are spontaneous reactions. Endergonic reactions gain free energy. Energy has to be supplied from surroundings. Metabolic energy drives endergonic reactions in cells. Most biochemical reactions are reversible. When reaction reaches dynamic equilibrium, rate of forward and reverse reactions are equal. For example: Adenosin triphosphate (ATP) is the universal currency of free energy in cells, which links catabolic reactions and anabolic reactions. ATP is a nucleotide consisting of an adenine, a ribose and a triphosphate unit. Because its triphosphate unit contains two phosphoanhydride bonds, ATP is an energy rich molecule. When ATP is hydrolyzed to adenosine diphosphate (ADP) and orthophosphate (Pi), about 32 kJ/mol of free energy is liberated [10].
ATP energy is used to drive endergonic reactions. ATP is constantly formed and a typical cell has ten times more ATP than ADP. However, ATP can not be stored for long since it is always being used. 10 million ATP's are made and used in every second in every cell. In turn, ATP is formed from ADP and Pi when fuel molecules are oxidized in chemotrophs or when light is trapped by phototrophs. This cycle is the fundamental mode of energy exchange in biological systems.

Calorimetry is the science of measurement of the amount of heat evolved or absorbed in a chemical reaction, change of state, or formation of a solution. Since, most biological reactions involving the breakdown of complex molecules are more or less always exothermic in nature, these can be investigated using calorimetric analysis. Further, calorimetric measurements by themselves are not reaction specific and can thus be used with a wide range of materials. Specificity to identify or perform a selective assay can be achieved by coating the calorimeter with specific catalysts such as enzymes, antibodies or even single stranded oligonucleotides for DNA sequence analysis [11]. When the analyte is exposed to the catalyst, the biochemical reaction begins and its evolution in terms of total amount of heat generated and kinetics is proportional to the reactants concentration as well as the rate constants of the reaction.

Thus, when an exothermic biological reaction is performed on a micromachined, thermally isolated structure, the small thermal mass and good thermal isolation will allow the temperature of the structure to rise. This rise in temperature can be very accurately measured in real time using a micro thermal sensors integrated on the structure. The
microcalorimeters have high sensitivity and fast response time for real-time analysis of very small quantities of analytes. In addition, heat transport can be studied with the same structure if a heat source is integrated.

1.3.2 Heat Transport Studies on Biological Materials

The three-dimensional structures of biological molecules such as proteins are vital to the function of bimolecules within a living system. As will be demonstrated in this work, understanding the energetic basis of these structures has not only fundamental but also practical significance. Study of thermal properties of biological molecules and assays can be used for monitoring the conformational change and binding events. Thermal properties such as the thermal conductivity, specific heat and thermal diffusivity implicitly contain information about the various degrees of freedom of the molecular species, and how they transport heat energy.

For example, the study of conformational or phase changes in proteins through the measurement of the changes in specific heat allows us to understand their structure and function. The most interesting feature of proteins is that in the functional, so called native state, they are folded into unique conformations. And these structures are responsible for the unique ability of biological molecules to recognize their functional partners and thereby fulfill their work. All literature is in agreement that hydrophobic interaction is the major contributor to the stability of the native state of the protein [12, 13]. In terms of the hydrophobic interactions and their affect on stability, the heat capacity of nonpolar compounds dissolved in water is found to be directly proportional to the surface area of number of solvated water molecules in the first solvation shell. It
suggests that the hydrophobic contribution in stabilizing the native protein could be evaluated from the change in the heat capacity combined with the temperature parameters.

Thus, if heater structures are integrated with a calorimeter, the propagation of a heat pulse through the analyte can be studied. Since the propagation of the heat pulse depends on the thermal conductivity and specific heat of a given material, these will change as the material changes from being predominantly reactants to becoming predominantly products as a biochemical reaction or “energetic” change in a biochemical molecule of interest occurs. This provides a new and independent method to investigate biological reactions and their products.

Fundamental thermal properties of interest and their relationship to each other are briefly described next.

1.3.2.1 Thermal Conductivity

Heat conduction in solids can be thought of as a simple molecular exchange of kinetic energy. The basic law defining the propagation of heat in a one-dimensional homogenous solid is given by:

\[
\frac{dQ}{dt} = -\kappa A_p \frac{dT}{dx}
\]

the quantity of heat \( dQ \) conducted in the \( x \)-direction of a uniform solid in time \( dt \) is a product of the conducting area \( A_p \) normal to the flow path \( x \), the temperature gradient \( dT/dx \) along this path, and the thermal conductivity \( \kappa \) of the conducting material. The negative sign indicates the fact that heat always flows from regions of higher temperature.
to those of lower temperature. Thermal conductivity is an intrinsic property of a conducting material defined for a homogenous conducting solid by eq. 1.6. It has the S.I. units of W/m-K. In general, thermal conductivity is not constant, but is dependent on, among other things, temperature, pressure, and porosity of a conducting material.

1.3.2.2 Specific Heat

The specific heat is the amount of heat per unit mass required to raise the temperature of a given sample by one degree Celsius. However, to make the specific heat independent of the volume/mass/quantity of the sample, specific heat is typically given as specific heat per unit mass (J/kg-K), molar specific heat (J/mol-K) etc. Expressed in these units, the specific heat capacity is a material property (constant) for a given temperature condition and does not depend upon it shape or size. The relationship between heat and temperature change of a sample is usually expressed in the form shown below where \( c \) is the specific heat and \( m \) is the mass. The relationship does not apply if a phase change is encountered, because the heat added or removed during a phase change does not change the temperature.

\[
dQ = m c dT
\]  

1.3.2.3 Thermal Diffusivity

The thermal diffusivity is a measurement of heat propagation speed during the change of temperature over time and is defined as the thermal conductivity divided by the product of specific heat times the density of a material.
A large value of $D$ indicates a rapid diffusion of the heat through the material. A high value of thermal diffusivity can arise either due to a high thermal conductivity or due to a small heat capacity value of the material.

1.4 Calorimetric Biosensor

1.4.1 Biological Kinetics

Metabolic pathways are sequences of bioreactions. Almost all cell reactions involve enzymes. Most enzyme-catalyzed reactions are exothermic, which may be used as a basis for measuring the rate of reaction and analyte concentration (Table 1.3).

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Analyte</th>
<th>$-\Delta H$ (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>Hydrogen Peroxide</td>
<td>100</td>
</tr>
<tr>
<td>Glucose Oxidase</td>
<td>Glucose</td>
<td>80</td>
</tr>
<tr>
<td>Penicillinase</td>
<td>Penicillin G</td>
<td>67</td>
</tr>
<tr>
<td>Lactate Dehydrogenase</td>
<td>Na pyruvate</td>
<td>62</td>
</tr>
<tr>
<td>Ureasu</td>
<td>Urea</td>
<td>61</td>
</tr>
<tr>
<td>Cholesterol Oxidase</td>
<td>Cholesterol</td>
<td>53</td>
</tr>
<tr>
<td>Uricase</td>
<td>Uric Acid</td>
<td>49</td>
</tr>
<tr>
<td>Trypsin</td>
<td>Peptides</td>
<td>30-10</td>
</tr>
<tr>
<td>Invertase</td>
<td>Sucrose</td>
<td>20</td>
</tr>
<tr>
<td>Chymorypsin</td>
<td>Esters</td>
<td>16-4</td>
</tr>
</tbody>
</table>

Table 1.3: Molar enthalpies of enzyme-catalyzed reactions [14][2].
The rate of reaction catalyzed by a soluble enzyme can be described by *Michaelis-Menten* kinetic equation [15].

\[
A + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EA \longrightarrow \overset{k_2}{P + E} \tag{1.9}
\]

where \(E\) is the enzyme, \(A\) is the analyte, \(P\) is the product and \(EA\) is the enzyme-analyte complex. For a bath reaction, the balances for \(A\) and \(EA\) are written as:

\[
\frac{dA}{dt} = -k_1AE + k_{-1}(EA) \tag{1.10}
\]

\[
\frac{d(EA)}{dt} = k_1AE - (k_{-1} + k_2)(EA) \tag{1.11}
\]

with initial condition at \(t=0\): \(A=A_0\), \(E=E_0\), \((EA)=0\).

The enzyme concentration is usually much lower than the substrate; most of enzyme is present during the reaction in the form of enzyme-analyte complex, \(EA\). And it is possible by assuming a quasi-steady state.

\[
\frac{d(EA)}{dt} = 0 \quad (E<<A_0) \tag{1.12}
\]

\[
E_0 = E + (EA)
\]

Then \(EA\) and \(E\) concentration can be solved from eq. 1.11 and 1.12. Substituting for \(E\) and \(EA\) in eq 1.10 gives the *Michaelis-Menten* equation.

\[
r_A = -\frac{dA}{dt} = \frac{r_{max}A}{K_M + A} \tag{1.13}
\]
where $r_A$ is the reaction rate, maximum reaction rate $r_{\text{max}} = k_2E_0$, and the Michaelis-Menten constant $K_M = (k_{-1} + k_2)/k_1$. The typical value for $K_M$ is between $10^{-6}$ and $10^{-1}$ M. The Michaelis-Menten equation exhibits three distinct regions for the reaction rate (Fig. 1.3). The low concentration region ($A \ll K_M$) can be approximated by first-order kinetics: $r_A = -dA/dt = r_{\text{max}}A/K_M$. The rate of depletion of reactant is proportional to the instantaneous concentration of sample. The reactant concentration decays exponentially: $A = A_0 \exp(-r_{\text{max}}/K_M)$. For high substrate concentration ($A >> K_M$), the relation approaches zero-order: $r_A = r_{\text{max}}$. And the rate of reaction is independent of substrate concentration and is constant at the maximum value. In the intermediate substrate concentration range ($0.1 K_M < A < 10 K_M$), the full Michaelis-Menten equation must be used to guarantee an accurate $r_A$.

---

![Graph](image)

**Figure 1.3:** Reaction rate vs. substrate concentration for the Michaelis-Menten equation [15].
1.4.2 Calorimetry Classification

There are three general types of calorimeters classified by how heat transfer takes place between the reaction vessel and surroundings [16].

i. Heat conduction calorimetry: Heat exchanges between chamber and isothermal heat sink surrounding. Temperature changes are measured as voltage output of a thermoelectric sensor between chamber and the sink. Heat sink is kept isothermal to keep heat transfer coefficients constant. The temperature of reaction chamber is proportional to heat generation.

ii. Isoperibol (Adiabatic) calorimetry: Nearly no heat transfer occurs. The heat quantity evolved during an experiment is equal to the product between the measured temperature changes and the heat capacity of the vessel and its content.

iii. Isothermal (power compensation) calorimeter: Temperature of the reaction vessel is kept constant by heating or cooling.

Other ways of classification is by the mode of operation:

i. Static: isothermal, isoperibol, adiabatic

ii. Dynamic: scanning of surrounding, isoperibol scanning, adiabatic scanning

and the construction mode:

i. Single measurement

ii. Twin or differential measurement

For each form of calorimetry, if it is used in solution condition, there are three types of reactant mixing method. These are titration, batch addition and flow mixing. In titration calorimetry, one reactant is titrated repeatedly or continuously into the other
reactant. Batch addition means mixing of the total volume of both reactants in one operation. Most flow calorimeters have a reaction zone where the two flow reactants mix to form product stream. In all the cases, the temperature change or the heat produced in the system is measured.

Heat conduction calorimetry is relatively simple to fabricate and operate because neither feedback temperature control system nor complex thermal isolation structure is needed. Most microscale calorimeters are heat conduction calorimeters. They have been used to study (bio)chemical reactions, material thermal properties, gas/fluid type and gas pressure. Some work has been done on power compensation calorimetry for material thermal properties and biomolecule binding studies.

1.5 State of the Art

Calorimetry is one of the oldest techniques, which began at the end of 18th century during the studies of animal respiration. It suffered from long equilibration (response) times and solution calorimetry only became a routine analytical tool in the last 30 year. Sensitivity, small sample volume size and high throughput are some of the areas of recent focus in calorimetric instrument development.

1.5.1 Large Sensors

1.5.1.1 Enzyme Thermistor (ET)

The “enzyme thermistor” (ET) concept pioneered by Mosbach in 1974 is the highest sensitivity calorimeter until now. Up to 60~100μl sample can be studied per hour [17].
The simplest design for this concept is thermal enzyme probe (TEP), which is a temperature transducer with enzyme directly attached to it. However, since most heat evolved in the enzymatic reaction is lost to the surroundings, this approach suffers from low sensitivity. TEP is primarily intended for batch operation.

The most efficient detection method for an enzyme thermistor contains a column with immobilized enzyme, where the heat from the reaction is transported by the liquid passing through to a temperature sensor mounted at device outlet (Fig. 1.4). The commercial product by Thermometric, Jarfalla, Sweden is called thermal assay probe (TAP). It has two identical column ports in a carefully thermal stabilized aluminum jacket. The two ports can be used independently or one of them can be used as a reference channel. The thermistors are connected to a Wheatstone bridge with maximum sensitivity of 100 mV/m°C. The major limitation of the temperature resolution is the temperature fluctuation due to friction and turbulence in the column. For a reaction with enthalpy change of 80 kJ/mol, a temperature change of 0.01°C for 1ml of 1mM sample flowing at 1ml/min can be expected. The enzyme immobilization is usually done by using propylamino-derivatised CPG or Eupergit C. The column life can be several months with good method to prevent microbial growth in the solutions and low lines.

ET has been used in many biochemical analysis fields. For application based on immobilized enzyme, glucose and urea are the most studied subjects. Glucose is the most commonly used clinical analyte. When glucose reacts with glucose oxidase (usually co-immobilized with catalase), the working concentration range is from 1µM to 1mM, which is limited by the oxygen supply. Only about 0.25mM oxygen is soluble in water at 25°C. By using more soluble electron acceptor, the working concentration range can be
increased to > 75mM. For glucose/hexokinase reaction, the working range is 0.1-25mM. For urea/urease reaction, the working range is 0.01-200mM.

To measure reactions of low enthalpy change or low substrate concentration, adding sequentially acting enzymes can increase the total temperature signal. This is called enzyme amplification. Instead of immobilizing the enzyme, cells can also be attached to columns. This automatically handles the coenzyme regeneration. It also allows ET apparatus to be used to determine enzyme activity and cell metabolism.

Figure 1.4: Schematic diagram of a calorimetric biosensor. The sample stream (a) passes through the outer insulated box (b) to the heat exchanger (c) within an aluminium block (d). From there, it flows past the reference thermistor (e) and into the bioreactor (f), containing the biocatalyst, where the reaction occurs. The change in temperature is determined by the thermistor (g) and the solution is passed to waste (h). External electronics (i) determine the difference in the resistance, and hence difference in temperature, between the thermistors.
For the detection of large molecules, such as hormones and antibodies, thermometric enzyme linked immunosorbent assay (TELISA) has been designed. The standard procedure is that unlabeled antigen mixed with a fixed amount of enzyme labeled antigen is applied to the immunosorbent column mounted inside the ET. Labeled and unlabeled antigens compete to bind to the column. The amount of labeled antigen bound to the column is determined by measuring the enzymatic reaction heat when a substrate is injected. Finally, the bound antigen is washed away from the immunosorbent and the column is regenerated for next assay.

1.5.1.2 Other Calorimeters

Beside ET, there are other bench top calorimetric instruments, such as stepwise batch titration calorimeters, flow mixing calorimeters and differential scanning calorimeters.

The titration calorimeters are produced by MicroCal, Nortampton, USA; Thermometric, Jarfalla, Sweden; and Hart Scientific, Pleasant Grove, USA. The typical solution volume of reaction vessel is 0.5-3ml with 1-10µl is injected at each step. The isothermal titration calorimeter (ITC) from MicroCal is a hybrid between an adiabatic and a power compensation calorimeter. A thermocouple plate surrounded by two reaction vessels is used as a differential thermometer. The whole structure is thermally isolated from surroundings. During measurement, the reference vessel is heated by a constant power, the thermocouple controls a feedback circuit supplying heat to the reaction vessel and forces the temperature difference between the vessels to be zero. An exothermic process will cause a decrease of the feedback power and an endothermic reaction has the
opposite effect. Thermometric’s titration calorimeter (part of the Thermal Activity Monitor, “TAM”) and Hart Scientific’s titration calorimeter are all heat conduction calorimeters employing insertion reaction vessels.

Commercial flow calorimeters are from Thermometric (Part of TAM) and Sodev, Sherbrook, Canada. The Sodev’s instrument is a semi adiabatic instrument with a thermopile as a temperature sensor between the flow vessels. The two streams of solution, one with constant concentration and one with changing concentration, flow at a typical rate of 5~20ml/hr and are brought together at the heat sensitive area of the flow vessel for reaction.

Differential Scanning Calorimetry (DSC) measures the heat changes that occur during controlled increase (or decrease) in temperature. In a DSC, the difference between heat flow to the sample and a reference held at the same temperature is recorded as a function of temperature. From DSC curves, changes in the heat capacity of the biological materials and the enthalpy associated with certain processes can be obtained. The DSC from MicroCal has cell volume ~ 0.5 ml. Both solution and solid samples can be tested. The instrument has scan rates in the range of 0ºC to 90ºC per hour and can be operated in the temperature range of −10ºC to +130ºC. As little as 12.5 micrograms protein can be used to determine thermodynamic parameters.

1.5.2 Micromachined Sensors

1.5.2.1 Enthalpy Sensors

*Herwaarden* [18] fabricated the first micro thermal biosensor by using an aluminum/polysilicon thermopile on a freestanding membrane. The membrane cavity was
used as reaction chamber. Enzymes were immobilized on the backside of the membrane. The thermopile was integrated with a 100X on chip amplifier. The device is less affected by external thermal effects and can be operated without environmental temperature control. For glucose/glucose oxidase reaction, the device reported a detection range of 20 µmol to 3 mmole with a sensitivity of 45 µV/mmol. (Fig. 1.5)

This device is now commercially available from Xensor, Delft, Netherlands. There are several improved versions of this device currently available. They have different size

Figure 1.5: Cross section of thermopile and flow injection setup [18].
mono-crystalline silicon membrane and numbers of heaters and are encapsulated in ceramic pin-grid- array (PGA) with a hole in the bottom. (Fig. 1.6)

*Calvert* microcalorimeter is one of the best examples of heat conduction calorimeters [19]. The reaction volume in such a device is usually 15 – 100ml, the sensitivity is at the microwatt level and the long-term stability is high. Such microcalorimeters have been used for thermal and energetic studies of cellular biological systems. More recently, miniaturized devices have been described by *Zanini* [20], *Zieren* [21], *Muehlbauer* [22], *Bataillard* [23] and *van Herwaarden* [24] based on thin film thermopiles for the detection of glucose and urea. *Xie* presented design based on enzyme thermistor (ET). The thermistor array was fabricated on silicon [25] or quartz [26]. Different enzymes were immobilized on each thermistor, allowing multiple analysis be done at the same time.

---

**Figure 1.6**: Xensor liquid nano calorimeter NCM-9924 (thermopile side) [27].

---

1.5.2.2 Thermal Properties Sensors
Wang presented a MEMS differential calorimeter with integrated microfluidics for measuring structural transitions of biomolecules in solution [28]. The device features two identical freestanding membranes, resistive temperature sensors, heaters, and a thermopile differential temperature sensor between the two membranes. Integrated PDMS channels allow handling small volumes (~1µl) of liquid samples. During measurement, the temperature of the liquid sample and reference materials in the microfluidic chambers are scanned over a continuous range of temperatures with on-chip temperature control and the differential thermal response is monitored by the thermopile. The differential heat capacity of sample and reference $\Delta C$ is found to be

$$
\Delta C = \frac{\Delta Q}{dT/dt}
$$

where $\Delta Q$ is the differential heat power and $dT/dt$ is the time rate at which the chamber temperature is changed. This device has been used to measure unfolding of Lysozyme at concentration 300mg/ml. Yu fabricated a thin-film DSC for investigations of melting properties of small metal particles and glass transition of polymer films [29]. The calorimeter operates at high heating rates (15-200k/ms) and is very sensitive (30pJ/K).

A thermal conductivity sensor has been developed by Volkein to study the thermal conductivity of CMOS polysilicon [30]. The sensor consists of two beams with identical heating and temperature measurement parts. One beam is a sandwich structure with a polysilicon layer in between two dielectric layers, the other beam has no polysilicon layer. The two beams are connected. The thermal conductivity of the polysilicon can be obtained by calculating the difference of temperature response of the two beams for heat inputs.
Xensor also use similar configuration described in the enthalpy sensor part as a thermal conductivity gauge to do vacuum measurement and determine gas type.

1.6 Project Plan

Prior to this research, thermistors and thermopiles have been used to fabricate MEMS calorimeters to detect enzymatic reactions and measure reactant concentrations. However, a major limitation in existing MEMS thermal devices for liquid samples is the lack of integration with microfluidics. Samples are typically loaded either with a pipette or injected into conventional flow cells. This requires a large sample; which is a hindrance for measuring biomolecular transitions. This kind of set-up is not real lab-on-chip design and makes multiply detection very difficult. By using FEM simulation (the model detail will be described in chapter 4), integration of microchamber on top of thermopile is compared with using backside cavity as reaction chamber. The front side glass chamber is set to be 600µm * 600µm * 30µm. The backside cavity has membrane size 400µm * 300µm and depth of 500µm. Assuming all the analytes in both chambers are fully reacting, the front side design has sensitivity of 4.31mK/M-nl and the back side design has sensitivity of 3.87mK/M-nl for urea/urease reaction. The front side integration design has higher molar sensitivity for unit volume because the heat loss through bulk silicon is less in this case. The integration design is also more compact and requires less sample volume.

Prior work was focused on measuring the enthalpy change during a reaction. Very little work has been done on biochemical thermal properties. The only standard technique is monitoring specific heat change during bimolecular phase transition with DSC. As a
biochemical reaction proceeds, the thermal conductivity and specific heat of a given fluid will change as the content changes from being predominantly reactants to becoming products. This provides a new and independent method to investigate biological reactions in real-time.

The biosensor in this project is based on heat conduction calorimetry. It is a thin film thermopile fabricated on a silicon chip using micromachining techniques. The thermopile has been fabricated on a freestanding membrane. The cold junctions of thermopile are placed on the bulk of the silicon chip, which serves as an isothermal heat sink at room temperature. In order to transport the analyte, microfabrication techniques were used to integrate the fluidic channels and the reaction chamber onto the thin film thermopile sensor. In its basic mode of operation, this device seeks to measure the heat of biochemical reactions. Planar heaters have also been incorporated in the path of the fluid. And the device can also be configured as a thermal conductivity sensor. The most novel aspects of this work include:

i. The first demonstration of an integrated microfluidic reaction chamber directly on the freestanding membrane thermal sensor.

ii. Analyte sample volumes of ~15 nl. This represents a factor of 50 – 100 times reduction in the sample volumes.

iii. A thermal sensor capable of two modes of operation: (a) as enthalpymeter and (b) thermal properties measurement.

iv. Accurate measurement of the thermal conductivity of insulating samples including most commonly used microelectronic/MEMS polymer materials.
v. Application of the sensor for the measurement of the thermal properties of biochemical reactions in real-time including enzymatic and antibody-antigen reactions.
REFERENCES


14. Webpage, *Calorimetric biosensors*


Chapter 2

Thermoelectric Calorimetric Sensor Design

This chapter reviews the principles of thermal sensing, thermoelectric effects and design of the calorimetric biosensor. Section 2.1 introduces and compares the various methods for thermal sensing. Section 2.2 describes the three thermoelectric effects. Definition and discussion of the thermocouple and thermopile are given in section 2.3. Seebeck coefficients of different materials are discussed next. Section 2.5 and section 2.6 explains working modes, sensitivity and noise consideration for a ideal thermal sensor. Finally, the standard design rules of a thermal sensor are presented. How to adapt the rules to design particular sensor structures for bio-measurements is discussed.

2.1 Temperature Sensors

Temperature is a fundamental thermodynamic quantity and its measurement involves two steps: (i) bringing an object with a temperature dependent property into contact with the object whose temperature is to be measured, and (ii) allowing the two objects to reach thermal equilibrium. The object with the calibrated temperature dependent property is known as the thermometer or thermal sensor. An important condition is that the thermometer or thermal sensor needs to be small enough in relation
to the object whose temperature is being measured so that the flow of heat between the two systems during equilibration process does not significantly perturb the state of the object under investigation. Practical thermometers need to be simple, reproducible, adequately sensitive, and easy to use. If the relationship between the physical quantity and temperature can be derived from fundamental physical principles, the thermometer is called a primary thermometer. In this case, the device operation is based upon the fundamental measurements of thermodynamic temperature. Examples of such thermometers include gas thermometer, acoustic thermometer, dielectric constant thermometer, noise thermometer, total radiation thermometer, magnetic thermometer etc [1]. These thermometers when used with care can be used for accurate measurement of the absolute temperature. However, these devices can be quite cumbersome and often require considerable experimental skills and integration of exotic materials for the accomplishment of such measurements. Thus, except for fundamental calibration experiments where these thermometers are used as calibration standards, these techniques are very rarely implemented as thermal sensors in the micro and nanoscale devices. The most practical implementation of thermal sensors is based upon effects which have no simple theoretical derivations but can be well characterized empirically. In Table 2.1 some of the most popular temperature measurements effects used in miniaturized sensors will be described and compared.
<table>
<thead>
<tr>
<th></th>
<th>Working Principal</th>
<th>Working range</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermo-mechanical</td>
<td>Difference in thermal expansion of bimorph causes temperature dependent curvature.</td>
<td>-130 ~220 °C</td>
<td>Robust, relatively cheap, Highly sensitive measurement technique.</td>
</tr>
<tr>
<td>Thermo-resistive</td>
<td>Material’s resistance changes with temperature.</td>
<td>-200 ~850°C</td>
<td>Thermistor: high signal to noise ratio, high repeatability. Self-heat effect</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thermistor: &lt;100 °C</td>
<td></td>
</tr>
<tr>
<td>Thermo-couples</td>
<td>The junctions of two dissimilar metals will produce a voltage proportional to the</td>
<td>-200 ~1700°C</td>
<td>Low cost, wide temperature range, reasonably short response time, reasonable repeatability and accuracy.</td>
</tr>
<tr>
<td></td>
<td>temperature difference between them.</td>
<td></td>
<td>Nonlinearity, need reference temperature.</td>
</tr>
<tr>
<td>Junction-based</td>
<td>p-n junction exhibits temperature dependence.</td>
<td>-55~185 °C</td>
<td>Can be incorporated within IC, Non-linear, self-heating error, uncertainty, need for individual calibration and lack in interchangeability</td>
</tr>
<tr>
<td></td>
<td>The diode I-V characteristics and V_{BE} of bipolar transistors are used.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acoustic</td>
<td>Self-resonant of SAW oscillator varies with temperature.</td>
<td></td>
<td>Millidegree resolution, good linearity, and low hysteresis, hermetic package needed to prevent unwanted mass loading.</td>
</tr>
<tr>
<td>Quartz/Resonant</td>
<td>Resonant frequency of quartz is sensitive to temperature due to piezoelectric</td>
<td>-280 ~250°C</td>
<td>High sensitivity and accuracy, high dynamic range,</td>
</tr>
<tr>
<td></td>
<td>effect</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The proposed device measures temperature based on thermoelectric effect. The rest of this chapter will discuss the material, design, and performance considerations of a thermal sensor based on thermoelectric effect.

2.2 Thermoelectric Effect

Thermoelectric effects occur when a temperature gradient exists in some parts of a conducting system. There are three fundamental thermoelectric effects named after their discoverers namely Seebeck effect (1826), Peltier effect (1834) and Thomson effect (1857) [2, 3].

2.2.1 Seebeck Effect

When a conductor is subjected to a temperature gradient, electrons at the hot end of such a conductor diffuse to states of lower energy at the cold end. This sets up an electric potential difference between the two ends of the conductor. Discovered by Johann Seebeck (1770-1831), this phenomenon is named after him and is known as the Seebeck effect. The electric potential builds up until a state of dynamic equilibrium is established between: (i) the number of electrons flowing towards the cold end due to the temperature difference and (ii) the number of electrons driven towards the hot end due to the electrostatic repulsion by the excess charge accumulated at the cold end. Thus, at equilibrium, the number of electrons passing in both the directions per second through a cross section normal to the flow are equal, although the velocities of the electrons from the hot end will be higher than those from the cold end. This difference in velocities
ensures a continuous transfer of heat (thermal conduction) along the temperature gradient without charge transfer after the establishment of dynamic equilibrium.

If we wish to measure the Seebeck potential, electrical connections to the two ends of the specimen under test must be made using a material different from the specimen. Otherwise the symmetry in the circuit will cause no net voltage to be detected. The distribution of the electric potential $V(T)$ in a pair of conductors of different materials $A$ and $B$, having the junction maintained at a temperature $T_1$ and the free ends maintained at a lower temperature $T_0$ is shown in Fig. 2.1. To measure the thermoelectric power, a voltmeter at a temperature $T_0$ is connected using an identical pair of conductors to the free ends of $A$ and $B$. We should note that Seebeck effect is in no way a junction phenomenon. Instead, it is a temperature gradient phenomenon and the magnitude of which, for strain-free homogeneous conductors, depends on the nature of the two conductors $A$ and $B$ and the temperature difference between the hot and the cold junctions. For a small temperature difference $\Delta T = T_1 - T_0$, the derivative

$$\frac{\Delta V_{AB}}{\Delta T} = S_{AB} \quad (33)$$

is called the thermopower of the couple or the Seebeck coefficient of material $A$ with respect to material $B$ and is a function of temperature $T$. $\Delta V_{AB}$ is the open circuit voltage for the given material combination and temperature difference. If material $B$ is a superconductor the absolute thermopower $S_A$ of material $A$ can be measured, because a superconductor below its transition temperature shows no thermoelectric effects. Size
effects in the Seebeck coefficient are likely to be important only if the physical dimensions of the devices are comparable to the electron mean free path.

2.2.2 Peltier Effect

Jean Peltier (1785-1845) discovered that if an electric current passes from one material to another, then heat may be evolved or absorbed in the junction region, depending on the direction of current flow. A Peltier coefficient \( \Pi_{AB} \) may be defined as
the rate of absorption or evolution of heat per unit current flowing from $A$ to $B$. If $\frac{dQ_{AB}}{dt}$ is the net rate of evolution or absorption of heat energy at the junction, then

$$\frac{dQ_{AB}}{dt} = \Pi_{AB} I$$

where $I$ is the current flowing from $A$ to $B$. $\Pi_{AB}$ is a function of the temperature at the junctions of the two conductors.

### 2.2.3 Thomson Effect

Thomson effect is related to the evolution or absorption of heat on the passage of an electric current through a single conductor in the presence of a thermal gradient. Thomson coefficient $\varsigma$ is defined as the heat generated per second per unit current flow per unit temperature gradient. It is a reversible heat. The relationship between absolute thermopower $S$, the Peltier coefficient $\Pi$ and Thomson coefficient $\varsigma$ are known Kelvin relations. For a material at a temperature $T$, they can be written as:

$$\Pi = T \cdot S$$

$$\varsigma = \frac{dS}{dT}$$

### 2.3 Thermocouples and Thermopiles

#### 2.3.1 Thermocouples

In order to measure the Seebeck potential, it is necessary to use of two different materials. Otherwise the symmetry in the circuit will cause no net potential to be detected. The configuration of two different conductors joined together in a series connection as shown in Fig. 2.1 is known as a thermocouple. A working thermocouple
thus consists of a sensing junction, at temperature $T_1$, and a reference junction, at temperature $T_0$. The voltage developed by the thermocouple is measured with a high input impedance voltmeter.

i. **Metal Thermocouples**

Any two dissimilar metals may be used to make a thermocouple. Thermocouples are classified into seven standardized types, mainly arising due to historical reasons, as (J, K, T, E, R, S or B) and exhibit a range of desirable features. (Table 2.2)

<table>
<thead>
<tr>
<th>ISA</th>
<th>Material (+ &amp; -)</th>
<th>Temperature Range (°C)</th>
<th>Sensitivity @ 25°C (µV/°C)</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>Chromel &amp; Constantan (Ni-Cr &amp; Cu-Ni)</td>
<td>-270~1000</td>
<td>60.9</td>
<td>LT:±1.67°C HT:±0.5%</td>
</tr>
<tr>
<td>J</td>
<td>Iron &amp; Constantan (Fe &amp; Cu-Ni)</td>
<td>-210~1200</td>
<td>51.7</td>
<td>LT:±2.2<del>1.1°C HT:±0.375</del>0.75%</td>
</tr>
<tr>
<td>K</td>
<td>Chromel &amp; Alumel (Ni-Cr &amp; Ni-Al)</td>
<td>-270~1350</td>
<td>40.6</td>
<td>LT:±2.2<del>1.1°C HT:±0.375</del>0.75%</td>
</tr>
<tr>
<td>T</td>
<td>Copper &amp; Constantan (Cu &amp; Cu-Ni)</td>
<td>-270~400</td>
<td>40.6</td>
<td>LT:±1~2% HT:±1.5% or ±0.42°C</td>
</tr>
<tr>
<td>R</td>
<td>Platinum &amp; 87% Platinum/13% Rhodium (Pt &amp; Pt-Rh)</td>
<td>-50~1750</td>
<td>6</td>
<td>LT:±2.8°C HT:±0.5%</td>
</tr>
<tr>
<td>S</td>
<td>Platinum &amp; 90% Platinum/10% Rhodium (Pt &amp; Pt-Rh)</td>
<td>-50~1750</td>
<td>6</td>
<td>LT:±2.8°C HT:±0.5%</td>
</tr>
<tr>
<td>B</td>
<td>70% Platinum/30% Rhodium &amp; 94% Platinum/6% Rhodium (Pt-Rh &amp; Pt-Rh)</td>
<td>-50~1750</td>
<td>6</td>
<td>LT:±2.8°C HT:±0.5%</td>
</tr>
</tbody>
</table>

Table 2.2: Properties of standardized metal thermocouples [4].
2.3.2 Thermopiles

When several thermocouples are connected in series, the device is known as a thermopile and is schematically shown in Fig. 2.2. The series combination of the individual thermocouples results in $M$-times higher voltage output, where $M$ is the number of thermocouples constituting the thermopile, so long as all the hot junctions are at the same temperature. The main drawbacks of this arrangement arise from the fact that, if any of the thermocouples is an open circuit the thermopile fails, alternatively, if any of the thermocouples are short circuited, the output voltage decreases dramatically.

Thermopiles have various attractive properties compared with other temperature difference measurement methods, such as thermistor and transistor pairs. It must be emphasized that since, thermoelectric effect is not a junction phenomenon but a fundamental transport property of a material in the presence of temperature gradient many of the noise problems relating to electrical contacts are mitigated in these devices.

To summarize,

i. Thermopile does not need any bias for operation.
ii. Thermopiles have no offset or self-heating effect.

iii. Thermopiles exhibit lower noise. There is no interference caused by power supplies.

Silicon thermopiles can be fabricated by CMOS compatible processes. In this case highly reproducible devices with little variations of the electrical parameter across the wafer can be obtained and thus result in very uniform thermopile sensitivity across many devices [5].

2.4 Thermoelectric Properties of materials

2.4.1 Seebeck Coefficient

In this thesis we are interested in accurately measuring the changes in temperature arising due to changes in enthalpy or changes in thermal properties during biochemical reactions. We will be using the Seebeck effect to convert changes in temperature into electrical signals. Therefore, a discussion only on the Seebeck coefficient of various materials and its phenomenological dependence is discussed. In the following sections a discussion on the thermoelectric effect in metals and silicon is presented as these are of most interest in thin film integrated sensors. Both bulk as well as thin film values will be discussed.

2.4.1.1 Bulk Materials

The Seebeck coefficient can be expressed as the derivative of the Fermi Energy $E_F$ with respect to the absolute temperature $T$ [2]:

$$S = \frac{1}{q} \times \frac{dE_F}{dT}$$
where \( q \) is the absolute value of the elementary charge.

i. Metals

For metals and degenerate semiconductors, neither carrier density nor Fermi level will change much with temperature. That is why the metals have much smaller Seebeck coefficient, which can be calculated by [2]:

\[
S = \frac{\pi^2 k_B^2 T}{q(E_F - E_C)}
\]  

where \( k_B \) is the Boltzmann constant. At room temperature the absolute value is between 0–10\( \mu \)V/K.

ii. Single Crystal Silicon

For non-degenerate silicon there are three main effects contributing to the Seebeck coefficient [6]:

With increasing temperature

a) The silicon becomes more intrinsic.

b) The charge carriers have a higher average velocity, leading to charge build-up on the cold side of the silicon. Scattering of charge carriers is usually temperature dependent, leading to charge build-up on the cold or hot side of the silicon. This depends on the hot carriers that can move more freely than the cold ones or are trapped by increased scattering.

c) A net flow of phonons flows from hot to cold side and drags the charge carriers at the same time. In temperature 10-500K, momentum transfer from acoustic phonons to the charge carriers can occur.

For non-degenerate silicon [7]:

\[
\]
where $N_C$ and $N_V$ are the effective densities of states of the conduction and valence band. $n$ and $p$ are the electron and hole concentrations. The parameter $s$ is the indicator for energy dependence of the relaxation time in the Boltzmann transport theory, the value of which is between $-1$ to $2$. $\phi$ is phonon scattering coefficient, which is related to doping density and temperature. At room temperature, equation 2.6 can be approximated as a function of electrical conductivity $\sigma$ as [8]:

$$S = 2.6 \frac{k_B}{q} \ln\left(\frac{\sigma_0}{\sigma}\right)$$

where $\sigma_0$ is a constant, $1/\sigma_0 \approx 5 \times 10^{-4} \, \Omega\cdot\text{cm}$.

iii. Polycrystalline Silicon (polysilicon)

For highly doped polysilicon ($>10^{18}\,\text{cm}^{-3}$), the phonon scattering can be neglected and only carrier scattering at the ionized dopants occurs [9]. The Seebeck coefficient is now given by:

$$S_{p/n} = \pm \frac{k_B}{q} \left[ \frac{4}{3} \cdot F_2(\zeta_{p/n}) \cdot g_{i1}(G_i) - F_2(\zeta_{p/n}) \cdot g_{ii}(G_i) - \zeta_{p/n} \right]$$

where $\zeta_{p/n}$ is the reduced Fermi energies:

$$\zeta_p = \frac{E_V - E_F}{k_B T} = -\ln\left(\frac{N_V}{p}\right)$$

$$\zeta_n = \frac{E_F - E_C}{k_B T} = -\ln\left(\frac{N_C}{n}\right)$$

$$S_p = \frac{k_B}{q} \left[ \ln\left(\frac{N_V}{p}\right) + \frac{5}{2} + s_p + \phi_p \right](p\text{-type})$$

$$S_n = -\frac{k_B}{q} \left[ \ln\left(\frac{N_C}{n}\right) + \frac{5}{2} + s_n + \phi_n \right](n\text{-type})$$
For non-degenerate silicon, $\zeta_{p/n}$ is negative. $F_i(\zeta_{p/n})$ is the Fermi integral:

$$F_i(\zeta_{p/n}) = \int_0^\infty \frac{x^i}{\exp(x - \zeta_{p/n}) + 1} \, dx \tag{2.10}$$

The ratio $F_3(\zeta_{p/n})/F_2(\zeta_{p/n})$ equals to 3.

The functions $g_0$ and $g_1$ describe the influence of the grain size $G_i$. For ionized impurity scattering, the ratio $g_1/g_0$ is approximately one. The Seebeck coefficient does not depend on polysilicon grain size. So equation 2.8 simplifies to [10]:

$$S_{p/n} = \pm 86 \cdot (4 - \zeta_{p/n}) \mu V / K \tag{2.11}$$

For polysilicon with doping level below $10^{18}\text{cm}^{-3}$, lattice scattering dominates. In this case, the Seebeck coefficient:

$$S_{p/n} = \pm \frac{k_B}{q} \left[ \frac{2 \cdot F_i(\zeta_{p/n})}{F_0(\zeta_{p/n})} - \zeta_{p/n} \right] = \pm 86 \cdot (2 - \zeta_{p/n}) \mu V / K \tag{2.12}$$

Both equation 2.11 and 2.12 can be unified by using parameter $s$:

$$S_{p/n} = \pm \frac{k_B}{q} \left( \frac{5}{2} + s - \zeta_{p/n} \right) \tag{2.13}$$

Experimental Seebeck coefficient of polysilicon as a function of doping can be found in Fig. 2.3
2.4.1.2 Thin-Film Materials

In eq. 2.8 the ratio of functions which describe the effect of film thickness and grain size is approximated by unity. However, in thin film the carriers are scattered by film imperfections or surface and grain boundaries. A distinct decrease in bulk Seebeck coefficient is observed [12]. By appropriately annealing, a considerable increase can be achieved in some cases.

2.4.1.3 Thin-film Thermopiles

Planar thermopiles have been fabricated using variety of thin film materials on various substrates. One of the first integrated thin film thermopiles using polysilicon and
gold was demonstrated by Lahiji [5] and was used as an infrared detector. More recently, thin film thermopile based infrared detectors, flow sensors, imagers etc. from CMOS materials have been demonstrated by van Herwaarden [13], Lengggenhager [14], Elbel [15], Moser [16], and Choi [17]. Thin film Bi-Sb thermopiles have been fabricated by Volklein [18] and Zieren [19]. One of the fastest thin film thermopiles with a response time of 20µs was demonstrated by Srinivas et al. using freestanding copper-constantan thermopiles and surface micromachining techniques. Table 2.3 compares the various thin film thermocouple materials. Typical thermopiles fabricated using silicon micromachining techniques and thin-film thermoelectric materials are capable of resolving temperature differences (ΔT) of ~10mK and have a response time (τ) of ~20ms [5].

Table 2.3: Comparison of various thermoelectric materials. (See section 2.4.2. for figure of Merit).

<table>
<thead>
<tr>
<th>Thermocouple Material</th>
<th>Thermopower (µV/K)</th>
<th>Figure of Merit (K⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper-Constantan</td>
<td>41</td>
<td>4.6 X10⁻⁵</td>
</tr>
<tr>
<td>Single Crystal n-Si / Aluminum</td>
<td>201</td>
<td>1.7 X10⁻⁵</td>
</tr>
<tr>
<td>p-Polysilicon / Aluminum</td>
<td>137</td>
<td>1.1 X10⁻⁵</td>
</tr>
<tr>
<td>p-Polysilicon / n-Polysilicon</td>
<td>200</td>
<td>1.4 X10⁻⁵</td>
</tr>
<tr>
<td>p-Bi₀.₅Sb₁.₅Te₃ / n-Bi₀.₈₇Sb₀.₁₃</td>
<td>330</td>
<td>1.3 X10⁻³</td>
</tr>
</tbody>
</table>
2.4.2 Thermoelectric Figure of Merit

The figure of merit $Z$ of a thermoelectric material is given by the expression

$$Z = \frac{S^2 \sigma}{\kappa} \quad \text{(2.14)}$$

where $S$ is the Seebeck coefficient, $\kappa$ is the thermal conductivity and $\sigma$ is the electrical conductivity of the material. Intuitively, this can be understood from the fact that in thermoelectric cooling and power generation applications, it is desirable to have a high Peltier coefficient which is in turn proportional to the Seebeck coefficient (eq. 2.3). At the same time, the electrical resistance and the thermal conductivity of the thermocouple need to be as small as possible to minimize Joule heating and to maintain the maximum temperature difference between the two junctions. However, for metallic systems, Wiedemann-Franz law states these two parameters are related as

$$\frac{\kappa}{\sigma T} \approx 2.45 \cdot 10^{-8} \, \text{V/°C} \quad \text{(2.15)}$$

Materials with high value of $ZT$ are found to be efficient thermoelectric materials for cooling and power generation applications. High figures of merit are typically observed in heavily doped semiconductors, the best known materials being the tellurides of antimony and bismuth. With the advent of semiconductors the efficiency of thermoelectric generators greatly increased. For a two material thermocouple, the the figure of merit $Z_{A/B}$ becomes

$$Z_{A/B} = \frac{S_{A/B}^2}{\left[\left(\frac{\kappa_A}{\sigma_A}\right)^{1/2} + \left(\frac{\kappa_B}{\sigma_B}\right)^{1/2}\right]^2} \quad \text{(2.16)}$$
when the geometric condition $\left( \frac{A_B}{A_A} \right)^2 = \frac{\left( \kappa_A \sigma_A \right)}{\left( \kappa_B \sigma_B \right)}$ is satisfied, where $A_B$ and $A_A$ are the cross sectional areas of the thermopile legs. Table 2.3 lists the thermoelectric figure of merit for some of the commonly used thermoelectric thin film thermocouple materials.

2.5 The Ideal Thermal Sensor

Fig. 2.4 illustrates the ideal model of a thermal sensor. The sensor consists of a sensitive area $A_s$ coupled via a thermal conduction path (thermal conductance $G$) to a heat sink of constant ambient temperature $T_0$. The ultimate minimum detectable signal is limited by the temperature fluctuation (temperature noise) on the sensitive area when there is no thermal input. The ideal sensor can be used in two modes of operation:

i. Measurement of the Heat Input

ii. Measurement of the Thermal Properties of the Sensing Element/Thermal Conduction Path

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Figure 2.4: Ideal Thermal Sensor.
2.5.1 Operation Mode 1: Measurement of Heat Input

When power is absorbed by the element, its time dependent temperature will change for a given conductance and specific heat. When a heating power $Q$ is absorbed by sensitive area $A_s$, the temperature increases to $T_1$. Neglecting convection or radiation heat loss, the heat flux is dissipated by thermal conduction via $G$. For small temperature differences $(T_1 - T_0)/ T_0<<1$, the steady-state temperature increase of the sensor is:

$$\Delta T = (T_1 - T_0) = \frac{Q}{G} = R_T Q$$ \hspace{1cm} (2.17)

where $R_T$ is the thermal resistance which is determined by the dimensions (length $l$, cross sectional area $A_p$) and the thermal conductivity $\kappa$ of the thermal path.

$$R_T = \frac{l}{\kappa A_p}$$ \hspace{1cm} (2.18)

A harmonic time dependent power input $Q(t)=Q + Q_\omega \exp(i \omega t)$ leads to a transient behavior with time constant $t = R_T C$, where $C$ is the heat capacitance of the sensor. The transient temperature is

$$\Delta T_\omega (t) = \frac{Q_\omega}{\sqrt{G^2 + \omega^2 C^2}} \exp(i \omega t + \phi)$$ \hspace{1cm} (2.19)

with phase $\phi = \tan^{-1}(\omega C / G)$. For a thermal detector to exhibit high sensitivity, $\Delta T$ must be as large as possible which, as eq. (2.19) shows, can be achieved by making $G$ as small as possible and $\omega$ sufficiently low so that $\omega C \ll G$. In other words both the thermal heat capacity of the detector element and its thermal coupling to the surroundings should be as
small as possible. The implication of minimizing \( C \) results in a sensing element of as small and as light a construction as practicable. It can be seen that the requirement for maximum sensitivity (i.e thermal conductance to be as small as possible) implies a large value for the time constant of the device. This is an inherent limitation present in thermal detectors and necessitates a compromise on their performance. The value of \( G \) also determines the magnitude of the temperature noise fluctuations, a small value being required for highest sensitivity. The smallest value for the thermal conductance is achieved when the thermal coupling of the sensing element to the heat sink is only through radiative exchange. The principal requirement of a thermal detector is therefore to optimize its interaction with the sensing input while reducing as far as possible all other thermal contacts with the surroundings.

**2.5.2 Operation Mode 2: Measurement of the Thermal Properties**

Alternatively, for an input power, if the thermal conductance is changed, the temperature of the device is also expected to change.

For a fixed power input, the temperature amplitude is

\[
\Delta T = \frac{Q_0}{\sqrt{G^2 + \omega^2 C^2}} \tag{2.20}
\]

If \( G^2 >> \omega^2 C^2 \), use Taylor expansion, eq. **2.20** can be approximate to

\[
\Delta T \approx \frac{Q_0}{G} [1 - \frac{1}{2} \frac{\omega^2 C^2}{G^2}] \tag{2.21}
\]

Taking logarithm and then differentiating both sides, eq **2.21** becomes
At low frequency, $\omega C$ is much smaller than $G$. Temperature amplitude can be treated as a function of $G$ only. By measuring temperature amplitude, material thermal conductivity can be obtained. Increase in the thermal conductance causes temperature amplitude to decrease. Information of specific heat and thermal diffusivity can be calculated based on frequency response and phase shift.

2.6 Noise Considerations and Ultimate Sensitivity of Thermal Sensors

All detectors are limited in the minimum absorbed power they can detect by some form of noise which may arise in: (i) the detector, (ii) the source (measured) or (iii) in the electronic system following the detector. An ideal thermal sensor is a device that samples the incident power and produces an electrical current/voltage proportional to the total power absorbed by detector surface. The objective of optimum detector design is to reduce the internal noise of the detector to a level at which only the noise arising from the source (measurand) to which the detector responds can be detected, though this may not be always possible. In the absence of any noise from the electronic system following the detector, the random thermal fluctuations of the detector element which is coupled to the heat sink via a thermal conductance $G$ and the random electrical fluctuations (Johnson Noise) arising due to the finite resistance $R$ of the detector sets the ultimate limits of thermal detectors.

$$\frac{\delta(\Delta T)}{\Delta T} = -\frac{\Delta G}{G} \left(1 - \frac{2\omega^2C^2}{2G^2 + \omega^2C^2}\right) \quad 2.22$$
2.6.1 *Johnson Noise*

In the absence of an electrical bias, the absolute minimum internal noise that exists is termed as *Johnson noise*. *Johnson* noise is found in all resistive materials. This form of noise arises from the random motion of the current carriers as a fluctuating voltage within any resistive material and is always associated with a dissipative mechanism. If an ideal voltmeter with infinite input resistance is used to measure the voltage signal, the equivalent mean squared temperature fluctuation arising due to the finite electrical resistance $\langle \Delta T_{ne}^2 \rangle$ of the detector is given by [20]:

$$\langle \Delta T_{ne}^2 \rangle = \frac{4k_B T R}{(MS)^2}$$

where $M$ is the number of thermocouples in the thermopile and $S$ is the Seebeck Coefficient of the thermocouple used and the noise is assumed to be measured in a 1 Hz bandwidth.

2.6.2 Temperature Fluctuation Noise

In most thermal detectors the noise limit of a detector is set by the spontaneous temperature fluctuations of the detector element due to the heat conductance $G$ to the surroundings. For the ideal thermal detector discussed in section 2.5.1, the mean squared temperature fluctuations and the minimum detectable power $P_{\text{min}}$ are given by [20]:

$$\Delta T_n^2 = \frac{4k_B T^2 G}{G^2 + \omega^2 C^2}$$

$$P_{\text{min}} = 2T \sqrt{k_B G}$$
where $G$ is the thermal conductance, $C$ is the thermal mass of the detector element and the noise is measured at a frequency $\omega$ in a 1Hz bandwidth. For the thermopile used here, the thermal conductance $G$ can be approximated to $5\times10^{-5}$ W/K, resulting in $P_{\text{min}} = 1.58 \times 10^{-11}$ W/Hz$^{1/2}$.

If the heat conductance $G$ is due to radiative heat exchange only, then

$$G = 4\varepsilon\sigma_{SB} T^3 A_s$$  \hspace{1cm} \text{(2.25)}$$

where $\sigma_{SB}$ is the Stefan Boltzmann constant, $A_s$ is the area of the detector element over which the radiative exchange occurs, and $\varepsilon$ is the emissivity of the detector element. For a thermal detector of area $A_s = 300 \mu m \times 400 \mu m$, $\varepsilon = 1$, $T = 300K$ and $B = 1$ Hz, the r.m.s. noise power is determined to be $P_{\text{min}} = 1.9 \times 10^{-12}$ W/Hz$^{1/2}$. So temperature fluctuation due to radiation can be ignored.

### 2.6.3 Ultimate Limit of Sensitivity of the Device

The noise equivalent power due to the finite resistance (electrical noise) and finite thermal conductivity (thermal noise) in the fabricated microcalorimeter can be calculated using the expression

$$P_{\text{min}} = \frac{\left[\langle T_m^2 \rangle + \langle T_{\text{ne}}^2 \rangle\right]^{1/2} S_v}{S_T}$$  \hspace{1cm} \text{(2.26)}$$

where $S_v$ is the responsivity of the thermopile in V/W, and $S_T$ is the temperature responsivity of the thermopile in V/K. For this thermopile design, $G = 5\times10^{-5}$ W/K, $R = 250k\Omega$, $S = 350\mu V/K$, $M = 16$, $S_v = 1$ V/W, $S_T = 5$ mV/K, and $T = 300$ K. The noise equivalent power of the device can be estimated to be $\sim 50$ nW/Hz$^{1/2}$. The enthalpy
change for oxidation of glucose using glucose oxidase is $\Delta H = -80 \text{ kJ/mol}$. A glucose oxidase molecule converts 1000 glucose molecules per second at the saturation concentration (1 mMole). If the reaction is limited by the availability of glucose molecules, $4 \times 10^{11}$ glucose molecules (0.6 pmoles) are required every second to generate the minimum detectable power. If the chamber volume is assumed to be 15 nl, the minimum detectable concentration of glucose is found to be 44 $\mu$M at a minimum flow rate of 15 nl/s. These calculations are based on 1 Hz bandwidth, however if the output of the enzyme thermopile is measured in dc conditions, the noise is expected to be much larger than estimated here. Additionally, this calculation does not consider any fluctuations due to fluidic mixing, fluidic flow, or pressure variations.

2.7 The Proposed Thermal Biosensor

Ideally, the thermal biosensor must be able to work in both the modes of operation described in Section 2.5. In order to accomplish this, a heater and a temperature-sensing element must be included in the design. The sensor structure should maximize the temperature difference between the hot and cold junctions. Because of the liquid testing requirement, integrated microfluidics is needed. For more complex unpurified bio-sample analysis, sensor arrays need to be considered.

The desired properties of thermopile are:

i. High sensitivity and linearity.

ii. Small time constant: Although reaction time for bioreaction is relatively long (minutes to hours), the device time constant should be in seconds to provide adequate real-time measurement resolutions.
iii. The electrical impedance, which generates Johnson noise, should be designed to provide maximum system signal to noise ratio.

Fig. 2.5 shows the schematic layout of the proposed microcalorimeter. The thermopile is located on top of a freestanding membrane. Thermopile hot junctions are located on the freestanding membrane whereas the cold junctions of the thermopile are located on the rim of the silicon chip. The large thermal mass and good thermal conductivity of the rim keeps the cold junctions at room temperature whereas the unconstrained membrane, where the reaction occurs, rises in temperature due to the heat of reaction. In addition a polysilicon heater was integrated onto the membrane. The polysilicon heater provides a technique for the calibration of the thermopile and for the measurement of the thermal properties of the fluid assays. A microfluidic channel was bonded onto the thermopile structure. The integration of microfluidic channels and reaction chamber on top of the freestanding membrane allows for the introduction of controlled quantities of the analyte and enzyme, which are made to mix on the hot junctions due to the reaction chamber design.
2.7.1 Thermopile Materials

The desired thermopile properties are not independent or simply related. First we must select a pair of material for the thermocouple. To maximize \((T_1 - T_0)\) with respect to the electrical circuit noise, both electrical conductivity \(\sigma\) and thermal conductivity \(\kappa\) should be minimized. This situation leads to the selection of thermocouple material in terms of maximum value of the figure of merit.

*Seebeck* coefficient \(S\) is inversely related to carrier concentration by \(S \approx c_V/(qN_c)\), where \(c_V\) is the specific heat of the charge carriers, \(N_c\) is the number of carriers and \(q\) is
the charge of each carrier. The electrical conductivity \( \sigma \) is approximately proportional to the carrier concentration. Thermal conductivity \( \kappa \) depends on charge carrier density too. The net result is a maximum \( Z \) as a function of carrier concentration between \( 10^{18} \) to \( 10^{20} \text{cm}^{-3} \). (Fig. 2.6)

![Graph showing the relationship between Seebeck Coefficient, Electrical Power Factor, Electrical Conductivity, and Thermal Conductivity with carrier concentration.](image)

**Figure 2.6:** Thermoelectric properties of metals, semiconductors and insulators [14].

Thus, p-type polysilicon with doping density of \( 10^{19} \text{cm}^{-3} \) was chosen as one of the thermopile materials. Although a p-type silicon-n-type silicon thermocouple would provide the highest sensitivity, metal (aluminum or chromium/gold) was chosen as the second material constituting the thermocouple in our design mainly for ease of fabrication. For this thermocouple, the expected Seebeck coefficient \( S_{p-poly-Cr} \), is \( S_{p-poly} [350 \mu \text{V/K}] - S_{chromium} [16 \mu \text{V/K}] = 334 \mu \text{V/K} \). Thermopile electrical resistance is very
important parameter. In this case is dominated by the polysilicon resistance. Polysilicon resistance can be calculated based on polysilicon sheet resistance \( R_{\text{sheet}} = 127 \Omega / \text{square} \) and thermopile design by eq. 2.27

\[
R = M \cdot R_{\text{sheet}} \cdot \frac{L}{W}
\]

2.27

\( M \) is the number of thermocouples in the thermopile, \( L \) and \( W \) are the length and width of thermopile. Resistances of the order of 10-100 k\( \Omega \) are high enough to allow a high sensitivity, while low enough to avoid too much noise.

2.7.2 Polysilicon Heater

Polysilicon resistor is used as a heating element. Because of the layout of the fluidic channel, the interconnect lines of the heater are long. To limit the heat generation on the interconnect wires, the heater section located on the freestanding structure has a width of 10\( \mu \)m compared to the 50\( \mu \)m and 100\( \mu \)m wide interconnection wires. The heater length is 400\( \mu \)m and falls exactly on top of the membrane while the interconnect wires are laid out on the bulk silicon. The heat generated in the interconnect wires is rapidly dissipated into the substrate, which acts like a heat sink.

2.7.3 Freestanding Structure

Sensor structure must be designed to maximize the temperature difference between the hot and cold junctions. Silicon is a very good thermal conductor \( (\kappa = 148 \text{ W/m-K}) \). In our design, bulk silicon serves as the heat sink, and the sensor active area on which the thermopile and the heater are supported is made up of a thin silicon oxide
and/or silicon nitride membrane. Silicon dioxide \((\kappa = 2.4 \text{ W/m-K})\) and silicon nitride \((\kappa = 15 \text{ W/m-K})\) have much lower thermal conductivity than silicon. By creating a 2-10\(\mu\)m thick membrane, the structure thermal resistance can be increased by two orders of magnitude, without degrading the electrical properties [21]. This is especially so when compared to wafer thickness of 500 \(\mu\)m. So does the sensor sensitivity, which is directly proportional to the thermal resistance. In our design, a sandwich structure membrane has been chosen, which includes a silicon oxide layer sandwiched in between of two silicon nitride layers. The two nitride layers have the same thickness and stress, so the whole structure is stress compensated such that a flat membrane is guaranteed after release etching.

In addition to the material, the geometry of the freestanding structure on which the thermopile and the heater are fabricated provides an additional design parameter and needs to be optimized. There are three possible membrane design geometries:

i. Closed membrane: The cold junctions of thermopile are located on the wafer-thick rim around the etched membrane, which defines the hot junctions region. The rim serves not only as the heat sink, but also as the mechanical suspension of the membrane and mechanical protection. This structure has the lowest thermal resistance and time constant. The thermal resistance is practically no larger than half of the thermal sheet resistance of the membrane. In our case, the membrane thermal sheet resistance is about \(10^5\)K/W. However, a high thermopile sensitivity can be still be obtained due to the fact that more pairs of thermocouples can be accommodated on this structure.
ii. Cantilever/bridge beam: The thermal resistance of the beam is proportional to the length-width ratio of the beam, which can be really high. Bridge structure has one quarter of the thermal resistance of the cantilever of the same dimensions. Because a bridge thermally equals to two cantilevers with half-length in parallel. Mechanically, the beam is more fragile than the closed membrane and more difficult to make and use. This structure has a medium thermal resistance, time constant and thermopile sensitivity (see Fig. 2.7 (a)).

iii. Floating Membrane: This structure consists of a large piece of membrane hanging by a few suspension beams (see Fig. 2.7 (b)). The thermal resistance can be very high, because the suspension beams are usually long and narrow. But the narrow beams limit the number of the thermocouples that can be accommodated, which leads to a low thermopile sensitivity. This structure is the most fragile one and special care need to be taken in design, production and use. The time constant of the structure is also high.

For the present device, the need for the integration of the microfluidic channel on the freestanding structure necessitated the compromise of using the closed membrane structure. The membrane size is 400µm*300µm.
2.7.4 Microfluidic Channel

For present device, the thermopile and the heater are constructed on a silicon substrate. The microfluidic components are realized on polymer-based (SU8 and PDMS) or a glass (Pyrex 7740) substrates by batch fabrication. Both the chosen polymer and glass are biocompatible, optically transparent and can be bonded onto the silicon substrate to result in conformal and hermetically sealed channels to the substrate. The polymer process is a low temperature process (<100 °C). The glass channels are more mechanically robust.

Size of small biomolecules is less than 1nm whereas; cells have size in the range of 10µm to 100µm. Thus, the smallest channel width was chosen to be 100µm, so most biofluids can flow through the system. To have a reasonable aspect ratio, the depth of the channel was chosen to be around 40µm. Because the device will be used to measure thermal properties of bio fluids, the reaction chamber covers the heater and the whole

Figure 2.7: Schematic drawing for (a) cantilever structure and (b) float membrane structure.
thermopile atop the membrane. The fluid in the chamber forms a thermal path from the heater to the hot and cold junctions of the thermopile. The chamber size is 600µm*600µm. In general, as channel cross-sectional dimensions are reduced to the 100-µm range, fluidic flows are laminar at the velocities that are practically achievable. This phenomenon has profound impact on microscale fluidics, because mixing of fluids in laminar flow is entirely a function of diffusion. The impact can be positive, by controlling the location of interface of enzyme and analyte, mixing and heat generation can be controlled to happen on top of the hot junctions. On the negative side, reactant consumption efficiency is low and is wasteful of analytes. Laminar flow offers the additional benefit of helping to prevent the formation of bubbles. The flow profile in the chamber must be parabolic. Its impact on sensitivity will be discussed in chapter 4.

2.7.5 Sensor Arrays

Using sensor arrays has two major advantages. First is to get repetitive information which can be used to confirm results or remove noise by averaging. Second is for detection of multiple analytes or multiple characteristics of one analyte. The creation of an array of such calorimeters is important for successfully accomplishing a realistic assay on biological samples. Each calorimeter in the array contains a specific enzyme or biocatalyst for detecting a specific biomolecule of interest. The reaction chambers are connected with fluidic channels so that for any given analyte, simultaneous assay for various molecules can be performed. Figure 2.8 shows a schematic representation of such a multianalyte calorimetric device.
Figure 2.8: An Array of the proposed microcalorimeters with specific immobilized catalyst in each reaction chamber (shown as a different colored patch) will allow for the simultaneous detection of various biochemical molecule of interest. The specificity of each pixel is achieved by the selectivity of the catalyst in each reaction chamber.
REFERENCES

Chapter 3

Sensor Fabrication and Calibration

This chapter describes the fabrication and calibration for the calorimetric biosensor. Section 3.1 starts with description of the mask layout of the sensor. Section 3.2 presents the overall fabrication process flow. Specific steps, such as choice of metal and patterning, anisotropic etch and channel bonding are explained. Calibration experiments on several relevant thermopile and sensor figures of merit are presented in Section 3.3. These include the thermopile resistance, power responsivity, and the response time of the device. Temperature sensitivity of the sensor is derived based on the power responsivity results and the thermal resistance simulated by finite element model of the sensor. Finally, hot water flow test has been performed on the device to check the sealing integrity and the linearity of the temperature sensitivity.

3.1 Mask Layout

Fig. 3.1 shows the mask layout of the device. Five masks are needed for the fabrication process. Fig 3.1(a) is the composite view of the device layout comprising of all the mask layers of one sensor. Fig 3.1(b) shows the thermopile and the heater inside the chamber. The red rectangle indicates the membrane area. Fig 3.1(c)–(g) are individual
mask for polysilicon etch (blue), metal patterning (light green), backside etch (gray), passivation layer patterning (purple) and channel (green).

Figure 3.1: Mask layout for sensor. (a) mask overview, (b) closer look of chamber area, (c) mask for polysilicon etch, (d) mask for metal etch, (e) mask for backside etch, (f) mask for passivation layer etch, (g) mask for channel fabrication.

The metal mask shown here is for etch process. Lift-off process requires an opposite polarity mask. The channel mask can be used for glass channel etch or making the mold for PDMS channel casting.
3.2 Processing

Fig. 3.2 shows the detailed fabrication process of the microcalorimeter. A stress compensated $2\mu m$ thick silicon nitride (0.3 $\mu m$)-silicon dioxide (1.4 $\mu m$)-silicon nitride (0.3 $\mu m$) composite layer is deposited on a double-side polished bare silicon wafer. Thereafter, $0.4\mu m$ thick LPCVD polysilicon ($10^{19}$ $/cm^3$, $p$-doped) layer is deposited on top of the silicon nitride layer. The polysilicon is patterned to define one of the legs of the thermopile and the integrated heater structure using a reactive ion etch (RIE) process terminating on the silicon nitride layer. The thermopile structure is completed by depositing $0.4 \mu m$ thick chromium/gold layer by patterning and evaporation using lift-off process. The mask layer for the creation of the dielectric membrane is then patterned on the backside of the wafer using double-side alignment and released by EDP etching of the silicon. The thermopile pattern on the front side of the wafer is protected during the backside wet etching of the silicon substrate. $0.5\mu m$ thick silicon oxide layer is sputter deposited on the thermopile and patterned to act as a passivation layer. The top view of the fabricated chip shows that the polysilicon layer covers all the areas where the glass microfluidic chip will be anodically bonded.
Separately, 30~50 $\mu$m microfluidic channels in Pyrex 7740 glass are patterned using a Cr/Au mask and etched using HF. Inlet and outlet holes of 0.5 mm diameter are drilled using diamond drill and finally the glass wafer is aligned and anodically bonded to

---

Figure 3.2: Schematic illustration of the fabrication process. (1) Starting substrate with 0.4$\mu$m polysilicon on 2$\mu$m of $\text{Si}_3\text{N}_4$-$\text{SiO}_2$-$\text{Si}_3\text{N}_4$ layer; (2) Polysilicon on top side is patterned to form the thermopiles legs and etch windows are opened on the backside; (3) 0.4$\mu$m Cr/Au is deposited and patterned to complete the microthermopile structure; (4) Substrate silicon is anisotropically etched from backside using Ethylene Diamine Pyrocatechol (EDP) etchant, (5) Thermopile is passivated using a 500 nm sputtered $\text{SiO}_2$ layer; and (6) Pyrex 7740 wafer with etched microfluidic channels is anodically bonded on the polysilicon layer.
the polysilicon layer. Since the polysilicon layer is floating on the dielectric layer, an electrical connection between the polysilicon layer and the silicon wafer has to be made for anodic bonding process to result in a good bond. Fig. 3.3 shows the completed device with the glass microfluidic channel anodically bonded onto the silicon microthermopile with the drilled inlet and outlet ports. The thermopile bonding pads are located such that they can be accessed for wire bonding after the glass microfluidic chip is attached. Finally, a PDMS layer is attached on top of the glass to seal and hold the connection tubings and complete the microfluidic system.

Figure 3.3: Optical photographs of the fabricated device. (a) Shows a close-up view of the microthermopile consisting of 16 Cr-p⁺ polysilicon thermocouples connected in series and the heater structure on the membrane. (b) Shows the complete microcalorimeter with the bonded glass chip and the bonding pads.

Another design layout is a circular thermopile consisting of 26 thermocouples in series with a heater in the center (see Fig. 3.4). The thermopile is circular; the membrane is still square because the anisotropic etch process used in this work. Although the
thermopile is circularly symmetric, the temperature profile contours exhibit rectangular symmetry due to the boundary conditions imposed by the rectangular shape of the freestanding membrane. This mismatch of thermopile layout and temperature profile led to considerable complexity in the interpretation of the subsequent results on the thermopile performance. Thus, in the rest of the thesis results on the rectangular design are reported and discussed.

![Circular microthermopile consisting of 26 Cr-p+ polysilicon thermocouples connected in series and the heater structure on the membrane.]

**Figure 3.4:** Circular microthermopile consisting of 26 Cr-p+ polysilicon thermocouples connected in series and the heater structure on the membrane.

Other than glass, polymers such as SU-8 and PDMS, are also used to build microfluidic channel because of their low cost and processing capability. To improve the electrical isolation of the thermopile from the biochemicals, vapor-phase deposition of parylene can be added as the last step. Specific steps needed to optimize and develop for this device fabrication will be discussed further.
3.2.1 Choice and Patterning of Metal

To build the metal leg of the thermocouple, different metals and methods have been used. Initially, aluminum was chosen, because it is inexpensive, easy to etch and capable of forming good ohmic contact with polysilicon. During etching of aluminum to a depth of 0.5µm (thickness of the deposited Al layer) to form one of the thermopile legs, decreased the line widths from 3µm to submicrons due to severe undercutting during the wet etching process (Fig. 3.5). This result is not desirable since it results in poor device yield, but has been since used to make submicron/nano structures. Additionally, aluminum has very low tolerance to any of the silicon anisotropic etchants and thus was attacked immediately even at the slightest exposure during the backside silicon etching process.

Figure 3.5: Aluminum line was patterned as wide as polysilicon line. But after etching, it became much thinner.
Chromium/gold has better resistance to chemical exposure such as EDP, HF and was thus used in the rest of the work presented here. But galvanic effects were observed during chromium wet etching [1]. These effects occur when silicon is contacted with a noble metal and immersed in an etching solution. The noble metal in this case is the evaporated chromium/gold film. The silicon/gold couple forms a galvanic cell. Silicon is oxidized and oxygen in solution is reduced at the gold electrode. In acid solutions, the galvanic current results in dissolution of the semiconductor. In alkaline solutions the galvanic current results in the growth of an anodic oxide layer, which passivates the silicon surface[2]. Both acid-based and alkaline-based chromium etchants had been tested. In both solutions, the metal lines disappeared. Eventually, a lift-off process for the patterning of gold with chrome as adhesion was used.

3.2.2 Anisotropic Etch

The freestanding membrane can be fabricated with deep reactive ion etching (DRIE) or anisotropic wet etch. The DRIE process is not restricted by the crystal planes of the silicon wafer and can be used to fabricate various shapes of cavities and mesas. DRIE results in much sharper slope of near vertical wall, which serves better as heat sink. Since the DRIE process was not available during the fabrication phase of the project, anisotropic wet etching of Si was used.

Two anisotropic etchants have been used to micromachine the membrane.

i. Alkaline solution based on potassium hydroxide (KOH)

ii. Solutions of ethylenediamine (NH₂(CH₂)₂NH₂), catechol (C₆H₄(OH)₂), pyrazine (C₃H₄N₂) and water
KOH has high etch rate, anisotropy and low toxicity. It is inexpensive, easy to use and dispose. However, its selectivity with respect to oxide and nitride is relatively low. Front side protection is very difficult. A layer of chemical vapor deposited nitride or oxide was found to be insufficient as a protection layer. Black wax and mechanical seal kits are the most common used methods. Black wax is cheap and easy to apply. Unfortunately it can only survive about 2-3 hours etching and for a through wafer etch process; new black wax has to be applied every two hours. Black wax residue is very hard to remove. After half an hour dip in Toluene and an hour oxygen plasma, the chip was still very dirty which made the later deposition of the passivation layer afterwards difficult. The alternative, mechanical protection and fixture made of Teflon is expensive. In the present work, some of the backside etches were done with 50% KOH solution at 80-85°C. Etch rate is \(~ 60\mu m/hr\).

Chemical reactions for the etching of silicon by Ethylene diamine pyrocatechol (EDP) is [3]:

\[
2NH_2(CH_2)_2NH_2 + Si + 3C_6H_4(OH)_2 \rightarrow 2NH_2(CH_2)_2NH_3^+ + [Si(C_6H_4O_2)_3]^{2-} + 2H_2
\]  

3.1

EDP has a high selectivity with respect to silicon dioxide and nitride layer. The etch ratio of (100) silicon to silicon dioxide is about \(10^4\). Highly boron doped (>5*10^{19}cm^{-3}) silicon is not etched significantly and can be used as an etch stop. Additionally EDP etch is CMOS compatible. But the use of EDP is not as practical as KOH because it is toxic and even suspected to be carcinogenic. When using EDP solution, safety and cleanness are imperative. For front side protection, silicon wafer was glued to a same size glass wafer with silicone rubber RTV66 (from GE) at the edge. The silicon rubber was cured for 24
hours in room temperature. After the etch, a careful cleaning is required and the silicone rubber can be removed with a blade. Since the highly doped polysilicon and chromium/gold all have good chemical resistance to EDP etchant, short time exposure to EDP due to silicone rubber failure did not usually harm the front side thermopile. Most anisotropic etches in this work were performed with an EDP solution containing 72ml water, 72g catechol, 1.35g pyraine and 225ml ethylenediamene at a temperature of 110°C. The etch rate for silicon is about 80µm/hr.

Table 3.1 compares the two anisotropic etchants used in this project.

<table>
<thead>
<tr>
<th></th>
<th>KOH</th>
<th>EDP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Etch Rate for (100) Silicon</strong></td>
<td>1~2 µm/min</td>
<td>0.75~1.25µm/min</td>
</tr>
<tr>
<td><strong>Anisotropy</strong></td>
<td>(111):(110):(100) 1:600:400</td>
<td>(111):(100) 1:35</td>
</tr>
<tr>
<td><strong>Mask Material</strong></td>
<td>SiO₂ (14Å/min), Si₃N₄</td>
<td>SiO₂ (2Å/min), Si₃N₄ (1Å/min), Au, Cr, Ag, Cu</td>
</tr>
<tr>
<td><strong>Dopant Dependence</strong></td>
<td>&gt; 10²⁰ cm⁻³ boron reduces etch rate by about 20</td>
<td>&gt;5*10¹⁹ cm⁻³ boron reduces etch rate by about 50</td>
</tr>
<tr>
<td><strong>CMOS Compatiblility</strong></td>
<td>Completely incompatible</td>
<td>Compatible</td>
</tr>
<tr>
<td><strong>Toxicity</strong></td>
<td>Relatively safe, non-toxic</td>
<td>Corrosive, carcinogenic</td>
</tr>
</tbody>
</table>

3.2.3 Channel and Integration

Fluidic components are very important for our device, which are critical for delivering small quantity analytes to the sensing element. The fluidic channel must be leak-tight, relatively tall, and biocompatible. Glass, silicon, and plastic are the most commonly used channel materials. Their advantages and disadvantages can be found in
Table 3.2. Because of the high cost and complex fabrication process, silicon was not chosen. The thermopiles are on planar substrate and have moderate high temperature tolerance. Glass channel can be anodically bonded to the device. Polymer channel fabricated by direct deposition or fabricated separately and integrated by bonding are also demonstrated.

Table 3.2: Comparison of three most commonly used microfluidic channel materials.

<table>
<thead>
<tr>
<th></th>
<th>Silicon</th>
<th>Glass</th>
<th>Plastic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Long, Complex Capillary Systems</strong></td>
<td>Sacrificial Etching Limitations</td>
<td>Yes</td>
<td>Both Sacrificial &amp; Bonded</td>
</tr>
<tr>
<td><strong>Large Sample Volume (µl-nl)</strong></td>
<td>Only on Bonded Systems</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Large Area (sample limited)</strong></td>
<td>Expensive</td>
<td>Moderately Expensive</td>
<td>Inexpensive</td>
</tr>
<tr>
<td><strong>Sample Contaminants (ionic)</strong></td>
<td>Needs Passivation</td>
<td>OK</td>
<td>OK</td>
</tr>
<tr>
<td><strong>Typically Disposable</strong></td>
<td>Expensive ($20/Wafer)</td>
<td>Expensive ($10/Wafer)</td>
<td>Inexpensive ($0.5/Wafer)</td>
</tr>
<tr>
<td><strong>On-chip Detection</strong></td>
<td>Yes</td>
<td>No (Si Hybrid)</td>
<td>Possible (Detector Patches)</td>
</tr>
<tr>
<td><strong>Metal Feed Throughs</strong></td>
<td>Hermetic Bond Difficult</td>
<td>Hermetic Bond Difficult</td>
<td>Easily Made (Soft Materials)</td>
</tr>
<tr>
<td><strong>Valve Actuators</strong></td>
<td>Cumbersome Mechanisms, Poor Seals</td>
<td>Difficult, Poor Seals</td>
<td>Good Seals, Novel Actuator Materials</td>
</tr>
<tr>
<td><strong>Processing</strong></td>
<td>Conventional</td>
<td>Conventional</td>
<td>Need New Techniques</td>
</tr>
</tbody>
</table>

3.2.3.1 Polymer Channel

Polymers in general are a good material option for fabricating microfluidic channels because they are easy to machine, inert to the conditions of the assay, and often
optically transparent. Low temperature fabrication and bonding process is an attractive feature of polymer based channels. It is also possible to pre-deposit chemical moieties into fabricated channels before enclosure. Most of the polymers used to date have favorable dielectric strengths, low thermal conductivities and the material surface is modifiable to suit the application.

i. SU-8 channel

SU-8 is a negative, epoxy-type, near-UV photoresist that has been originally developed, and patented by IBM. This photoresist can be as thick as 2 mm and aspect ratio of >20 have been demonstrated with standard contact lithography equipment. SU-8 is virtually insoluble in most chemicals if well crosslinked. Etching in O₂ plasma alone does not work well but by introducing F-radicals (SF₆ or CF₄) different authors have report etch rate of several µm/min [4, 5]. Piranha clean (H₂SO₄:H₂O₂) does attack SU-8 and can be used to strip even severely hard baked films where solvents fail, assuming the substrate allows. Another processing problem related to SU8 is that it is very difficult to bond the cured (crosslinked) SU-8 to other materials including substrates. This leads to poor sealing of and the fabricated microfluidic channels. For our device, SU8-10 is used to build 30~50µm thick channel walls (Fig. 3.6) and glass lid is glued on top with Torr Seal® two part epoxy glue from Varian Inc.
ii. PDMS channel

Poly-(dimethylsiloxane) (PDMS) is a silicone rubber. PDMS consists of an inorganic backbone of alternating silicon and oxygen atoms. Methyl groups are attached to the silicon atoms forming the repeating unit in the polymer. It has a unique flexibility, resulting in one of the lowest glass-transition temperatures of any polymer.

PDMS is an attractive material for imprinting application because of its elastomeric properties. PDMS can be cast against a positive relief master to form microfluidic networks. The master can be made using silicon micromachining techniques, in which the positive relief is transferred to the silicon wafer using a KOH-based etching process or SU-8 photoresist. PDMS has the interesting properties of being transparent (no byproducts of curing), permeable to a variety of liquids and vapors, fairly inert, and high gas permeability. This gas permeability property is for instance very important so far as the culturing cells which requires the supply of oxygen to the cells growing within the closed microfluidic system. To get good cell growth it is also very important to use non-
toxic materials and PDMS due to its structural and material composition adequately fulfils this requirement. Finally its optical transparency in its cured (solid) state enable easy observations of the cell activities or bio reactions. These observations can be very important in understanding the different behavior according a surface treatment or the response to stimuli during the cells culture or the efficiency of the desired bio reactions.

Cured PDMS has a very low surface energy. That is, it is difficult for chemical species to interact with the surface, and adhesion is poor. Changing the surface characteristics of the polymer to give it a higher surface energy enhance the adhesion characteristics of the polymer [6]. The PDMS surface may be oxidized with the discharge from a Tesla coil. This corona discharge electrically breaks down chemicals comprising the air. Some of the chemical species produced by this process can react with the PDMS surface to convert it to a less hydrophobic surface. A plasma discharge or UV exposure works the same way.

3.2.3.2 Glass channel

Thirty years ago, Wallis and Powerantz [7] discovered that if an electric field is added between a metal as anode and a glass as cathode, strong adhesion strength could be obtained with a lower temperature than using thermal bonding. This process is usually known as anodic bonding or electrostatic bonding. Compared to thermal bonding, anodic bonding has the advantage of lower process temperature (300°C~500°C) and less stringent requirement for surface quality. The bonding is hermetic, high yield and clean processing for the fabrication of micro fluidic channels and the strength is even higher than the substrate [8].
Glass such as Corning 7740 (Pyrex), Corning 7070, and soda lime 0080 are used in anodic bonding. In the case of Si, Pyrex 7740 is the most commonly used, because it has coefficient of thermal expansion curve which closely matches Si single crystal, especially for temperature $<300^\circ$C (see Fig. 3.7) [9]. Pyrex 7740 contains 80.5% SiO$_2$, 12.9% B$_2$O$_3$, 2.2% Al$_2$O$_3$, 0.4% B$_2$O$_3$, and 3.8% NaO. Glass transition temperature is 530$^\circ$C.

Figure 3.7: Thermal expansion of silicon and Pyrex 7740.

For anodic bonding, the working theory is to apply a positive (+) DC voltage to the Si wafer and negative (-) to the glass wafer while the wafers are pressed together and heated. The electric field drives Na$^+$ ions in a glass wafer away from the interface region. It leaves the less mobile oxygen ions at the sodium depleted zone near the interface. Oxygen ions then diffuse into silicon surface and react with silicon. Thus an amorphous layer of SiO$_2$ is formed in the bonded region, creating a permanent covalent bond. Anodic
bonding process needs an increased temperature, because the formation of silicon oxide and the mobility of oxygen and sodium ions is temperature dependent. Therefore, while holding pressure constant, the bonding time increases with decreasing temperature setting [8].

To decrease gold immigration and residual stress, 350°C was chosen in this work. A voltage of 450V and pressure of 25N/inch$^2$ was used. Bonding time is less than 5min. To form good bonding, wafer surfaces must be particle free and have a surface roughness of less than 0.1 µm. The native or thermal oxide layer on the Si must be thinner than 2000Å. And OH$^-$ solution treatment of surfaces before bonding is preferred.

3.3 Calibration

After the thermopiles were fabricated, we measured the electrical resistance, the responsivity and response time. By using finite element modeling (the detail of the model will be discussed in chapter 4), we also estimated the temperature sensitivity of the thermopile.

3.3.1 Electrical Resistance

Using semiconductor characterization system KEITHLEY 4200-SCS, Current-Voltage curve of thermopile was measured (Fig. 3.8). Voltage sweeps from -5 V to 5 V by a step of 0.1 V was used, and the current was limited to less than 1mA. In this design, thermocouple of $M=16$, thermopile length $L=370$ µm, width $W=3$ µm and depth $d=0.4$ µm was used. Thus, the expected resistance of the device can be given by: $R_{thermopile} = M*[Resistance\ of\ the\ Polysilicon\ leg] + (Resistance\ of\ the\ gold$
The measured resistances are in the range of 240 kΩ – 270 kΩ. Measured resistance matches the expected resistance quite well. The I-V curve shows good linearity.

![Thermopile I-V Curve](image)

**Figure 3.8:** Thermopile I-V curve. Voltage input sweeps from –5V to 5V at step of 0.1V. The current output has good linearity.

### 3.3.2 Responsivity

To determine the responsivity, the integrated polysilicon heater was used to dissipate heat by applying a voltage on it. The thermopile output was measured as the voltage applied on the heater was varied and yielded a responsivity ~0.94 V/W (Fig. 3.9). The thermopile output increased linearly with the dissipated heat and the standard deviation error is less than 1%. 

\[
[leg] = 16 \times [(127 \times 370/3) + (0.0244 \times 370/(3 \times 0.4))] = 250.7 \text{ kΩ.}
\]
3.3.3 Temperature Sensitivity

A more important parameter of thermopile is the temperature sensitivity. $10^{19}$-boron doped polysilicon has a Seebeck coefficient of $\sim 350 \, \mu V/\degree C$ [10] and chromium has a Seebeck Coefficient of $\sim 16 \, \mu V/\degree C$ at $20\degree C$ and the thermopile has 16 polySi-Cr thermocouples connected in series. Therefore, the nominal temperature sensitivity of the thermopile is expected to be $\sim 5.3 \, mV/\degree C$.

By using finite element modeling the temperature map of the thermopile hot and cold junctions for a specific level of power dissipated in the polysilicon heater located on the membrane was calculated. To calculate the thermal resistance, $1 \, \mu W$ heat was dissipated in the polysilicon heater. The temperature of the bottom of bulk silicon (where it is thermally attached to the package) was fixed at room temperature 298 K.

Figure 3.9: Integrated heater was used to calibrate the sensitivity of thermopiles.
Temperature distribution in the membrane due to the heat generated in the polysilicon heater was calculated. For 1 $\mu$W of heat, temperature profile along the lines on which of the hot and cold junctions of the thermopile are located was calculated (Fig. 3.10). For an input power of 1 $\mu$W, the average temperature difference of 0.2 mK was calculated between the hot and cold junction which gave a value for thermal resistance of 200 K/W. Dividing the slope of the line form Fig. 3.9, which is the experimentally measured responsivity of the thermopile with the simulated thermal resistance, we were able to obtain a temperature sensitivity of $\sim$4.7 mV/°C which agrees within 20% of the roughly estimated 5.3 mV/°C value discussed earlier. Given the simplifications used in the rough model and the comparison using theoretical Seebeck coefficient values for the $p$-polysilicon and chromium thin films, the agreement between the measured and predicted values can be considered to be reasonable.
3.3.4 Time Constant

The time constant of the device was measured by sinusoidally heating the diaphragm using the integrated heater and measuring the peak-to-peak output voltage as function of frequency. Fig. 3.11 shows the thermopile response for 0.5Hz and 10 Hz input respectively. Since both the positive and the negative part of the sine wave result in heating of the polysilicon heater, the temperature function detected by the thermopile exhibits an output signal at double the input voltage frequency. At 10Hz the thermopile output amplitude decreases and begins to approach the noise levels. Based on these observations a time constant of less than 100ms was deduced. A more accurate measurement of the time constant is discussed next.
The thermopile does not respond uniformly to all frequencies. For a heat incidence $Q(t) = Q_0 \sin(\omega t)$ the thermopile output signal is

$$V = \frac{V_0}{\sqrt{1 + (f / f_c)^2}}$$

where $f_c$ is the corner frequency, also called 3-dB frequency. Time constant is related to corner frequency by $\tau = 1 / f_c$. Fig. 3.12 shows the normalized output voltage amplitude of the thermopile $V/V_0$ as function of the applied signal frequency. The corner frequency is 15Hz corresponding to a time constant of 67ms.

Figure 3.11: Thermopile output waveform for 0.5Hz and 10 Hz voltage input on the heater.
3.4 Demonstration of Sensor Operation using Hot Water

In order to test the basic operation of the integrated microfluidic device and the linear range of the temperature sensor, a simple flow experiment was set-up. These experiments were designed to demonstrate the temperature sensitivity of the device using the integrated microfluidic channel.

3.4.1 Experiment Setup

First, a syringe pump was used to pumped water through a hot water bath. Then, the heated water flowed through the integrated microfluidic channel while the thermopile output voltage was monitored (Fig. 3.13).

Figure 3.12: Frequency response of thermopile. The corner frequency is at 15Hz, the time constant is 67ms.
3.4.2 Temperature Sensitivity

Fig. 3.14 shows the thermopile output for flow rate 0.1-5ml/hr and hot water bath temperature form 25°C to 50°C. Fig. 3.14 (a) shows the thermopile output at low flow rate in the range of 0.1-0.5ml/hr. In this flow range, the thermopile sensitivity shows no significant change as the flow rate changes. At higher flow rate in the 1 - 5ml/hr range, as the flow rate is increased the sensitivity of the thermopile was found to increase as shown in Fig. 3.14 (b). In this range, for all flow rates, the thermopile showed good linearity for temperature sensing.
For all the flow rates, the measured sensitivity of the thermopile (~50µV/°C for flow rate of 5ml/hr) was worse than the predicted maximum value of 4.7mV/°C. This can be due to two reasons: (i) water was made to flow through a constant temperature bath
after which it entered the microfluidic channel on the thermopile. The relatively slow flow rate can result in a reduction in the temperature of the water by the time it reaches the thermopile causing an underestimation of the temperature sensitivity of the device, and (ii) the layout of the microfluidic channel results in the hot water uniformly flowing over both the hot and cold junctions of the device. Although, the cold junction is attached to the substrate, its temperature is likely to rise. Therefore, the actual temperature difference between the two junctions will be lower than ideally possible with a perfect boundary condition and will result in a lower sensitivity. That also means the real linear temperature sensing range of the thermopile might be smaller. But most milliliter quantity biochemical reactions cause temperature change less than 1 °C. Our devices’ linear temperature sensing range is good enough for the application.

### 3.4.3 Dependence on Flow Rate

The output voltage dependency on the flow rate for different hot water bath temperature is shown in Fig. 3.15. The signal is proportional to the square of the flow velocity. The sensitivity increases as the hot water bath temperature increases. The increase in the output of the device at constant water bath temperature as a function of flow rate indicates that there is likely heat loss in the tubing connecting to the sensor and thus no calibrations on flow rate can be performed as such. However, these experiments clearly demonstrate the basic operation of the thermal sensor with integrated fluidic channel.
3.5 Summary

i. Fabrication of thermopile, release of freestanding membrane, fabrication and integration of microfluidic system are described. Several version of the device have been successfully fabricated and demonstrated

ii. Preliminary testing was performed to make sure that the devices functioned as planned.

a. Thermopile resistance was measured and compared with predicted value. Good agreement was found.

b. Thermopile responsivity was measured and converted into a temperature sensitivity of the device. Based on the measurements a temperature

Figure 3.15: Output voltage vs. flow rate at different temperature.
sentivity of 4.7mV/K was obtained and is sufficient to provide up to a mK resolution.

c. Flow testing using hot water showed that the device has a linear response as a function of temperature and quadratic dependence on the flow rate for a given temperature.

iii. Thus the basic feasibility and potential of the device has been demonstrated. In the next chapter detailed biochemical measurements will be discussed.
REFERENCES

Chapter 4

Heat of Reaction Measurement

This chapter presents the results on the measurement of heat of biochemical reactions (enthalpy) using the integrated micromachined thermopile sensor. The generation or absorption of heat during a biochemical reaction causes a change in the temperature of the membrane on which the hot junctions of the thermopile are located in turn leading to the generation of a voltage across the thermopile. The amplitude of this voltage is proportional to the reactant concentration. First, enzymatic reaction of glucose is performed on the microthermopile in open air to demonstrate the device functionality. In section 4.2, continuous flow tests are described. The tests include flow tests to determine the mixing region and flow tests for three different enzymatic reactions. Analytical and finite element modeling are used to analyse the measured sensitivity and investigate possible approaches for device improvement in section 4.3. Finally, dynamic behavior of sensor for periodic flow is studied.

The following chemicals were used in the tests conducted in this work: glucose oxidase (GOD, from ThermoDMA, 260U/mg), catalase (Bovine liver, from CalBiochem, 55300U/mg), urease (from CalBichem, 338U/mg); D(+) -glucose (anhydrous, for microbiology, from J. T. Baker), urea (ultrapure bioreagent, from J.T. Baker), hydrogen peroxide (30%, stablized, from J. T. Baker).
4.1 Liquid Batch Enzymatic Reaction

Initially, the thermopiles were used in an open channel configuration for the measurement of heat of enzymatic reaction. After packaging the freestanding thermopile in a standard ceramic dual-in-line package, a barrier structure using molded PDMS was attached around the thermopile membrane to confine the analyte to this region and prevent its spreading on to the bonding pads. 0.25mm O.D. tubing was attached on top of the PDMS barrier for dispensing the analyte on the diaphragm area. The opening of the tubing was aligned to be on top of the membrane of the thermopile. A PDMS lid was created to seal the package to reduce the evaporation of the substrate. The experimental set-up is schematically shown in Fig. 4.1.

Figure 4.1: Schematic illustration of the open-air, liquid batch, test set-up for monitoring the enzymatic reactions.
Catalytic reaction of glucose to gluconic acid by glucose oxidase is as follows:

\[
\text{Glucose} + O_2 \xrightarrow{\text{Glucose Oxidase}} \text{EA} \xrightarrow{} \text{Gluconic Acid} + H_2O_2 \quad 4.1
\]

where EA represents the enzyme-analyte complex. The enthalpy change for this reaction is \( \Delta H = -80 \text{ kJ/mol} \) [1]. In the measurement technique used in this work, a precisely measured volume of enzyme was first dropped over the thermopile membrane area and allowed to dry in ambient conditions. This was followed by a steady flow of the analyte on to the membrane area using the tubing attached to the device until it filled the entire open PDMS channel area. In the experiments 8 \( \mu l \) of glucose oxidase with concentration of 1 mg/ml and an activity 260 Units/mg was used. This implies that a total of \(~2\) Units of glucose oxidase was applied to the thermopile membrane area. Using a syringe pump, 1 \( \mu l \) substrate was dispensed on the active area at a flow rate of 0.5 ml/hr. Prior to dispensing the substrate; the device was covered with a PDMS lid and tightly sealed to create a vapor-saturated environment surrounding the thermopile during measurement.

Fig. 4.2 shows the time dependence of the output of the thermopile for glucose solutions with concentration from 5 mM to 500 mM and for water on glucose oxidase coated thermopiles. It can be seen that as the concentration of the glucose solution increases, the output signal also increases correspondingly. From Fig. 4.2, it can also be seen that the reaction time constant is \(~18\) s for the 0.5 M glucose solution. In saturation, 1 Unit of glucose oxidase catalyzes the oxidation of 1 \( \mu \text{mole} \) of glucose to gluconic acid per minute at 25°C. For 1 \( \mu l \) of 0.5 M glucose solution, the total glucose dispensed on the thermopile is 0.5 \( \mu \text{mole} \). At the rate given above, the dispensed glucose should be consumed in \(~15\) s which is consistent with the observed time constant.
It can be seen that as the concentration of the glucose solution increases, the output signal also increases correspondingly.

As discussed in chapter 1, enzymatic catalysis obeys Michaelis-Menten kinetics and the reaction rate \( r_s \) can be written as:

\[
r_s = -\frac{d[\text{Glucose}]}{dt} = \frac{r_{\text{max}}[\text{Glucose}]}{K_M + [\text{Glucose}]} \tag{4.2}
\]
$r_{\text{max}}$ is the maximum rate of the reaction and $K_M$ is the Michaelis-Menten constant. For this reaction, there are two regimes, which are of interest. The first occurs as soon as the glucose drop comes in contact with enzyme; at this instant for all the molarities used in the experiment, the initial concentration of glucose exceeds the concentration of the enzyme by at least two orders of magnitude and therefore the reaction occurs in saturation. In this state the maximum reaction velocity ($r_{\text{max}}$) is attained and is independent of the substrate concentration. Since the above test is not performed under continuous flow conditions, the finite amount of substrate dispensed will eventually be consumed and as the reaction proceeds, the concentration of the substrate becomes much smaller than the Michaelis-Menten constant. In this regime, the reaction rate will be proportional to the concentration of the substrate and therefore the substrate concentration (and the output voltage) decays exponentially as

$$\text{[Glucose]} = [\text{Glucose}]_{\text{Max.}} e^{-kt} \quad 4.3$$

In order to verify the predicted behavior from eq. 4.3 at low concentrations, an exponential decay function was fitted to the experimentally obtained data for 0.5 M concentration glucose solution. As can be seen from Fig. 4.3, the exponential function fits well with the observed decay at low concentrations and gives a rate constant of $k = (r_{\text{max}}/ K_M) = 0.36 \text{ s}^{-1}$. It must also be noted that, as expected, an appreciable departure from the exponential fit occurs at high concentrations (peak value) where the reaction rate occurs in saturation and is independent of the concentration. If we roughly estimate maximum reaction rate as $r_{\text{max}} = 0.5 \text{ M}/18 \text{ s} = 27.8 \text{ mM/s}$, then the apparent Michaelis-Menten constant $K_m = 77.1 \text{ mM}$. This number is larger than most reported value (3-
17mM [2-4]). While a variation in the Michaelis-Menten constant is expected due to environmental conditions, such as pH value, temperature and ionic strength, the observed difference is too large to be accounted for these reasons. A high $K_m$ indicates weak interaction between enzyme and substrate. In the experiments reported here, the enzyme was first dried on the thermopile followed immediately by dropping the analyte on top of it. Under these circumstances, the reaction is very likely controlled by diffusion of the analyte to the enzyme or vice versa which must considerably slow down the reaction. Unlike, the value of $K_m$ quoted in literature under active mixing/agitation conditions, this value represents a more realistic estimate of $K_m$ that can be expected in integrated microsensors. Thus, diffusion inhibition may be the cause of high $K_m$.

Figure 4.3: Thermopile output decays exponentially with time at low concentrations for glucose reaction.

In the glucose sensitivity measurements discussed above, in spite of covering with a PDMS lid, heat loss due to evaporation is expected to influence the magnitude of the
measured output. At low concentrations, a decay overshoot in the output is seen in Fig. 4.2. This may be explained as arising due to the variability in the sealing of the PDMS lid which in turn influences the relative magnitudes and the possible phase difference between the heat generation due to enzymatic reaction and the net heat loss due to evaporation. Another cause may be due to the variability in the diffusion of the analyte as the reaction proceeds. Enzyme covers the whole thermopile area. The tubing is attached to assure the analyte drop form and fall on the hot junctions. But there is possibility that as the reaction proceeds, the analyte-enzyme diffusion shifts the position of the maximum heat generation towards the cold junctions. Such an event can easily reverse the polarity of the observed output signal.

From these measurements, a preliminary estimate of the possible glucose detection limit of the device can be obtained. For 1 µl of 5 mM glucose solution, a peak output of 60 µV was experimentally obtained. The typical noise level in the device was measured to be ~6 µV which implies a detection limit of ~1 mM. In terms of molarity, this corresponds to a glucose sensitivity of 1 nmole since the measurements were performed with 1 µl of substrate.

4.2 Continuous Flow Enzymatic Reaction

4.2.1 Flow Test Setup

To perform flow tests, we used two syringe pumps to pump reactants onto the sensor. The waste liquid is collected in a beaker. Output signal from thermopile is detected by a voltmeter and recorded by a computer. The sensor is placed under a
microscope for observation. Every time flow of a new liquid is started, some air bubbles may be trapped in the flow system. It is important to make the system bubble-free for getting consistent results. If bubbles are observed under microscope, high flow rate was be used to remove them.

Most experiments are done with both pumps working continuously at a constant rate. Periodic flow measurements needed pump working at a periodic rate. The pumps used in this work, were capable of being programmed to pump liquid with a complex flow velocity profile using a RS-232 communication port. The analog signal of a function generator was converted to a digital one by a Keithley 2000 Multimeter, and then transferred to a computer. The PC then would use this voltage reading as a multiplier in conjunction with the user’s supplied input (specifically, the minimum and maximum flow rates desired), to determine what the fluid flow rate should be. Once the flow rate was calculated, the appropriate command was sent to the syringe pump. Fig. 4.4 shows the test setup.
4.2.2 Flow Testing

After the proof of concept experiments using the open channel testing, the thermopile calorimeter fabrication was completed to include the microfluidic reactor. For the fabricated microfluidic channel on the thermopile device, the flow through the sensor is expected to be laminar. This is because, the *Reynolds* number given by the expression

\[
\text{Re} = \frac{LV_{\text{avg}} \rho}{\mu}
\]

\[4.4\]
where $L$ is the channel dimension, $v_{\text{avg}}$ is average flow rate, $\rho$ is the fluid density and $\mu$ is the dynamic viscosity. Water flow in microchannel: $L = 100 \ \mu\text{m}$, $v_{\text{avg}} = 0.31 \ \text{mm/s}$, $\rho = 1000 \ \text{kg/m}^3$, $\mu = 0.001 \ \text{Pa/s}$. So $\text{Re}=0.031<<2000$. Thus, flow is completely laminar and no turbulence expected.

A PDMS layer was attached to the glass cap so that microfluidic connections could be easily made to the inlet and outlet ports. To initially observe the flow of liquid in the microfluidic reactor and the flow patterns, blue and red dyes were mixed in water and the two dyes were pumped using syringe pumps through the two inlet channels and observed under the microscope. The red dye was pumped at a constant flow rate of 0.5 ml/hr whereas the flow rate of the blue dye was varied between 0-0.5 ml/hr. Pictures in Figure 4.5 show the mixing pattern for blue dye flow rates of 0.1 ml/hr and 0.5 ml/hr. The effect of changing the flow rate of the blue dye is that of changing the location and the contour of the boundary at which the mixing of the two liquids occurs.
Before the enzyme calibration experiments were carried out, 30% hydrogen peroxide was pumped through one inlet port and enzyme catalase (400 U/ml) was pumped through the other inlet port. Keeping catalase pumping-rate fixed at 0.5 ml/hr, the flow rate of hydrogen peroxide was varied. Fig. 4.6 shows the dependence of the microcalorimeter output on the flow rate of hydrogen peroxide. A maximum output was obtained for a flow rate of ~0.6 ml/hr. This result can be expected since the output of the calorimeter not only depends upon the flow rate, but also on where the mixing of the

Figure 4.5: Flow pattern for two different flow rates of the blue dye. (a) The red dye was pumped at 0.5 ml/hr from left and the blue dyes are flowed at 0.1 ml/hr from top. The fluid interface occurs at the top left corner and barely covers half of the line across which the hot junctions are located. (b) The two dyes are pumped at 0.5 ml/hr, which moves the mixing interface to more uniformly cover the line across which the hot junctions are located.
enzyme and substrate occur with respect to the hot junctions. Initially as more substrate is pumped there is an increase in the output of the thermopile until the mixing interface boundary begins to move away from the hot junctions resulting in an overall decrease in the calorimeter output. The reduction in the residence time of the reactants as well as the movement of the enzyme-substrate mixing boundary away from the hot junction explains the overall reduction in the calorimeter output with increasing flow rate of the analyte.

![Graph](image)

**Figure 4.6:** Variation of the microthermopile output as a function of the flow rate of 30% hydrogen peroxide. Catalase flow rate was fixed at 0.5 ml/hr. For a flow rate of 0.6 ml/hr of hydrogen peroxide a maximum in the output was observed.

Liquid batch testing described in Section 4.1 suffered from lack in reproducibility and accuracy of dosing and positioning of the added reactant. Moreover, cooling effect from the evaporation of the liquid sample had suppressed the output voltage because of
the large free surface of the sample drop with respect to its volume. Continuous flow tests of the hydrolysis of urea by urease, the hydrolysis of hydrogen peroxide by catalase, and the oxidation of glucose catalyzed by glucose oxidase were performed on the completed devices. Both substrate and enzyme were pumped in the microchannel through the two inlets by syringe pump at same flow rate (0.5 ml/hr). The two liquids were made to mix and react in the microchamber. Reaction heat was detected by the thermopile. The reacted product flowed out through the outlet port.

4.2.3 Continuous Flow Enzymatic Testing

Continuous flow tests were done for hydrolysis of urea. The reaction is given by:

\[ \text{CO}(\text{NH}_2)_2 + 2\text{H}_2\text{O} \xrightarrow{\text{Urease}} 2\text{NH}_4^+ + \text{CO}_3^{2-} \]  

The enthalpy change is 61 kJ/mol [1]. Fig. 4.7 shows the test result of urea reacting with 250 U/ml urease. Each measurement was made by averaging the output over a period of 5 minutes. The output voltage is linear to the substrate concentration. The sensitivity is 17 µV/M.
Continuous flow tests were next performed on the hydrolysis of hydrogen peroxide using catalase enzyme. The reaction is as follows:

$$2H_2O_2_{\text{Catalase}} \rightarrow 2H_2O + O_2$$  \hspace{1cm} (4.6)

The enthalpy change for the hydrolysis of hydrogen peroxide by catalase is 100 kJ/mol [1]. Fig. 4.7 indicates a linear response of the sensor to the hydrogen peroxide concentration in the range of tested concentration. The tests were performed with enzyme concentrations of 800 U/ml. The sensitivity is 22.3 µV/M. With the

Figure 4.7: Output of the microthermopile as a function of urea and hydrogen peroxide concentration catalyzed by Urease and Catalase.
enthalpy ratio for the urease/catalase reactions being $100/61=1.64$, thus $17 \, \mu V/M$ for urease/urea reaction should imply $17*1.64=27.9 \, \mu V/M$ for hydrogen peroxide /catalase reaction which is reasonably close to the observed value of $22.3 \, \mu V/M$.

Finally, the oxidation of glucose by glucose-oxidase (GOD) was performed in the thermopile microreactor (eq. 4.1). Since the oxidation of glucose by glucose-oxidase requires a mole of oxygen for every mole of glucose, the reaction could not proceed due to the unavailability of such a large quantity of oxygen. The lack of oxygen quenched the reaction and consequently no thermopile output was obtained. Other means of dissolving oxygen in the analyte by aerating the analyte before pumping did not result in enough oxygen to sustain the reaction. The test was therefore accomplished by producing molecular oxygen in the reaction chamber by catalyzing hydrogen peroxide using catalase enzyme. Mixture of one part of 1 M hydrogen peroxide and one part of glucose of varying concentration was mixed and pumped into the reaction chamber through one of the inlets. This hydrogen peroxide concentration was chosen to assure sufficient oxygen supply for all the glucose concentrations used in experiments. A mixture of one part catalase (1600 U/ml) and one part glucose oxidase (480 U/ml) was pumped through the other inlet. Fig. 4.8 shows the output voltage increasing linearly with the concentration of glucose. The device has a sensitivity of $53.5 \, \mu V/M$. At the beginning of the experiment, high concentration of H$_2$O$_2$ was used by mistake. For 30% (9.77M) hydrogen peroxide, sensitivity of the device to glucose was found as high as $395 \, \mu V/M$. The exact mechanism of this enhancement and effect of using different molarities of hydrogen peroxide for glucose sensing still need to be investigated.
4.3 Modeling

4.3.1 Analytical Modeling

A simple analytical model for the temperature profile of the membrane assuming a uniform heat generation over the whole membrane area was developed. Assuming that the boundaries of the membrane are maintained at ambient temperature, the temperature profile of the silicon nitride-silicon dioxide-silicon nitride membrane was modeled by neglecting any radiation or convection heat losses. The heat conduction equation for the two dimensional membrane can be written as
where $\Delta T(x, y)$ is the temperature difference between the membrane at $(x, y)$ and the rim of the membrane, $\dot{Q}$ is the heat generated per unit time per unit area on the membrane surface, $d$ is the total thickness of the membrane, and $\kappa_{||}$ is the effective planar thermal conductivity for the multilayer membrane material made of thin layers of silicon nitride and silicon dioxide in alternate layers, and can be expressed as

$$\kappa_{||} = v_{ox} \kappa_{ox} + v_{nit} \kappa_{nit}$$  \hspace{1cm} (4.8)

$v$ is the volume fraction of the two components, $\kappa$ is the thermal conductivity, and the subscripts $ox$ and $nit$ represent the values for silicon dioxide and silicon nitride respectively [6]. Using the boundary conditions, $\Delta T(x=0, y) = 0$, $\Delta T(x=a, y) = 0$, $\Delta T(x, y=0) = 0$, $\Delta T(x, y=b) = 0$, the solution of the heat conduction eq. (4.7) can be written as

$$\Delta T(x, y)|_{membrane} = \sum_{n,m} \frac{16 \dot{Q} a^2 b^2}{\kappa_{||} d \pi^4 (m^3 na^2 + n^3 mb^2)} \sin \left( \frac{n \pi x}{a} \right) \sin \left( \frac{m \pi y}{b} \right)$$  \hspace{1cm} (4.9)

where the summation is over $n=1, 3, 5, ...$ and $m=1, 3, 5, ...$. In the present case, $a = 400 \mu m$ and $b = 300 \mu m$ and the total thickness of the membrane is $2 \mu m$. The hot junctions of the thermopile are physically located along a straight line parallel to the $x$-axis between $x = 36.5 \mu m$ and $x = 351.5 \mu m$ at $y = 168.5 \mu m$. The effective thermal conductivity of the membrane $\kappa_{||}$ is calculated to be $6.0 \text{ Wm}^{-1}\text{K}^{-1}$. The heat generated on the surface of the membrane is due to the enzymatic reaction. The concentrations used in the experiments are much large than the Michealis-Menten constant, which is $25 \text{ mM}$ for hydrogen peroxide[7], $24 \text{ mM}$ for urea [8] and $4-17 \text{ mM}$ for glucose, thus the reaction rate is
always at the maximum. Assuming all the analyte is consumed before chamber being refreshed, so the heat generated per unit time unit area can be calculated as

$$\dot{Q} = \frac{\Delta H \cdot M_o \cdot d_c}{\tau_r}$$  \hspace{1cm} \text{4.10}

where $\Delta H$ is the enthalpy of the reaction in J/mol, $M_o$ is the molarity of the reactant in moles/liter, $d_c$ is the depth of the reaction chamber, and $\tau_r$ is the time required to refresh the entire volume of the reaction chamber in seconds which depends on the flow rate used in the experiment. Using the value of $Q$ from eq. 4.10 in eq. 4.9, the temperature profile along the hot junctions is calculated from which the molarity sensitivity ($S_M$) of the microthermopile is calculated as

$$S_M = \frac{\Delta T \cdot S_T}{M_o}$$  \hspace{1cm} \text{4.11}

where $S_T$ is the temperature sensitivity of the microthermopile which for the current device is 4.7 mV/K. Implicit in this derivation are the assumptions that the enzymatic reaction occurs uniformly over the entire membrane area, that the reaction is not saturated i.e., the reaction chamber volume is entirely refreshed before the consumption of the reactants occurs. Additionally, conductive heat losses due to the presence of the thin film thermopile itself have been ignored. While these assumptions make the developed model simple, the predictions from it are expected to overestimate the performance of the device. The model does not consider any effects due to the flow of the reactants on the membrane in terms of heat transfer arising due to mass transfer.

Using the ideal model developed here for the hydrolysis of urea by urease, if all the urea molecules in the enclosed reaction chamber are completely reacted, 1 M solution
of urea will generate a total heat of $6.1 \times 10^7$ J/m$^3$ of heat. For the typical flow rates used in the enzymatic testing, $\tau_r = 0.27$ s. Using the depth of the reaction chamber of 30 $\mu$m, the heat generated per unit area per second on the membrane can be estimated to be $6.8 \times 10^3$ W/m$^2$. Using this value of $Q$, the temperature profile of the membrane was calculated and plotted as a contour map in Fig. 4.9(a). The figure also shows the location of the hot junctions of the thermopile and the integrated heater on the temperature profile. Fig. 4.9(b) is a plot of the temperature along the line where the hot junctions are located. The average temperature on the hot junction is 38 mK higher than the rim of the membrane where the cold junctions are located. The temperature sensitivity of the thermopile has been already determined to be 4.7 mV/K, which gives molarity sensitivity of $182 \mu$V/M for the urea-urease reaction. The predicted sensitivity is more than a factor 10 greater than the measured sensitivity and is primarily thought to arise due to the poor mixing of the enzyme and the substrate solutions in the microreactor. The low Reynolds number of the reaction chamber results in a laminar flow, causing the reaction to only occur in a small region around the interface of the reactants, which is at least an order of magnitude less than the volume of the microreactor and can easily account for the difference. Further, it is known that the sensitivity of thermopile drops as the square of the distance when power dissipation moves away from hot junction [9]. Since the dynamics of the boundary layer location and mixing of the enzyme and substrate as well as other simplifying assumptions could not be quantified in the experiments performed, the model was not improved any further. Table 4.1 lists the experimentally measured and predicted sensitivities for the three reactions.
Figure 4.9: (a) Shows the temperature profile on the membrane calculated using the simplified analytical model. The thermopile hot junctions and the polysilicon heater are schematically shown on the temperature profile. (b) Shows the calculated temperature profile across the line along which the hot junctions of the microthermopile are physically located.

Table 4.1: Predicted and measured molar sensitivity of the microcalorimeter. The predicted sensitivity was based on a simple thermal model of the membrane with no consideration of the microfluidics of the flow channels and reaction chamber.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Predicted Sensitivity (µV/M)</th>
<th>Measured Sensitivity (µV/M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urease/Urea</td>
<td>181.9</td>
<td>17</td>
</tr>
<tr>
<td>Catalase/Hydrogen Peroxide</td>
<td>303.2</td>
<td>22.3</td>
</tr>
<tr>
<td>(Glucose Oxidase + Catalase)/Glucose</td>
<td>545.7</td>
<td>53.5</td>
</tr>
</tbody>
</table>
4.3.2 FEM Modeling

For more accurate three dimension study of the device, finite element method has been used.

The FEM simulations performed using Coventorware® software; the system is modeled as a multilayer composite with boundaries capable of exchanging heat and continuous temperature distribution across them. The modeling device size is 800µm*800µm and has following characteristics (see Fig. 4.10):

i. The bulk silicon thickness is set to be 50µm and the bottom temperature is fixed at 300K.

ii. Membrane layer thickness is 2µm and the effective thermal properties are calculated based on eq. 4.8 and

\[
(\rho c)_{\text{eff}} = \nu_{ax} \rho_{ax} c_{ax} + \nu_{nit} \rho_{nit} c_{nit}
\]

iii. To simplify the calculation, we assumed thermopile layer is continuous layer spread out across the whole thermopile area width. To compensate the additional width, the corresponding layer thickness was reduced accordingly.

iv. The polysilicon heater layer thickness is 0.4µm. Only the parts surrounding the thermopile area were considered.

v. The passivation layer consists of 0.5µm silicon dioxide and 1µm parylene.

vi. 30µm water layer covers the whole thermopile and heater area.

vii. The chamber layer thickness is set to be 50µm and the top temperature is fixed at 300K.
The model used parabolic tetrahedrons mesh and has 3327 elements. The material thermal properties used in the model are shown in Table 4.2. This model is used for both enthalpy detection simulation and thermal conductivity measurement simulation. In order to estimate quantity sensitivity, volume heat generation input was added to certain range in the fluid or the heater layer. And average temperature difference of hot and cold junctions is calculated. In the next sections, volume heat generation will be calculated based on experimental conditions and temperature output will be discussed.

Figure 4.10: Cross-section view of FEM device.
4.3.2.1 Flowing Enzyme

In continuous flow experiment, heat generation range depends on mixing caused by diffusion. Diffusion can be described by Fick’s second law. Fick’s second law states that the time rate of concentration change is related to the second derivative of the concentration gradient through the diffusion coefficient as given by [10]:

$$\frac{\partial C_o}{\partial t} = D_0 \nabla^2 C_o$$  \hspace{1cm} 4.13

where $C_o$ is the concentration, $D_0$ is diffusion coefficient. For small molecule, a typical diffusion coefficient $D_0$ is about 1-5*10^{-9} \text{m}^2/\text{s}. Mixing requires three degrees of freedom, since microfluid flows are fundamentally two-dimensional, the third dimension for mixing is time. The estimated mixing length was calculated based on diffusion coefficient of 10^{-9} \text{m}^2/\text{s} and linearized Fick's second law [11]. For the flow rates used in the test, it takes \(\tau_r = 0.27\) second for one reactant flowing from inlet through the chamber to the outlet. The diffusion length \(L_D = (D_0\tau_r)^{1/2} = 16\ \mu\text{m}\). The mixing region can be

<table>
<thead>
<tr>
<th>Material</th>
<th>Thermal Conductivity (W/m*K):</th>
<th>Specific Heat (J/g*K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silicon Dioxide</td>
<td>2.4</td>
<td>0.84</td>
</tr>
<tr>
<td>Silicon Nitride</td>
<td>15</td>
<td>1.26</td>
</tr>
<tr>
<td>Poly-Si</td>
<td>30</td>
<td>0.8</td>
</tr>
<tr>
<td>Gold</td>
<td>297</td>
<td>1.29</td>
</tr>
<tr>
<td>Si</td>
<td>148</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Table 4.2: Material property data used in FEM simulation
simplified as a 600 µm*16 µm triangle (see Fig. 4.11(a)). The lateral dimension of the reaction chamber is much greater than the height. The flow profile in vertical direction is parabolic (see Fig. 4.11(b)). At the chamber surface, the flow rate is zero and there is limited mixing. So mixing region depth is not the whole depth (30µm) of the reaction chamber. Mixing depth of 15µm is used in the simulation model. Heat of reaction is not directly generated on top of hot junctions, but travels through liquid to heat the hot junction. To understand the impact of the mixing location, mixing region is placed at the chamber bottom (right on top on hot junctions), middle of the chamber and at the top (right under chamber wall). Glass and polymer are used as chamber material. Heat of reaction for 1 M urea/urease reaction (6.1x10^7 J/m^3) is used to calculate the average temperature difference between hot and cold junctions. (see Table 4.3).

Figure 4.11: (a) Estimated mixing region used in FEM modeling, (b) Parabolic flow profile in vertical direction in the reaction chamber and the three mixing location used in FEM modeling.
Mixing in the middle of the chamber is more close to the reality than the other two cases. Mixing in the middle of chamber instead of the bottom causes ~25% decrease in sensitivity in both chambers. With temperature sensitivity of 4.7mV/K, glass chamber device has molarity sensitivity of 15.32µV/M for the urea-urease reaction, which is reasonably close to the measured result of 17µV/M. Polymer chamber device is expected to have a has molarity sensitivity of 21.8µV/M. Simulation results show that polymer is a better chamber material.

### 4.3.2.2 Enzyme immobilization

Because of the parabolic flow profile, the heat generation is not right on top of the hot junctions of the thermopile, but travels through liquid to the hot junctions. Instead of flowing both enzyme and substrate, flowing substrate to enzyme coated hot junctions will bring heat generation directly on top of hot junctions. But enzyme immobilization is much more complex than flowing directly. It will limit the fabrication process can be used afterwards. Immobilized enzyme’s lifetime controls the device lifetime. FEM simulation was done to estimate the sensitivity for immobilization test.

<table>
<thead>
<tr>
<th></th>
<th>Glass Chamber</th>
<th>Polymer Chamber</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Top</strong></td>
<td>2.01 mK</td>
<td>3.60 mK</td>
</tr>
<tr>
<td><strong>Middle</strong></td>
<td>3.26 mK</td>
<td>4.64 mK</td>
</tr>
<tr>
<td><strong>Bottom</strong></td>
<td>4.33 mK</td>
<td>5.43 mK</td>
</tr>
</tbody>
</table>
Per square meter and per monolayer of enzyme molecules approximately $2 \times 10^{16}$ enzymes can be attached to the sensor surface. Assuming that effectively there are three layers of molecules of each enzyme active in the coating and one enzyme molecule can converts 3 substrate molecules, there will be maximally $6 \times 10^{19}$ conversions/m2-s, equal to $10^{-4}$ mol/ m2-s. For 315µm*20µm enzyme coating (right on top hot junction), 15nl reaction volume contains at 1mM, the substrate will be depleted after 24s. For flow rate 0.5ml/hr, every 0.27 second, the reaction volume is refreshed. Therefore, enzyme molecules will operate at saturation for substrate concentration larger than 10.8µM. When the enthalpy is 61 kJ/mol, the heat will be 6.1 W/ m$^2$. The temperature difference between hot and cold junction is only 1.7 mK. Increasing the enzyme coating area to 315 µm*140 µm (from 10 µm below hot junction up to the border of membrane), the temperature difference increased to 2.3 mK. Immobilization does not improve sensitivity due to the limited supply of enzyme molecules. But it makes the device more compact, which is especially important for multi-analyte detection in sensor arrays.

4.4 Periodic Flow Testing

Previously, we tested using a steady flow of enzyme and analyte to heat the hot junction of the microthermopile. Since dc measurements need large bandwidths, the overall noise in such measurements is high and therefore limits the accuracy and precision of the measurements. In Chapter 2, the ultimate sensitivity of the device had been calculated. To achieve this kind of sensitivity, AC measurement is necessary. So dynamic response of the devices to a period ac flow was investigated. To determine the frequency of periodic flow, sensor response was investigated upon stopping the flow of
reactants and is shown in Fig. 4.12. The response time is about 150 second for 0.5 M hydrogen peroxide and 210 second for 0.5 M glucose. This low response time is because the remaining reactants which is about 90% of the chamber volume still continue to react even after the flow is stopped.

![Graph showing output voltage over time](image)

Figure 4.12: Thermopile output signal after termination of reactant pumping to the fluidic channels.

Once, the active pumping of the reactants is stopped, as discussed in section 4.1, the reaction rate starts to decay exponentially as the concentration of the substrate drops to below the *Michaelis-Menten* constant. Here we analyze the curve for hydrogen peroxide-catalase reaction since the reaction kinetics of the glucose-glucose oxidase reaction are complicated by the simultaneous presence of the hydrogen peroxide-catalase reaction which was mixed in this case for the production the oxygen. Fig. 4.13 shows the
exponential decay for the hydrogen peroxide-catalase reaction with an exponential fit to the observed data. From the fit, the exponential curve has a decay constant $k = (r_{max}/ K_M) = 0.011 \text{ s}^{-1}$. Estimating the maximum reaction rate $r_{max} = 0.5 \text{ M/150 s} = 3.3 \text{ mM/s}$, the apparent Michaelis-Menten constant can be calculated to be $K_M = 303 \text{ mM}$. This number is much larger than the reported value of 25 mM. Once again, the small chamber volume and the highly diffusion limited kinetics of the reaction lead to a much higher value of $K_M$. In fact, comparing the estimated value of $K_M$ obtained for liquid batch testing (Section 4.1) with the continuous flow case, the estimated $K_M$ is even higher for the continuous flow case. Here, as we discussed earlier, the reaction is occurring in 15 nl chamber along the contact interface of the enzyme and the analyte. The mixing of the reactants is severely diffusion limited in this case and is expected to result in a very high value of $K_M$. Overall, it can be concluded that one of the major challenges in miniaturization of biosensors is this reduction in sensitivity arising from the diffusion enhancement of the Michaelis-Menten constant.
The dynamic behavior of the thermopile-based calorimeter was measured. During the test, one pump pumps 30% hydrogen peroxide at constant rate of 0.5 ml/hr; the other one pumps catalase (400U/ml) as a square wave. Square wave flow rate changes between 0 and 0.5 ml/hr at certain frequency. For 0.01 Hz, the output signal can follow the flow pulse (Fig. 4.14). The output voltage has a delay from the flow input. For 0.05 Hz, the output signal does not have enough time to go back to the base line and can not follow the flow pulse anymore. Periodic output shown in Fig. 4.14 were first observed using a voltmeter. Following these measurements, a lock-in amplifier was used to improve the signal to noise ratio of the device. The function generator used to drive the syringe pump provided the reference signal for the lock-in amplifier. However, the periodic output from the sensor could not be tracked by the lock-in amplifier since the phase lock was

Figure 4.13: Thermopile output decays exponentially with time at low concentrations for hydrogen peroxide reaction.

\[ E(t) = E_0 e^{-kt} \]

Where: 
- \( E(t) \) is the output voltage at time \( t \). 
- \( E_0 \) is the initial output voltage. 
- \( k \) is the decay constant. 
- \( t \) is the time. 

The graph shows the exponential decay of the thermopile output over time for the hydrogen peroxide-catalase reaction. The exponential fit of the data is given by the equation 

\[ E(t) = -22.92 + 23.87e^{-0.01069t} \]
constantly lost by the lock-in amplifier. The large capacitances due to the inlet and outlet ports, long fluidic tubings and the various resistances involved in fluidic systems, make these measurements very challenging. However, integration of a micropump integrated directly on to the device might make such lock-in detections possible. These measurements were however not attempted in this work.

Figure 4.14: Thermopile output for pulsing catalase to hydrogen peroxide at 0.01 Hz.
REFERENCES


Chapter 5

Measurement of Thermal Conductivity

In this chapter, application of the micromachined thermopile-based sensor for the measurement of thermal properties of polymer samples and biochemical molecules will be presented. In this application, the integrated polysilicon heater is used to generate a heat wave which travels to the thermopile. Since the test material is in intimate thermal contact with the membrane on which the heater and the thermopile hot junctions are located, the signal detected by thermopile is a function of the thermal properties of the test material in the micro channel. First, the working principle of the device is explained with a lumped-element model. In section 5.2, liquids with known thermal conductivities are tested to obtain the relationship between output amplitude and thermal conductivity. Then analytical and FEM modeling are used to analyse the data. Commonly used polymer materials in microfabrication were tested and their thermal conductivities were calculated based on the fitted curve. In addition, the proposed technique was applied for monitoring biochemical reactions in real-time as the thermal properties changed from that of the reactants to products in section 5.4. Initial experiments on enzymatic and antibody-antigen reactions show clear evolution of the signal based upon the change in thermal properties. Interesting results have been observed and are compared with other
standardized techniques. This is a completely novel way of biosensing with potential for high sensitivity immunosensing capabilities.

The following chemicals were used in the tests conducted in this work: glucose oxidase (GOD, from ThermoDMA, 260U/mg), urease (from CalBichem, 338U/mg); D(+)-glucose (anhydrous, for microbiology, from J. T. Baker), urea (ultrapure bioreagent, from J.T. Baker ), bovine serum albumin (10^{-4}M (2.5mg/ml) BSA, min 99%, form SIGMA), rabbit anti-bovine serum albumin (anti-BSA antibody (3.6mg/ml), from SIGAMA), human fibrinogen (2.5mg/ml), anti-human fibrinogen antibody (>1million, from Hybridoma and Cell Culture Laboratory, Penn-State Univ.)

5.1 Measurement Principle

Thermal conductivity and specific heat capacity are fundamental material properties for a given temperature condition and do not depend upon material shape and size. Studying these thermal properties is important for microfluidic systems design and may provide a novel method for biochemical reaction monitoring. Several methods have been developed for the measurement of the thermal conductivity of poor thermal conductors [1-4]. In most of these methods, heat is made to primarily flow through the sample under test and the temporal and spatial variation of the resulting temperature is measured [5]. Using silicon microfabrication techniques, thin film heaters and temperature sensors can be easily integrated on freestanding membranes and structures for the measurement of thermal properties of thin films. These techniques have been
recently used to demonstrate microsensors for the determination of the thermal properties of CMOS materials [6, 7] and fluid samples [8].

Figure 5.1: (a) Schematic cross-sectional view along the A-A line shown in the top-view (b) top-view of the microcalorimeter showing the heater and thermopile on a freestanding membrane. The inside boundaries of the integrated microfluidic channel is also illustrated (dotted area). Sinusoidal current of frequency $f$ is used to generate a periodic heat signal at frequency $2f$. The output voltage and phase is continuously monitored using a lock-in-amplifier.

Fig. 5.1(a, b) shows schematic cross-section and top view of the device for the measurement of thermal properties of different materials. Heat generated on the heater (labeled in the top view) is conducted to the temperature sensor (thermopile hot junctions) through the material under investigation and other thermal paths. In principle,
the heat can be transferred by any of the mechanisms to the ambient. However, under typical operating conditions of the thermal microsensors, heat flux through convection and radiation is calculated to be less than 1% [9]. Heat conduction is the primary heat transfer mechanism. Lumped element thermal simulations can be modeled as electrical networks composed of grounded capacitors (thermal mass) and resistors (thermal resistance). Temperature difference corresponds to electrical potential difference (voltage) and heat flux corresponds to electrical current. Thermal resistance and thermal capacitance link these two together as electrical resistance and electrical capacitance link voltage with current. Thermal resistance and thermal capacitance are determined by thermal properties and dimensions of the thermal conduction structure. For the thermal system of our device, the temperature difference between hot and cold junctions can be calculated as

\[ T(x_1) - T(x_2) = Q \ast (R_{\text{therm}} - \frac{j}{\omega C_{\text{therm}}}) \]  

where \( Q \) is heat flow, \( R_{\text{therm}} \) is thermal resistance and \( C_{\text{therm}} \) is the thermal capacitance, which equal:

\[ R_{\text{therm}} = \frac{L}{\kappa A_p} \]  
\[ C_{\text{therm}} = \rho v c \]  

\( L, A_p, \kappa \) are the length, cross-sectional area and thermal conductivity of the thermal conductor respectively. \( \rho, v, c \) are the density, volume and specific heat of the conductor.

Accounting for all the possible paths for heat transfer, several idealized direct thermal resistances need to be considered such as the resistance due to: (i) the air under the
membrane, (ii) the membrane ($R_m$), (iii) the fluid ($R_f$) from heater to thermopile. Heat also travels indirectly through fluid to the PDMS/glass channel and then back to fluid. Of all these four paths, the thermal resistances of fluid and membrane are the dominating ones. A simplified lumped-element model of the sensor is shown in Fig. 5.2. The temperature difference is frequency dependent and has a low-pass filter characteristic. At low frequency ($\leq 1$Hz), the thermal system can be treated as a steady state (thermally equilibrated) system. The thermal capacitors can be treated as open circuit and only the thermal resistance of membrane and fluid are considered. The resolution of this technique depends upon the thermal influence the fluid has in relation to the structures constituting the sensor such as the membrane. If the fluid is a perfect thermal conductor (with $\kappa = \infty$ W/m-K), it forms a short path for thermal conduction, the temperature difference becomes zero. If the fluid is a perfect thermal insulator (i.e., $\kappa = 0$ W/m-K), it equals to an open thermal path and the temperature difference only depends on the membrane. The real fluid is somewhere in between. For an accurate thermal characterization of the fluid, a significant amount of heat must flow through the fluid. The design and materials of the sensor must be chosen to maximize this possibility.
5.2 Liquid and Polymer Measurement

Determination of the thermal properties of thin film polymeric materials is of increasing interest due to the rapid developments in microfluidic systems and micro fuel cells from which such systems are typically constructed. Furthermore, the design and performance of such systems is also influenced by the thermal properties of the nanoliter quantities of fluids flowing through them.

The fabricated calorimeter was used for the investigation of the thermal properties of the liquid samples and polymeric materials. In order to accomplish this, the integrated polysilicon heater is used to introduce a periodic heat signal which travels through the freestanding membrane and the test sample on the membrane to the thermopile hot junctions. The magnitude of the signal for periodically varying heat input was used to determine the thermal conductivity of the material under investigation respectively. A syringe pump was used to flow the test fluid onto the heater-sensor system. A sinusoidal

\[ Q = Q_0 \sin(2\omega t + \Phi) \]

Figure 5.2: Lumped-element model consisting of thermal resistance of channel, fluid, membrane and air.
ac current without dc offset was used to input Joule heat onto the membrane where the hot junctions of the thermopile are located. The frequency of the thermal signal in the device is at twice the heating signal frequency. Thus the thermopile exhibits an output signal at double the input heating current frequency. An SRS830 lock-in amplifier was used to measure the output voltage amplitude of the thermopile. The input signal to the polysilicon heater of frequency $f$ was used as the reference signal and the $2f$ signal from the microthermopile was detected. This method naturally rejects any noise at frequency $f$ as a result of direct electrical coupling.

For a fixed amplitude of the heating signal in the polysilicon heater, the output voltage of the microthermopile was measured at increasing heating frequencies in the presence of the test fluids, fluid mixtures, and polymers on the device. Measurements were carried out in quasi-stationary fluids using a very small flow rate of 20 µl/hr. This low flow rate allows for the validity of the steady state conditions and at the same time refreshes the fluid sample to reduce the influence of change in the thermal properties of the fluids due to the temperature increase of the sample. Fig. 5.3 shows the thermal Bode plot for the device with the thermopile output showing the expected low-pass filter characteristic. A corner frequency of ~5 Hz for the test liquids and ~15 Hz for air was observed.
The device was also used for the measurement of the thermal conductivity of five different polymers commonly used in microfabrication processes. In this case instead of bonding the microfluidic channel, the polymer layer was drop deposited and cured before making the measurements.

5.3 Modeling

5.3.1 Analytical Modeling

The experiment was analytically modeled using 1-dimensional heat conduction model since the thermopile hot junctions were located only 40 µm away from the polysilicon heater (located at \(x=0\)) and parallel to it. Although the microchamber filled the space above the cold junction, FEM simulation showed for the heat generation used
in the test, cold junction’s temperature remained at room temperature. Using this approximation and assumption, the steady state temperature \( T(x,t) \) on the membrane at the hot junctions for a periodic heat flux, \( Q = Q_0 e^{i\omega t} \), can be written as [10]

\[
T(x,t) = \frac{Q_0 D_{\text{eff}}}{2i\omega \kappa_{\text{eff}}} e^{\left(-\frac{x}{\sqrt{D_{\text{eff}}}}\right)} e^{i\omega t}
\]

where \( \kappa \) is the thermal conductivity, and \( D_{\text{eff}} = \kappa_{\text{eff}}(\rho c)_{\text{eff}} \) is the thermal diffusivity, \( c \) is specific heat per unit mass, and \( \rho \) is density of the material. For the calculation of the temperature of the hot junctions, an effective heat capacity per unit volume \((\rho c)_{\text{eff}}\) and effective thermal conductivity \((\kappa_{\text{eff}})\) given by [11]

\[
(\rho c)_{\text{eff}} = V_{\text{membrane}}\rho_{\text{membrane}}c_{\text{membrane}} + V_{\text{sample}}\rho_{\text{sample}}c_{\text{sample}}
\]

\[
\kappa_{\text{eff}} = V_{\text{membrane}}\kappa_{\text{membrane}} + V_{\text{sample}}\kappa_{\text{sample}}
\]

have been used. \( v \) is the volume fraction of the two components - membrane and sample which constitute the two major thermal conduction paths of heat to the thermopile. For the sample on the membrane, the ratio of the volume of the fluid/polymer to the membrane scale in the ratio of their thicknesses i.e., 30 \( \mu m \):2 \( \mu m \) respectively since the surface area of both the components is equal. Thus the volume of the test sample was typically 15 times larger than the volume of the membrane. Although the exact solution of the problem in this work requires a three-dimensional finite element analysis of the membrane-sample system, the analytical solution approximates quite closely to the observed results for high thermal conductivity samples.

From eq. 5.3, the thermopile output amplitude depends not only on the thermal conductivity but also on the thermal diffusivity of the test sample. However, the effective
thermal diffusivity of the sample-membrane system calculated using eq. 5.4 was dominated by the thermal diffusivity of the membrane and resulted in a value of \( \sim 2 \times 10^{-6} \) m\(^2\)/s with a variation of less than 1% for the fluids tested in this work. The analytical curve is calculated using this value of the thermal diffusivity. Analytical model predicts the observed output quite well for conditions where the thermal diffusivity of the test layer is comparable to the membrane. However, considerable deviation is seen for low thermal conductivity samples such as 100% IPA and air where the mismatch is high. This suggests that for these conditions; the magnitude and the assumed isotropy of the effective diffusivity/conductivity calculated using eq. 5.4 is not valid and therefore predicts a much higher output. The \textit{rms} amplitude of the output signal at 0.5 Hz frequency corresponding to the thermal conductivity \( \kappa \) of the various fluids on the sensor membrane and analytical solution are shown in Fig. 5.4.
5.3.2 FEM

The FEM model from Chapter 4 is used here again with different input conditions. Instead of heat generation in the mixing region, Sinusoidal heat generation is set on heater structure. Average temperatures of hot and cold junctions are calculated. In both analytical and finite element simulations, a thermopile temperature sensitivity of 3.8

Figure 5.4: Thermopile output voltage as a function of the thermal conductivity of the test fluid. Measurements were performed at 0.5Hz frequency. Analytical solution uses an effective thermal diffusivity value of $2 \times 10^{-6}$ m$^2$/s for all the fluids tested. A large mismatch in the thermal properties of the membrane and fluid results in a poor fit of the simple model to the observed value. The FEM simulations were found to exactly match the observed output since they are not limited by these simplifying assumptions.
mV/K has been used here instead of 4.7 mV/K in chapter 4 since these measurements were made on a different device with a lower temperature sensitivity.

The FEM model parameters were matched to the measured output of the device for a sample of known conductivity and diffusivity (water). Using these settings in the model, the thermopile output as a function of thermal conductivity was calculated between $\kappa = 0.1 - 0.6$ W/m-K. Fig. 5.5 shows the output of the thermopile as a function of thermal conductivity and for the different polymers tested. The results of the predicted thermal conductivity are listed in Table I and compared to available literature for polyimide (~2%) [12] and SU-8® (~10%) [13] polymers.

![Graph](image)

**Figure 5.5:** Finite element simulation of the output against the thermal conductivity of 30µm thick various polymer thin films. The simulation curve was used to predict the values of the thermal conductivity of the polymers and is listed in Table 5.1.
5.4 Bioreaction Measurement

During a biochemical reaction, when the reactants turn into products, the chemical structure and/or the vibration modes of these molecules are expected to change as well. This change causes the thermal conductivity to change of the resulting sample and leads to a method for real-time bioreaction monitoring. Experiments are done for determining the feasibility of this method and the possibility of doing quantitative determination of biofluid concentration according to their thermal conductivity value. The major challenge for this technique is that most biofluids are water solutions. Since the water molecules domain the thermal conduction, their thermal conductivities are very close to water value. Study of thermal conductivity change during bioreactions was performed for both enzymatic and antibody-antigen binding reactions. All the measurements were done at 1Hz with liquid batch set-up similar to the one described in

<table>
<thead>
<tr>
<th>Material</th>
<th>Measured $\kappa$ [W/m-K] (From Literature*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyimide</td>
<td>0.098 (0.1)</td>
</tr>
<tr>
<td>Shipley 1827 Photoresist</td>
<td>0.195</td>
</tr>
<tr>
<td>SU-8 Negative Photoresist</td>
<td>0.2 (0.225)</td>
</tr>
<tr>
<td>BPRS-100 Photoresist</td>
<td>0.205</td>
</tr>
<tr>
<td>Spin-on-Glass</td>
<td>0.55</td>
</tr>
</tbody>
</table>
section 4.1. Experimental results show that for thermal conductivity measurement, liquid batch and close channel provide same output amplitude vs. liquid thermal conductivity relationship. The thermal conductivities of biofluids were calculated based on the FEM model presented in the previous section. Before the experiment, we estimated the ultimate thermal conductivity sensitivity for the device to motivate the feasibility of the technique.

5.4.1 Ultimate sensitivity of the Thermal Conductivity Monitoring Mode of Operation

In chapter 2, we derived the expression for change of temperature difference due to the change of thermal conductance of the heat path. For \( G^2 >> \omega^2 C^2 \), eq. 2.22 can be approximated as:

\[
\Delta G = -G \frac{\delta(\Delta T)}{\Delta T}
\]

For bandwidth = 1 Hz, the equivalent temperature fluctuation (minimum detectable temperature change) of our device is 10 µK. For water and water solutions, the thermal conductance \( G \) of the system can be approximated to \( 5 \times 10^{-5} \) W/K. \( \Delta T \) is about 0.2K for the power used in our experiment. So the sensitivity limit for thermal conductivity change is \( 4.1 \times 10^{-5} \) W/m-K. Since the thermal conductivity of water is \( \sim 6.0 \times 10^{-1} \) W/m-K, this gives a resolution of \( \sim 68 \) parts per million.

To improve this value further, we need to increase the thermal influence of test sample over membrane. Using lumped element model shown in Fig 5.2, for a fixed heat
flux $Q$, the relationship between the temperature difference $\Delta T$ and liquid thermal resistance $R_L$ can be written as

$$\Delta T = Q\left(\frac{R_L R_M}{R_L + R_M}\right)$$

$$\frac{\delta(\Delta T)}{\Delta R_L} = Q\left(\frac{R_M}{R_L + R_M}\right)^2$$

$$\Rightarrow \Delta G_L = -\left(G_L + G_M\right)^2 \frac{\delta(\Delta T)}{Q}$$

For the present device, $R_L \approx R_M$, so $\delta(\Delta T)/\Delta R_L=0.25Q$. If we increase the membrane thermal resistance 10 time, $R_L \approx 0.1 R_M$, then $\delta(\Delta T)/\Delta R_L=0.68Q$. This is ~3 time increase in resolution. Compared to silicon dioxide and silicon nitride, polymers have an order less thermal conductivity and are better materials for membrane. Fig 5.6 shows FEM results for membrane with thermal conductivity of 0.2 W/m-K compared to our present device. Polymer membrane curve has a slope of 0.67 K/(W/m-K) at around 0.6 W/m-K, which is almost twice the value of current device (0.375 K/(W/m-K)). To decrease the thermal conductance of present membrane, attempt to open a slot on membrane between heater and hot junctions has been made. But the opening made the membrane weak and caused it to crack when liquid was introduced on it.
5.4.2 Enzymatic Reaction

The more exciting application of the device presented is for monitoring real-time biochemical reactions as the thermal properties change with time due to the composition of the test fluid changing from reactants to products. Measurements were first performed for enzymatic reactions; the results are shown in Fig. 5.7 and Fig. 5.8. Fig. 5.7 (i)-(iii) are the results for glucose/glucose oxidase reaction and Fig. 5.8 (i)-(iii) are the result for urea/urease reaction. All the experiments started with water as the base line and the letters in the figures indicate the time of introduction of a new fluid.

i. Glucose/Glucose Oxidase Reactions
Fig. 5.7(i): The sensor was initially loaded with ~15 µl of water. At (a) an additional ~5 µl water was injected into the chamber. As expected, no change in the thermal conductivity of water of 0.601 W/m-K was observed. At (b) ~ 5 µl of 1 M glucose solution was added to the water. The resulting 0.2M glucose solution thermal conductivity changed to ~0.583 W/m-K. At (c) 1 µl glucose oxidase solution (240 unit/ml) was injected and the enzymatic reaction was allowed to proceed. The final product thermal conductivity is seen to be ~0.587 W/m-K.

Fig. 5.7(ii): The sensor was initially loaded with ~20 µl of water. At (a) an additional ~1 µl glucose oxidase solution was injected into the chamber. There is no change in the thermal conductivity. At (b) ~ 5 µl of 1 M glucose solution was added. The thermal conductivity changed to ~0.584 W/m-K.

Fig. 5.7(iii): The sensor was initially loaded with ~15 µl of water. At (a) ~ 10 µl of 1 M glucose solution was added to the water. The resulting 0.4M glucose solution thermal conductivity changed to ~0.567 W/m-K. At (b) 1 µl glucose oxidase solution (240 unit/ml) was injected and the enzymatic reaction was allowed to proceed. The final product thermal conductivity is seen to be ~0.572 W/m-K.

Note that the addition of any reactants to the device reaction chamber is accompanied by the large spikes in the output which are completely unrelated to the experiment and so have been truncated in the graphs to emphasize on the results of interest. Experiment show that glucose solutions have lower thermal conductivity than water. The thermal conductivity decreases as concentration increases. The final reaction products were observed to always have a slightly higher thermal conductivity than the
reactant. However, this value is still lower than the thermal conductivity of water, which implies that the final solution still contains some of the reactants or other partially reacted product molecules since the final products of oxidation of glucose result in water and CO₂. More importantly, the device is clearly able to track the reaction in real-time.

Figure 5.8: Real-time measurement of the thermal conductivity of glucose+glucose oxidase reactions.

ii. Urea/Urease Reactions

Fig. 5.8(i): The sensor was initially loaded with ~20 µl of water. At (a) an additional ~5 µl of 1M urea was injected into the chamber. The resulting 0.2M urea
solution thermal conductivity changed to ~0.593 W/m-K. At (b) 1 µl urease solution (250 unit/ml) was injected and the enzymatic reaction was allowed to proceed. The final product thermal conductivity is seen to be ~0.595 W/m-K.

**Fig. 5.8(ii):** The sensor was initially loaded with ~20 µl of water. At (a) an additional ~1 µl urease solution was injected into the chamber. The resulting solution thermal conductivity changed to ~0.592 W/m-K. At (b) ~5 µl of 1M urea was injected into the chamber and the enzymatic reaction was allowed to proceed. The final product thermal conductivity is seen to be ~0.595 W/m-K.

**Fig. 5.8(iii):** The sensor was initially loaded with ~15 µl of water. At (a) an additional ~5 µl of 1M urea was injected into the chamber. The resulting 0.25M urea solution has thermal conductivity changed to ~0.591 W/m-K. At (b) another ~5 µl of 1M urea solution was added to the solution. The resulting 0.4M urea solution thermal conductivity changed to ~0.587 W/m-K. At (c) 1 µl urease solution (240 unit/ml) was injected and the enzymatic reaction was allowed to proceed. The final product thermal conductivity is seen to be ~0.590 W/m-K.

Experiment shows that once again urea solutions have lower thermal conductivity than water, although the values are much closer to the thermal conductivity of water than for the same concentration glucose solutions. Once again, the final reaction products were observed to always have a slightly higher thermal conductivity than those of the reactants. In all cases tested, the value of the thermal conductivity of the final product of reaction was lower than thermal conductivity of water.
5.4.3 Enzymatic Reaction for Different Power Input

The resolution of the thermal conductivity, from eq. 5.6 is seen to be directly proportional the heat flowing between the heater and the temperature sensor. Thus, the sensor output amplitude change (Temperature amplitude change) for higher heat flow would be larger resulting in a higher resolution in the thermal conductivity. From eq. 5.6, we can see for higher temperature amplitude (higher power input), same thermal conductance change causes higher temperature amplitude change (output amplitude change). Power input and output amplitude change should have linear relationship. The

Figure 5.8: Real-time measurement of the thermal conductivity of urea+urease reactions.
output of the sensor was recorded at four different input powers with (a) 20 µl of water on the sensor, (b) after addition of 5 µl of 1 M glucose solution to the water (resulting in an effective glucose solution of 0.2 M), and (c) after the equilibration of the reaction upon addition of 1 µl of glucose oxidase. The output amplitude changes between different liquids against power input are shown in Fig. 5.9. The results match the theoretical prediction.

Figure 5.9: Output amplitude changes between different liquids vs. power input.

All the former experiments were done at lower power. In those experiments, the noise level for water is about 4 mW/m-K. This means our real thermal conductivity resolution is smaller than the ultimate sensitivity as predicted by the expression given by eq. 5.6 by a factor of 100. By using 9 time higher power, we expect 9 time larger signal and thus better resolution. Fig. 5.10 shows the result for same experiment (water turning
to 0.2M glucose to final product) done at different input power. For input power = 45 mW, the noise reduce to 1mW/m-K.

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**Figure 5.10**: Water, 0.2M glucose and final product thermal conductivity measured at different power input level.

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### 5.4.4 Antibody-Antigen Reaction

Initially static measurements were performed for the following biochemicals: (i) 2.5mg/ml bovine serum albumin (BSA), (ii) 3.6mg/ml rabbit anti-bovine serum albumin (anti-BSA antibody), (iii) human fibrinogen (iv) 2.5mg/ml anti-human fibrinogen antibody, (v) completely bonded anti-BSA with BSA, and (vi) completely bonded anti-human fibrinogen with human fibrinogen. The thermal conductivity of anti-BSA was 25 mW/m-K lower than BSA and the amplitude of their products fell in between. Human fibrinogen and anti-human fibrinogen have very close thermal conductivity. But their
product has ~8.2 mW/m-K lower thermal conductivity. The same experiment was repeated with the reaction performed in liquid batch chamber by adding 10 µl antigen to the 10 µl antibody or the opposite. Agitation is required for these experiments. The thermopile output was monitored in real-time as the reaction occurred and converted into thermal conductivity as shown in Fig. 5.11. As expected the output change from reactant value to product value in all cases. The reaction time can be estimated to be ~100 seconds and ~50 seconds for BSA and human fibrinogen respectively.

In both these experiments, the final value of the thermal conductivity of the product solutions seems to be approaching a value closer to the buffer solution value. This might be due to the fact that as the antibody-antigen bind into a single molecule, the effective concentration of the product is half that of the initial reactants and this effective dilution of the product solution leads to a thermal conductivity value closer to that of the buffer solution. Also note that the thermal conductivity of the buffer solution is slightly lower than that of water once again showing that the addition of dissolving molecules such as glucose and salt into water lowers its thermal conductivity.
Figure 5.11: Real-time measurement of the thermal conductivity change of anti-BSA+BSA reactions and human fibrinogen reactions and compared with static results.
5.4.5 Verification of the above measurements using a Quartz Crystal Microbalance (QCM) Measurement

QCMs are known to provide very sensitive mass measurement because of the resonance frequency change upon the deposition of a given mass on the QCM electrode. Thus, QCMs can be used to monitor real-time basis change of the mass given by the inter-molecular reaction, such as DNA hybridization and antigen-antibody reaction [14-16]. The piezoelectric immunosensor is thought to be one of the most sensitive analytical instruments developed to date, being capable of detecting antigens in the picogram range [17]. Same BSA/anitBSA binding and human fibrinogen/antihuman fibrinogen binding experiments were done with a commercial bench top QCM to compare results.

The QCM (AFFINIXQ4) developed by ULVAC has four channels with vertical agitation system and temperature control system. The sensors have fundamental frequency of 27MHz, frequency resolution of 1Hz and sensitivity of 0.6ng/cm². The sensor cell has a volume of 600 µl.

Before the experiment, sensor cells must be cleaned carefully with piranha solution, then rinsed and blow dried. First step of the experiment is to add 500 µl PBS buffer to the sensor cell and agitate, followed by frequency stabilization for about 15 to 30 minutes. Second step is injection of antigen (1 µl) into the sensor cell. Protein will bind to the surface of gold electrode which causes the frequency to decrease. After the frequency becomes stable again, antibody (1 µl) was injected. This frequency change is caused by the antigen-antibody reaction. The frequency data for BSA and human fibrinogen binding experiment are shown in Fig. 5.12.
In Fig. 5.12, the first drop of frequency is because the antigen attached to gold electrode. The second drop is because of the antigen-antibody binding reaction, which is about 500 Hz for human fibrinogen and 600 Hz for BSA. For affinixQ4, 30 pg mass causes 1Hz frequency shift. So about 15 ng anti-human fibrinogen bonded to human fibrinogen and 18 ng antiBSA bonded to BSA during the reactions. The reaction time for both reactions is about 5 minutes, which is longer than we observed through our calorimeter experiment. It is because the sensor cell holds 500 µl PBS buffer, which means the much lower real reactants concentration and longer reaction time.

Figure 5.12: QCM frequency decreases because of protein fixing and antigen-antibody binding.
5.5 Discussion and Conclusion

The thermal conductivity of water solutions and its dependence on concentration have not been adequately studied. Riedl [18] proposed a simple equation for the thermal conductivity of water salt solutions and acid solutions at 20°C

\[ \kappa_s = \kappa_w + \sum_i \alpha_i C_{0i} \]  \hspace{1cm} 5.6

where \( \kappa_s \) is the thermal conductivity of solution; \( \kappa_w \) is the thermal conductivity of water; \( C_{0i} \) is the molar concentration of electrolyte; \( \alpha_i \) is coefficient characteristic for each ion and determined from experiment data, which is negative value for all cations and most anions and typically in the range of -0.0375~0.018. So most salt and acid water solutions have lower thermal conductivity than water and its value decreases with concentration. This confirms with our observation during the enzymatic reactions.

Antibody and antigen are much bigger molecules. Their solutions are more like liquid suspensions. The effective thermal conductivities of liquid suspensions depend the thermal conductivity of particle, the particle concentration, particle shape (structure) and the interactions among particles [19]. The mechanism of thermal conductivity change during antibody-antigen reaction needs more investigation.

Heat flux in a liquid can be reduced to the transfer of all types of motion: translation, rotational and vibrational, form particles at a higher energetic level to particles with lower energy level. In solutions the transfer of heat by the solvent is predominant in the overall balance, and the fraction of the dissolved substance carries only little weight. This is confirmed by the insignificant difference in the thermal conductivity of water solutions form water. The dissolved particles can serve not only as
direct heat carrier, but can also provide bonding or disruptive effect on the structure of the solvent, thus changing the ability of the solvent to transfer thermal motions.

Until now, all the calorimetric immunosensors need some kind of enzymatic reaction as a signal amplifier, because immunoreactions do not generate enough heat [20]. This work demonstrates a new approach to measure the reaction directly. In summary, we have demonstrated the overall capability of the technique and expect to improve upon the design and accuracy of the measurements further. Future work on the sensor will involve improvements of the design of the sensor for more sensitive measurements of the thermal conductivity of biochemical fluids at lower concentration.
REFERENCE


Chapter 6

Conclusion and Future Work

6.1 Conclusion

A micromachined thermopile-based calorimetric sensor integrated with a microfluidic channel has been fabricated. The fabricated microthermopiles have a responsivity of 1 V/W, temperature sensitivity of ~4.7 mV/K, and a time constant of less than 100ms. The thermopiles showed good linearity over a large temperature range. The devices can also be configured as thermal flow sensors.

The devices were used to detect enzymatic reactions and measure reactant concentrations. Enzymatic catalysis of glucose, hydrogen peroxide and urea, were performed using glucose oxidase-catalase, catalase, and urease respectively in continuous flow configuration using the integrated microfluidic channel. A sensitivity of 53.5µV/M for glucose, 22.3 µV/M for hydrogen peroxide and urea a sensitivity of and 17µV/M was obtained respectively. Detection limit for glucose in the continuous flow mode is ~ 2 mM (30 pmole). This moderate molarity sensitivity is primarily due to the small volume of the analyte used and relatively poor mixing region in the reaction chamber, which depends on the diffusion coefficient and time. For nanoliter analyte, the heat generated by biochemical reaction is small. More sensitive temperature sensing mechanism is required to achieve micromolar concentration detection limit. To improve mixing, pumping
sample liquid, building channel structure to force mixing or immobilizing enzyme on micromotor are some of the methods.

The device was also used to measure the thermal properties of insulating polymers and biochemical samples. In this application, a heat wave is generated using the heater and travels to thermopile. The signal detected by thermopile is thus a function of the thermal properties of the liquid or polymer on the sensor. We used the device for the measurement of the thermal conductivity of five different polymers commonly used in microfabrication processes. The results of the predicted thermal conductivity are compared to available literature for polyimide (\(\sim 2\%\)) and SU-8® (< 10%) polymers. The device is also used for monitoring real-time biochemical reactions as the thermal properties change with time due to the composition of the test fluid changing from reactants to products. Real-time enzymatic reaction for (glucose, urea) and antigen-antibody binding reaction (BSA, human fibrinogen) have been observed. The results proved the device can track the progress of biochemical reactions in real time. Quantitative analysis of biochemical sample concentration is difficult, because the thermal conductivity value of the samples are very close to that of water value especially in the low concentration region. Using lower thermal conductivity membrane material is expected to further improve the device thermal conductivity sensitivity. This is the first demonstration of monitoring biochemical reactions in real-time using this technique.
The following archival publications have resulted from this work:


2. **Yuyan Zhang and Srinivas Tadigadapa**, “Calorimetric Biosensors with Integrated Microfluidic Channels” Biosensors and Bioelectronics, **19**(12), pp. 1733-1743, 2004


This device has also been used as a temperature control and temperature detection platform to construct a MEMS hydrogen sensor by Ramanathan [1]. A simple palladium resistor was fabricated on top of thermopile, and its response to various concentrations of hydrogen was analyzed. The temperature of this resistor was controlled, using the underlying heater and thermopile, and subjected to identical tests. These results were analyzed to reveal an improvement in the pattern of response and an increase in the range of detectable concentrations at elevated temperatures. The sensor was demonstrated of sensing concentrations below 3000 ppm at temperatures in the vicinity of 50 °C.
6.2 Future Work

6.2.1 Integrated Pump

Integration of pump with the device has several advantages. It makes the system more compact for a realistic lab-on-a-chip design. Without the extra connecting tubing to external pumps, smaller reactant volumes are required. By pumping periodically, the sample can be mixed better. All the heat of reaction measurements in this work were performed in dc conditions, which require the use of wide measurement bandwidth in turn resulting in low signal to noise ratio. To improve sensitivity figure, making ac measurements is desirable. In chapter 4, we proved by pumping one reactant periodically, ac heat signal (voltage) can be generated. But due to the large fluidic capacitances and resistances involved in the fluidic systems, this periodical signal is difficult to measure. AC measurements can be more readily achieved if a micropump is integrated onto the device. It may then be possible to achieve an order of magnitude higher sensitivity than what has been reported here.

During recent years, a lot of mechanisms have been used for pumping actuators such as piezoelectric, electrostatic, thermopneumatic, electrochemical, bimetallic, shape memory alloy, and electromagnetic [2]. A pump based on the electroactive polymer with high electrostrictive strain, has the advantage of large displacement and relative high frequency responses. Several kinds of electroactive polymers (EAPs) have developed, which show very high strain level compared with conventional piezoelectric ceramics [3-5]. Among them, poly(vinylidene fluoride-trifluoroethylene) (P(VDF-TrFE)) based electrostrictive copolymer and terpolymer show very high strain and elastic energy density with high load capability [4, 5], which are desired for the actuator application.
Due to their flexibility, low cost and biocompatibility, PVDF based EAPs are ideal for developing all-polymer based disposable BioMEMs and microfluidic devices.

Xia [6] has demonstrated a microfluidic pump by integrating nozzle and diffuser type microdiode with the electroactive polymer, which can pump methanol with a flow rate of 25 µl/min at 60 Hz. The backpressure of this pump can reach about 35 mm of water, and the flow rate of this pump can also be easily adjusted by controlling electrical field. The nozzle/diffuser type pump consists of a chamber, and two planar nozzle and diffuser at each side with a preferential flow direction to direct the flow. A diaphragm is mounted on the chamber, and when it bends up and down, the fluid is pumped through the channel, as schematically shown in Fig. 6.1(a). In Xia’s design, the polymer bimorph diaphragm was bonded to the glass substrate using epoxy glue to form the pump. This design can be readily integrated with the calorimeter described in this work. The top view of such an integrated system is shown in Fig. 6.1(b).
The microthermopile device presented in this thesis was measured to have a temperature sensitivity of 4.7 mV/°C, making it possible to achieve a temperature resolution of ~10⁻³°C. Fabricated sensors could monitor biochemical reactions by the measurement of thermal signals in real-time from both the heat of reaction as well as from the evolving thermal properties of the reacting fluids. For the measurement of heat of reaction, the ultimate sensitivity of this sensor for glucose catalyzed by glucose
oxidase enzyme was estimated to be ~30pmole. Since the volume of the integrated reaction chamber was ~15nl, this gave an ultimate molar sensitivity of ~2 mM.

The above discussion briefly highlights a fundamental problem of laboratory-on-a-chip biosensor arrays. As the test sample volumes are reduced, achieving high molar sensitivities can only be possible if a sensing property with high phenomenological sensitivity is used. The only alternative to this approach would be to integrate an amplification step whereby the number of biomolecules of interest in a given sample volume are selectively increased using processes such as polymerase chain reaction (PCR) for DNA strands or by chromatographic and electrophoretic techniques for other biomolecules. This however is achieved at the cost of greater system complexity.

It is well known that shear-mode quartz resonators made from certain crystal cuts can be used as very sensitive temperature sensors with unprecedented resolutions of up to µK [7-9]. Table 6.1 lists the temperature sensitivity of the resonance frequency with temperature for different quartz crystal cuts [10]. This phenomenological sensitivity of quartz crystals represents 2-3 orders of magnitude improvement in temperature sensitivity in comparison to other similar temperature dependent phenomena such as the Seebeck effect on which a thermopile device is based. The unprecedented temperature sensitivity along with the low noise performance that can be achieved in quartz crystal oscillators can be used to realize a micro-calorimeter for monitoring of bio-chemical reactions. Fig. 6.2 shows a schematic diagram of the proposed quartz resonator based calorimetric biochemical sensor.
A simple analytical model for the temperature profile of the quartz membrane assuming a uniform heat generation over the whole membrane area is developed to show

Table 6.1: Temperature dependence of the resonance frequency of different cuts of Quartz crystals.

<table>
<thead>
<tr>
<th>Quartz Crystal Cut</th>
<th>Temperature Dependence of the Resonance Frequency (ppm/°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC-Cut</td>
<td>20</td>
</tr>
<tr>
<td>LC-Cut</td>
<td>35.4</td>
</tr>
<tr>
<td>Y-Cut</td>
<td>90</td>
</tr>
<tr>
<td>SC-Cut (b-mode)</td>
<td>-25.5</td>
</tr>
<tr>
<td>SC-Cut (dualmode)</td>
<td>80-100</td>
</tr>
<tr>
<td>NLSC-Cut</td>
<td>14</td>
</tr>
</tbody>
</table>

Figure 6.2: (a) Shows the top view of a single pixel of the proposed quartz oscillator based calorimeter with the top and bottom electrodes. (b) Shows a cross-sectional view of a single pixel.
the feasibility of the idea. Assuming that the boundaries of the membrane are maintained at ambient temperature, the temperature profile of a circular quartz membrane was modeled by neglecting any radiation or convection heat losses. The heat conduction equation for the two dimensional membrane can be written as [11]

$$\kappa \frac{\partial}{\partial r} \left( r \frac{\partial \Delta T}{\partial r} \right) = -\frac{\mathcal{G}_t}{d} \quad 6.1$$

where $\Delta T(r)$ is the temperature difference between the membrane at $r$ and the rim of the membrane, $\mathcal{G}_t$ is the heat generated per unit time per unit area on the membrane surface, $d$ is the total thickness of the membrane, and $\kappa_\parallel$ is the planar thermal conductivity for the membrane material.

Using the boundary conditions, $\Delta T(r=a) = 0$, the solution of the heat conduction eq. 6.1 can be written as

$$\Delta T(r)_{\text{Membrane}} = \frac{\mathcal{G}_t (a^2 - r^2)}{4 \kappa_\parallel d} \quad 6.2$$

The average temperature of the electrode which covers the membrane from $r=0$ to $r=a/2$ equals

$$\Delta T(r)_{\text{avg}} = \frac{11 \mathcal{G}_t a^2}{48 \kappa_\parallel d} \quad 6.3$$

The thermal conductivity of the Y-cut quartz membrane $\kappa_\parallel$ is $1.38 \text{ Wm}^{-1}\text{K}^{-1}$. Assuming the heat generated on the surface of the membrane is due to the enzymatic reaction, the heat generated per unit time unit area can be calculated the same way described in chapter 4. 1 M solution of urea will generate $6.8 \times 10^3 \text{ W/m}^2$ heat per unit area.
per second on the membrane. Using this value of $Q$, the average temperature was calculated and plotted as for different membrane thickness and diameter in Fig. 6.3.

![Graph showing temperature vs membrane thickness for different diameters.](image)

**Figure 6.3:** Average Temperature on electrode for different membrane diameter and thickness.

To reduce the stress caused by fluid viscosity, the membrane could be recessed by ~1-2µm via etching. This thin layer of water is will act as a boundary layer and will not be affected by the forced flow. However, this layer will reduce the membrane surface temperature. The effect of 1 µm water on 0.5mm and 1mm diaphragm temperature profile was calculated by FEM and shown in Fig. 6.4. For 1mm, 5µm membrane, the introducing of water of 1µm water will cause temperature drop 30.5% and 2µm water will cause a drop of 32.8%.
Simulation results show that for the same heating condition, quartz temperature sensors have higher temperature rise than the present device by a factor of 100 or more. Y cut quartz also has higher temperature sensitivity. The quartz based calorimeter can detect much lower enzymatic reactant concentration and have applications in immunosensing, metabolism study and more.

Figure 6.4: Average electrode temperature with and without 1µm water on top.
REFERENCES

Yuyan Zhang

Yuyan Zhang was born in Beijing, China in 1973. She received a Bachelor’s of Science degree from the Tsinghua University, Beijing, China in Electrical Engineering along with a minor in Mechanical Engineering in 1997. In 2000, Yuyan obtained a Master’s of Science degree in The Institute of Electronics, Chinese Academy of Sciences, Beijing, China for development of a microthermopile for microwave power detection. Her doctoral work involved the development of micromachined calorimetric sensor for biochemical sensing and polymer characterization.