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A Calorimetric Biosensing System for Quantification of Urinary Creatinine

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ABSTRACT: In this work, we demonstrate the quantification of creatinine in human urine samples using a microcalorimetric sensing system. The calorimetric sensor is based on an array of microfabricated Y-cut quartz resonators. The piezoelectric quartz is etched down to a thickness of 10 μm and exhibits a bulk acoustic resonance of 166 MHz. The temperature sensitivity of this high-frequency quartz resonator is 14,600 Hz/K due to the high phenomenological sensitivity of quartz. Most importantly, the quartz sensors and the analyte fluidics are decoupled providing a significantly more robust calorimetric sensing system than directly contacted chip calorimeters. A reference resonator, consisting of a suspended structure held by four arms, was realized to achieve a thermally isolated from the bulk quartz by using focused ion beam etching. We employ alginate entrapped creatinine deiminase to transduce urinary creatinine into temperature signatures, permitting the quantification of creatinine. Fairly good agreement to the measured creatinine values in the 5 urine samples using calorimetric and HPLC methods is obtained.

The medical community is increasingly relying on affordable, miniature biosensors to evaluate and guide treatments. These biosensors must be capable of analyzing small quantities of samples, have fast response times, and good operational stability. Biosensors based on amperometric, potentiometric and fluorescent biosensors have been extensively developed, however, none of these have the broad applicability for biochemical transduction as calorimetric detection. The general applicability of calorimetric sensing arises from the fact that all biochemical reactions and interactions are associated with an enthalpy of reaction which either produces or consumes heat. Therefore, a single calorimetric transducer can provide a universal platform to quantify a variety of biomarkers, where specificity is achieved through reactions with of biomarker specific macromolecules such as enzymes, proteins, and antibodies.

Commercial calorimeters are the gold standard for monitoring biological interactions and can be easily used as biosensors. However, they are very expensive, too large, and a single measurement can take up to 30 minutes to complete, especially when quantifying very low concentrations. To address these issues, miniaturized chip calorimeters have been explored [1, 2, 3, 4]. Chip calorimeters typically consist of thermally isolated reactor incorporating thin film thermistor or thermopile based temperature sensors and include microfluidics to guide the solution of interest to the sensing region [5, 6, 7]. Due to the integrated configuration of the thermometers and the microfluidic reactors containing immobilized biocatalysts, chip calorimeters must be disposed of after each use. No matter the economy of scale, these devices are eventually not so inexpensive especially when compared to screen printed enzyme electrodes used in electrochemical glucose and other biosensors [8]. Therefore, the development of a robust microcalorimeter with disposable fluidics containing the recognition element is of immense interest. Acute kidney injury (AKI) occurs in 5-7% of hospitalized patients [9] and results in a mortality rate of about 50%.[10] while chronic kidney disease (CKD) has also become a significant global health problem, with prevalence rates in the United States of 13% [11]. CKD describes the gradual loss of kidney function ultimately resulting in end state renal disease and dialysis treatment. Thus, a critical need exists to develop clinically reliable biosensing systems for the detection of early acute kidney injury. Creatinine is largely excreted by glomerular filtration and is a good, though not perfect, marker of renal function. Accordingly, the renal clearance of creatinine is commonly used as an index of GFR. In this paper we employ Y-cut quartz resonators as ultrasensitive temperature sensors and remotely couple the heat of reaction arising of the deamination of creatinine mediated by creatinine deiminase enzyme. Physical separation between the microfluidics and the temperature sensor allows for realizing disposable fluidic cartridges with no resulting fouling or degradation of the performance of the resonator temperature sensors.

Bulk acoustic wave Y-cut quartz resonators can be configured as a ultrahigh sensitivity temperature sensors due to the very high resonator quality factors and phenomenological temperature-frequency sensitivity of +90 ppm/K [12]. Micromachining quartz results in bulk acoustic wave resonators with reduced thermal capacity enabling faster reacting (response time) thermal device as well as higher frequency shifts due to the higher operating frequency. In our previous work, we demonstrated a micromachined, non-thermally isolated, single, Y-cut quartz resonator and remotely coupled it to a fluidic channel containing immobilized urease for the calorimetric detection of urea [13]. These measurements showed that a differential measurement set-up was required to minimize the effects of ambient temperature fluctuations and mechanical stresses arising from the analyte pumping. In this paper we advance the capabilities of this device for the quantification of creatinine in human urine samples. To eliminate undesirable signals arising due to thermal drift and mechanical artifacts, a second thermally isolated quartz resonator reference sensor is used here. In the previous work we utilized a layer-by-layer electrostatic immobilization process for urease [14]. However, this layer-by-layer process was found to be incompatible with creatinine deiminase. In this work we demonstrate an alginate entrapment method to immobilize the creatinine deiminase for the purpose of developing the creatinine biosensor. We present the first microcalorimetric creatinine measurements in human urine samples and benchmark these against HPLC measured values.

EXPERIMENTAL SECTION

Biosensor Fabrication and Operation. The temperature sensor in the microcalorimeter is a thermally sensitive Y-cut quartz crystal resonator (QCR). The micromachined resonators were fabricated on a 100 µm thick, 1" diameter Y-cut quartz crystal substrates. The detailed description of the fabrication process of these resonators is described elsewhere [15]. Briefly, using an inductively coupled plasma etch, the quartz substrate was thinned to ~10 µm in defined regions. These thin 10 µm regions were thereafter patterned with gold electrodes on both the faces as shown in Figure 1(a), which defines the quartz bulk acoustic wave resonator. To achieve further thermal isolation, a focused ion beam etching system, FEI Helios Nanolab 660, was used was used to mill away regions of the thinned quartz leaving the resonator region suspended by four legs as shown in Figure 1(b). The thermally isolated device was used to quantify all common mode signals from stress or thermal drift and was used as the reference sensor. The devices were packaged onto a 100 µm thick stainless steel plate and wire bonded and electrically connected for measurements using SMA coaxial RF-connectors. Detailed description of the packaging process may be found elsewhere [13].

The resonance characteristics of the quartz resonators were monitored using an Agilent 4395A/E5061B impedance/network analyzers. The 10 µm thick quartz resonators displayed resonance at 166 MHz. The susceptance and conductance curves at resonance are

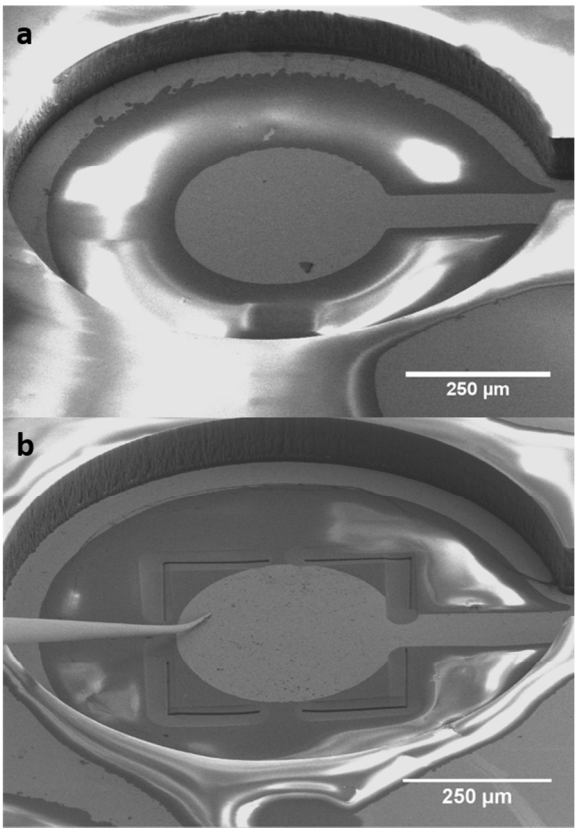

Figure 1. (a) SEM Image of a micromachined quartz resonator with a thickness of 10 µm (b) The resonator area is thermally isolated by using focused ion beam etching leaving the central region suspended by the four arms.

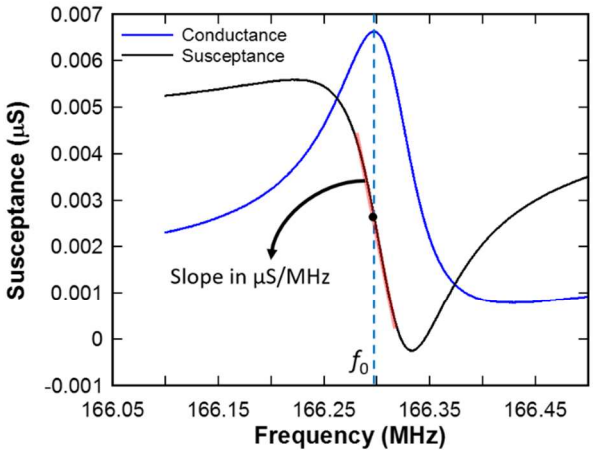

Figure 2. The Admittance and Susceptance characteristics of the resonator about resonance frequency. The slope of the linear part of the susceptance curve is used to convert real time monitoring of susceptance changes into frequency change.

shown in Figure 2. Fixing the frequency of the impedance analyzer at the peak of the conductance, an increase in temperature results in the entire susceptance curve to move to right, i.e. manifests as an increase in the

susceptance at this fixed frequency. Dividing the measured change in susceptance at a fixed frequency by the slope of the linear region of the resonator susceptance-frequency curve, in units of μS/Hz, gives the frequency shift.

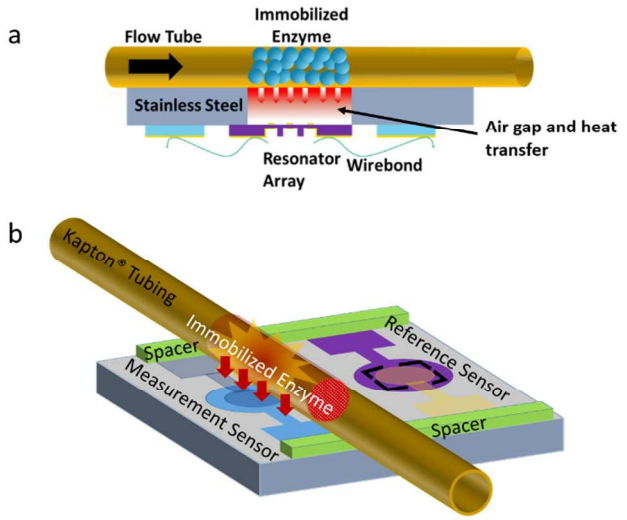

Figure 3. Schematic of the biosensor. (a) A schematic of the calorimetric demonstrating heat transfer through the air gap (b) The primary and reference sensors are displayed. The reference sensor is thermally isolated.

The schematic in Figure 3a illustrates the cross-sectional view of the construction of the calorimetric biosensor. A flow tube is suspended 100 μm above the quartz resonator. The 100 μm thick stainless steel plate acts as a spacer which defines the space between the fluidics and the sensor. The stainless steel plate is milled such that an air gap exists between a section of the polyimide tubing and the QCR. Alginate beads are packed into the flow tube to permit the flow of analyte over the creatinine containing beads and placed right above the quartz resonator.

The alginate beads were formed by spraying an alginate solution containing creatinine deiminase into a solution of calcium chloride. Six hundred microliters of 1.33% w/v low molecular weight Alginic acid (Sigma) in DI water was mixed with 10 mg of lyophilized microbial creatinine deiminase (MPbio) and shaken for 5 minutes. A solution of 300 mM calcium chloride in DI water was created and placed onto a stir plate with magnetic stir bar spinning at 600 rpm. The enzyme containing alginate solution was added to a syringe equipped with a 32-gauge hypodermic needle with 90-degree bend (EFD). This syringe was loaded onto a syringe pump with the hypodermic needle placed directly over the calcium chloride solution. The syringe was pushed at 2.9 mm/min until all 600 microliters of the alginate solution are sprayed into the calcium chloride solution, forming alginate beads with diameters of 200-300 μm.

A differential measurement was performed by placing the polyimide tubing containing these beads on the 100 μm thick stainless steel plate such that the alginate beads were located exactly over the primary QCR, while the thermally isolated reference resonator received no direct heat from the biochemical reaction, as shown schematically in Figure 3b. As the solution containing creatinine is injected through this fluidic tubing containing immobilized creatinine deiminase, the creatinine is hydrolyzed and heat is produced. The transfer of heat to the quartz resonator via conduction through air, stainless steel plate, and radiation results in the resonance frequency of the primary QCR to shift. The frequency shift is monitored as a function of time to quantify the concentration of creatinine in solution. The thermally isolated reference QCR is used to eliminate all common mode noise. Since both the resonators are monolithically integrated, thermal isolation from the rest of the quartz prevents the undesired conductive heat transfer of the heat coupling from the analyte reaction tubing over the primary sensor through the quartz. Since the thermally isolated resonator has no channel above it, the frequency shift of this resonator is primarily influenced by thermal fluctuations and other common mode noise inputs such as mechanical noise from the miniaturized pumps.

The thermal fluctuation of the system was determined by measuring the frequency shift of the resonator over a 5 minute period without flow. The Allan deviation of the resonator, under these conditions, was calculated to be 2.18 Hz with a time constant of 10 seconds. In comparison, an AT-cut (temperature compensated) resonator with similar frequency showed an Alan deviation of 0.25 Hz. Determination of the noise from the mechanical pumping was generated by recording the frequency shifts during pumping operation. The Allan deviation under these condition with the same time constant was 8.36 Hz. The first case represents only thermal fluctuation, while the second case incorporates both thermal fluctuation and mechanical pumping. These data suggest the mechanical pumping is the source of approximately 75% of the noise while the thermal fluctuation results in about 25%. The differential signal under same conditions of pumping and temperature yielded an Allan deviation of 3.16 Hz. The incorporation of the reference sensor provides a 236% improvement in the signal to noise ratio. The response of the system at 1 mM creatinine has been measured to be 60 Hz. Therefore, using the Allan deviation as the noise floor, the theoretical limit of resolution of the current device can be calculated to be 50 μM. However, the practical limit of detection is expected to be somewhat higher due to lack of complete compensation and randomness is the signals between the two resonators.

Enzyme Immobilization and Characterization. The enthalpy of the hydrolysis of creatinine catalyzed by creatinine deiminase has not been thus far reported. Therefore, the enthalpy of this reaction was experimentally deduced using a Microcal isothermal titration calorimeter (ITC). The ITC chamber was filled with 400 μl of 200 nM creatinine deiminase and 0.1 M phosphate buffer. To the ITC chamber, 1 μl of a 3 mM creatinine solution was injected to produce a ~7.5 μM

solution. After complete hydrolysis of the creatinine this 1 µl injection was repeated two additional times. The calorimetric response of the Microcal ITC to the hydrolysis of 7.5 µM creatinine catalyzed by creatinine deiminase is displayed in Figure 4(inset). The experiment was performed for three molar ratios between creatinine and the enzyme through a series

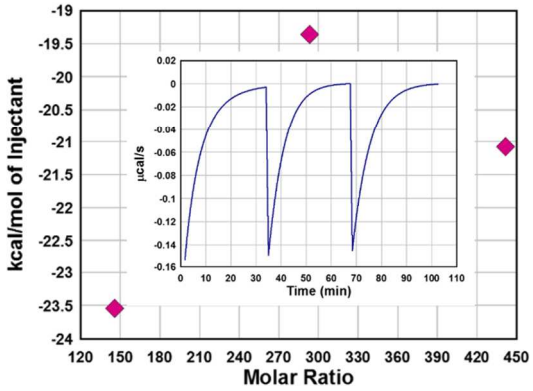

Figure 4. Isothermal Calorimetric determination of hydrolysis creatinine by creatinine deiminase. The real time raw data of the evolution heat for 7.5 µM creatinine catalyzed by 200 nM creatinine deiminase at 23 °C is shown in the inset.

of creatinine injections. The average enthalpy of the reaction from these measurements was determined to be 91 kJ/mol ± 6.8 kJ/mol and is shown in Fig. 4.

A fluorescence assay was utilized to independently investigate the alginate immobilization technique prior to incorporation into our microcalorimetric biosensor. This fluorescence assay utilized a pH sensitive dye 8-hydroxypyrene-1-3-6-trisulfonic acid (HPTS). The fluorescent dye was excited with 454 nm light and emits light at 520 nm, with an absorption spectrum over the range of 6 to 9 pH units. The maximum amount of creatinine used was 40 mM. Therefore, a maximum of 40 mM NH_3 can be produced, limiting the maximum pH of a 0.1 M phosphate buffered solution to 6.975 pH units. This pH limit ensures that the solution is well within the linear regime of the fluorescent dye. For conversion of the output from the plate reader to pH units, the fluorescence intensity of HPTS at various pHs is required. In all cases, the solution was initially set to a pH of 6.2, such that the data obtained from the plate reader may be converted into moles of substrate consumed or NH_3 produced. For this, solutions of 2 mM HPTS were set to various pH values in the range of 6.2 – 7.4 and their intensity recorded with the plate reader. In this 'linear region' where the intensity changes linearly with pH, we determined that for every unit of pH change, the dye will emit fluorescence equal to 2440 A.U. from the plate reader.

For actual calibration of the efficacy of the enzymatic catalysis of alginate entrapped creatinine deiminase, we found that during fluorescent measurement the bead move and created a considerable fluctuation in the fluorescent signal. Thus, for the measurement of the alginate entrapped creatinine deiminase we entrapped the enzyme in square alginate hydrogels patches 4 x 4 cm^2 and 1 mm thick rather than beads. A 1.33% (w/v) alginate solution containing 10 mg/ml creatinine deiminase was pipetted into PDMS mold, which defined the square hydrogel. Alginate solutions lower than 1.33% w/v were not used due to their low mechanical stability. A solution consisting of 300 mM calcium chloride solution in distilled water was pipetted on top of the alginate and enzyme solution. The alginate hydrogel was allowed to form for 30 minutes prior to use. The alginate hydrogels were then rinsed with DI water and removed from the PDMS mold. The alginate hydrogel pieces were placed on a 96-well plate. The enzymatic activity was determined using the fluorescent assay.

Kinetic data was extracted from the fluorescence intensity of the solutions monitored by the plate reader. The change in pH of the solution may be converted to a reaction rate by applying the Henderson-Hasselbalch equation and knowledge of the products of hydrolysis. Since ammonia is a weak base (pKa ~32.5), it may be ignored from the Henderson-Hasselbalch analysis. Further, the dye's (pKa 7.1) contribution was determined to be negligible since its concentration, 2 mM, is much lower than that of the 100 mM phosphate buffer (pKa 6.8). The Henderson-Hasselbach equation applied to this system can be written as

$$pH = 6.8 + log\left(\frac{[A-]}{[HA]}\right) \quad (1)$$

$$0.1M = [A-] + [HA] \quad (2)$$

where [HA] is the weak acid and [A-] is the conjugate base. From these, [A-](pH), the concentration of the conjugate base at a particular pH can be written as

$$[A-](pH) = \frac{0.1\times10^{pH-6.8}}{1+10^{pH-6.8}} \quad (3)$$

For creatinine deiminase, the concentration of reacted creatinine was then calculated using the following reaction

$$Reacted\ Creatinine = \left([A-](6.2) - [A-](pH)\right) \quad (4)$$

RESULTS AND DISCUSSION

The production of ammonia over time from creatinine hydrolysis of solutions containing 2.5 mM, 5 mM, 10 mM and 20 mM creatinine is shown in Figure 5(a). The hydrolysis of creatinine produces 1 ammonia molecule for every molecule of creatinine. The Michaelis-Menten constants were determined for creatinine deiminase entrapped in the hydrogel using a Lineweaver-Burke double reciprocal plot of the concentration of creatinine and the velocity of reaction. The double reciprocal plot is displayed in Figure 5(b). A linear fit to these data points indicates the Michaelis constant is 27.0 mM, and the maximum velocity, V_{max}, is 135 nmol NH_3/min. Following the successful demonstration of the immobilization technique with creatinine deiminase, the immobilized enzyme was incorporated into the calorimetric system.

Calibration samples of known quantities of creatinine were prepared in a 0.1 M phosphate buffered solution at

pH 6.8. These calibration samples were introduced into the column, and the response of the quartz resonators was recorded over three sample injections. A flow injection analysis technique was used to measure the microcalorimetric response to the enzymatic catalysis of creatinine. Using miniaturized-pumps 100 µl of creatinine containing solution was passed through the immobilized enzyme, followed by 100 µl of 0.1 M phosphate buffered solution at a volumetric flow rate of 100 µl/min. This process of injecting creatinine followed by the buffer was

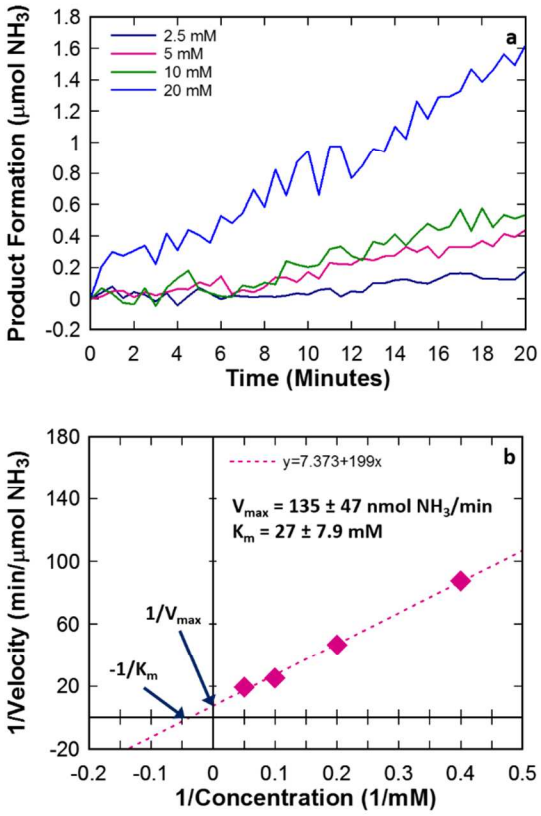

Figure 5. Fluorescent assay of creatinine hydrolysis. (a) The output of the plate reader converted to ammonia product formation (b) The Lineweaver-Burke plot of creatinine hydrolysis.

performed 3 times per test. The response of the primary QCR and reference QCR are plotted in Figure 6(a).

The primary and reference resonators were slightly different in frequencies which results in a commensurate difference in temperature sensitivities. Therefore, the response of the reference QCR was scaled by the ratio of the natural resonance frequency of the primary sensor, f_p, to that of the reference sensor, f_r and the differential signal, Δf_d, is now calculated by subtracting the change in frequency of the primary sensor, Δf_p, and the scaled change in frequency of the reference sensor, Δf_r, i.e.,

$$\Delta f_d(t) = \Delta f_p(t) - \frac{f_p}{f_r}\Delta f_r(t) \qquad (5)$$

The differential measurement produced by using this technique is displayed in Figure 6(a). The differential response eliminates the noise caused by the fluidic pumps, seen as the downward bumps at the top of the primary QCR, as well as thermal fluctuation of the QCRs. The elimination of the spurious artifacts in the output signals permits a more accurate and reproducible system. The differential response of the calorimetric system to creatinine hydrolysis of 5, 12.5 and 25 mM solutions is plotted in Figure 6(b). These plots demonstrate that more concentrated creatinine solutions produced a larger calorimetric response, both regarding the maximum frequency shift and the width of the response.

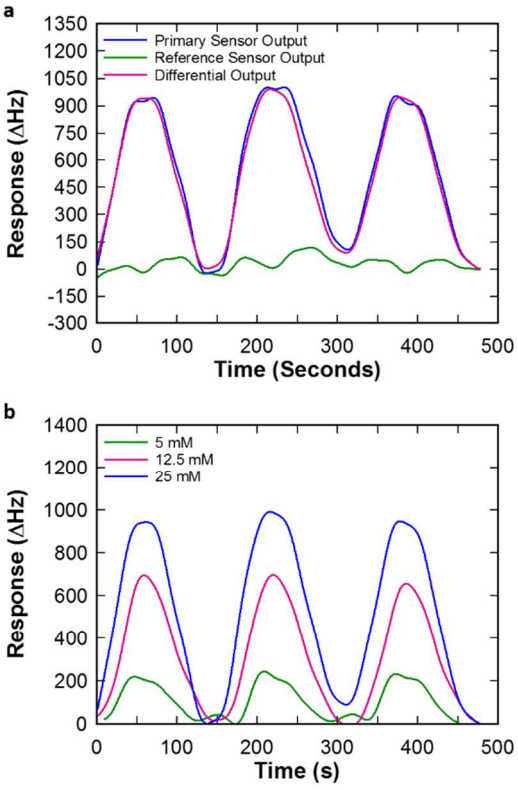

Figure 6. Calorimetric response. (a) The primary and reference sensors response to the reaction of 25 mM solutions of creatinine, as well as the difference between the two sensors. (b) The differential response to the hydrolysis of 5, 12.5 and 25 mM creatinine solutions.

The physiological range of urine creatinine lies between 1-30 mM. Therefore, nine solutions of various creatinine concentration between 1 and 30 mM were used to calibrate the microcalorimetric system. These creatinine solutions were formed using a 0.1 M phosphate buffer adjusted to a pH of 6.8. The calibration curve, which relates creatinine concentration to the response of the calorimetric system is displayed in Figure 7(a). The calibration curve was fit using Michaelis-Menten kinetics, such that equation may be used to relate the response of the microcalorimetric system to an unknown quantity of creatinine. However, rather than using the typical maximum velocity, $V_{max,}$ the maximum response of our microcalorimeter, R_{max}, was substituted into the equation. Subsequently, the velocity of the reaction for a particular substrate concentration, v, was changed to the response of the calorimeter, R, according to the following equation:

$$R = \frac{R_{max}}{\left(1+\frac{K_m}{[S]}\right)} \quad (6)$$

where K_m is the Michaelis constant, and $[S]$ is the concentration of the creatinine used. The obtained data were fit to this modified Michaelis-Menten equation using a least square error method in MATLAB®, which yields a Michaelis constant of 33.6 mM and a maximum response of 2060 Hz. The standard error of the maximum response was determined to be 160 Hz and standard error of the Michaelis constant was determined to be 2.9 mM. In the

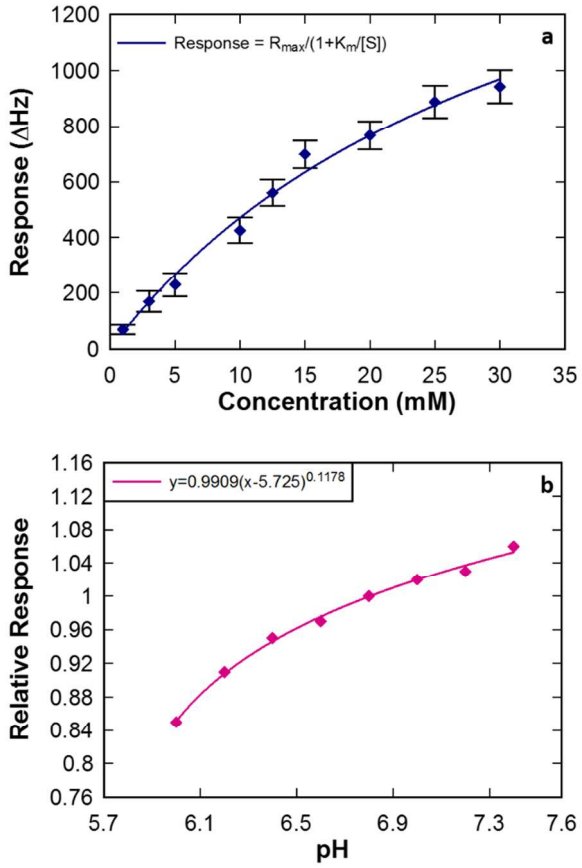

Figure 7. Calorimetric calibration. (a) The differential response of the calorimetric system to FIA of various creatinine concentrations. This data is fit using Michaelis-Menton kinetics. (b) The calibration of sensor response to solutions of various pH levels.

lower concentration range (< 20 mM), the response is nearly linear, whereas the Michaelis-Menten kinetics become more prevalent in the higher concentration ranges.

Enzyme activity is known to be heavily dependent on the pH of the solution, in which the enzyme resides. Moreover, the pH of random urine samples is likely to vary widely over a broad range. For example, the typical pH of urine in a balanced body is slightly acidic in the morning, (pH = 6.5 - 7.0). However, urine becomes more alkaline (pH = 7.5 - 8.0) by evening in healthy people primarily because no food or beverages are consumed while sleeping. The pH of the analyte solution is therefore critical since it influences the enzymatic activity and consequently will affect the response of the calorimetric system. To understand how a change in pH will affect our calorimeter a calibration was performed using 25 mM of creatinine in 0.1 M phosphate buffer solution of various pH from 6-7.4 and the results areshown in Fig. 7(b). The optimal pH of creatinine deiminase was reported by the manufacturer to be 8.5. The relative response of this creatinine solution was scaled to that of the creatinine solution at pH 6.8, the same pH at which calibration was performed. The results demonstrate the response monotonically rises from pH 6 to pH 7.4. Further, the response of a solution at pH 6 is 15% less than that of the same solution at pH 6.8.

Following the establishment of these calibrations, the microcalorimetric system was used to quantify creatinine in human urine samples. The urine samples were collected at The Pennsylvania State Hershey Medical School. The creatinine concentration in these urine samples was quantified using high-pressure liquid chromatography (HPLC) to provide a benchmark for the calorimetric sensing system. The HPLC experiments were also performed at Penn State Hershey facilities.

Before testing the urine was filtered using a 0.5 mm syringe filter unit (Millipore). Following filtration, the flow injection analysis technique described previously was used to introduce 100 µl of urine into the microcalorimeter followed by 100 µl of 0.1 M phosphate buffer. Each sample of urine was tested five times, and the peak height of the responses was quantified as described previously. The response, R, of the calorimeter was inserted into eq. (6) to determine the concentration of the urine sample.

Table 1 displays the concentration of creatinine in each urine sample as determined by the HPLC method and by the calorimetric method. The concentration of creatinine determined through the calorimetric technique is consistently lower than that of the HPLC method. The only exception is sample #1, which is higher due to the increased pH of the sample. The response of the calorimeter will not only be influenced by the pH variation of the urine samples but also interfering molecules. These molecules will typically decrease the activity of the creatinine deiminase enzyme when compared to the pure calibration sample. The observed differences may be attributed to differences in interfering molecules between samples as well as to the differences in pH levels. Table 1 contains the pH of each urine sample. The pH of each sample was determined using a Symphony pH meter. The pH calibration curve shown in Fig. 7(b) was used to compensate for changes in enzyme activity due to pH fluctuations. In this manner, we can determine whether pH is the main cause of the observed deviation from the HPLC analysis or other endogenous molecules play a role.

The pH-adjusted measurements are displayed in Figure 8. In all cases the pH-adjusted measurements are closer to

the HPLC measurement than the non-adjusted, however, the influence of pH does not completely explain the differences between the HPLC analysis and the calorimetric measurement. This difference is most likely due to interfering endogenous molecules in the urine samples, which influence the enzymatic activity of the enzyme and subsequently the response of the calorimeter and need further investigation.

Table 1. Comparison of the calorimetric to HPLC quantification of creatinine

Sample #	HPLC (mM)	Calorimeter (mM)	pH
#1	20.37	25.5 ± 3.2	7.45
#2	6.22	3.1 ± 2.5	6.25
#3	12.44	9.2 ± 2.8	6.43
#4	12.16	10.2 ± 3.1	6.52
#5	13.15	10.3 ± 4.3	6.70

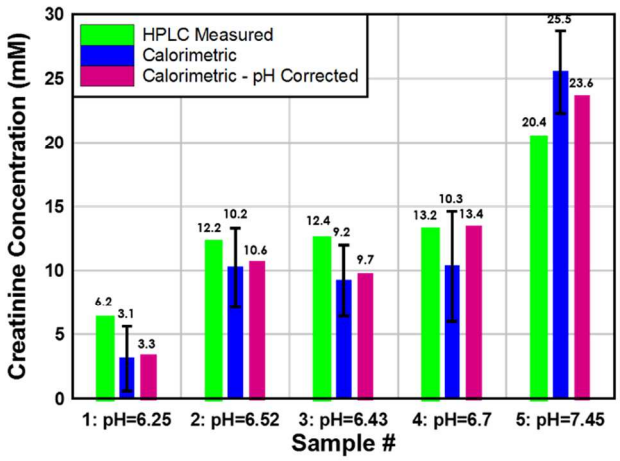

Figure 8. The HPLC, Calorimetric method and pH adjusted calorimetric measurement are compared for each of the 5 urine samples.

At present, the most widely used methods for the quantification of creatinine are based upon the use of alkaline picrate also known as the Jaffe method and its variations [16, 17], enzymatic assays [18, 19, 20], and high performance liquid chromatography (HPLC) methods [21, 22, 23]. The Jaffe method typically suffers from significant errors especially due to interfering proteins which can result in 15 – 25% overestimation [24]. Furthermore, several endogenous and exogenous substance can result in a lack of specificity in this technique. Specifically interference from glucose and acetoacetate can significantly suppress the measured results. Several inorganic chemical–based methods have also been investigated as alternatives to the Jaffe method, however their efficacy has not been proven to be significantly better [24]. Enzymatic methods due to their improved specificity and fewer interferents have been explored as possible alternative but studies have shown some interference effects even in these methods. HPLC is a very sensitive analytical method for the quantification of creatinine. However, several chromatographic approaches are available. In all these cases, it has been shown that sample deproteinization improves the specificity [24]. Thus, HPLC is has been considered as the standard method for comparison of creatinine concentration especially for calibration purposes. What is critical to note is that in the method described in this paper, we did not use any sample preparation technique besides using a 5 µm particle filter. Thus, the differences in the obtained results could be either due to enzymatic interference with other components in the urine or due to suppressed enzyme activity upon repeated exposure to significant fluctuations in the pH of the samples. These effects need further investigation. What is significant is that even without any sample preparation or purification and enzyme immobilization optimization, the obtained results with the error bars are comparable to HPLC results. Further improvements in the quantification of creatinine and approaches towards reduction in the overall variability of the calorimetric response will be explored further in future work.

CONCLUSIONS

This paper demonstrates the first calorimetric measurement of creatinine in five human urine samples. The microcalorimeter performance has been improved through the incorporation of a thermally isolated reference sensor. This paper demonstrated the use of this microcalorimeter for the quantification of prepared creatinine samples with concentrations between 1-30 mM. Further, the pH dependence of the response of the microcalorimeter was investigated. These two calibrations enabled investigation of human urine samples. The response of the calorimeter to human urine samples was used to interrogate the concentration of creatinine in these samples. The quantification of creatinine from the calorimeter was compared to an HPLC technique. We conclude from this work the calorimeter is biased from both endogenous compounds as well as the pH of the urine sample which influence the enzymatic activity. A compensation technique to eliminate the influence of pH did not fully adjust the calorimetric measurement to that of the HPLC. However, through this work we have demonstrated a robust microcalorimetric sensing system for the quantification of creatinine in human urine samples and further investigations to understand and improve the response of the calorimetric output will be reported in future work.

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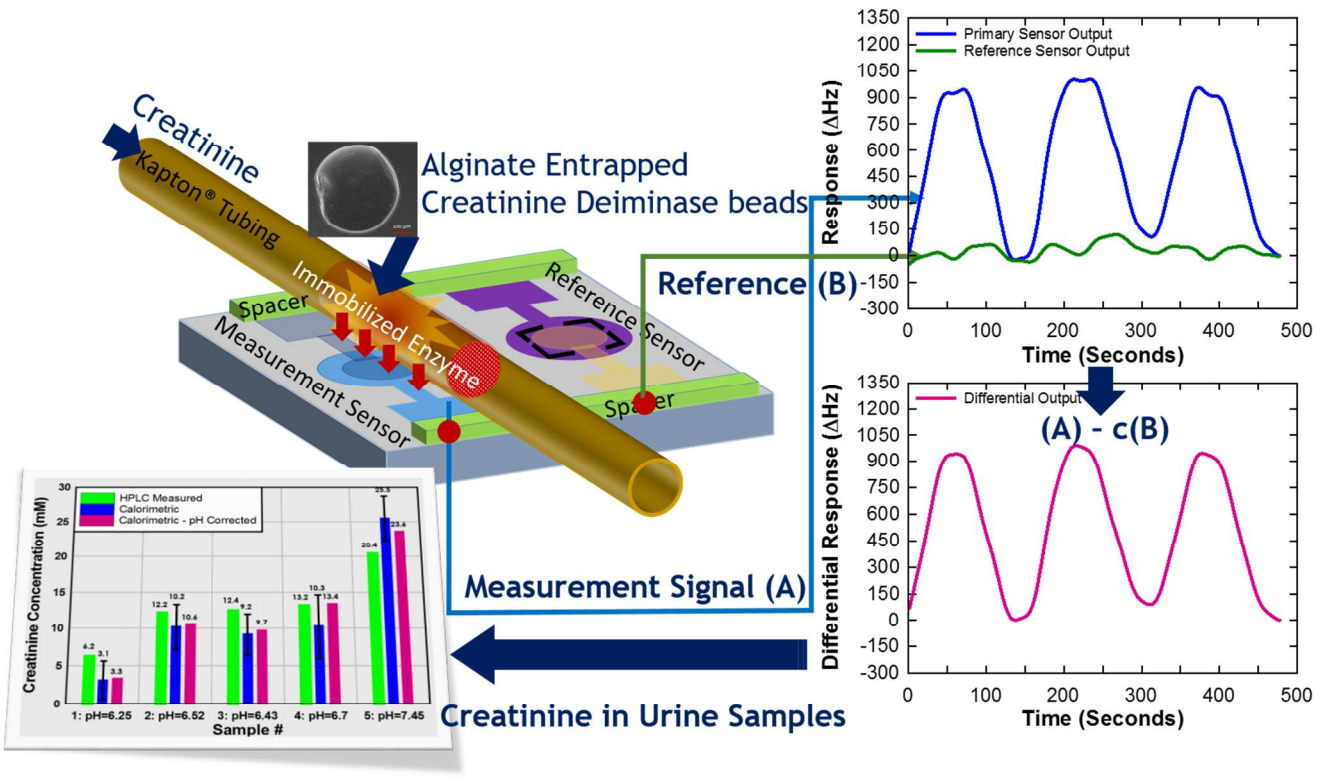
Creatinine
Kapton® Tubing
Alginate Entrapped
Creatinine Deiminase beads
Immobilized Enzyme
Spacer
Reference Sensor
Measurement Sensor
Reference (B)
Measurement Signal (A)
Creatinine in Urine Samples
Primary Sensor Output
Reference Sensor Output
Response (ΔHz)
Time (Seconds)
Differential Output
(A) - c(B)
Differential Response (ΔHz)
HPLC Measured
Calorimetric
Calorimetric - pH Corrected
Creatinine Concentration (mM)
1: pH=6.25
2: pH=6.52
3: pH=6.43
4: pH=6.7
5: pH=7.45
Sample #