Liquid and gas micro-calorimeters for (bio)chemical measurements

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Abstract

Micro-calorimeters are offsetless and often highly selective chemical sensors that measure concentrations of substances in gases and liquids by detecting the heat of reaction. In this paper we present the results of a comprehensive study of the design, operation and performance of integrated-silicon and poly-silicon micro-calorimeters for use with enzymatic and electropolymerized reactive coatings.

Introduction

Thermal-resistance micro-calorimeters

In the thermal-resistance micro-calorimeter, the heat of a chemical reaction is measured by converting it into a temperature difference across a structure of known thermal resistance [1]. The resistive micro-calorimeter is a self-generating sensor operating in four steps.

(i) The concentration C (in mol/m^3) of a substance in a carrier fluid (gas or liquid) is converted into a reaction rate Q (in mol/s) by bringing the mixture into contact with the catalyst or enzyme:

$$Q = C(\mathrm{d}K/\mathrm{d}t) \tag{1}$$

The chemical-conversion efficiency $\mathrm{d}K/\mathrm{d}t$ (in m^3/s) depends upon the chemically active layer (catalyst or enzyme) and the particular experimental set-up.

(ii) The reaction rate Q of the concentrate is transduced into heat of reaction P (in W) by the change in enthalpy:

$$P = Q(-\Delta H) \tag{2}$$

The enthalpy change $-\Delta H$ is the energy freed by the chemical reaction (in J/mol) and is physically determined.

(iii) The heat of reaction is converted into a temperature difference ΔT (in K) by a thermal resistance:

$$\Delta T = PR_{\mathrm{th}} \tag{3}$$

R_{th} is the thermal resistance across which the temperature difference is created and measured (in K/W). It is determined by the structure of the sensor, and it can be influenced by the experimental set-up.

(iv) The temperature difference is transduced into an output voltage U (in V) by a thermopile:

$$U = \Delta T N\alpha_{\mathrm{s}} \tag{4}$$

$N\alpha_{\mathrm{s}}$ is the sensitivity of the thermopile (in V/K), and this is determined by the design and technology of the sensor.

The overall transfer to the thermal-resistance micro-calorimeter is now given by:

$$U_{\mathrm{tp}} = -(\mathrm{d}K/\mathrm{d}t)\Delta H R_{\mathrm{th}} N\alpha_{\mathrm{s}} C \tag{5}$$

where U_{tp} is the output signal of the thermopile (in V). Note that U_{tp} will be zero if C is zero, and so the sensor has no offset.

To optimize the micro-calorimeter, we must optimize each of the four steps above and assure the compatibility of the steps with each other. We have to develop deposition techniques of chemically active layers that do not destroy the fragile silicon or silicon nitride membranes, have optimum chemical-conversion efficiency, and give minimum deterioration of the thermal performance of the device. We have to find the best

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thermal structure to convert the heat into a temperature difference. The geometry and technology of this structure depend upon the application (gas or liquid) and the layers to be deposited. The structure also influences the choice and the design of the temperature-difference sensing element. We have chosen thermopiles for their offsetless character, resulting in offsetless overall sensor characteristics.

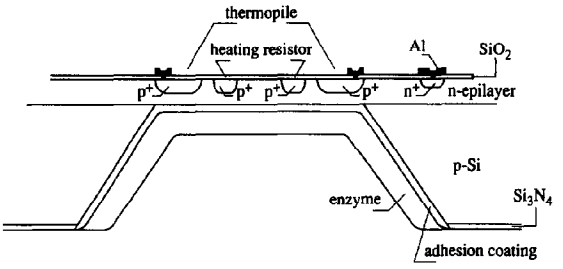


Fig. 2. Cross section of the closed-membrane liquid micro-calorimeter, with adhesion layer and enzyme layer indicated.

Liquid micro-calorimeter

Two basically different sensors have been developed, one micro-calorimeter for gases and another for liquids. We will discuss the liquid micro-calorimeter in more detail, because this sensor has been developed further.

Operation in flow-injection analysis

The liquid micro-calorimeter is to be used in flow-injection analysis (FIA), in which samples are injected into the main stream of a carrier fluid and led past the sensor. This allows on-line monitoring of (bio)chemical reactions. The main objectives for the FIA set-up are a small reaction volume and a good chemical-conversion efficiency. Convenient handling of the sensors is also desirable. This has been found in a three-component system. At the bottom is a zero-force connector for PGA (pin-grid array) housings. In the middle is the micro-calorimeter chip itself, mounted in a PGA housing, see Fig. 1. Because of availability of technology, we made a choice for integrated silicon sensors, and for thermal optimization a structure with a thin silicon membrane within a 0.5 mm thick rim was chosen, see Fig. 2 for a cross section of the sensor chip itself, with the enzyme layer and an adhesion layer also indicated. The PGA with the sensor chip is inserted in the zero-force connector. The PGA is a ceramic housing with a flat, ceramic top surface of 29×29 mm,

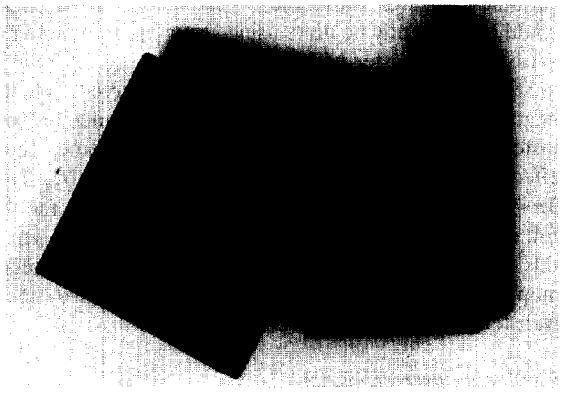
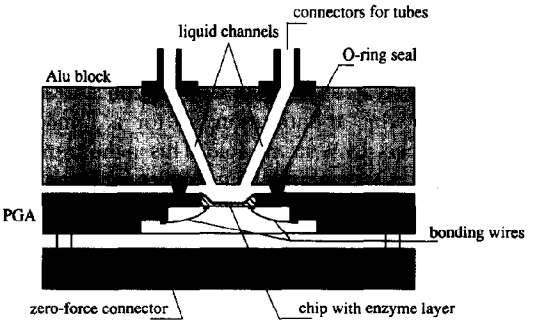


Fig. 1. Ceramic 68-pins PGA with hole, with and without micro-calorimeter sensor chip glued in it.



Flow-Injection Analysis (FIA) set up

Fig. 3. Schematic cross section of the flow-injection analysis set-up with the liquid micro-calorimeter chip in PGA housing.

in which for our purposes a hole of 6×6 mm has been made in the middle. In this hole, the 5×5 mm sensor chip is glued, the etch-cavity of the etched membrane is located at the flat ceramic top surface, and forms the reaction volume for the liquid.

Figure 3 gives a schematic cross section. At the top of this PGA rests an aluminum block with channels that lead the liquid past the sensor. An 'o'-ring around these channels at the PGA–aluminum interface makes a liquid-tight seal around the etch-cavity of the sensor chip. In this way, reaction volumes of the order of 10 μl are obtained. Thus, the physical set-up which determines how the sample solution comes into the reaction volume is defined.

Reaction rate and heat-of-reaction density

We will make an estimate of the reaction rate for this set-up, the factor dK/dt, and the heat of reaction. We will do so for the combination of the glucose oxidase and catalase enzymes, with an enthalpy change $-\Delta H$ of 180 kJ/mol. A glucose oxidase molecule converts slower than the catalase and is the limiting factor for the reaction rate. Typically, it converts maximally 1000 glucose molecules per second, a rate which is obtained at the saturation concentration, which is of the order of 1 mMole (1 Mole = 1 mol/litre = 10^{-3} mol/m^3). And per square metre and per monolayer of enzyme mol-

ecules approximately 2×10^{16} glucose oxidase enzymes can be attached to the sensor surface. Assuming that effectively there are three layers of molecules of each enzyme active in the enzyme coating, there will be maximally 6×10^{19} conversions/m^2 s, equal to 10^{-4} mol/m^2 s. A 10 μl reaction volume contains, at 1 mMole, about 10^{-8} mol glucose molecules. At a surface of 10 mm^2 and a reaction rate of 10^{-4} mol/m^2 s, the supply of glucose molecules in the reaction volume will be depleted after 10 s. But at a flow rate of 1 ml/min the reaction volume is refreshed every 0.6 s, when less than 6% of the glucose is converted. One may, therefore, expect that the enzyme molecules will operate close to maximum conversion speed for a saturation concentration. When the enthalpy is 180 kJ/mol, the heat-of-reaction density P'' as a function of the concentration C will be 18 W/m^2 at saturation, so for a 1 mMole solution we expect 18 W/m^2/mMole. This is the transfer of eqns. (1) and (2) combined, but not yet for a specific sensor surface area.

Thermal design

For the structure of the liquid micro-calorimeter a closed silicon membrane is the optimum choice for several reasons. It separates the liquid and the electronics side, as can be seen in Figs. 2 and 3. The effective thermal resistance is not degraded unacceptably by the heat transfer to the liquid flow in the FIA set-up, and a silicon membrane of 4 to 8 μm thick is relatively strong, compared to dielectric membranes of silicon nitride or silicon oxide. This is of importance for the deposition of the enzyme layers. In the membrane, a silicon–aluminum thermopile is integrated to measure the temperature difference between the middle and the rim. With a homogeneous heat production P'' all over the circular membrane, the temperature increase $T(r)$ (in K/Mole) at a circle of any radius r with respect to the outer rim at radius r_{rim} is, in first approximation, given by [1]:

$$T(r)=P''R_{st}(r_{rim}^2-r^2)/(4+G''R_{st}r_{rim}^2) \qquad (6)$$

In eqn. (6) P'' is the heat-of-reaction density as derived above. R_{st} is the thermal sheet resistance of the silicon membrane, which is 1667 K/Wsq for a 4 μm thick membrane, and the factor $G''R_{st}r_{rim}^2/4$ takes into account the heat transfer to the flow, G'' (in W/Km^2) is the heat-transfer coefficient. For non-flowing water, G'' is 1200 W/Km^2 over a distance of 0.5 mm – the height of the reaction volume – while for flowing water G'' can be much higher.

The sensitivity of the thermopile, measuring between the cold rim (at r_{rim}) and an inner circle with radius r, is proportional to the square root of the ratio of its width and length, when rectangular strips are used. The width is proportional to $(r_{rim}+r)$, while the length is roughly given by $(r_{rim}-r)$. Optimizing the product of the temperature increase and the thermopile sensitivity as a function of r gives an optimum for r of $0.5r_{rim}$. The same optimum is found for a radially-designed thermopile. This optimum, $r=0.5r_{rim}$, leads to the following approximate expression for the thermal resistance R_{th} of the micro-calorimeter:

$$R_{th}=T(0.5r_{rim})/P=R_{st}/(16+4G''R_{st}r_{rim}^2) \qquad (7)$$

In eqn. (7) the heat of reaction P is given by the heat-of-reaction density times the sensor surface, $P''\pi r_{rim}^2$. Without water, R_{th} can be of the order of 100 K/W. With non-flowing water present, the factor $G''R_{st}r_{rim}^2/4$ is of the order of 0.6, giving a loss of 40%. With flow, the loss is higher, in practice up to a factor of 6. Then eqn. (7) is not accurate anymore. Note that in reality, our membranes are square, so eqns. (6) and (7) are fundamentally approximative in the first place.

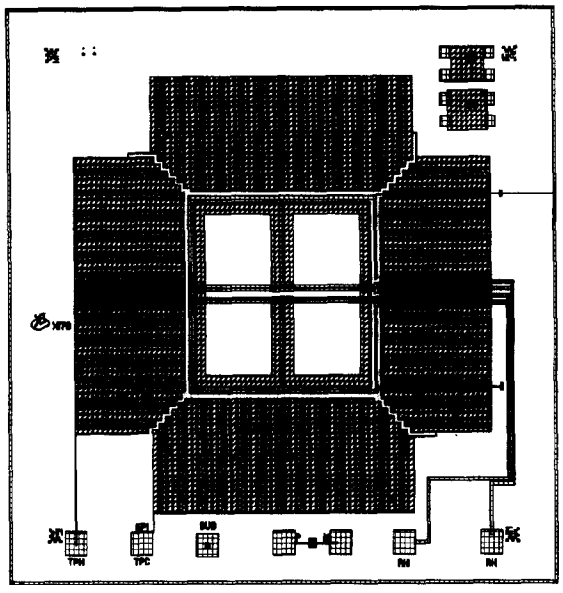
Design and fabrication of the sensor chips

The sensor chips have been fabricated in a 2 μm bipolar process on 4″ ⟨100⟩ p-type wafers with 4 and 8 μm thick n-type epilayers and p-type implanted thermopiles and heaters. The thermopiles, which consist of 120 implanted silicon strips with aluminum as counter material, have an estimated sensitivity of 50–70 mV/K, and a resistance of 150 kΩ. In the centre of the membrane, a 1 kΩ heating resistor is integrated to calibrate the transfer of the micro-calorimeter, see Figs. 2 and 4 for a schematic drawing and photograph. Because the sensor sensitivity is proportional to the area of the membrane, the sensor is not made too small. It is not made too large either, to prevent an oversized reaction volume and to limit the fabrication costs. The compromise came out at 5×5 mm overall chip size, giving a membrane of 3.5×3.5 mm.

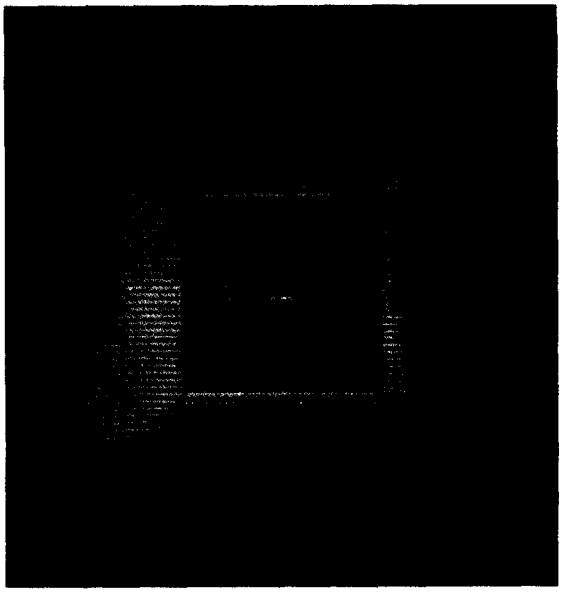
The wafers were ECE etched in KOH to obtain 4 or 8 μm thick silicon membranes. Then, they were sawn and the chips were encapsulated in the PGA ceramic housings with holes. Testing of the sensors could conveniently be done using the integrated heater. With this, a transfer of 8 V/W in air down to 3.6 V/W in still water and 1.3 V/W in FIA was observed. When heating with a resistor in the centre of the membrane instead of homogeneously over the membrane, the effective thermal resistance is higher, $R_{st}\ \ln(r_{rim}/r)/2\pi=R_{st}/9$. Because the membrane thickness for the 4 μm epilayer sensors becomes, in practice, nearly 5 μm, this comes down to about 150 K/W. Assuming a thermopile sensitivity of about 55 to 60 mV/K gives the transfer of 8 V/W measured in practice.

Enzymatic layer deposition

In our experiments, the enzymes were immobilized on the backside surface of etched membranes, the side



(a)



(b)

Fig. 4. (a) Drawing of the liquid micro-calorimeter chip X178 (5×5 mm). (b) Photograph showing the etched membrane using backside illumination.

in contact with the liquid. The techniques used for this are spinning and drop-dispense techniques. Important is the use of an intermediate layer between the enzyme and the silicon membrane, which gives good adhesion of the enzymes, but also allows easy removal with methanol after the enzymes have lost their activity, see Fig. 2. More details on this procedure can be found in ref. 2.

Experimental results

In the FIA set-up with the micro-calorimeters, various enzymes have been tested [2]. Using glucose oxidase in combination with catalase for the detection of glucose results in an enthalpy change $-\Delta H$ of 180 kJ/mol. Using urease for the detection of urea results in $-\Delta H$ of 61 kJ/mol, and using β-lactamase for the detection of Penicillin G results in $-\Delta H$ of 67 kJ/mol. A typical measurement result for glucose solutions using the combination of glucose oxidase and catalase enzymes is shown in Fig. 5 for various concentrations of the glucose. With the FIA technique used here, the actual glucose concentrations were approximately a factor of 60 lower than indicated, due to dilution. With an enzyme surface of 10 mm^2, a transfer of 0.75 V/W for homogeneous heating (equivalent to 1.3 V/W with the heater), and the heat-of-reaction density of 18 W/m^2 at saturation, we expect an output signal of 135 μV at saturation. From Fig. 5 we find an output at saturation of about 70 μV, taking into account a 100× electronic amplification. Apparently, the activity of the glucose oxidase/catalase enzyme coating is equivalent to about 1.5 monolayers instead of 3 monolayers. In the linear region we find a transfer of about 45 μV/mMole, taking into account the 60-fold dilution. Extrapolation indicates a saturation asymptote of 3 mMole. With the sensitivity of 45 μV/mMole and a noise level of below 1 μV, the detection threshold is of the order of 20 μMole. The results in Fig. 6 for different substances give an idea of the selectivity of the sensor, while the base lines give an idea of the offsetless character.

More recently, results for creatinine have been obtained, using the enzyme creatinine deiminase (or creatinine iminohydrolase). The determination of creatinine is of interest in diagnosing kidney disfunctions. In blood, the creatinine concentration is usually 30–40 μM, but it will rise up to 1000 μM in the case of

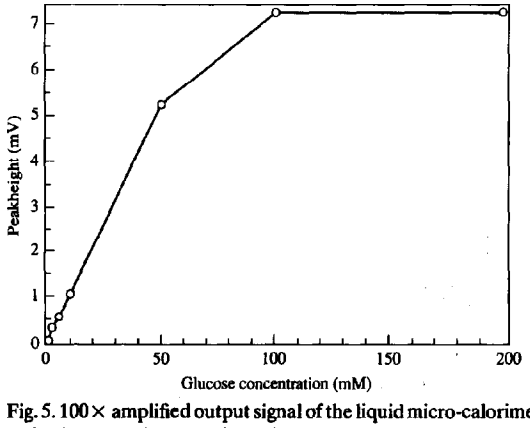


Fig. 5. 100× amplified output signal of the liquid micro-calorimeter with glucose oxidase and catalase enzymes for various glucose concentrations.

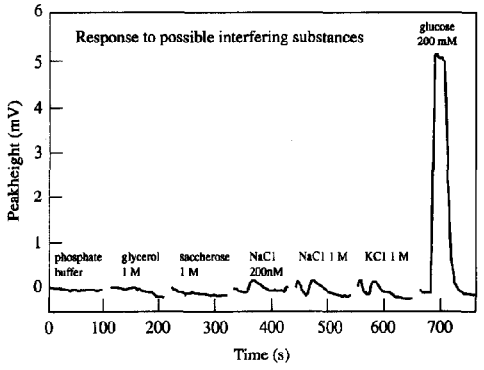


Fig. 6. Output of liquid micro-calorimeter with glucose oxidase and catalase enzymes for different substances.

kidney disfunction. In urine the concentration is usually 4–18 mM, rising up to 40–50 mM in the case of kidney disfunction. Up till now, creatinine has been measured indirectly using calorimetric methods, or by using multi-enzyme methods. In both cases, other substances present in physiological fluids (such as blood and urine) cause interference. That is why the calorimetric method based on only one, creatinine-specific enzyme, and measuring the concentration of creatinine directly, has definite advantages over the other methods. In Fig. 7, the results for measurements of creatinine samples in phosphate buffer (7.0 pH) using a FIA set-up are depicted. In this case, the sample is not previously diluted. This shows a sensitivity of the order of 1 mV/mM. From the peaks of Fig. 7 we can estimate a detection threshold of the order of 5–10 μM, comparable to that for glucose. More elaborate results will be published in the near future.

Gas micro-calorimeter

Design considerations

Besides the liquid micro-calorimeter, a gas micro-calorimeter has been designed, along the same operational principles, i.e. a heater and thermopile on a thin membrane. Here, we used 0.4 μm thick low-stress LPCVD silicon nitride membranes, with n-type LPCVD polysilicon elements (0.4 μm thick, 25 Ω/sq sheet resistance), suspended in a 0.5 mm thick silicon rim. Because of the much better thermal isolation of dielectric membranes compared to that of silicon membranes, we can now use smaller chips to obtain a similar performance. Figure 8 shows a lay-out drawing and a photograph of the gas micro-calorimeter chip, which measures 4×3 mm. The silicon nitride membrane is 2.6×1.6 mm large. The central region of about 1.4×0.35 mm contains an inter-digitated transducer electrode (IDT) made of polysilicon. This IDT has 10 μm wide electrodes with a spacing of 5 μm. It has an aspect ratio of 7000, the length of all electrode fingers combined versus the electrode spacing. Directly around the IDT

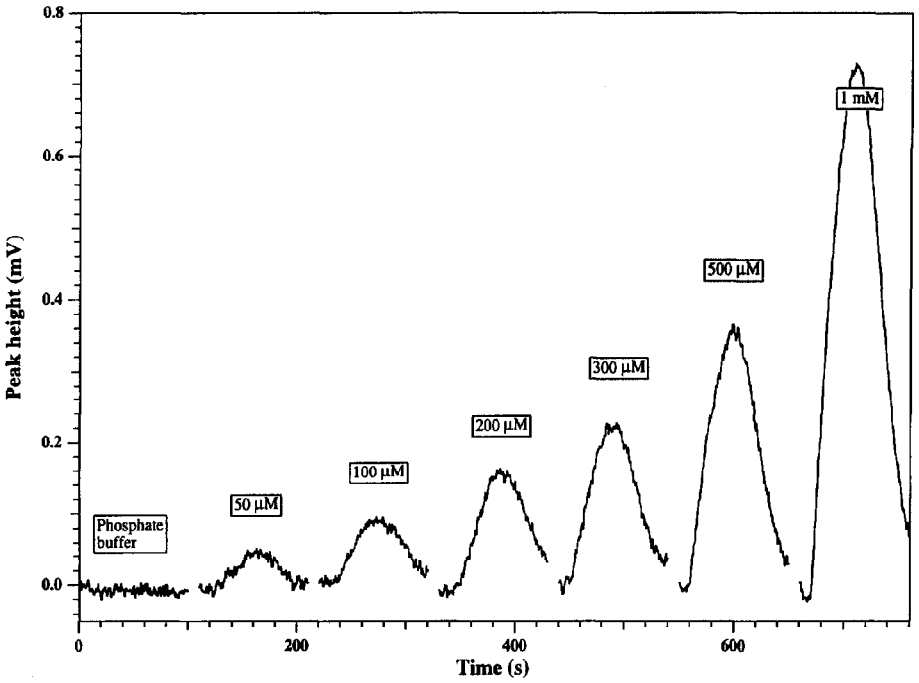
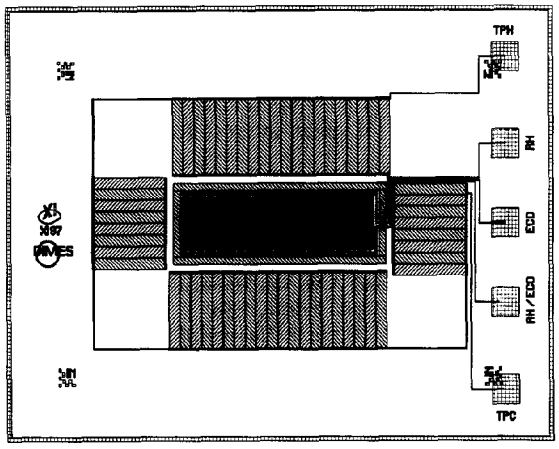
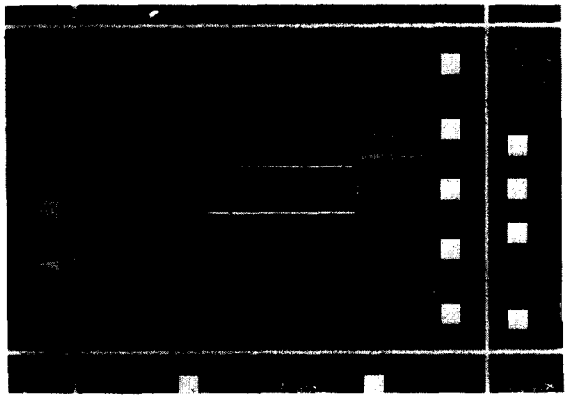


Fig. 7. Output of the liquid micro-calorimeter for various concentrations of creatinine.



(a)



(b)

Fig. 8. (a) Drawing of the thin-film gas micro-calorimeter (4×3 mm). (b) Photograph of the sensor chip with backside illumination.

is an 18 kΩ polysilicon heater, to heat the IDT up to several hundreds of kelvins, for calibration or for measurements at elevated temperatures. The polysilicon thermopile measures the temperature difference between the heater (and the IDT), and the cold rim of the sensor. It is made of 56 n-type polysilicon–aluminium strips, with an estimated sensitivity of 10 mV/K and a resistance of 85 kΩ.

Thermal design

The measured thermal transfer in air of the device is 26 V/W, indicating a thermal resistance of about 2.5 kK/W. The thermal characteristics of the gas micro-calorimeter are different from those of the liquid micro-calorimeter, if we assume that heat of reaction is generated in the centre of the membrane only. In the centre, there is direct heat transfer from the membrane via the gas to the ambient. This loss is due to conduction. With the thermal conductivity of air $\kappa_{air}=26$ mW/Km and a gap of 0.5 mm, the heat-transfer coefficient G'' is 100 W/Km^2, assuming two-sided heat transfer. For the 1 mm^2 central region alone this gives a conductance of $G_{air}=100$ μW/K. The polysilicon thermopile almost entirely covers the outer half of the nitride membrane. We assume for the thermal conductivity of polysilicon, low-stress silicon nitride and aluminum the following values: $\kappa_{poly}=30$ W/Km, $\kappa_{SiN}=3$ W/Km, $\kappa_{Al}=150$ W/Km. This results in conductivities for the membrane components of $G_{SiN}=15$ μW/K, $G_{Al}=30$ μW/K and $G_{poly}=90$ μW/K. In total, the conductance by the membrane (in vacuum) will be about 135 μW/K. In addition, there is heat loss from the thermopile to the surrounding air. With a thermal sheet resistance of the polysilicon/nitride sandwich of about 70 kK/Wsq, and with the thermopile strip length L of 0.5 mm, we find a loss factor for the strips [1] of $G''R_{st}L^2/3=0.3$. So, the effective thermal conductance of the thermopile is about 200 μW/K. Combined with the conduction of the centre this gives 300 μW/K, a thermal resistance of 3.3 kK/W, which agrees fairly well with the value of 2.5 kK/W estimated on basis of the measurements.

Calorimeter and resistive sensor

In gases, the chemically active layer and the electronic elements can be located at the same side, since there is no risk of short-circuit by liquids. This opens up the way to utilize the device as a resistive sensor as well. The resistive gas-sensor operation is based on the IDT, which is used to measure the resistance of conductive polymer layers or other layers on top of it. In this operation, the membrane centre can be heated to a fixed temperature, the constant-temperature operation like a pellistor, by using the heater and thermopile in a feedback loop. But, because of the fast time constant of the sensor, 16–20 ms in air, it can also be temperature-cycled to characterize the shape of the reaction profile, an exciting possibility.

Chemically active layer deposition

The gas micro-calorimeter can be used with a variety of chemically active layers. Because the sensor has all its bond pads on one side, and because of the high aspect ratio of the IDT, the substrate can be dipped into a Langmuir–Blodgett trough to deposit, for instance, substituted phthalocyanine films for NO_2 detection [3] or odour sensitive biological mono-layers [4]. The use of a metal oxide active layer is entirely feasible as well, due to the high aspect ratio of the IDT. The layer could be physically sublimed or sputtered onto the IDT.

We are currently exploring the electrochemical deposition (ECD) of substituted heterocyclic conjugated polymers onto the IDT for gas and odour detection, see ref. 5 for background information. The residual presence of aluminum in the IDT has made the ECD

of a polymer film difficult. We hope to overcome this problem in the near future.

The temperature range of the gas micro-calorimeter, and the option to deposit various chemically active layers with different methods, permits detection of a variety of environmentally hazardous and flammable gases, such as CO, NO_2, H_2 and some hydrocarbons.

Conclusions

In this paper we have presented two promising chemical sensor devices, based on a thermal measurement. The liquid micro-calorimeter is capable of measuring a variety of substances in a liquid solution by means of enzymatic chemical conversion and measurement of the heat of reaction. The offsetless and selective character of these sensors makes them attractive for biochemical research, and in future perhaps for on-line measurement of biomedical quantities, for instance such as glucose concentration in blood. The gas micro-calorimeter shows possibilities not only for calorimetric measurement of combustible gases. It can also be applied for gas measurements using conductive polymer layers, with the additional feature that it can be temperature-cycled with any desired temperature profile as a function of time. This is of great interest for chemical layer research.

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