Journal of Thermal Analysis and Calorimetry, Vol. 57 (1999) 241-251

RECENT DEVELOPMENTS IN INTEGRATED CIRCUIT CALORIMETRY

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Abstract

In the present paper the properties of a new class of calorimeters, so called integrated circuit calorimeters (IC calorimeters) are discussed. IC calorimeters are constructed on the basis of micro-sized silicon chips with integrated thermopile and heater. The applicability of integrated circuits for calorimetric purposes strongly depends on their sensitivity and on an optimized area of the sensitive sphere. Thus the sensitivity has to be much higher than that of the heat power transducers of conventional calorimeters. The parameters of different integrated circuits are compared corresponding to their use in calorimeters. Different constructions of IC calorimeters are possible: batch and flow-through calorimeters operating at isoperibol or temperature scanning mode. As an example a batch type IC calorimeter for liquid samples with a resolution in heat measurement of <100 μJ is described in more detail. The applications of this calorimeter are represented in the paper with the investigation of enzyme catalysed reactions, i.e. the hydrolysis of urea and the oxidation of glucose catalysed by the enzymes glucose oxidase and catalase.

Keywords: enzyme catalised reactions, inhibitors, integrated thermopiles, micro calorimeter

Introduction

For the time being calorimetric arrangements are available for measurements with sample mass between some kilo- and milligrams. Samples with larger mass or volume are applied mostly to investigate the technical representative systems and to determine relevant parameters for the safety of chemical reactions.

The application of calorimeters for scientific problems in different realms such as biology, pharmacy, food- and agrochemistry is highly required because of the experimental and time expenditure necessity. Beside the resulted availability from larger mass sample, an increase of the time for thermostating and measurement is required which cause to higher requirements on the stability of the arrangement as well as on the data evaluation.

Integrated circuits from microelectronics have been used to construct our calorimeters. Therefore, calorimeters of this class are abbreviated as IC calorimeters. IC calorimeters operate with unusual small sample mass, high resolution for a succession of thermal events and with higher sensitivities, compared to the well-known arrangements. The expenditure should be minimised for the preparation, realisation and for the evaluation of calorimetric investigations. For these purposes, we have selected integrated circuits with a thermopile and a heater on a thin Si-chip which is

1418–2874/99/ $ 5.00

Akadémiai Kiadó, Budapest
Kluwer Academic Publishers, Dordrecht

assembled inside a mechanical stable chip carrier. Our calorimeters have compared with well-known classical calorimeters. As a result of this comparison significant advantages have been detected such as unusual small heat capacity of the active, sample holding place on the Si-chip and a very small time constant for the heat exchange between the sample and the heat sink. The great number of thermocouples in the pile is regarded to be a very valuable supposition to achieve a sufficient sensitivity. The properties of the integrated circuits are an extremely important precondition to accomplish a good dynamic behaviour of the calorimeter, simplified kinetic evaluations as well as high resolution for the thermal impulse successions.

The aim of this publication is to depict the advantages and disadvantages of the application of IC calorimeters for investigations with liquids using a batch version. Investigations with IC calorimetry and mass flow of the liquid components are in preparation.

The integrated circuits for calorimeter constructions

For calorimetric applications we have tested different types of integrated circuits which are originally produced for radiation detection purposes. Such integrated circuits consist of a thin Si-chip with a rim for the stabilisation, assembled in a massive chip carrier (Fig. 1) [1, 2]. The Si-chip is a planar structure with a self-supporting membrane. The sensitive sphere for the heat detection is in the middle of the membrane and contains a heater resistance suitable for calibration purposes. Between the sensitive sphere and the rim a thermopile is integrated in the membrane to measure the temperature difference given as consequence of the heat flow through the membrane with the thermocouples. For the application of an integrated circuit as calorimeter, it is an important precondition that the heat flow takes place mainly through the membrane.

As a result of the decreasing sample mass at constant thermodynamic and kinetic conditions of the investigated system the substance turnover per time unit decreases in turn with the decrease in the heat power. Therefore, we regarded the heat power sensitivity of the Si-chip as a very important property of an integrated circuit for the application in calorimetry. Determining parameters for the heat power sensitivity are

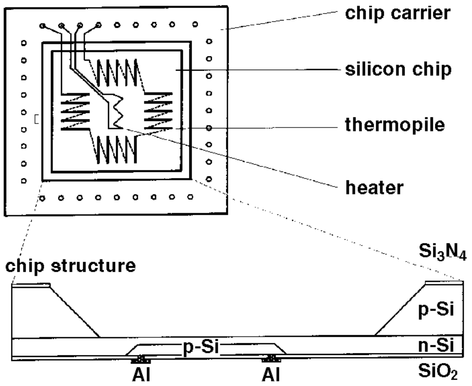

Fig. 1 Scheme of a silicon chip with integrated thermopile and heater

the thermal resistance R_{th} of the thin membrane, the Seebeck-coefficient α of the chosen thermocouple combination and the number of couples n in the thermopile $\varepsilon = \alpha n$. A small heat capacity of the membrane is highly required for the dynamic behaviour and for small thermal trouble proneness [3]. Temperature fluctuations of the chip carrier and/or of the rim cause only a low heat flow. In spite of the high R_{th}-value the proper time constant τ of the Si-chips tend to be a very small and the resolution for succession of thermal events is rather high.

Table 1 Parameters for different types of silicon chips usable for IC calorimeters

No.	Type	ε/ V K^{-1}	R_{th}/ K W^{-1}	S_P/ V W^{-1}	A/ mm^2	$S_{P,A}$/ V mm^2 W^{-1}
1	LCM 2506	0.07	67	4.7	4	19
2	LCM 2524	0.07	35	2.45	16	39
3	MKS 76	0.01	300	3.0	38	114
4	ETH	0.067	179	12	0.12	1.44

ε: temperature coefficient; R_{th}: thermal resistance; S_P: heat power sensitivity; A: calorimetric sensitive area; $S_{P,A}$: area – heat power sensitivity parameter;
LCM 2506, LCM 2524: Silicon thermopile chips, manufactured by Xensor Integration, Delft, NL, [11]; MKS 76: Silicon thermopile chip, developed by IPHT Jena, D, [2]; ETH: Silicon thermopile chip, developed by BALTES, ETH Zurich, CH, [4]

The specific parameters of Si-chips, which we have already tested for the calorimetric applications, are given in Table 1. As it can be seen from the table, the heat power sensitivity $S_p = \varepsilon R_{th}$ of the tested integrated circuits is significantly different. This difference is caused by different values of ε and R_{th} respectively. For R_{th} an optimal value must be chosen, since as a result of the rising value of R_{th}, a simultaneous increase in the proper time constant as well as in the influence of the undesirable heat flow through the contact media are given.

A significant finding could be seen from Table 1, which is the size of the sensitive sphere A of the thermopile equal to the calorimetric active area. If we assume a similar heat exchange per unit of A then the product $S_{pA} = AS_p$ is determining the magnitude of the signal from the thermopile. The possibility of increasing undesirable heat flow with rising A value leads to the necessitate to the optimisation of A.

The interpretation of the values appeared in Table 1 must take in to the consideration that for calorimetric application the A value as well as S_{pA} value of the integrated circuit 4 should be too small. The circuit 4 has compared with circuit 3 a significantly high sensitivity S_{pA}. But as consequence of the large active area an undesirable heat flow is observable through the contact media which accompany with lower stability of the transfer function of the constructed calorimetric arrangement.

Construction of different types of IC calorimeters

One of the aims of the present publication is to describe our calorimeters on the basis of a specific integrated circuits. The calorimeters are mainly used for chemical applications, which worked in the batch mode as well as in the mass through-flow conditions respectively. According to the low time constant, the IC calorimeters can be classified [5] in usual terms as heat-exchanging calorimeters operating in isoperibol or temperature scanning mode. Figure 2 shows schematically the different constructions of IC calorimeters using Si-chips as integrated circuit.

The simplest way is to make a batch type calorimeter. The sample is located on the sensitive sphere of the thermopile and the reaction can be initiated by dropping a second component onto the sample. The construction and the application of the batch type IC calorimeter are described in more details in the following sections.

For the purposes of investigations of thermal activated processes, calorimetric equipment was developed which possesses the possibility of a temperature scanning. In contrast to the conventional scanning calorimeters a twin system is not necessary for the compensation of parasitic heat flow, since the heat capacity of the inertia is very small. As it is shown in [6], by means of such type of IC calorimeters the tran-

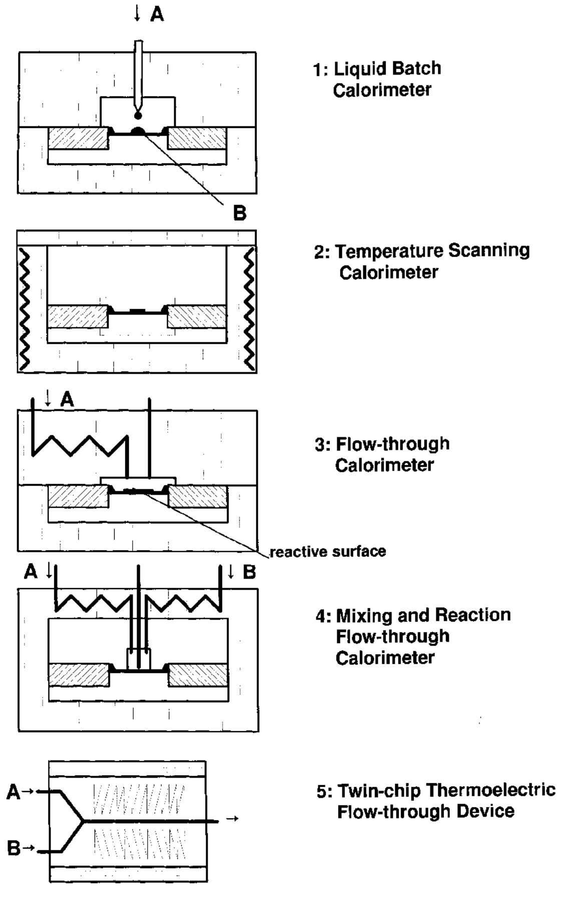

Fig. 2 Schematic view of different constructions of IC calorimeters (A, B solutions with reactive components)

sition enthalpies can be determined with high accuracy for samples in the sub-mg range at temperatures between 250 and 400 K.

Simple flow-through constructions are applied for the study of solid-gas interactions and to monitor the heat production of enzyme catalysed reactions. In the latter case the enzyme is immobilised at the surface of the chip and a solution containing the substrate passes the coated Si-chip continuously [7]. Using the device in the measurement of the heat of interactions between a solid deposited on the chip and a gas flow, the outstanding impulse resolution of IC calorimeters tend to be one of the most important advantage of the applications of such IC calorimeters. Especially in the case of periodically forced reactions a power resolution of 50 nW have been achieved [8]. Introducing an additional inlet channel and a thermal isolated mixing zone attached to the surface of the Si-chip, the simple through-flow device grows up to a mixing and reaction calorimeter for fluid reactants (Fig. 2).

The last example depicted in Fig. 2 is a Si-chip device, especially designed for mixing and reaction calorimetry with fluid reactants [9]. It consists of two sealed planar chips. The basic Si-chip with a 0.8 μm membrane contains the thermopile and a heater. A 0.5 mm thick glass chip with etched Y-shaped channels is glue bonded to the Si-chip to give a compact flow-through calorimeter which is capable of being manufactured in an easy way. In a following publication [10] we describe the application of such calorimetric devices for the multiple flow injection analysis.

A new batch-type IC calorimeter for investigations with liquid samples

A former report [6] includes the demonstration of the advantages of IC batch calorimeters, such as the considerable timesaving management of measurements, the small mass sample necessary for an investigation and the excellent dynamic behaviour which are useful for kinetic considerations [6, 11]. On the other hand, some difficulties are raised from the small mass sample. Assuming comparable heat power per unit volume, a limitation for further decrease of the mass sample is the drop of the signal. Such drop cannot be completely compensated by the remarkably high thermal sensitivity of the Si-chips. Moreover, as a result of the lack in reproducibility and accuracy of dosing and positioning of the added reactant further errors might be occurred. Finally, the evaporation of the liquid sample has to be suppressed to a great extend because of the large free surface of the sample drop related to its mass.

To overcome these problems we have reconstructed our IC batch calorimeter. The main goal of the new reconstruction is to accomplish in faster and more stable adjusting of the vapour pressure within the interior of the calorimeter. In further work we will improve the dosage of the second component by use of piezoelectric micro-pipettes. Figure 3 shows the reconstructed calorimeter in more detail. The calorimeter consists of two axial connected cylindrical aluminium blocks. The integrated circuit is located in the centre of the apparatus. An axial hole in the upper cylinder serves for the mounting of a Hamilton syringe, which holds the second reactant before the adding process. The moist ring attached to the top of the interior of the

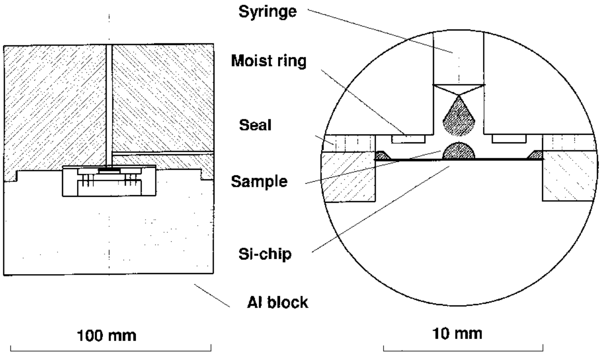

Fig. 3 IC batch calorimeter for liquid samples

calorimeter is used for a faster adjusting of the vapour pressure. This decreases the rate of the evaporation from the sample placed at the surface of the silicon membrane and therefore leads to a faster stabilisation of the signal base line. An extremely thin circular gauge with a diameter of 4 mm is used to ensure an exact positioning of the liquid drop (3 μl) of the sample in the centre of the surface of the chip. It helps also to increase the reproducibility of the size of the contacting area between drop and membrane surface. After placing the reactant onto the chip the calorimeter is closed. Then, the syringe is filled with the second component (i.e. 4 μl) and is introduced into the hole of the upper block of the calorimeter. The radial hole, which serves for pressure balance, is closed after introduction of the syringe. Thus, only minor gas exchange between interior of the calorimeter and the surroundings is possible. The good thermal contacts between the block and the chip as well as the syringe ensure sufficient low temperature differences between both reactants. In comparison with the old design of the IC batch calorimeter the following improvements has been done:

– The volume of the interior of the calorimeter was decreased to a minimal size. The free volume is now 90 μl instead of 1100 μl.

– A circular piece of filter paper is used as moist ring to increase the reproduction of the moist surface.

– The gas exchange with the surroundings is suppressed.

Figure 4 demonstrates the considerable role of the heat of evaporation in an IC batch calorimeter with open positioned micro-litre samples. In the corresponding experiments a drop of 4 μl water was put down to the dry surface of the Si-chip. The bigger signal (b) was obtained without use the moist ring. After some time when the gas atmosphere within the calorimeter is saturated a nearly steady state and slowly varying signal is obtained. The remaining heat power is caused by a lack of water vapour i.e. through the gap between syringe and aluminium block. The signals have a small negative slope probable because of the decrease of the surface due to the loss of the sample mass. The decrease of the surface reduces the rate of evaporation. The calculated overall heat exchange shows that the signal is really due to evaporation of the water. The data correspond sufficiently to the amount of evaporated water. As it

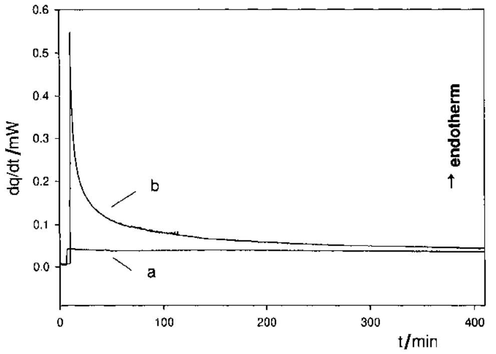

Fig. 4 Comparison of the course of the base lines after dropping of 4 μl water down to the surface of the Si-chip applying (a) and without applying (b) a moist ring

can be seen from the Fig. 4 the use of a moist ring helps to accelerate the steady-state conditions. Furthermore, the remaining heat power is lower, because the lack of water vapour is partially compensated by the evaporation from the moist ring. It is evident from comparable investigations with mercury that the evaporation of the sample has the determining influence on the position and stability of the base line.

The time necessity to perform one measurement depends only on the equilibration time and on the kinetics of the reaction. As a consequence of the minimised interior of the calorimeter accompanied by an improved partial pressure stabilisation a decrease of equilibration time from 30 to 15 min could be obtained. Thus, it is possible to carry out so many measurements per a day.

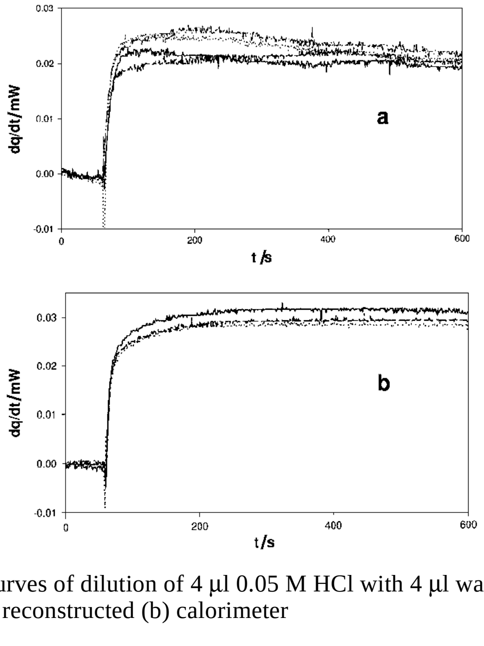

Fig. 5 Calorimetric curves of dilution of 4 μl 0.05 M HCl with 4 μl water measured using the old (a) and the reconstructed (b) calorimeter

In Fig. 5 two sets of curves are given. All plots represent the dilution of a 4 μl drop of 0.05 M HCl with 4 μl water measured using the old (a) and the reconstructed calorimeter (b). The dilution increases the vapour pressure. Therefore, a base line shift to higher heat power is observed. The reproducibility of the base line strongly depends on the defined conditions of the evaporation. Using the new calorimeter the scatter of the base line shift was decreased from 6 to 1 μW approximately.

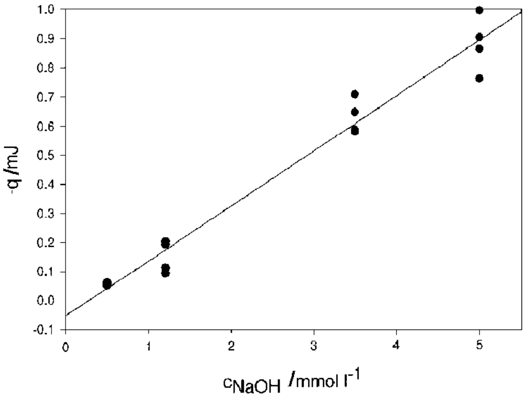

Fig. 6 Heats of neutralisation of 3 μl 0.1 M HCl by 4.4 μl NaOH of varying concentrations

The stability and reproducibility of the base line is the main restriction for the limit of heat detection. In Fig. 6 the measured heats of neutralisation of 3 μl 0.1 M HCl by 4.4 μl NaOH in a low range of concentration (0.5 to 5 mmol l^{-1}) are plotted. The graph shows that in the case of fast reactions the heat detection is limited to approximately 100 μJ.

The accuracy of the heat measurement was tested with the TRIS protonization reaction. The molar reaction enthalpy of –47.6 kJ mol^{-1} obtained from measurements of 0.1 M TRIS solution with HCl solutions in the concentration range from 0.02 mol l^{-1} to 0.05 mol l^{-1} agrees very well with the corresponding value (–47.4 kJ mol^{-1}) from the literature [12].

Applications of batch-type IC calorimeters

The described IC batch calorimeter is a powerful tool for thermokinetic investigations of enzyme catalysed reactions. For instance, we have studied the hydrolysis of urea catalysed by the enzyme urease. The reaction kinetic at the formation of ammonium carbamate in the first step is described by the Michaelis-Menten equation. Several hydrolysis equilibria are summarised in the second reaction step. For this step a first order reaction can be assumed. Figure 7 shows a measured heat-flow signal from the investigated reaction. The same figure depictes the corresponding heat-power curve obtained by an inverse filtering procedure of the measured curve, using a 3 s time constant. It is obvious from the graphs in Fig. 7 that the deviation between both curves is only remarkable in the first part of the curve. This demonstrates the special suitability of the IC calorimeter for the kinetic applications.

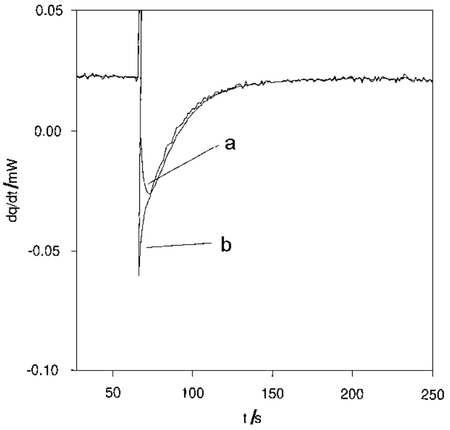

Fig. 7 Enzyme-catalysed hydrolysis of urea: a) Measured signal after adding of 4 μl solution of urease (3.5 μg) to 3 μl urea (3.5 mmol l^{-1}); b) Reconstructed heat-power signal

From the Michaelis-Menten equation a relation between the peak height *h* of the deconvoluted calorimetric curve and the enzyme activity can be derived:

$$v_{max} = hn(c_o + K_M)/c_o \qquad (1)$$

The Michaelis-Menten constant K_M and the maximum reaction rate v_{max} are parameters of this equation. The parameter v_{max} is directly related to the enzyme activity. The factor *n* in Eq. (1) is determined by the sensitivity of the heat-flow sensor, by the volume of the investigated sample and by the enthalpy of the reaction. The concentration of the urea is represented by the parameter c_o. Figure 8 depicts the dependence of the peak height on the amount of enzyme obtained from reactions with equal concentration c_o of urea. From the slope of the curve we have calculated an en-

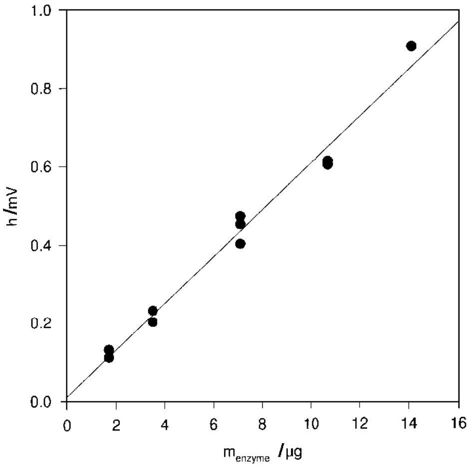

Fig. 8 Dependence of the peak height of the signal on the amount of enzyme obtained for a constant concentration of urea (7 mmol l^{-1})

zyme activity of 94 U mg^{-1}. An independent electrochemical measurement of the enzyme activity provided a value of about 80 U mg^{-1}.

Because of the defined causality between the measured heat power and the activity of enzymes, IC calorimeters are also applicable for the detection of inhibiting species. Thus, the decrease of the rate of the urea hydrolysis with increasing concentration of cadmium ions as inhibitor was measured with the IC calorimeter [11]. The inhibition of the enzyme peroxidase by cyanide ions is strong enough to detect the concentrations of cyanide in the ppb range with the calorimetric method.

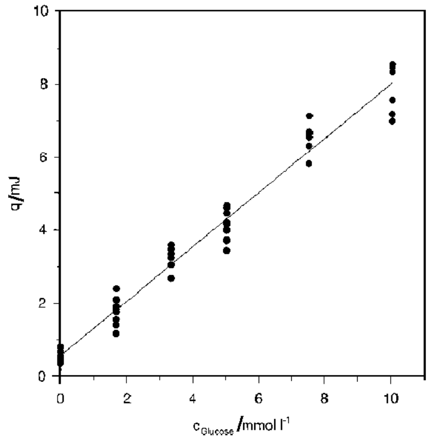

Fig. 9 Heats of oxidation of glucose catalysed by glucose-oxidase (GOD) and catalase (GOD: EC 1.1.3.4 from Biozym, 180 U mg^{-1}, 2 mg ml^{-1}; catalase: EC 1.11.1.6 from Serva, 1950 U mg^{-1}, 0.2 mg ml^{-1}; 0.1 M phosphate buffer, pH=6.86)

In contrast to the heat power as a measure of the catalytic activity of an enzyme, the integral heat exchange is related to the amount of substance involved in the reaction. In this connection IC batch calorimeters are useful for the direct and quantitative determination of analytes important e.g. in biochemistry and in medicine. As an example the calorimetric determination of glucose is given. According to Fig. 9 the limit of detection is approximately 1 mmol l^{-1}, this range is regarded to be a very interesting for the application of the calorimetric detection of glucose. The reason of the higher scatter of the data compared with those from the neutralisation measurements is that, the reduction of the signal-to-noise ratio occurred according to a lower rate of reaction.

Summary

Integrated circuit calorimeters as a new class of calorimetric devices are constructed on the basis of micro-sized silicon chips with integrated thermopiles and heaters. The high Seebeck coefficients of the series connected thermocouples and an optimised thermal isolation between reacting zone and a bulk heat sink ensure a sufficient high sensitivity which is necessary because of the very small sample mass.

Some interesting features of IC calorimeters as low sample mass, the simple and robust construction as well as the outstanding dynamic behaviour bring into focus new applications for thermochemical investigations.

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