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Chip reactor for microfluid calorimetry[1]

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Abstract

A microchip module with integrated flow channel and highly sensitive thin-film thermoelements of $Bi_{0.87}Sb_{0.13}$ and Sb was developed in order to realize a microcalorimeter for small volumes and flows in the μl and μl/min range, respectively. It was designed according to the principle of flow-injection analysis (FIA) and prepared using means of micromachining technology. The flow channel comprises two inlets, a mixing region, a measurement region and one outlet. In this way, the initiation of chemical reactions by interdiffusion takes place in the closest possible contact with the sensing elements. Three thermopiles (i.e. thermoelements connected in series) are arranged on a thin-film membrane covering the fluid channel. A thin-film heater of an NiCr alloy is integrated for internal electrical calibration. The microreactor was first tested in the neutralization reaction between sodium hydroxide and sulphuric acid for different NaOH concentrations, flows, and sample volumes, investigating for the present the integral signal of the three thermopiles connected in series. From the collected data, the minimum detectable power, minimum detectable NaOH concentration, and the reaction enthalpy were calculated. The results characterize the module as a sensitive chip calorimeter, showing the suitability of the micromachining technology for calorimetry. © 1998 Elsevier Science B.V.

Keywords: Calorimetry; Chemical microanalysis; Flow-injection analysis; Thermoelectrical transduction; Thin-film thermopiles

1. Introduction

Thermal transduction principles are widely used for the characterization of chemical substances and processes, due to the fact that nearly every chemical reaction, phase transitions, every mixing or wetting process is accompanied with exchange of heat. In addition, chemical compounds can be distinguished by different specific heats, and phase transitions of higher order are often marked by a change of the specific heat or a change in the dependence of specific heat on temperature. Therefore, it is not surprising that thermal characterization is used in a number of classical analytical methods as well as in miniaturized procedures, particularly in the thermoresistive transduction of analytical reactions [1–6]. In addition to the thermoresistive principle, thermoelectrical transduction was shown to be an efficient method for the measurement of small amounts of process enthalpies. It is an important advantage of thermoelectrical transduction that no electrical power is consumed in the

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formation of the signal. As for example, calorimetry was performed in classical flow systems for Zn^{2+} detection [7].

Thin-film thermopiles are under investigation as thermal transduction elements for microcalorimetry and for thermoelectrical biosensors [8–11]. The use of single chip elements in direct immersion in fluids or in fluid cells led to analytically interesting signals, but the signal level remained comparatively low. Higher output voltages were observed using extraordinarily sensitive BiSb/Sb thin-film thermopiles on free-standing membranes [12,13]. Calorimetry in single droplets on free-standing membranes was performed successfully, but considerable disturbance by the environment was observed [14–17]. Comparatively good analytical results were obtained, when highly efficient thin-film thermopiles on free-standing membranes were inserted in a flow device.

Glucose could be calorimetrically detected with a resolution of 90 μM and it was possible to measure in blood directly [15]. The disadvantage of this arrangement was its rather large channel volume of 19 μl and the separation of location of reaction and location of heat detection.

Preliminary test measurements with a newly developed device, integrating highly efficient BiSb/Sb thermopiles in a microfluid arrangement with a channel volume of ca. 2 μl and a very close thermal contact of the reaction zone with the transducing thin-film stack have been reported earlier [18]. Here, the concept and the preparation of this chip reactor are given in detail and the efficiency of the device in detecting low-concentration sodium hydroxide in a neutralization reaction is shown.

2. Technology

2.1. Thin-film deposition

The $Si_3N_4/SiO_2/Si_3N_4$ sandwich system used as carrier membrane is deposited on (100)-Si by plasma-enhanced chemical vapour deposition (PECVD).

NiCr- and Al-films are deposited by magnetron sputtering and are meant to serve as material for an integrated heater and bond pads, which are essential for electrical connection of metallic microstructures to a printed circuit board (PCB). Layers of $Bi_{0.87}Sb_{0.13}$ and Sb are formed by a thermal evaporation process in order to obtain metallic films of a columnar morphology, which is necessary for high thermoelectrical power. Photoresist films are deposited by the common spin-on procedure on a commercial device (CONVAC) and are used as electrically insulating intermediate layers between metallic films as well as covering layer. Precuring occurs at 80°C, the post-bake temperature is 210°C for the first film, then decreases in steps of 10°C for each following resist film to yield maximum stability in all the metallic deposition processes and not to risk damage of the pervious polymer layers.

2.2. Photolithography and etching

The photoresist AZ 1514-H (Hoechst) is used in all photolithographic procedures. It is exposed by a mask aligner Al 6-2 (Electronic Vision). A precise orientation of the thin-film patterns to the Si deep-etched area is realized by a double side alignment.

The microfluid channel is etched in a glass chip using an HF-solution of 39% and an NiCr layer as etch mask. Anisotropic wet etching of (100)-Si is carried out in aqueous solution of NaOH at 80°C. The $Si_3N_4/SiO_2/Si_3N_4$ sandwich stack is patterned by use of an SiO_2-mask and etched in concentrated phosphoric acid at 180°C. Al, NiCr, BiSb, and Sb are structured in specific wet etching baths.

2.3. Chip connection

The module is formed by connecting the silicon chip carrying the transducer thin films and the glass chip. Due to the incompatibility of anodic bond temperatures and the low melting point of bismuth, it is not possible to utilize an anodic bond process for the chip connection. Therefore, a glue-bonding procedure using two-component epoxy glue is preferred. The interface elements for the three fluid ports are also attached by glue bonding. After chip connection, the completed chip calorimeter is mounted on a printed circuit board (PCB). The electrical connections between chip and board are realized by wire bonding.

2.4. Calorimetric measurement

Calorimetric measurements are carried out with p.A. graded chemicals. A nanovoltmeter (Keithley 182) is used for the voltage measurement of the thermoelectrical signal. The calibration heater is supplied by a DC-source (Yokogawa 7651). Liquid flow is installed by a double-syringe infusion pump (Bioblock Scientific KDS 200), an additional handmade screw-device to clamp in one syringe is employed to yield an accurate injection FIA-style into one of the two steady streams produced by the pump.

3. Calorimeter design

3.1. Calorimeter concept

It was the goal of the development of a microflow calorimeter to realize a measurement device for reaction heats in case of mixing of two liquids. Therefore, the microfluid channel is formed in the shape of a 'Y', i.e. it possesses two inlets and one outlet. The inlet channels lead separate liquid streams to the junction of a longer central channel. The mixing of liquids and the reaction should proceed in this central channel, which then leads the reaction mixture to the outlet (Fig. 1). The foremost challenge is the appropriate arrangement of the transducing elements. Microelements are not predestined for an ideal thermal insulation. But it is comparatively simple to achieve well-controlled heat flows by choosing particular materials and geometries in microthermal elements. Any heat flow in a microsystem leads to a temperature gradient. Thermoelectrical transducers are well-suited for the detection of temperature differences corresponding to temperature gradients. A precondition of every thermoelectrical transducer is the sufficient definition of both ends of the temperature gradient, i.e. the heat source and the heat sink, and the establishment of a thermally well-controlled region in-between.

The heat source of this microflow calorimeter is given by the central fluid channel, that one might divide into three regions. The first region of this heat source is identical with the mixing zone of both inlet channels, wherein two streaming liquids get into contact and a chemical reaction can be initiated by the mixing. The second part of the heat source is the channel zone behind the orifice of the inlet channels and the mixing zone, and the third region consists of the last section of the channel immediately before the channel outlet. Each of these three regions is equipped with a separate thermopile.

The heat flow between the central and the silicon frame of the chip acting as heat sink is controlled by the membrane area. Avoiding the involvement of

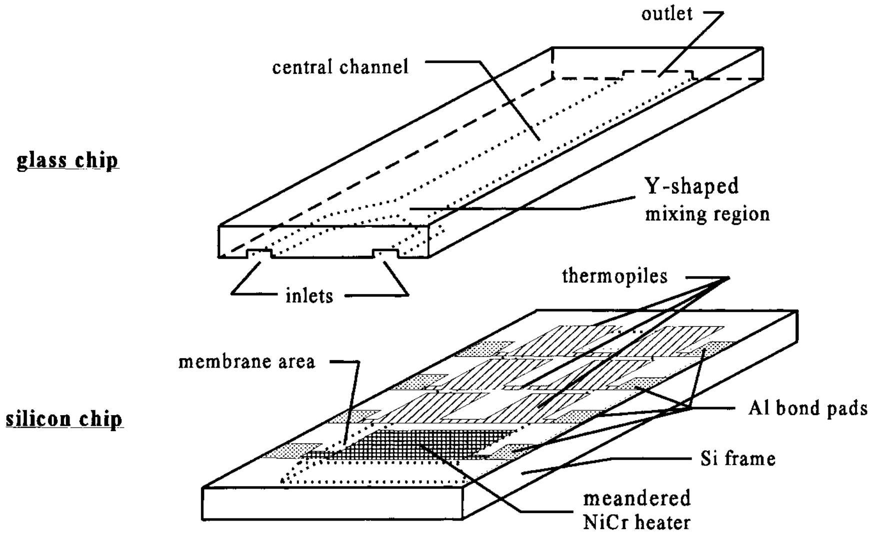

Fig. 1. Construction principle of the thermoelectrical microflow calorimeter.

silicon in the path of heat flow from heat source to heat sink ensures only a small heat loss via the attached glass chip due to its much smaller heat conductivity compared to silicon. The frame is thermally connected to the PCB. A temperature gradient between the active junctions in the central part of the chip element and the reference junctions in contact with the frame, and therefore a thermoelectrical signal, should be formed in every case of heat consumption or heat evolution in the central fluid channel. In this way, reaction enthalpies should become measurable.

The flow rate determines the residence-time of the reaction mixture in each of the three measurement zones. Therefore, the difference between the thermoelectrical signals in these zones provides information about the temporal change of the rate of heat evolution. This information can be used for studies on reaction kinetics.

It is hardly possible to calculate the thermal behaviour of the microflow calorimeter completely. That is the reason why it is more useful to calibrate the whole system. Such a calibration is very easy if thin-film resistors are used as microheaters, deposited in the same close thermal contact with the liquid as the active junctions of the thermopiles. This heater for calibration is located in the first region of the mixing zone. It is possible to calibrate the response of the calorimeter for any type of liquid and for different flow rates very quickly by giving an electrical pulse on the heater, which is converted into heat. In addition, this heater can be used for the modulation of temperature in the microchannel.

The materials of the calorimeter are selected considering their heat conduction coefficients, their electrical properties and their compatibility to the microfabrication process in its entirety. Silicon is used as chip material due to its good thermal conductivity, which is desired for the equalization of temperature in the outer frame of the chip. A second advantage of silicon is the existence of well-established methods of anisotropic etching for the preparation of the membrane area and the compatibility to all preparation steps of the thin-film stack for the transducers. The second half shell of the microchannel calorimeter should be made from glass in order to involve a material, in which a small fluid channel can be integrated by isotropic wet etching, and which features a low thermal conductivity in order to avoid rapid heat transport from the reaction region to the frame. The carrier membrane for the thermolegs was made of a $Si_3N_4/SiO_2/Si_3N_4$-multilayer, a combination with low tensile stress serving as a good electrical insulator. Aluminium is used for electrical microwiring and for preparation of the contact pads, because it possesses a high electrical conductance and can make good contact by wire bonding. NiCr is a very stable material with comparatively high specific resistance, therefore well-suited as heater material for moderate heating power, and additionally used to improve the adhesion of the Aluminium bond pads to the membrane material on the silicon; moreover, its temperature coefficient of electrical conductivity is very low. Therefore, no change of electrical resistance in dependence of temperature has to be considered in calibrations. $Bi_{0.87}Sb_{0.13}$ and Sb are chosen because of the exceptional thermoelectrical power that can be achieved by this material pairing, already used in a family of miniaturized physical thermoelectrical sensors [13,14].

3.2. Preparation

A 0.5 mm thick 4"-wafer of (100)-silicon is double-sidedly polished and deposited with a layer system of $SiO_2/Si_3N_4/SiO_2$. Then this system is partly removed from the back side of the wafer in a chemical wet etching process. The remaining layer structure serves as etch mask during the following silicon-removing anisotropic etch process, yielding free-standing membranes of 4.4×13 mm^2 on the front side of the wafer, each defining one chip element. NiCr and Al are deposited on the front side in 0.1 μm and 1 μm thick layers, respectively. $Bi_{0.87}Sb_{0.13}$ and Sb are deposited in 0.4 μm and 0.3 μm thick layers, respectively, and structured. As an electrical isolator, a 1.6 μm thick photoresist layer is deposited between BiSb and Sb. A covering resist layer of 0.5 μm is added for protection (Figs. 2 and 3).

The thermoelements are arranged in three piles of 48 thermocouples each, reaching onto the membrane area with one end in thermal contact with the silicon frame (Figs. 2 and 3). One heater meander is positioned on the membrane where, after connection of silicon and glass half shell, the mixing region will be located (Fig. 4). Eventually, single chips measuring 9×17.6 mm^2 are separated.

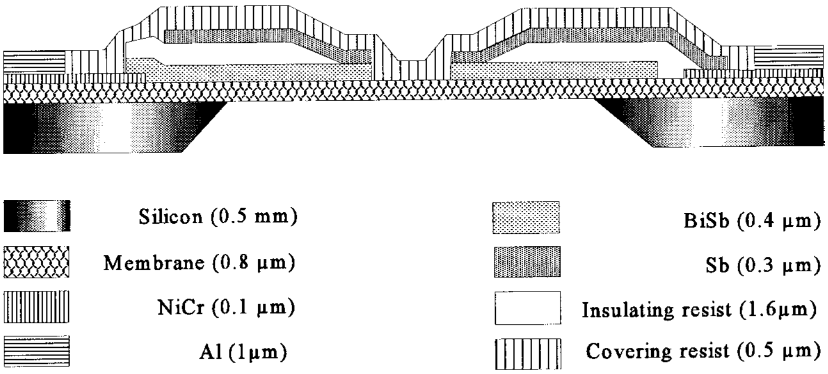

Fig. 2. Thin-film stack for the thermoelectrical transduction (film thickness in brackets).

The glass half shell of the calorimeter, measuring $7\times17.6\ \mathrm{mm}^2$, is prepared from a boron-doped, isotropically wet etched, and eventually separated 0.5 mm thick 4"-glass wafer. The etched Y-shaped channel is 0.1 mm deep. Both Y-arms are of 1 mm in width, joining after ca. 5 mm to form 2 mm wide central channel of 12.6 mm length.

The two half shells are connected by glue bonding, aligning both parts along the axis of the channel, avoiding bubbles within the thin glue layer as well as glue within the channel and on the bond pads. The Al-pads along the sides of the silicon chip are not covered with glass (Fig. 5). The inlet and outlet interface elements are attached with equal care. After curing, the module is mounted on a PCB and electrically connected by wire bonding (Fig. 6).

The microcalorimeter is equipped with hoses (inner diameter 0.4 mm) by connecting them to its interface elements and is then inserted into the insulation chamber. The hoses of the inlet channels are affixed on the syringes of the double-syringe infusion pump, an identical syringe clamped into the handmade device is equipped with a short piece of hose connected to a T-fork-element that has been inserted into one of the two aforementioned main hoses. Establishing two steady flows by the pump it is now possible to inject accurate volumes of a third liquid into one stream, enabling us to carry out flow-injection analysis. The hose volume between T-element and calorimeter has to be chosen considering the sample volume and the flow rate in order to avoid any disturbance of the evolving signal by the injection procedure. The channel outlet of the module is equally equipped with one hose, through which the product is removed. Electrical wiring soldered to the printed circuit board of the calorimeter leads to both DC-source and nanovoltmeter, which are connected to a PC for data acquisition, evaluation, presentation, and storage (Fig. 7).

4. Calorimeter testing: Results and discussion

A threefold calibration was carried out. First, the empty calorimeter was heated by applying various defined heating pulses, leading to knowledge about the thermal behaviour of the sole module (Fig. 8). The time to reach 95% of the signal maximum is t_{95}=6 s.

Secondly, heating was performed on the calorimeter filled with pure water at zero flow (Fig. 9). Here, the t_{95} is ca. 15 s; moreover, thermal signal fluctuation due to the presence of water is observable. To fill the module, the two syringes in the infusion pump were supplied with water, the pump was engaged and the microfluid channel was filled avoiding any bubbles. The additional syringe and the handmade device were not needed. Syringes are always to be filled with bubble-free liquid, without any gaseous buffer.

Thirdly, the heating procedure was repeated with water at different flows. Variation of flow at constant heating power as well as variation of heating power at

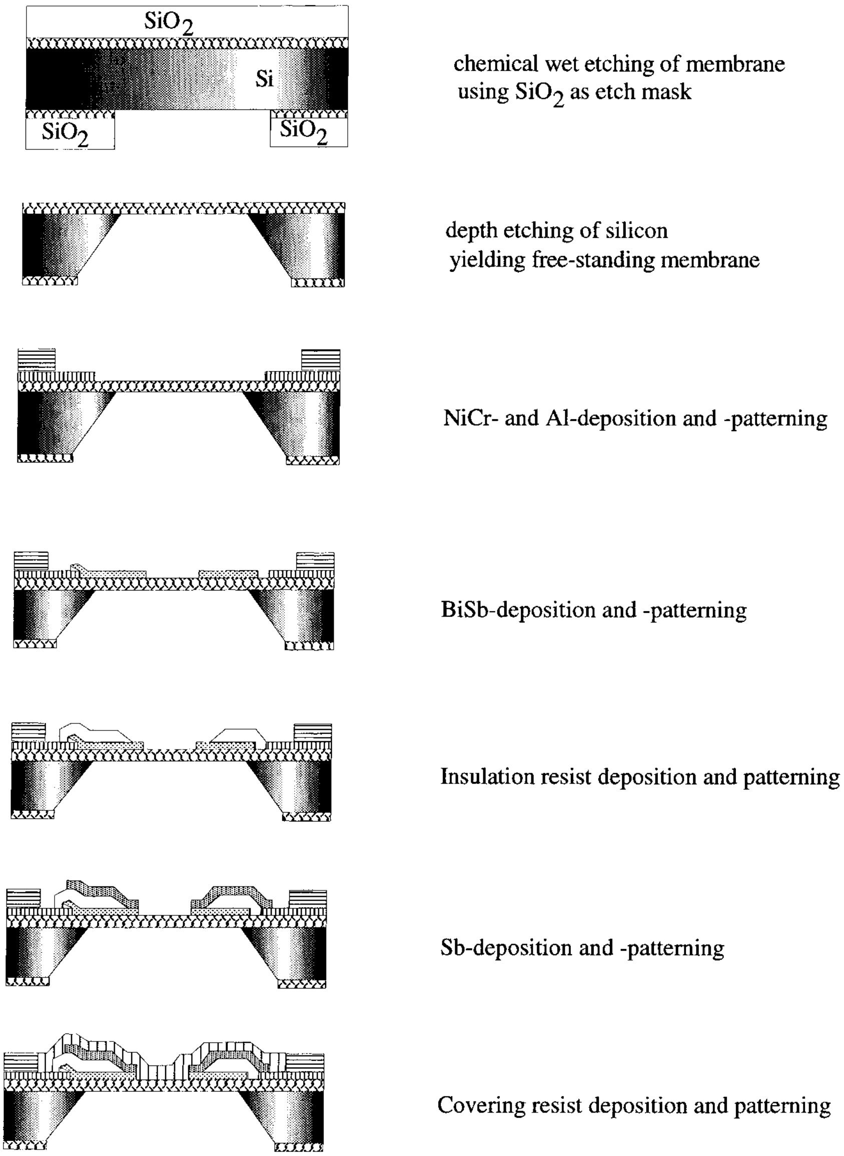

Fig. 3. Main steps of the preparation of the silicon half shell (not to scale).

constant flow (Fig. 10) was investigated. The results are predictable: less flow yields lower signals and larger t_{95}, less heat evolution leads to lower signals.

The responsivity, S, defined as ratio of thermovoltage to applied heating power is calculated; e.g. S=0.75 V/W at a flow of 5 μl/min.

As test reaction, the neutralization of sulphuric acid and sodium hydroxide was chosen due to simple handling, good establishment, and small reaction time. For this purpose, one syringe in the pump was filled with sulphuric acid at a concentration of 5% corresponding to ca. 1 mol/l. The other one was again filled with pure water, the syringe in the special device was

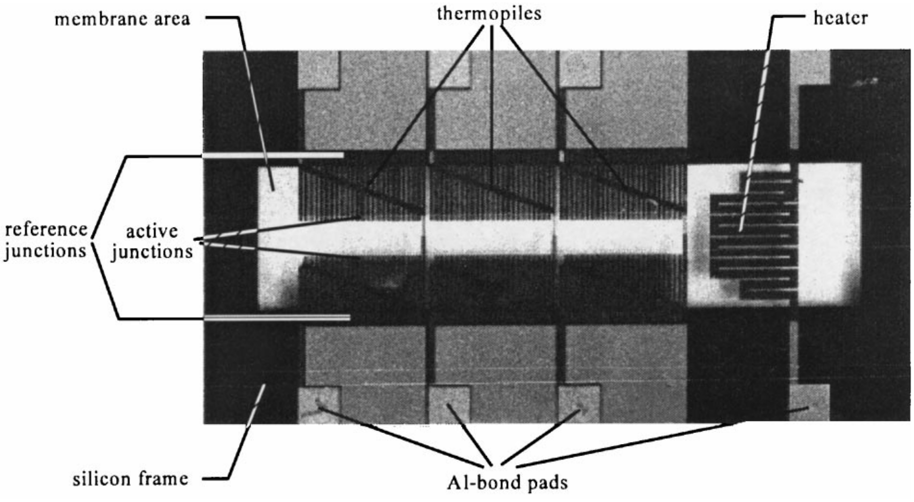

Fig. 4. Completed silicon half shell of the micro-flow calorimeter.

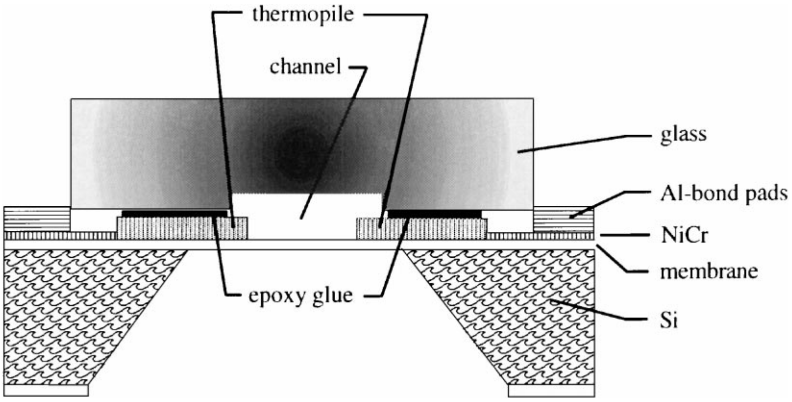

Fig. 5. Cross-sectional view of the calorimeter (thermopile section) after glue bonding of the silicon and glass half shells (not to scale).

filled with sodium hydroxide as was the short piece of hose leading to the T-fork element. After establishing a certain flow of both water and H_2SO_4 through the microfluid channel, NaOH was injected into the water stream by simply screwing the special device and moving the syringe piston. The volume of injected solution is determnined by the number of drills of the screw with a sufficient accuracy for volumes down to 1 μl. The screwing itself yielded a thermoelectrical signal due to the higher flow rate. One has to assure that the injection is completed and the zero-level of the thermoelectrical signal is established again before the NaOH-sample enters the mixing region of the calorimeter and the heat of reaction is detected.

In this way, several combinations of NaOH sample volume, NaOH concentration, and flow rate were chosen for the investigation of evolution of heat.

Fig. 11 shows the thermoelectrical signal of the neutralization reaction involving a flow of 5 μl/min, applying 2 μl NaOH of a concentration of 5 mmol/l, i.e. a total amount of 10 nmol NaOH. The graph shows

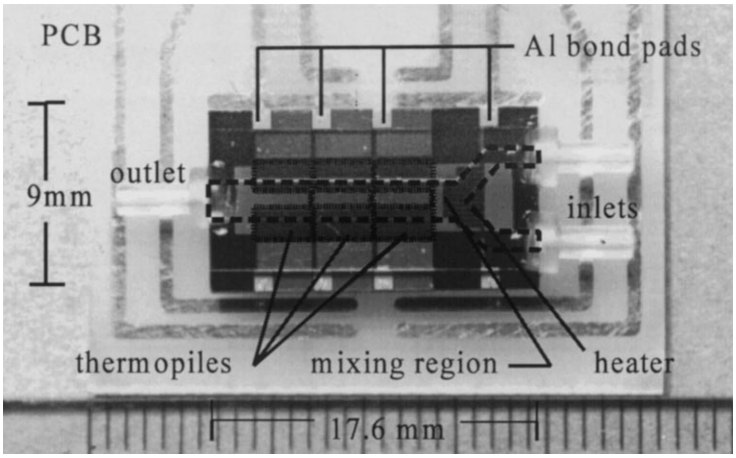

Fig. 6. Completed microflow calorimeter.

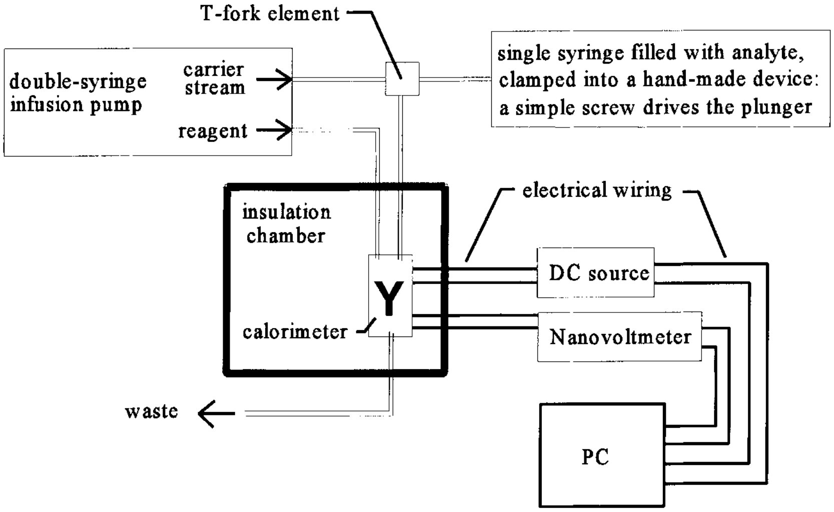

Fig. 7. Measurement arrangement.

a significant signal peak after 50 s and a signal duration of 100 s.

Only if the residence-time exceeds the reaction time, indicated by different slopes of signal rise and decline, the reaction is totally monitored.

It has to be stressed here, that a sample volume of 2 µl equals the channel volume, and dilution to a factor of two occurs in the mixing zone, in this case actually leading to the detection of 5 nmol NaOH.

Using the calibration data, the detected amount of evolved heat of reaction and the calorimeter efficiency, defined as the ratio of detected heat and expected heat of reaction, based on sample volume and concentration can be calculated after signal integration (Table 1). An efficiency of ca. 70% is yielded using a comparably high concentration of 100 mmol/l NaOH at low flows. Investigating neutralization at a low concentration of 5 mmol/l NaOH, it is found that the calorimeter efficiency equals ca. 70±8.4% at 5 µl/

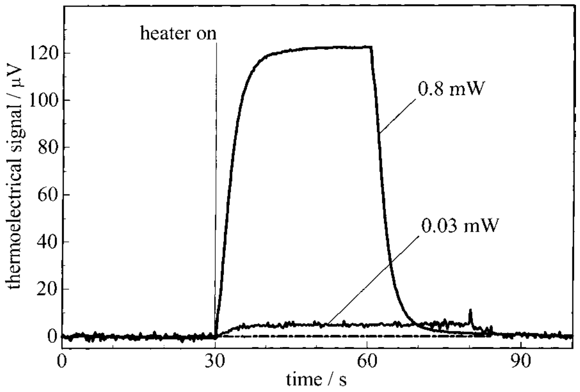

Fig. 8. Thermoelectrical response of the thermopile closest to the heater in case of electrical heating by the integrated thin-film resistor at heating powers of 0.8 mW and 0.03 mW: without liquid.

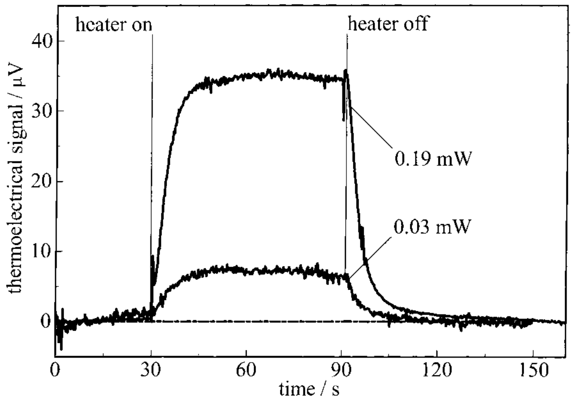

Fig. 9. Thermoelectrical response of the thermopile closest to the heater in case of electrical heating by the integrated thin-film resistor at heating powers of 0.19 mW and 0.03 mW: channel filled with water at zero flow.

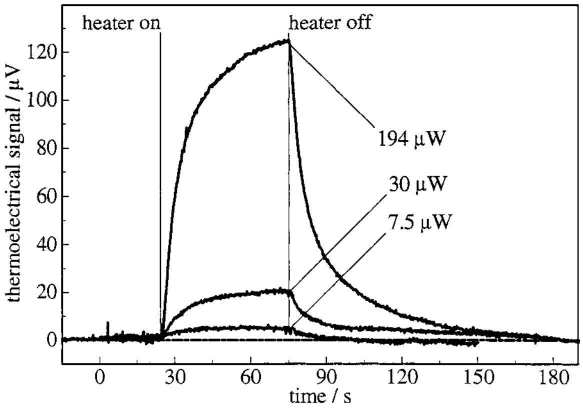

Fig. 10. Thermoelectrical response of thermopiles in series in case of electrical heating by the integrated thin-film resistor: channel filled with water at a flow rate of 5 µl/min in each inlet channel at heating powers of 194, 30 and 7.5 µW.

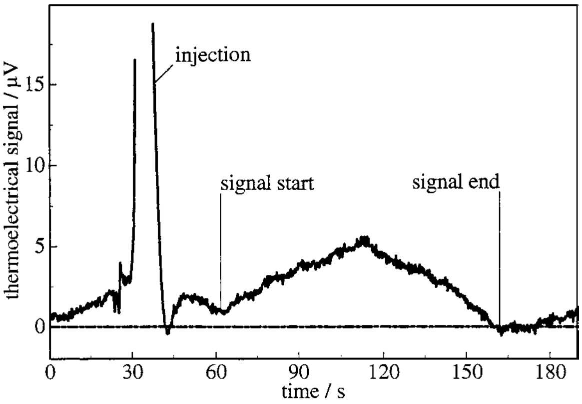

Fig. 11. Thermoelectrical detection of the neutralization of ca. 1 mol/l H_2SO_4 with 2 µl of 5 mmol/l NaOH, totalling 10 nmol NaOH, at a flow rate of 5 µl/min in each inlet channel (thermopiles in series).

min flow and rises with decreasing flow and prolonged residence-time. The experimental data indicate, that the module is suited for the investigation of low-concentration reagents in small sample volumes of down to 1 µl and at low flow rates in the µl/min-range. Sample volume and flow rate have to be suitable to yield an optimized residence-time and to monitor the entire reaction over time.

More accurate data might be achieved by further optimization of the calorimeter design (heater placement) and optimized experimental arrangement.

5. Conclusions

1. It is possible to construct a microflow calorimeter by using microfabricated chips.

Table 1
Detected heat of reaction and molar reaction enthalpy drawn from experiments investigating the neutralization of sulphuric acid and sodium hydroxide with various combinations of sample volume, sample concentration, and flow rate

Sample volume (μl)	4	1	2	2	2	2
Concentration (mmol/l)	100	100	5	5	5	5
Flow (μl/min)	5	5	3	5	7	10
Molar reaction enthalpy for neutralization of H_2SO_4 and NaOH, ΔH_{mol} (kJ/mol) [19]	65	65	65	65	65	65
Expected experimental reaction enthalpy (ΔH/mJ)	26	6.5	0.65	0.65	0.65	0.65
Detected heat (W/mJ)	18.23±0.66	4.52±0.49	0.61±0.08	0.44±0.06	0.17±0.02	0.22±0.03
Efficiency $W/\Delta H$ (%)	70±2.5	70±7.6	88±11.1	67±8.4	26±3.3	34±4.3

2. High sensitivity of such a microflow calorimeter can be achieved by the arrangement of highly efficient thin-film thermopiles (Bi/Sb/Sb) in close contact with microfluid channels.
3. A thermal insulation between the measurement zone of the fluid channel and the frame of the chip calorimeter can be realized by etching of a large chamber, which is covered by a dielectric thin-film membrane supporting the legs of thermocouples in order to realize sufficient temperature gradients between hot and cold junctions of the thermopiles.
4. Thin-film resistors for internal electrical calibration and thermal modulation can easily be integrated in the microflow calorimeter.
5. The constructed microflow calorimeter is mechanically stable enough for the liquid flow.
6. Two liquids can be mixed in the flow calorimeter by infusing two flows into the Y-like shaped channel configuration.
7. The microflow calorimeter can be calibrated internally very quickly by using an integrated thin-film heater.
8. The feasibility of the microflow calorimeter could be demonstrated by the neutralization of NaOH with H_2SO_4. A concentration of 5 mmol/l NaOH was detected in a volume of 2 μl, corresponding to an absolute amount of 10 nmol NaOH.
9. The data indicate the suitability of the presented calorimeter for sample volumes of 0.1 up to 1 μl at flow rates of 5 μl/min or less.
10. The microflow calorimeter should be suited for investigations on reaction kinetics in small sample volumes at low concentrations using the measurement of local gradients of heat evolution in the microflow and/or the thermal modulation by the integrated thin-film heater.

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