

A microcalorimetric platform for studying the heat produced by chemical reactions in microliter volumes

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Abstract

We describe the development of a microcalorimetric platform for accurate measurement of the heat power generated by chemical/biochemical reactions in liquid volumes of a few 10 μL , which we used to measure the heat of mixing of 1-propanol in deionized water. The system allows fast overall stabilization to a setpoint temperature with a stability of ± 1 mK. The combination of an open-well sample reservoir with a commercial nanocalorimetric chip (Xensor Integration) makes the platform easy to use and very versatile. The open-well format allows preloading a sample by pipetting, while fluidic interconnections enable injecting subsequently additional reagents.

Keywords: calorimetry, thermopile, temperature stabilization, chemical reaction, mixing enthalpy.

Introduction

Calorimetry is an analytic method which allows the direct measurement of heat released/absorbed during a chemical or biochemical reaction. Its miniaturized version, typically called nanocalorimetry, is very attractive for life science applications and extensive literature is available on the subject [1]. In particular, thermopile-based differential nanocalorimeters working with very small sample volumes requiring sophisticated microfluidic interconnections were reported [2] [3]. Thermistor-based nanocalorimeters were also reported [4], [5] and allowed high-sensitivity detection in small sample volumes. Relatively larger volumes (a few 10 μL) were used for the study of slow metabolic processes and a precise temperature stability was a prerequisite for good signal resolution. Such type of application, based on a rather sophisticated temperature controlled system, was already investigated in literature [6]. The platform presented here, instead, is designed in order to provide a flexible and straightforward micro/nanocalorimetric solution that allows analyzing very conveniently chemical/biochemical reactions over a wide range of reagent concentrations.

Theory and experimental

A three-dimensional schematic of the developed microcalorimetric platform is depicted in Fig.1A, showing the assembly of the different parts around the thermopile-based nanocalorimeter, on the top of which a polycarbonate open-well reservoir is placed. The nanocalorimeter is a commercial sensor (Xensor Integration, XEN-NCM9924) made on a 22 μm silicon membrane consisting of an integrated silicon-aluminum thermopile and aluminum resistive heaters. The temperature of the aluminum holder is regulated by a proportional-integral-derivative (PID) temperature controller (LakeShore Cryotronics), allowing to set a target temperature with stability down to ± 1 mK by means of a flat heat source on top of the platform. As shown in Fig.1B, the reservoir has an inner size of 5×5 mm² and is glued to a 25 μm polystyrene membrane which avoids contamination of the sensor. The reservoir allows loading the liquid sample and is interfaced with the surrounding aluminum holder by fluidic connections enabling subsequent liquid injections. In addition to the resistive heater integrated on the silicon membrane, an external resistive heater can be applied directly in the reservoir filled with water (50 μL) for calibration.

Heat transfer simulations were carried out in order to optimize the platform's design, some results of which are shown in Fig.2. In particular, transient simulations for the platform temperature after switching on the flat heat source on top of the platform indicate a stabilization time constant of the whole platform of 30 minutes (Fig.2B), while the transient simulation after switching on a heat source

located in the reservoir indicates a time constant of about 10 seconds for developing a steady temperature difference between the hot and cold junctions of the thermopile (Fig.2C).

Results and discussion

Electrical calibration tests were run in order to quantify the power sensitivity (PS) of the microcalorimetric platform. A typical thermopile voltage signal (VTP) over time is shown in Fig.3A after baseline correction, which consists in putting to zero the baseline signal. The calibration is done by applying periodic and increasing currents to the resistive heaters, either the integrated one or the external one applied into the reservoir filled with 50 μL of water. Comparison of the calibration done using the two different configurations is shown in Fig.3B, while a close-up view on the 0-10 μW heat power range highlights a limit of detection of 500 nW. In particular, the electrical calibration using the external heater placed in the reservoir allows simulating more accurately the heat power generation within the sample.

The applicability of this platform to the study of a chemical reaction was tested by measuring the heat of mixing (better known as enthalpy of mixing H) of 1-propanol in deionized water in the concentration range from 0% to 69% of 1-propanol, the results of which are shown in Fig.4. In particular, literature data [7] showing the enthalpy of mixing H of 1-propanol over the mole fraction (x) are plotted in Fig.4C, while Fig.4D shows the comparison of the differential heat of mixing data points and the literature curve obtained by differentiation of the $H(x)$ behavior.

Conclusion

We believe this microcalorimetric platform provides a straightforward approach and solution to study many heat-generating reactions in a few 10 μL volumes, and that its versatility will make it applicable to studying a variety of biochemical and biological processes.

Acknowledgements

Funding of this work was provided by the EU Ideas program (ERC-2012-AdG-320404).

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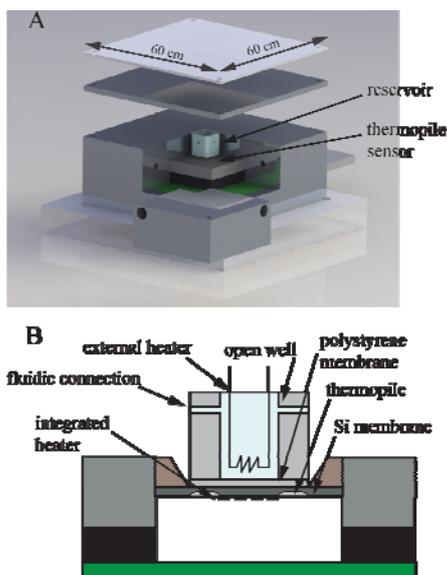


Figure 1: Microcalorimetric platform description. A) 3D schematic view of the platform, showing the location of the thermopile sensor and of the open-well reservoir. B) Cross-sectional view showing the thermopile and integrated heater on the silicon chip membrane, the open-well reservoir with the polystyrene membrane, the fluidic connections, and the external resistive heater.

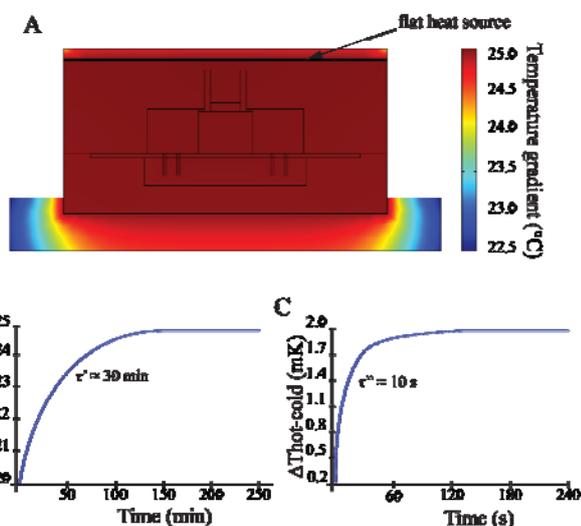


Figure 2: Computational heat transfer simulations using Comsol Multiphysics. A) Small temperature gradient in the microcalorimetric platform, when using a constant heat source on top of the platform (20 W), resulting in a steady temperature of about 25°C. B) Transient simulation for the platform temperature after switching on the flat heat source. C) Transient simulation after switching on a heat source (40 μ W) located in the reservoir starting from a stabilized platform temperature of 25 °C.

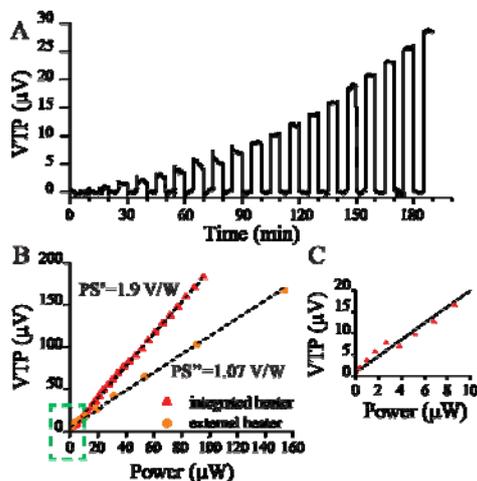


Figure 3: Thermo-electrical calibration of the platform using the Joule heating effect. A) Example of thermopile voltage signal (VTP) measured when applying periodic and increasing currents to the integrated heater. B) Comparison of the calibration carried out using the integrated heater and the external heater in the reservoir. The power sensitivity coefficient (PS) is estimated to be 1.9 V/W for the first case and 1.07 V/W for the second case. C) Close-up view of the integrated heater calibration curve for low heat power.

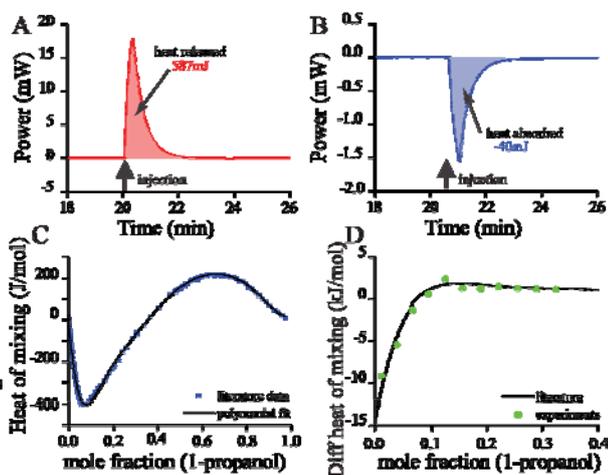


Figure 4: Measurements of the heat of mixing of 1-propanol in water. A) Heat power signal for the injection of 5 μ L of pure 1-propanol in 45 μ L of pure water. Time integration of this peak provides the total heat release (PS of 0.58 V/W). B) Heat power signal over time for the injection of 5 μ L of pure 1-propanol in a 45 μ L 27% v/v 1-propanol/water solution. C) Literature data for the molar heat of mixing of 1-propanol in water [7]. D) Differential heat when mixing 1-propanol in water. The points were obtained by integrating over time the heat power signal peaks and normalizing by the total number of moles, while the full curve was obtained by differentiating the curve of C).