Journal of Thermal Analysis, Vol. 42 (1994) 895-906

A HIGH SENSITIVITY TITRATION CALORIMETER USING PYROELECTRIC SENSOR

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(Received April 9, 1993)

Abstract

A new sensitive isothermal titration microcalorimeter using polyvinylidene difluoride (PVDF) as detector has been developed. Heat pulses of less than 0.4 μJ can be detected and the baseline noise level (*p-p*) is 40 nW. The calorimeter is constructed with one reaction cell (0.7 ml) insulated by several radiation shields inside a vacuum chamber. The performance of the instrument was examined by measuring the heat of protonation of Tris with HCl. The enthalpy of reaction was found to be -49 ± 1 $kJ\cdot mol^{-1}$, in good agreement with the reported value of -47.5 $kJ\cdot mol^{-1}$.

Keywords: microcalorimeter, pyroelectric sensor, titration calorimeter

Introduction

Calorimetry has been very useful in characterizing ligand-macromolecule and macromolecule-macromolecule binding reactions [1, 2]. Several industrial and academic laboratories are involved in the development of analytical procedures to obtain thermodynamic information about biological systems [3–5]. Among the techniques used, isothermal titration calorimetry is the most recent and measures directly the energetics of biochemical reactions at constant temperature. Here, the heat associated with the interaction of the sample (protein) upon stepwise addition of the reactant (ligand) is determined in terms of reactant concentration. From such a binding isotherm, one obtains in a single experiment, the binding constant, the enthalpy and the entropy of binding and the stoichiometry of the reaction.

Recently, sensitive instruments using solid-state thermoelectric modules as detectors and capable of measuring heat effects arising from reaction of only

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0368–4466/94/ $ 4.00

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nanomole amounts of reactants have been developed [6–10]. These advances have made possible detailed studies of the energetics of ligand-macromolecule and macromolecule-macromolecule interactions having dissociation constants in the range 10^{-3} to 10^{-8} *M*. Although many biological binding processes fall in that category, one would like to have calorimeters capable of studying association processes exhibiting higher affinity binding constants. In addition, the amount of sample required for complete determination of binding isotherms is still relatively large. This shortcoming often impedes the application of the technique to study biological materials under exploratory research because of their limited availability. Thus, it is highly desirable to develop a fast and super-sensitive calorimeter that can measure, with lesser amount of sample required, dissociation constants in the range of 10^{-9} *M* or lower.

For a detector to be sensitive, its heat capacity should be low, i.e., it should have a small mass and be made out of a material with low specific heat. For these reasons, pyroelectric sensors have been extensively used in radiation detection, especially in infrared and in laser power measurements. Initially, these devices were based on the use of poled ceramics such as lead zirconate titanate or single crystals such as triglycine sulfate. In 1971, Bergman *et al.* [11] made the first observation of pyroelectricity in a poled polymeric film, polyvinylidene fluoride (PVDF). Since then, this polymer have been extensively used in different applications [12–17]. Some of the most important features of this polymeric sensor are: fast response, low thermal diffusivity, extremely thin film, large surface areas, flexibility for conformity to curved surfaces and low cost. Unfortunately, pyroelectric detectors are also piezoelectric. They are sensitive to mechanical stresses, pressure changes and acoustical waves. These effects interfere with each others and, despite the many advantages, only few non-radiation heat detection applications are known with PVDF [18–20]. Also, because of the finite time constant RC, PVDF film is more suitable to dynamic measurements.

Pyroelectric and piezoelectric activities cannot be totally separated [21], but one can maximize pyro sensitivity by working at very low frequency, i.e., by using a low pass filter and by choosing proper electrical and mechanical designs. In this paper, we describe the approaches we took in the design of a titration calorimeter using PVDF. This calorimeter is capable of measuring thermal power in the submicrojoule level.

Electrical and mechanical considerations

Electrical considerations

Depending on the type of application, several electronic schemes can be designed. For low frequency applications such as thermal detection, it is desirable to have a long time constant. This can be obtained by using a high input resistance and capacitance. However, a high capacitance reduces the signal and a high resistance increases noise. Thus, the signal to noise ratio should be optimized when choosing these values. Since PVDF film is very sensitive to piezo activities at high frequencies, a low-pass active filter can be used to reduce these undesirable effects. This result can also be achieved by applying the common mode rejection concept, i.e., wiring two equal areas of a film out of phase to cancel mechanical and acoustical vibrations that stimulate both. In addition, digital filters can be designed to further reduce high frequency signals.

Mechanical considerations

The performance of a pyroelectric sensor depends on the thermal capacity of the calorimeter, and the thermal losses to the surroundings. A relatively large thermal load gives a fast but less sensitive detector. For this reason, the reaction cell should be as small as possible and should present a large surface of contact to the detector. Also, the PVDF film should be as thin as possible to assure uniform heating of the detector.

Thermal losses can be eliminated by several means. Heat loss through radiations can be reduced by using thermal shields while heat loss through convection can be eliminated by mounting the detector in a vacuum. Conduction losses can be minimized by using extremely fine lead attachment wires. The vacuum will also eliminate pressure changes of the surroundings and thus improve the signal to noise ratio. Finally, vibration mounts should be used to reduce pump vibrations and other mechanical interferences.

Instrument design

The considerations in the preceding paragraphs provided the guidelines for the construction of the instrument. It consists of several stages with cylindrical symmetry. A schematic diagram illustrating the calorimeter with the titration cell is shown in Fig. 1.

A 28 μm poled PVDF film (Atochem, King of Prussia, PA) coated with Ni-Cr is attached to each face of a gold lollipop shaped cell using a thin layer of conductive epoxy (3 *M*). In order to reduce the mechanical sensitivity of the detector, the film is folded in half and the inner surfaces are bonded together also with the epoxy. Each film has a capacitance of approximately 0.5 nF. A small piece of copper foil with preapplied conductive adhesive is used as lead attachment.

The electrical heat of calibration of the instrument is supplied with a resistive heater. An extremely fine Manganin wire, size 44 AWG, obtained from MWS Inc., Westlake Village, CA., is wound non-inductively on the cylindrical body of the reaction cell. The nominal resistance of the heater is 108 ohms. Good contact of the heater to the cell is assured by using GE varnish 7031. The heat sink is an aluminum block (a) that splits into two halves to allow easy assembly of the cell (b) and the sensors. The stainless steel tubing (c) attached to the titration cell fits tightly into the appropriate groove in the block over a length of about 3 inches allowing the incoming solution to be in good thermal equilibrium with the surrounding before mixing. A small copper block (d) containing the electronic components is tightly attached to the heat sink. This block elimi-

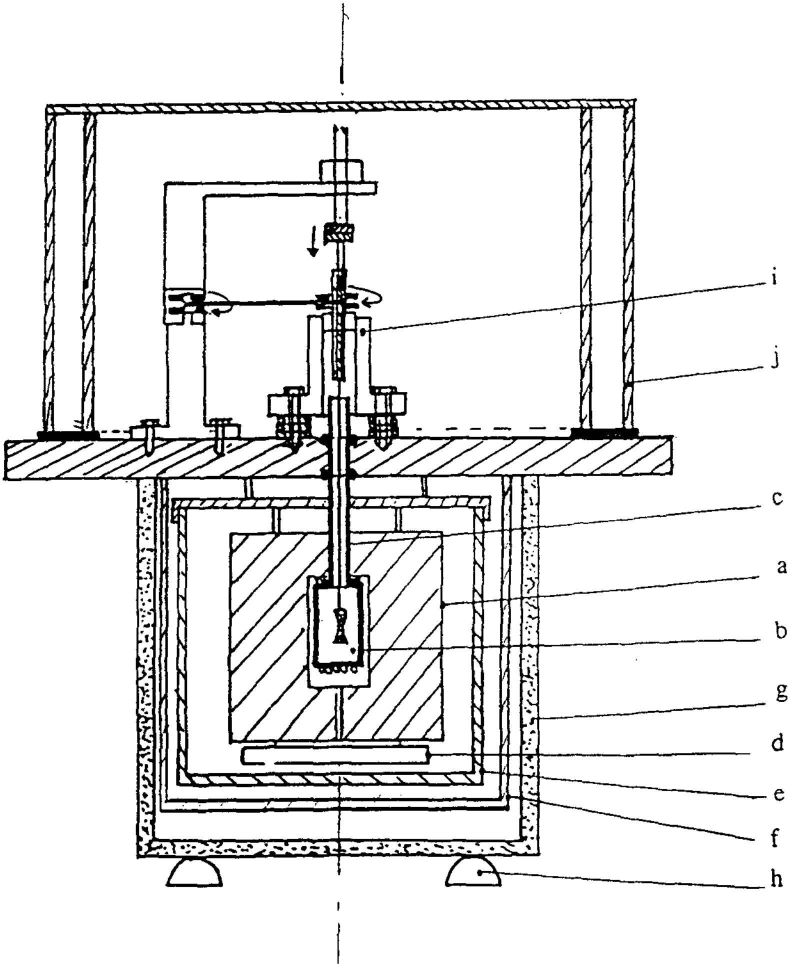

Fig. 1 Schematic diagram of the pyroelectric titration calorimeter. (a) aluminum heat sink; (b) titration cell and PVDF films attached to both sides of the cell. The heater wire for electrical calibration is wound around the body of the cell; (c) stainless steel tubing; (d) copper block containing the electronic circuit; (e) copper canister used as radiation shield; (f) second radiation shield; (g) vacuum chamber; (h) rubber mounts; (i) injection-stirrer assembly; (j) aluminum cover

nates internal temperature gradients and is connected to the common ground of the circuit.

This whole assembly is inserted into a floating copper cylinder (e) which serves as a first radiation shield and also as an electrostatic guard. A second radiation shield (f) made of aluminum is used as an outside jacket. The calorimeter is mounted inside a vacuum chamber (g) which in turn is supported by rubber mounts (h) to reduce mechanical vibrations. All electrical wires are connected to the outside using a vacuum tight feed-through connector (not shown in figure). The whole calorimeter may be assembled or disassembled easily within a few minutes.

Reactant delivery and the subsequent mixing of the solutions in the reaction cell is achieved with an injector-stirrer (i) assembly supplied by Microcal Inc., Northampton, Mass. The paddle of the syringe-stirrer is filed shorter to fit the cell and thorough mixing is accomplished at 400 rpm as determined using a glass cell with similar dimensions.

To further reduce the effect of pressure changes and acoustical waves through the opening of the reaction cell, a large aluminum cover (j) is seated on a rubber gasket on top of the instrument.

As mentioned above, for better sensitivity it is necessary to evacuate the whole calorimeter assembly. This is accomplished with a rotary pump connected to the vacuum chamber (g). A vacuum of 10^{-3} torr is usually sufficient to eliminate most of the noise.

Electronic circuit

A variety of pyroelectric detector signal processing electronics are available and have been discussed in many publications [13, 14, 21–25]. For our application, we chose a special operational amplifier to interface with the detector. This amplifier operates in a charge coupled mode which, by definition, is a current operated circuit with zero input impedance. The charge is quickly transferred from the piezo film to the capacitor in the amplifier's feedback loop and no voltage is generated across the film. This eliminates the effect of time constants of both the sensor and the connecting cable.

The signal processing system requires an operational amplifier having high input resistance and extremely low bias current. The op-amp selected here is a FET input Burr-Brown OPA-111BM operated in current to voltage mode. An input current of 10 pA will produce an output offset of 1 volt. The basic components of our electronic system are shown schematically in Fig. 2. The input resistance (2 kΩ), the feedback capacitance (2 pF) and the feedback resistance (10^{11} Ω) were determined experimentally to provide the best signal to noise ratio, to reduce the high frequency cutoff and to stabilize the baseline.

The layout of the amplifier's circuit is critical. The op-amp is inserted in a copper casing (Fig. 1d) which is well grounded and placed very close to the sensor. The inputs are guarded and connected to the same ground as the casing. Also, to avoid leakage, the input line is connected to a well insulated stand-off connector. Output signals from the modified charge coupled preamplifier pass to a buffer amplifier (OP07) to increase the gain and to a 3 pole low pass filter with a cutoff frequency of 1 Hz.

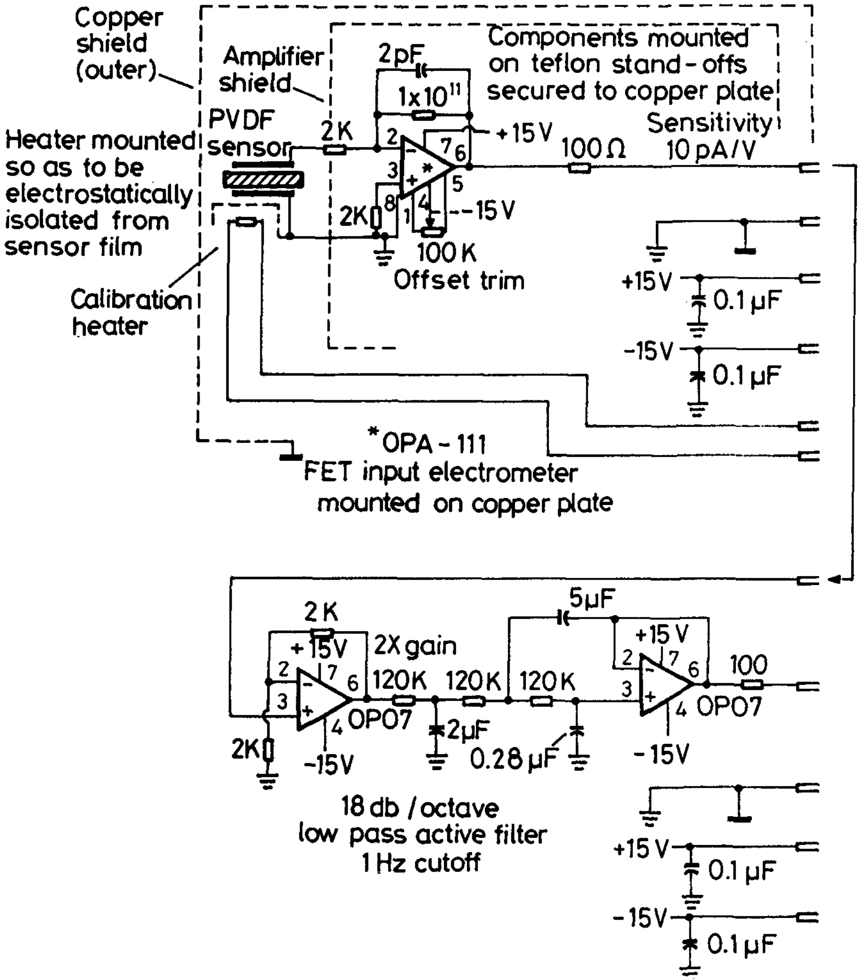

Fig. 2 Circuit block diagram of the calorimeter. As explained in the text the signal processing consists of three main functional elements: (1) preamplifier OPA-111BM, (2) gain amplifier OP07 and (3) analog filter

Principle of measurements

When thermal energy is absorbed, the PVDF film expands. This results in a detectable deformation and a corresponding charge output. The output of the detector is thus, proportional to the rate of the spatially averaged temperature rise, $d<T>/dt$, of the film [22]:

$$V(t) = ApRG(d<T>/\mathrm{d}t) \tag{1}$$

where A is the effective area of the sensor, p the pyroelectric coefficient of the PVDF film, R the feedback resistance of the OPA-111 amplifier and G the closed loop gain of the OP07 amplifier (Fig. 2).

The heat is flowing from the cell to the sensor. Using the continuity equation for energy flow and taking the one dimensional approximation, i.e., assuming that the lateral heat flow in the material is much less than the heat flow through the surface, the heat flux per unit area q^{*} is given by:

$$q^{*} = \mathrm{d}k\delta^2<T>/\delta z^2 = C\mathrm{d}\delta<T>/\delta t \tag{2}$$

where k and C are the apparent thermal conductivity and heat capacity of the cell, respectively. The parameter d is the longitudinal distance traveled by the heat from the reaction point to the cell. Substituting in the above equation, the output voltage becomes,

$$V(t) = K\mathrm{q}^{*} \tag{3}$$

where $K = (ApRG)/(Cd)$, and can be determined experimentally. The thermal energy change, $\mathrm{d}Q$, involved in the reaction can then be estimated by integrating Eq. 3 with respect to time.

$$\mathrm{d}Q = (1/K)V(t)\mathrm{d}t \tag{4}$$

On the other hand, the heat effect associated with the binding of n moles of ligand to a macromolecule after each injection [6] is given by:

$$\mathrm{d}Q = n\Delta H \tag{5}$$

where ΔH is the enthalpy of the reaction. The total cumulative heat absorbed or release upon binding is thus

$$Q = \nu\Delta HL_{\mathrm{b}} \tag{6}$$

where v is the total volume of the cell and L_b is the total concentration of the bound ligand.

Experimental results

Figure 3 shows the response of the sensor to pressure changes when small volumes are injected into the reaction cell. The first peak reflects the increase of pressure at the start of the injection. The signal comes back quickly to the baseline before going to the opposite direction when the injection is stopped. The amplitude of the peaks depends on the speed of the stepping motor and the separation between the peak depends on the volume of the injection. As can be seen in Fig. 3, the peaks are quite reproducible.

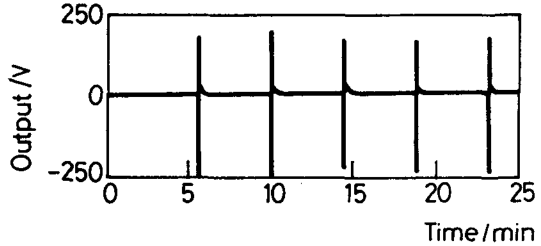

Fig. 3 PVDF response to pressure changes inside the reaction cell. The peaks correspond to injections of 4 μl of water into water. No thermal heat is involved here. The first peak corresponds to a positive pressure change (start of the injection) and the second peak to negative pressure change (end of injection). The magnitude of the peaks depends on the speed of the the stepping motor and the distance between the first and the second peak depends on the volume of the injection

A typical experiment is conducted in the following way. The cell and the injection syringe are first loaded with the same buffer solution. Several injections of known volumes are made and the data are saved. The cell and the syringe are then emptied, cleaned and dried with nitrogen purge after rising with methanol. The cell is then loaded with the sample and the syringe with the ligand in the same buffer. Injections of same volume and at same speed as in the control experiment are then made and the data saved in a different file. The two files are superimposed and subtracted from each other to yield the net signal due to the heat of reaction. This resulting signal is then processed by a special software developed in our laboratory. The software uses Fourier transform analysis to reduce the high frequency noise until the optimal signal-to-noise ratio is achieved. The difference between a non-processed data and a processed data is shown in Fig. 4. Each peak is then corrected for the baseline and integrated. The areas of the individual peaks are calibrated against electrical heat pulses of approximately the same magnitude.

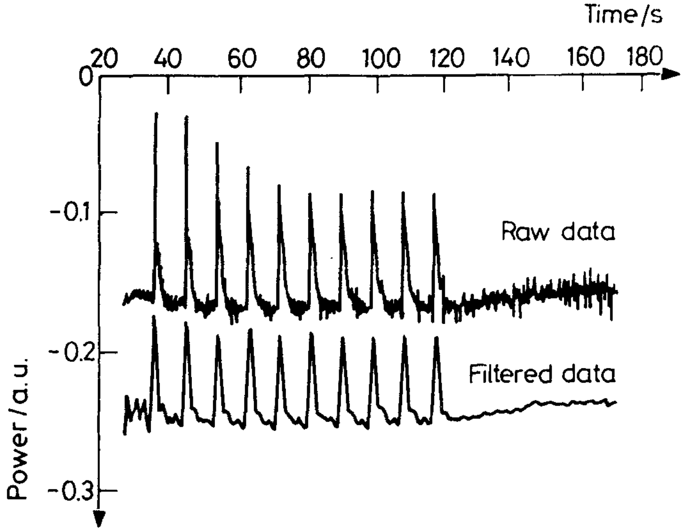

Fig. 4 Response to electrical heat pulses. Top trace represents unprocessed data. Bottom trace shows the filtered data using our noise reduction software

It is generally known that electrical calibration can lead to a significant systematic error due to the position of the heater relative to the sensor. For this reason, we test our electrical calibration with a chemical system with known enthalpy of reaction. The chemical reaction selected is the protonation of a 0.02 *M* Tris solution, *pH* 8.2, by HCl (0.0015 *M*). The enthalpy of protonation is well known and is reported to be –47.5 kJ·mol^{-1}. As mentioned above, the control experiment was obtained by injecting a buffer solution into the same buffer solution (Tris). The cell was then filled with the sample solution (Tris *pH* 8.2). A series of 7 μl HCl solutions were injected into the cell at the same speed and at intervals of 5 minutes. Figure 5 shows the responses to the heat of reaction after subtracting the signal of the control experiment. The areas under the peaks were calibrated with a 418 μJ heat pulse and the average of the 8 consecutive injections was determined to be –515±8 μJ. The enthalpy of reaction is thus calculated to be –49±1 kJ·mol^{-1}, which is very close to the reported value.

The sensitivity of a calorimeter is determined by introducing small, but known amounts of electrical powers to the calibration heater [6–8]. Figure 6 shows the responses to successive heat pulses with the cell completely filled with water (about 0.7 ml). The trace represents results obtained after data processing. The noise level as measured from peak-to-peak, is slightly less than 40 nW. The 10–90% response of an electrical step power input into the filled cell is less than 2 s. The first pulse corresponds to 4.2 μJ heat, and then the power is reduced by a factor of two each time for the following peaks. Electrical heat pulses in the 4 μJ range can be measured with a standard deviation of ±0.3 μJ. Figure 6 shows also that heat pulses as small as 0.25 μJ can be measured with a standard deviation of ±0.2 μJ. We estimated, however, that to measure a heat of chemical reaction with good precision, a minimum heat of 4–10 μJ should be applied.

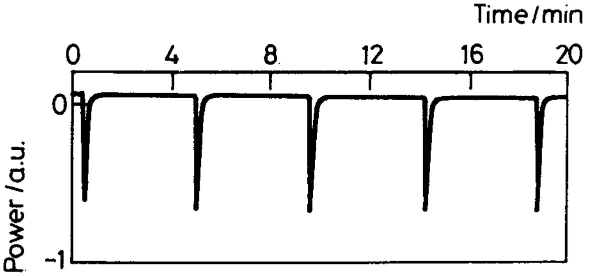

Fig. 5 Heat of protonation of a 0.02 *M* Tris solution. The power output in arbitrary units is shown as a function of time and was obtained from a series of 7 μl injections of a 0.0015 *M* HCl solution

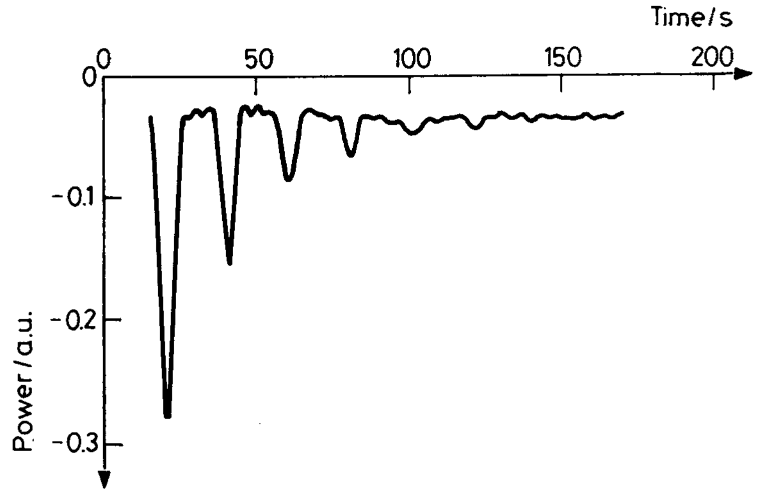

Fig. 6 Thermal power *vs.* time response obtained from successive electrical heat pulses into the titration cell containing 0.7 ml of water. The first peak corresponds to a 4.2 μJ pulse. The power is then divided by half for each successive pulse

Discussion

Most high sensitivity calorimeter are constructed with twin-cell design. The use of two cells connected in opposition permits erroneous signals due to environmental irregularities such as temperature or pressure perturbations to be canceled and hence, produces a better baseline performance. In addition, this differential mode is needed for the power compensation mechanism which improves the time response of the instrument. This mechanism is not necessary in our case because PVDF has a very short response time and the limiting factor is the thermal conductivity of the aqueous solution in the cell. For this reason, our calorimeter is constructed with one reaction cell only.

The calorimeter described here has the high sensitivity needed for most biochemical interaction studies and is suitable for small sample experiments. It does not require expensive equipment such as a nanovoltmeter. Furthermore, PVDF can be cut and fabricated into different shapes and sizes so that it can be adapted to different cell designs. Finally, because of the sensitivity of the polymeric film to pressure changes, an improved delivery system which can further reduce or eliminate completely the disturbance during reactant (ligand) introduction is needed to realize the absolute sensitivity of the calorimeter.

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Zusammenfassung — Unter Verwendung von Polyvinylidendifluorid (PVDF) als Detektor wurde ein neues isothermisches Titrationsmikrokalorimeter mit hoher Empfindlichkeit entwickelt. Damit können Wärmeimpulse noch unter 0,4 μJ registriert werden, der Rauschpegel der Basislinie (*p-p*) liegt bei 40 nW. Das Kalorimeter wurde mit einer Reaktionszelle (0,7 ml) gebaut, die – in einer Vakuumkammer – von verschiedenen Strahlungsschilden umgeben ist. Die Funktion des Gerätes wurde anhand der Messung der Protonierungswärme von Tris mit HCl untersucht. Als Reaktionsenthalpie fand man – in guter Übereinstimmung mit Literaturangaben (–47.5 $\mathrm{kJ\cdot mol^{-1}}$) – einen Wert von –49±1 kJ/mol.