

## Effect of a stirring process in an isothermal titration microcalorimeter

F. Socorro<sup>a</sup>, I. de la Nuez<sup>b</sup>, L. Alvarez<sup>a</sup>, M. Rodríguez de Rivera<sup>a,\*</sup>

<sup>a</sup> Departamento de Física, ETSII, Universidad de Las Palmas de Gran Canaria, Campus de Tafira, E-35017 Las Palmas, Spain

<sup>b</sup> Departamento de Ingeniería Electrónica y Automática, ETSII, Universidad de Las Palmas de Gran Canaria, Campus de Tafira, E-35017 Las Palmas, Spain

Received 29 July 2003; accepted 30 October 2003

### Abstract

In this paper, it is analysed the way in which the thermodynamic results obtained in an isothermal titration microcalorimeter, with continuous injection of a liquid and a variable mass are affected. Two aspects are presented, the first one refers to a variation in the baseline that takes place when the liquid mass is increased in the laboratory cell, this variation is due to the change in the thermal coupling between the stirrer and its contact with the thermostat. The second aspect is the analysis of the effect of the stirring process and the injection velocity on the homogenization of the remaining mixture in the laboratory cell.

The aim of the study of these two previous aspects is to increase the accuracy of the thermodynamic measures carried out. The variation of the baseline affects in a percentage from 1 to 2% of the total energy developed. It is also shown the necessity of a stirring process according to the injection velocity in order to obtain a homogeneous mixture at every instant. This fact allows to carry out a deconvolution of the calorimetric signal and to obtain directly the power developed in the mixture process in terms of the amount of mixed substance [Meas. Sci. Technol. 1 (1990) pp. 1285–1290; J. Thermal Analysis 41 (1994) pp. 1385–1392].

© 2004 Published by Elsevier B.V.

**Keywords:** Stirring process; Baseline correction; Injection velocity; Isothermal titration calorimeter; Liquid injection calorimeter

### 1. Introduction

Isothermal titration microcalorimeters (ITC) are utilized to study solid-liquid and liquid-liquid processes (enthalpies of solution, enthalpies of dilution, enthalpies of reaction in solution, enthalpies of mixing, etc.). If the experimental equipment provides a continuous and controlled liquid injection system, these calorimeters allow to determine, through a sophisticated treatment of the calorimetric signal, the power developed at every instant in the mixture process [1].

In any case, the accuracy of the obtained energetic results depends on the consideration of all those aspects that may affect the previous working hypotheses. In previous works some aspects have been analysed that we have called “micro-effects”. This type of effects may alter the final energetic result, that is why they have to be evaluated in the calibration of these experimental equipments [2–3].

In this paper, we analyse the stirring process according to two aspects: the first one refers to the variation in the baseline due to the thermal coupling of the stirrer with the thermostat. The second aspect refers to the homogeneity of the mixture produced by the stirrer, but this second aspect will be analysed in a different way, in this case, for a fixed stirring velocity, we will vary the injection velocity. To develop this work, we began by describing the experimental equipment, its representative model and the experimental measures carried out. We will make a theoretical and experimental study about the change in the baseline and an analysis on the effect of the injection velocity in the homogeneity of the mixture calculating the enthalpy of the mixture in terms of the mixed amount of substance.

### 2. Experimental equipment and stated model

The calorimeter used is a Thermometric TAM2277-204/2250 and the calorimetric signal is read directly by a

\* Corresponding author. Fax: +34 928 452922.

E-mail address: [rivera@step.es](mailto:rivera@step.es) (M. Rodríguez de Rivera).

digital multimeter with a 10 nV resolution. The injection system permits to inject step by step 0.0832  $\mu\text{l}/\text{step}$  of the motor that, through a micrometric screw, moves a billet which pushes the piston of a 50 ml Hamilton syringe; by programming the number of steps of the motor in each sampling ( $\Delta t$ ) we obtain the injection flow; in this case, the utilized injection flows have been: 1.51, 2.27, 3.03 and 5.29  $\mu\text{l}/\text{s}$  ( $\Delta t = 1.1$  s).

In order to model the calorimeter, we will use the localized-constants model that has shown to be ideal to represent the performance of these thermal measuring instruments with different configurations [4–7].

Some electrical calibrations of the system are carried out to determine the size of the model. They are used to check if a given model for a transference function of two poles is enough to reconstruct accurately the calorimetric curves. For this reason, and taking into account that the energetic dissipation can be produced in two places: in the calibration resistance and in the cell where the mixture takes place, a model made up of three bodies is proposed. In Fig. 1, the corresponding diagram is represented. In this model, the first body with a heating capacity  $C_1$ , represents the place where the calibration resistance is located; the second body, with a heating capacity  $C_2$ , represents the place where the mixture is accomplished, and a third body, with a heating capacity  $C_3$ , represents the detector system.

The energetic balance for each body gives the equations of the model. In this case, the power developed in a domain,  $W_i$ ; is equal to the absorbed power,  $C_i dT_i/dt$ , plus the losses by conduction to the neighbouring domains,  $P_{ik}(T_i - T_k)$ , and to the thermostat,  $P_i(T_i - T_0)$ . The term  $g(T_2 - T_0)$  represents the power due to the liquid injection that has the thermostat temperature and enters the cell that has the temperature  $T_2$ ; of course,  $g$  depends on the value of  $\rho c_p f$  of the injected liquid ( $\rho$  is the density,  $c_p$  is the heat-

ing capacity,  $f$  is the injection flow). The equations are the following ones:

$$\begin{aligned} W_1 &= C_1 \frac{dT_1}{dt} + P_1(T_1 - T_0) + P_{12}(T_1 - T_2) \\ W_2 &= C_2 \frac{dT_2}{dt} + P_2(T_2 - T_0') + P_{12}(T_2 - T_1) \\ &\quad + P_{23}(T_2 - T_3) + g(T_2 - T_0) \\ 0 &= C_3 \frac{dT_3}{dt} + P_3(T_3 - T_0) + P_{23}(T_3 - T_2) \end{aligned} \quad (1)$$

In these equations, we consider that all the parameters  $C_i$ ,  $P_i$  and  $P_{ij}$  are constant (They do not depend on time) apart from  $C_2$  and  $P_2$ . The capacity  $C_2$  depends on the heating capacity of the mixture and its value increases during the injection; the coupling  $P_2$  also increases because the thermal conductivity of the body  $C_2$  through the stirrer increases with the level of liquid in the cell. We consider that, the experimental output is equal to the difference of temperatures  $T_3 - T_0$ . The dissipated powers  $W_1$  and  $W_2$  are the ones developed in the calibration resistance and in the mixture, respectively.

As the capacity of the body where the calibration resistance is located is very low with regard to the other ones, we make:  $C_1 \approx 0$ ; so we also make:  $\theta_i = T_i - T_0$  and  $\Delta T_0 = T_0' - T_0$ . Replacing in Eq. (1), we obtain:

$$\begin{aligned} W_1 &= (P_1 + P_{12})\theta_1 - P_{12}\theta_2 \\ W_2 + P_2\Delta T_0 &= C_2 \frac{d\theta_2}{dt} + (P_2 + P_{12} + P_{23} + g)\theta_2 \\ &\quad - P_{12}\theta_1 - P_{23}\theta_3 \\ 0 &= C_3 \frac{d\theta_3}{dt} + (P_3 + P_{23})\theta_3 - P_{23}\theta_2 \end{aligned} \quad (2)$$

Finding  $\theta_1$  in the first equation and replacing in the second equation, we obtain an expression in which we can replace the value of  $\theta_2$  found in the third equation. The result is only one differential equation that relates the developed powers with the output  $y(t) = \theta_3(t)$ :

$$W_2 + \alpha W_1 + P_2\Delta T_0 = a_2 \frac{d^2 y}{dt^2} + a_1 \frac{dy}{dt} + a_0 y \quad (3)$$

In this equation the coefficients  $a_2$ ,  $a_1$  and  $a_0$  depend on the time when the injection is taking place because they depend on  $C_2(t)$  and  $P_2(t)$ ; when there is no injection, they are constant in time but they depend on the heating capacity of the liquids ( $C_2$ ) and on the thermal coupling  $P_2$ .

With the obtained final equation (Eq. (3)) the following considerations can be made:

1. The variation of  $P_2$  produces a change in the baseline if  $\Delta T_0 \neq 0$ .

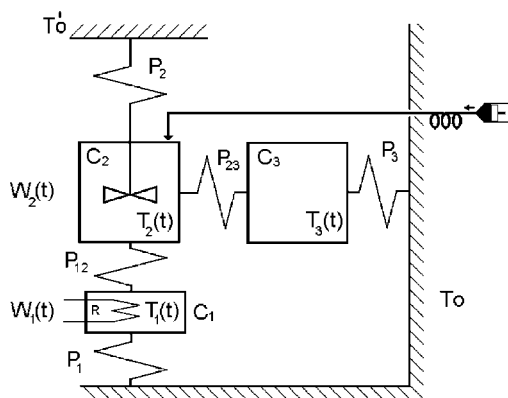


Fig. 1. Representative model of the calorimeter. The first body (with a capacity  $C_1$ ) represents the place where the calibration resistance is placed, the second body (with a capacity  $C_2$ ) represents the place where the mixture is carried out, and the third body (with a capacity  $C_3$ ) represents the detector system.

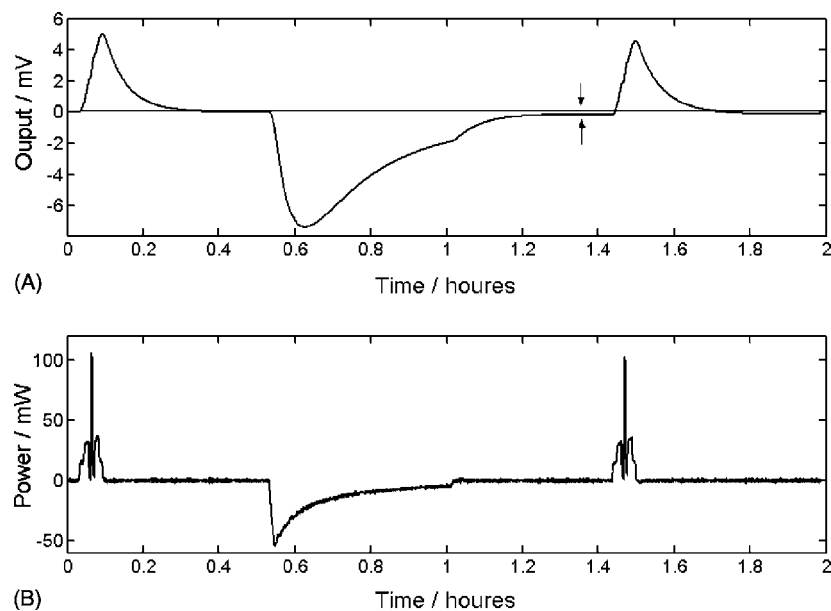


Fig. 2. (A) Thermogram corresponding to a cyclohexane injection ( $1.51 \mu\text{l/s}$ ) on initial  $1 \text{ cm}^3$  of benzene, before and after the mixture Joule dissipations are made, the arrows indicate the baseline jump produced. (B) Reconstruction of the developed powers with the corrected baseline.

2. The correction of the baseline must be always made after the deconvolution.
3. The sensitivity depends on the dissipation place.
4. The effect of the injection  $\rho c_p f \Delta T$ , term  $g(T_2 - T_0)$ , affects the coefficients  $a_2$ ,  $a_1$  y  $a_0$ .
5. If the injection flow is low, during the injection process it is possible to consider the calorimeter as a slightly variable linear system. In this case, to reconstruct the input signal it can be applied a variable reverse filter [1] in which the time constants used are slightly lower than the corresponding to the invariant situation in which the

cell has the same content of liquid (same heating capacity and same coupling) [4].

### 3. Evaluation of the stirring process

To study the effects of changing the baseline with the level of the liquid in the mixture cell, and to evaluate the effect of the injection velocity over the homogeneity of the mixture, measurements of electrical and chemical calibration have been carried out. For chemical calibration, we have realized

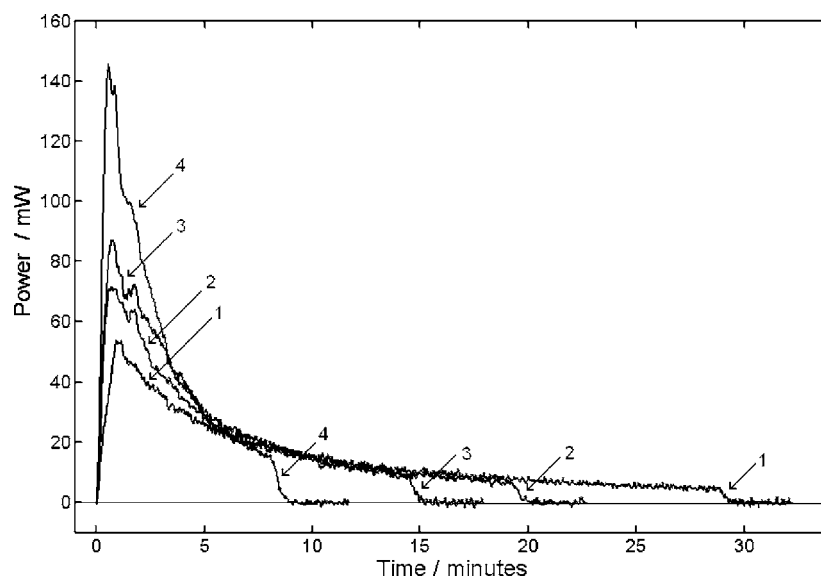


Fig. 3. Thermogenesis corresponding to the cyclohexane injection on initial  $1 \text{ cm}^3$  of benzene, with different injection flows:  $1.51 \mu\text{l/s}$  (curve 1);  $2.27 \mu\text{l/s}$  (curve 2);  $3.03 \mu\text{l/s}$  (curve 3) and  $5.29 \mu\text{l/s}$  (curve 4). In all cases the baseline has been corrected after the reconstruction of the input signal.

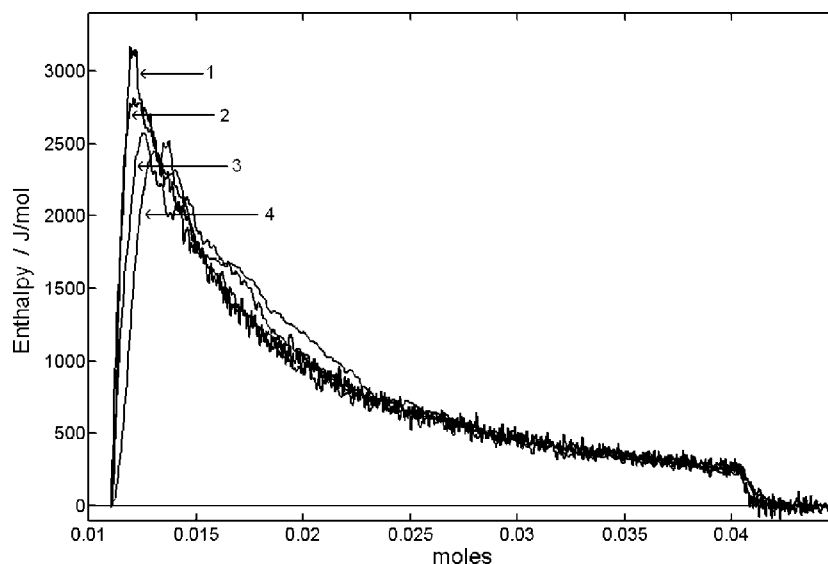


Fig. 4. Excess Partial molar enthalpy of cyclohexane according to the total number of moles. Curves corresponding to the cyclohexane injection on initial  $1 \text{ cm}^3$  of benzene, for different injection flows:  $1.51 \mu\text{l/s}$  (curve 1);  $2.27 \mu\text{l/s}$  (curve 2);  $3.03 \mu\text{l/s}$  (curve 3) and  $5.29 \mu\text{l/s}$  (curve 4). The stirring velocity was 60 rpm in all cases.

cyclohexane injections on hexane with different injection flows (from 1.5 to  $5.3 \mu\text{l/s}$ ). The stirring velocity has been the same in every measurement (60 rpm). Electrical calibrations are made up of a train of pulses with different amplitude and duration carried out before and after each injection. In Fig. 2 the corresponding thermograms and the reconstruction of the developed power are shown.

The identification method of the curves of electrical calibration has been referred to [7]. The obtained sensitivity is  $K = 312 \text{ mV/W}$ , and the time constants are  $\tau_1 = 205 \text{ s}$ ,  $\tau_2 = 16 \text{ s}$  for the dissipation previous to the mixture, and  $\tau_1 = 230 \text{ s}$ ,  $\tau_2 = 13 \text{ s}$  for the dissipation subsequent to the mixture.

The reconstruction of the power developed in the mixture process is made through a variable reverse filtering process [1] after which it is applied a second-class lowpass Butterworth filter with a cutoff frequency  $\nu_c = 0.11 \text{ Hz}$  ( $\Delta t = 1.1 \text{ s}$ ) [8]. The coefficients of the differential equation (Eq. (3)) are determined starting from the time constants obtained in the electrical calibrations before and after the mixture. The sensitivity utilized was  $K = 300 \text{ mV/W}$  and it was obtained starting from the reference values [9] after calculating the excess molar enthalpy [10]. In Fig. 3 it is shown the reconstruction of the power developed for each injection flow.

The total energy developed in the mixture process, for any injection flow, is  $\approx 25.0 \text{ J}$ , and the corresponding energy to the baseline jump varies from 0.3 to 0.5 J; i.e., the baseline correction affects in a percentage from 1.2 to 2.0% of the total developed energy.

To evaluate the homogeneity of the mixture according to injection flow and for a fixed stirring velocity (60 rpm), it has been represented the excess partial molar enthalpy of the cyclohexane according to the total number of moles (Fig. 4).

The obtained results show a deviation of the curves towards the right-hand side as the injection flow is increased. This deviation can be explained as follows: when increasing the injection flow, the mixture does not appear instantaneously because the stirring is not sufficient. The direct observation of the thermogenesis according to the time (Fig. 3) does not allow to notice these effect because, when increasing the injection flow, the amplitude of the signal also increases and it does not permit to see what really happens in low concentrations.

#### 4. Conclusions

The variation of a baseline in an ITC is explained because, when injecting, the liquid volume in the mixture cell increases and the thermal coupling with the thermostat, through the stirrer, changes. The slight temperature differences which exist between the different points of the thermostat produce the change in the baseline that would not take place if the whole thermostat was at the same temperature.

The correction of the baseline must be realized after a reverse filtering process of the calorimetric signal; in the studied cases, this correction implies up to 2% of the total energy developed in the mixture process.

To obtain a homogeneous mixture at every instant, it is necessary an adequate stirring process for each injection velocity. Reducing the injection velocity has three advantages: reducing the so-called injection effect ( $\rho c_p f \Delta T$ ), obtaining a homogeneous mixture at every instant, and the possibility of getting energy values of mixture at very low concentrations.

With this and other similar works, it is shown that the modelling process using the method called "localized-constants" is a real help to understand the performance of these thermal

measuring devices and, consequently, to obtain the highest performance from them.

## References

- [1] F. Socorro, M. Rodríguez de Rivera, J.P. Dubès, H. Tachoire, V. Torra, *Meas. Sci. Technol.* 1 (1990) 1285–1290.
- [2] M. Rodríguez de Rivera, H. Tachoire, V. Torra, *J. Therm. Anal.* 41 (1994) 1385–1392.
- [3] F. Socorro, M. Rodríguez de Rivera, *J. Therm. Anal.* 52 (1998) 729–737.
- [4] M. Rodríguez de Rivera, F. Socorro, J.P. Dubès, H. Tachoire, V. Torra, *Thermochim. Acta* 150 (1989) 11–25.
- [5] A. Amengual, F. Marco, M. Rodríguez de Rivera, V. Torra, *Thermochim. Acta* 188 (1991) 63–76.
- [6] F. Socorro, M. Rodríguez de Rivera, Ch. Jesús, *J. Therm. Anal.* 64 (2001) 357–366.
- [7] F. Socorro, I. de la Nuez, M. Rodríguez de Rivera, *Measurement* 33 (2003) 241–250.
- [8] The MathWorks Inc., *Signal Processing Toolbox*, version 3.0, 1993.
- [9] R.H. Stokes, K.N. Marsh, R.P. Tomlins, *J. Chem. Thermodyn.* 1 (1969) 211.
- [10] H. Tachoire, V. Torra, *Thermochim. Acta* 110 (1987) 171.