

## SIGNAL PROCESSING AND UNCERTAINTY IN AN ISOTHERMAL TITRATION CALORIMETER

M. Rodríguez de Rivera\* and F. Socorro

Departamento Física, Universidad de Las Palmas, 35017 Las Palmas de Gran Canaria, Spain

In this paper, it is made a study of the accuracy of an isothermal titration calorimeter in the operating mode of ‘continuous injection’. The experimental equipment has been a TAM2277-201/2250 by Thermometric AB and the liquid mixtures used in the calibration have been the mixture cyclohexane+benzene and the mixture water+ethanol. The calibration contemplates different effects that affect the uncertainty in the determination of the sensitivity, the effect of the liquid injection, the treatment of the calorimetric signal, the variation of the experimental baseline and the different noises included in the calorimetric signal.

**Keywords:** calibration, isothermal titration calorimetry, liquid mixtures, uncertainty

### Introduction

Isothermal titration calorimeters (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.). In the case of liquid media, the experimental method consists in the injection of a liquid on another which is in the mixture cell; there are two use ways. In the first one, a known amount of substance is injected on another amount of substance also known. The mixture dissipation produces a calorimetric output that returns to experimental zero, when this happens, the mixture energy is determined by integrating the experimental output and dividing by the calorimeter sensitivity (in  $V/W$ ). This process can be continued until the cell capacity is completed. This use way is classical and it is very widespread [1].

The second ITC use way consists in the ‘continuous injection’ (at very low velocity) of a liquid on another and, from a calorimetric signal processing, to determine the power that is developed in the mixture process in terms of the concentration; this piece of information is of great interest for the elaboration of liquid media behaviour models, for example, for the study of micellization processes [2, 3], etc. This ITC operating mode began to be used long time ago [4, 5] but, at present, it has little use due to the difficulty that the treatment of the calorimetric signal has; however, it provides a thermodynamical information about the thermal process under study superior to the one obtained by other techniques.

This paper pretends to reach two objectives: the first one is to establish a simple calibration methodol-

ogy of the ITC in the ‘continuous injection’ mode that helps to the correct use of this calorimetric technique, and the second one is to determine the uncertainty of the calibration carried out and, as a consequence, of the resulting thermal measures. It is indicated that the sensitivity obtained in this use way is the same as in the classical way.

Besides, the determination of the uncertainty of the calorimetric measures is an increasing concern nowadays [6, 7], and this paper contributes to encourage the evaluation of all those aspects that participate in the energetic balance of the thermal process that is intended to be measured.

### Experimental

The instrument used in this study is an isothermal heat conduction calorimeter TAM2277-204/2250 by Thermometric. The mixture cell has a 2.5  $\mu\text{L}$  capacity. The calorimetric signal is directly read by a HP3457A digital multimeter (10 nV resolution). The Joule calibration is carried out through a resistance of 50  $\Omega$  placed in the base of a cell and the feeding is done through a HP6633A programmable source; the dissipated power is determined at every time by measuring the tension in the terminals of a standard 10  $\Omega$  resistance located in series with the Joule calibration resistance, this voltage is measured with a HP3478A multimeter (100 nV resolution). The injection system allows to inject, step by step, 0.0832  $\mu\text{L}$  per step of the motor, which, through a micrometric screw moves a billet that pushes the piston of a 50 mL Hamilton syringe; programming the number of steps of the motor at ev-

\* Author for correspondence: mrodriguez@dfis.ulpgc.es

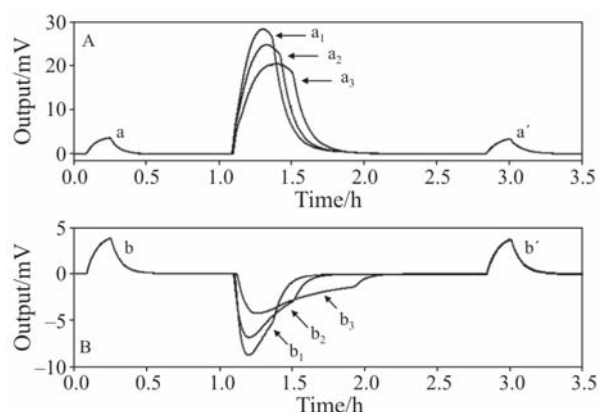
ery sampling period ( $\Delta t$ ) we obtain the injection flow; in this case, the injection flows used have been: 0.5, 1.0, 1.25 and 1.5  $\mu\text{L s}^{-1}$  ( $\Delta t=1.0$  s). A study of the injected volumes provides an uncertainty lower than 0.3%. All the described elements are connected to a PC through the GPIB bus.

In order to assure that the injected liquid acquires the thermostat temperature, it has been disposed a coil previous to the mixture cell, the coil capacity is 1.5  $\text{cm}^3$ . The stirring system (120 rpm) assures a homogeneous mixture at every time.

For the calibration of this instrument, it has been chosen the liquid systems water+ethanol and cyclohexane+benzene, these systems are recommended for the calibration of these calorimeters [8] and the mixture enthalpies are well known [9, 10].

The experimental method consisted in injecting 1.5 mL of the first liquid on initial 1 mL of the second. For the injection of water on ethanol, the utilized flows were 1.0, 1.25 and 1.5  $\mu\text{L s}^{-1}$ , and, for the injection of cyclohexane on benzene, the utilized flows were 0.5, 1.0 and 1.5  $\mu\text{L s}^{-1}$ . Although the injection velocities are low, special care has been taken to keep the injected liquid at the same temperature as that of the thermostat, this is the reason why it has been injected a volume equal to the capacity of the temperature homogenizing coil which is on the mixture cell and submerged in the thermostatic bath. All measures carried out in this work have been made with a thermostat temperature  $T_0=298.15$  K. During the measurement, the thermostat temperature is kept with a constancy of  $\pm 4$  mK.

The liquids used in the calibration have been: cyclohexane (Sigma-Aldrich, 99.9% HPLC grade),



**Fig. 1** Experimental curves corresponding to: A – the injection of 1.5 mL of water on initial 1 mL of ethanol, and B – the injection of 1.5 mL of cyclohexane on initial 1 mL of benzene, with the following injection flows:  $a_1, b_1 - 1.5 \mu\text{L s}^{-1}$ ,  $a_2, b_2 - 1.0 \mu\text{L s}^{-1}$  and  $a_3, b_3 - 0.5 \mu\text{L s}^{-1}$ . Before and after each injection, it is made an electrical calibration (curves a, a', b and b') in which it has been dissipated 10.45 mW for 600 s

benzene (Sigma-Aldrich, 99.9% HPLC grade), and ethanol (Merck, 99.8% grade).

Before and after each injection measure, it is carried out a calibration measure by Joule effect consisting in the dissipation of a constant power of  $10.4475 \pm 0.0015$  mW for 600 s. Figure 1 shows the experimental curves corresponding to the liquid mixtures measures and the electrical calibrations.

Stirring causes a noise in the baseline of the experimental output of  $\pm 1 \mu\text{V}$ , so the relationship signal/noise of the electrical calibration curves is 3820 (71.6 dB). In the curves corresponding to mixtures, the relationship signal/noise depends on the signal amplitude, for the case of injecting cyclohexane on benzene at 0.5  $\mu\text{L s}^{-1}$ , the relationship signal/noise is 4250 (72.6 dB). However, for the case of injecting water on ethanol at 1.5  $\mu\text{L s}^{-1}$ , the relationship signal/noise is 28340 (89 dB).

Apart from the noise produced by the stirring, there is another low frequency noise due to the temperature oscillations of the laboratory where the calorimeter is located; these oscillations are only visible when it is carried out the deconvolution of the calorimetric signal corresponding to the liquid injection.

## Transfer function

The experimental system can be modelled by means of a transfer function (TF) that relates the power dissipated in the cell to the experimental output. An identification of the experimental curves corresponding to the electrical calibrations provides a TF of three poles and a zero; however, the returns to zero of the curves corresponding to the mixtures only allow to identify three poles.

The previous paragraph suggests a model with three bodies as the one shown in Fig. 2. This modellization called 'at localized constants' is utilized in calorimetry and it is a way that allows to define the transference function of the experimental system and to incorporate the inherent and different characteristics of each instrument in the system operation equations [11, 12].

Each model equation is an energetic balance of each domain in which the dissipated power in each domain,  $W_i(t)$ , is equal to the power stored in itself,  $C_i dT_i/dt$ , plus the losses by conduction to the thermostat and to the neighbouring domains,  $P_{ij}(T_i - T_j)$ .

The first domain represents the mixture cell and its balance equation incorporates the term  $\rho c_p f(T_1 - T_0)$  that represents the 'injection effect', i.e., the power due to the temperature change of the injected liquid that varies from the thermostat temperature to the mixture cell temperature ( $\rho c_p$  is the volumetric heat capacity of the injected liquid and  $f$  is the injection flow).

The second domain represents the portacell where the calibration electrical resistance is located, and the third domain represents the detector, so the system output will be the temperatures difference  $T_3(t)-T_0$ . The input to the system will be the mixture power,  $W_{\text{mix}}$ , and the power dissipated in the calibration electrical resistance,  $W_{\text{elect}}$ .

The model equations are the following ones:

$$\begin{aligned} W_{\text{mix}} &= C_1 \frac{dT_1}{dt} + P_{12}(T_1 - T_2) + P_1(T_1 - T_0) + \rho c_p f(T_1 - T_0) \\ W_{\text{elect}} &= C_2 \frac{dT_2}{dt} + P_{12}(T_2 - T_1) + P_{23}(T_2 - T_3) + P_2(T_2 - T_0) \quad (1) \\ 0 &= C_3 \frac{dT_3}{dt} + P_{23}(T_3 - T_2) + P_3(T_3 - T_0) \end{aligned}$$

Applying Laplace transform to the previous equations and, defining the variables  $\theta_i(s)$  as Laplace transform of the temperatures difference  $T_i(t)-T_0$  and  $W_i(s)$  as Laplace transform of the power  $W_i(t)$ ,

$$\begin{aligned} W_{\text{mix}}^*(s) &= (sC_1 + P_{12} + P_1)\theta_1 - P_{12}\theta_2 \\ W_{\text{elect}}(s) &= -P_{12}\theta_2 + (sC_2 + P_{12} + P_{23} + P_2)\theta_2 - P_{23}\theta_3 \quad (2) \\ 0 &= -P_{23}\theta_2 + (sC_3 + P_{23} + P_3)\theta_3 \end{aligned}$$

being,

$$W_{\text{mix}}^* = W_{\text{elect}} - \rho c_p f \Delta T \quad (3)$$

Finding  $\theta_3(s)$ , it can be defined two transference functions that relate the output Laplace transform to the input powers:

$$\begin{aligned} H_{\text{mix}}(s) &= \frac{\theta_2(s)}{W_{\text{mix}}^*(s)} = \frac{K_{\text{mix}}}{(1+s\tau_1)(1+s\tau_2)(1+s\tau_3)} \quad (4) \\ H_{\text{elect}}(s) &= \frac{\theta_2(s)}{W_{\text{elect}}(s)} = \frac{K_{\text{elect}}(1+s\tau_1^*)}{(1+s\tau_1)(1+s\tau_2)(1+s\tau_3)} \end{aligned}$$

## Identification and deconvolution

Table 1 shows the values of the transfer function parameters  $H_{\text{elect}}(s)$  (Eq. (4)) obtained from the different electrical calibration curves. The main time constant  $\tau_1$  increases with the content heat capacity of the mix-

ture cell content:  $\tau_1$  varies from 237 s for a content in the cell of 1 mL of ethanol ( $\rho c_p \approx 1.93 \text{ J K}^{-1}$ ) to 307 s for a content in the cell of 1 mL of ethanol and 1.5 mL of water ( $\rho c_p \approx 8.20 \text{ J K}^{-1}$ ).

The sensitivity also undergoes changes depending on the liquid that is going to be injected: if the injection tube is full of cyclohexane, the sensitivity is superior due to the fact that the thermal conductivity of the cyclohexane ( $k=0.123 \text{ W K}^{-1} \text{ m}^{-1}$ ) is lesser than that of the water ( $k=0.607 \text{ W K}^{-1} \text{ m}^{-1}$ ). According to the proposed model, this means that the thermal coupling to the thermostat  $P_1$  is lesser in the first case. On the other hand, the sensitivity variation with the liquid volume decreases slightly due to the fact that the thermal coupling to the thermostat through the stirrer increases when the liquid level goes up.

The technique utilized in the identification and the deconvolution has been Inverse Filtering that consists in applying to the experimental output derivative filters and/or integrating to compensate the TF and to rebuild the input signal [13]. For the treatment of the experimental curves corresponding to the mixtures, four steps will be followed:

- filtering of the experimental curve
- correction of the baseline
- correction of the injection effect (Eq. (3))
- determination of the thermodynamical property

Three derivative filters will be applied on the curves corresponding to the mixtures. According to the proposed model (Eq. (4)), it will be used the same time constant obtained in the electrical calibrations carried out before and after each mixture. During the injection, the system can be considered linear and slowly variable and an adaptative filter is applied in which the first time constant will adapt linearly from the initial to the final value.

As dealing with an isothermal calorimeter, all the thermostat points should be at the same temperature and a jump in the experimental baseline should not take place, but the experimental reality is something different. When increasing the liquid volume in the cell, it also increases the thermal coupling of the stirrer to the thermostat and a jump in the baseline happens due to the fact that all the thermostatic bath points are not at the

**Table 1** Parameters of the Transfer Function (Eq. (4)) obtained from the electrical calibrations carried out before and after each mixture measure (Fig. 1)

Case of injection of water on ethanol	$K_{\text{elect}}/\text{mV W}^{-1}$	$\tau_1/\text{s}$	$\tau_2/\text{s}$	$\tau_3/\text{s}$	$\tau_1^*/\text{s}$
Before the injection (1 mL ethanol)	388.6±0.6	237	25	15	40
After the injection (2.5 mL ethanol+water)	386.7±0.3	307	25	15	40
Case of injection of cyclohexane on benzene	$K_{\text{elect}}/\text{mV W}^{-1}$	$\tau_1/\text{s}$	$\tau_2/\text{s}$	$\tau_3/\text{s}$	$\tau_1^*/\text{s}$
Before the injection (1 mL benzene)	400.1±0.8	239	25	15	40
After the injection (2.5 mL benzene+cyclohexane)	400.1±0.7	259	25	15	40

same temperature [14]. This is the reason why, after the filtering of the calorimetric output, a linear correction of the baseline will be carried out.

Next, the 'injection effect' (Eq. (3)) must be corrected. This effect has already been evaluated for this instrument in a previous work [15], its correction is realized through the expression:

$$y_{\text{corrected}} = y(1 + 0.0073\rho c_p f) \quad (5)$$

where  $y$  is the deconvoluted calorimetric signal,  $\rho c_p$  is the volumetric heat capacity of the injected liquid and  $f$  is the injection flow ( $\rho c_p f$  in  $\text{mW K}^{-1}$ ).

Dividing the signal resulting from the previous steps by the sensitivity, it is obtained an approximation of the power developed at every instant. From the power, it is determined the excess enthalpy per mixture mole,  $H^E$ , and the partial molar enthalpy of the injected component,  $H_1^E$ , with the following expressions [4],

$$H^E(x) = \frac{1}{n_1 + n_2} \int_0^t W_{\text{mix}}(t) dt \quad (\text{J mol}^{-1}) \quad (6)$$

$$H_1^E = \frac{E_{\text{mix}}(t)}{\dot{n}_1}$$

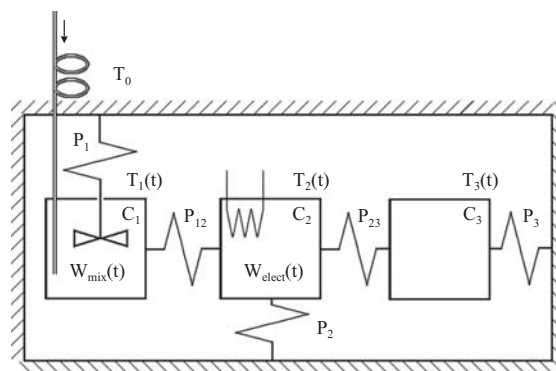
being  $\dot{n}_1$  the molar rate of injection, the molar fraction can be calculated for every instant:  $x = \dot{n}_1 t / (\dot{n}_1 t + n_2)$ , where  $t$  is the time and  $n_2$  are the initial moles of the component that is primarily in the mixture cell.

## Results and discussion

In this section, it is calculated the instrument sensitivity and, with the obtained value, it will be determined the excess enthalpies and the partial molar enthalpies of the injected liquid. In each case, it will be evaluated the uncertainty of the obtained thermodynamical result.

To obtain the sensitivity  $K_{\text{mix}}$  defined in the TF of the Eq. (4), it has been followed the four steps specified in the previous section, except for dividing by the sensitivity because it is not known. To obtain  $K_{\text{mix}}$ , the reference enthalpy is divided by the result of the deconvolution realized and it is looked for the value of  $K_{\text{mix}}$  that minimizes a quadratic error criterion. For the mixture water on ethanol, it is obtained a value of  $K_{\text{mix}} = 382.1 \text{ mV W}^{-1}$  (the standard deviation  $\sigma = 11 \text{ J mol}^{-1}$ ) and for the case of injecting cyclohexane on benzene, it is obtained  $K_{\text{mix}} = 399.9 \text{ mV W}^{-1}$  (the standard deviation  $\sigma = 8 \text{ J mol}^{-1}$ ). Once again, as in the case of electrical calibrations, it is observed that the sensitivity is lesser when injecting water due to the fact that the thermal coupling to the thermostat through water is greater than through cyclohexane.

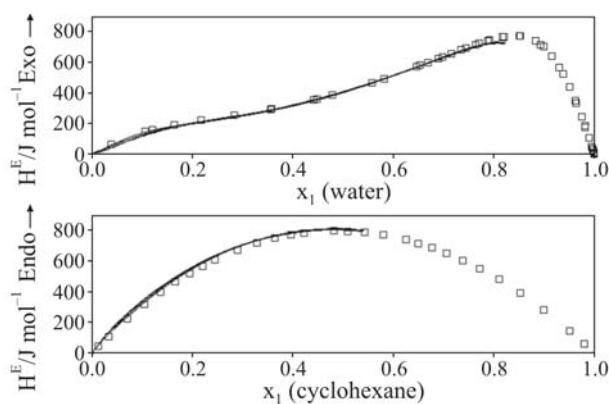
In the experimental practice, it is necessary to propose a unique value of  $K_{\text{mix}}$  to be used for every liquid mixture, taking as a sensitivity the center of the limits,



**Fig. 2** Scheme of the calorimetric model.  $C_1$  is the body heat capacity that represents the mixture cell,  $C_2$  the one of the portacell that includes the calibration electrical resistance at its base, and  $C_3$  the detector system capacity. The calorimetric output of the model is the temperatures difference  $T_3 - T_0$ .  $P_1$ ,  $P_2$ ,  $P_3$ ,  $P_{12}$  and  $P_{23}$  are the thermal coupling between domains and with the thermostat

and considering a rectangular distribution for the calculation of the uncertainty, we have:  $K_{\text{mix}} = 391 \pm 8.9 \text{ mV W}^{-1}$ . Besides, considering a coverage factor similar to 2, it can be concluded that the proposed sensitivity value has an uncertainty lesser than 5% [16]. With this sensitivity value, it is recalculated the excess enthalpy of the mixture and it is obtained a standard deviation on the reference values of  $\sigma = 17.6 \text{ J mol}^{-1}$  for the mixture water+ethanol (7502 experimental points) and of  $\sigma = 17.3 \text{ J mol}^{-1}$  for the mixture cyclohexane+benzene (10921 experimental points). Figure 3 represents the values obtained from the excess enthalpy for all those measures carried out and for each one of the two mixtures used in the calibration.

Figure 4 represents the results of the deconvolution of the experimental curves. The application of the derivative filters has amplified the noise contained within the signal and has shown the thermal oscillations of the laboratory; the width of these oscillations is greater in the



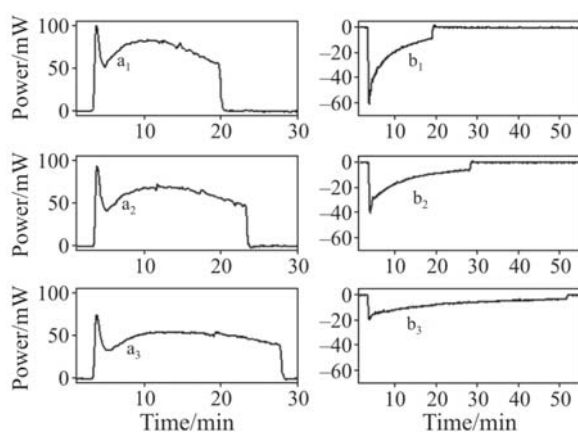
**Fig. 3** Excess enthalpy (Eq. (6)) for the two liquid systems utilized in the calibration. The solid lines are the experimental results of this work, and the square dots are the reference values [9, 10]

case of injecting water as this is proportional to the heat capacity of the injected liquid (Eq. (3)).

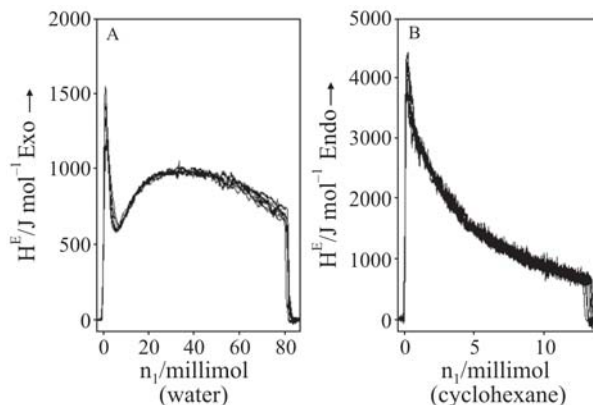
Figure 5 shows the other evaluated thermodynamical magnitude: the excess partial molar enthalpy of the injected component (Eq. (6)). To evaluate the uncertainty of the experimental result, it is built an average curve between the different measures and it is determined the standard deviation of the experimental curves over the average curve. The calculated standard scatterings are  $\sigma=40.7 \text{ J mol}^{-1}$  for the case of water on ethanol and  $\sigma=80.4 \text{ J mol}^{-1}$  for the case of cyclohexane on benzene. Over the average value of the calculated energy (890 and  $1500 \text{ J mol}^{-1}$ ), there is a 4.6% in the first case and a 5.3% in the second case. Within these results, it is included the different uncertainty sources inherent to these measures: the ones from the signal treatment, the ones from the high and low frequency noises, etc.

It is indicated that the injected liquids utilized in this work have different thermal properties. The case of injecting water is very unfavourable as it has high heat capacity and high thermal conductivity, this makes the injection effect be greater and the sensitivity diminish. However, cyclohexane has low heat capacity and low thermal conductivity, being then in a more favourable situation.

It is also shown the advantages of reducing the injection velocity. The first one is that the 'injection effect' is lesser and the second one is that there is a higher mixture energy resolution at low concentrations. However, when reducing the injection velocity, the signal width and the relationship signal/noise decrease and, when applying derivative filters to rebuild the input signal, the noise amplifies (Fig. 4).



**Fig. 4** Results of the experimental curves deconvolution corresponding to the injection of 1.5 mL of water on initial 1 mL of ethanol, with the following injection flows:  $a_1 - 1.5 \mu\text{L s}^{-1}$ ,  $a_2 - 1.25 \mu\text{L s}^{-1}$  and  $a_3 - 1.0 \mu\text{L s}^{-1}$ . Results of the experimental curves deconvolution corresponding to the injection of 1.5 mL of cyclohexane on initial 1 mL of benzene, with the following injection flows:  $b_1 - 1.5 \mu\text{L s}^{-1}$ ,  $b_2 - 1.0 \mu\text{L s}^{-1}$  and  $b_3 - 0.5 \mu\text{L s}^{-1}$



**Fig. 5** Partial excess molar enthalpy (Eq. (6)) of the injected component in terms of the injected component moles. All the measures utilized in the calibration are represented. A – Case of injecting water on initial 1 mL of ethanol,  $f=1.0, 1.25$  and  $1.5 \mu\text{L s}^{-1}$ . B – Case of injecting cyclohexane on initial 1 mL of benzene,  $f=0.5, 1.0$  and  $1.5 \mu\text{L s}^{-1}$

## Conclusions

The mixtures water+ethanol and cyclohexane+benzene utilized for the calibration are adequate as they have very different characteristics: the first one is exothermal and the second one is endothermal, the heat capacities and the thermal conductivities of the injected liquids are also very different; in these conditions, it has been determined the sensitivity with an uncertainty lesser than 5%.

The results of the excess enthalpies,  $H^E$ , of each mixture have been obtained with a standard deviation, with respect to the reference values, of  $\sigma \approx 17.5 \text{ J mol}^{-1}$ , over a maximum of  $H^E \approx 800 \text{ J mol}^{-1}$  (2.5%). And the results of the partial excess molar enthalpies of the injected component of each mixture have been obtained with a standard deviation,  $\sigma$ , with respect to an average curve, of  $\sigma=40.1 \text{ J mol}^{-1}$  for water+ethanol, and of  $\sigma=79.3 \text{ J mol}^{-1}$  for cyclohexane+benzene; this is equal in both cases to a  $\approx 5\%$  over the average value of the partial excess molar enthalpy of each mixture.

## References

- 1 A. Ababou and J. E. Ladbury, *J. Mol. Recognit.*, 19 (2006) 79.
- 2 H. Jelesarov and H. R. Bosshard, *J. Mol. Recognit.*, 12 (1999) 3.
- 3 S. Tai and K. C. Tam, *Colloids. Surf. A: Physicochem. Eng. Aspects*, 229 (2003) 157.
- 4 H. Tachoire and V. Torra, *Thermochim. Acta*, 110 (1987) 171.

- 5 F. Socorro, M. Rodríguez de Rivera, J. P. Dubès, H. Tachoire and V. Torra, *Meas. Sci. Technol.*, 1 (1990) 1285.
- 6 V. Torra and H. Tachoire. *J. Therm. Anal. Cal.*, 52 (1998) 665.
- 7 I. Wadsö and L. Wadsö. *J. Therm. Anal. Cal.*, 82 (2005) 553.
- 8 R. Sabbah, A. Xu-Wu, J. S. Chickos, M. L. P. Leitão, M. V. Roux and L. A. Torres, *Thermochim. Acta*, 331 (1999) 93.
- 9 M. J. Costigan, L. J. Hodges, K. N. Marsh, R. H. Stokes and C. W. Tuxford, *Aust. J. Chem.*, 33 (1980) 2103.
- 10 R. H. Stokes, K. N. Marsh and R. P. Tomlins, *J. Chem. Thermodyn.*, 1 (1969) 211.
- 11 A. Isalgue, J. Ortín, V. Torra and J. Viñals, *An. Fis.*, 76 (1980) 192.
- 12 R. Kirchner, M. Rodríguez de Rivera, J. Seidel and V. Torra, *J. Therm. Anal. Cal.*, 82 (2005) 179.
- 13 F. Marco, M. Rodríguez de Rivera, J. Ortín, T. Serra and V. Torra, *Thermochim. Acta*, 102 (1986) 173.
- 14 M. Rodríguez de Rivera and F. Socorro, *J. Therm. Anal. Cal.*, 80 (2005) 769.
- 15 M. Rodríguez de Rivera and F. Socorro, *J. Therm. Anal. Cal.*, 85 (2006) 477.
- 16 *Guide to the Expression of Uncertainty in Measurement*, International Organization for Standardization (ISO), Geneva, Switzerland 1995.

---

DOI: 10.1007/s10973-006-8104-2