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Thermochimica Acta 414 (2004) 95–100
thermochimica acta
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Short communication

Rebuttal to communication critical of the use of pressure perturbation calorimetry for measuring volumetric properties of solutes [Thermochim. Acta (2003) 75–80]

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Received 10 June 2003; received in revised form 1 October 2003; accepted 4 November 2003

Abstract

A recent communication by Randzio [Thermochim. Acta 398 (2003) 75] was highly critical of the design and operation of a new commercial instrument used to carry out pressure perturbation calorimetry (PPC), and questioned the accuracy of data obtained from the instrument. Each of the technical objections by Randzio on design and operation is rebutted in this communication and data are presented to support the performance and accuracy of the instrument. Direct comparison of coefficients of thermal expansion measured by PPC with corresponding values obtained by density measurements on well-defined systems shows excellent agreement between the two methods within the accuracy of the density data. In our opinion, there is no evidence to support Randzio's criticism of the instrument's design, operation, or accuracy.

Keywords: Pressure perturbation calorimetry; Coefficient of thermal expansion; Differential calorimeters; Cell mismatch; Pressure effects; Calorimetric control experiments

1. Introduction

A commercial calorimeter, capable of carrying out pressure perturbation calorimetry (PPC), was introduced several years ago [1]. By measuring the differential heat produced when small pressure changes (500 kPa) are applied above the sample and reference solutions in a differential calorimeter, it is possible to obtain precise estimates of the coefficient of thermal expansion α_P of the partial volume of solutes dissolved in solution at low concentration. The first publication of results from this instrument was by Kujawa and Winnik [2], who determined α_P for poly(*N*-isopropylacrylamide) and related polymers in H_2O and D_2O over a temperature range which included a thermal structural transition whose volume change ΔV_P was determined. In early 2002, a paper from the manufacturer was published [3] which provided more details on the design and operation of the instrument as well as results from numerous PPC studies on dilute protein solutions and individual amino acids in several solvents.

Randzio published a communication in this journal [4] which was critical of the PPC instrument, and of certain procedures recommended by the manufacturer and used by Kujawa and Winnik. He was also critical of the inadvertent omission of reference to earlier studies by himself and others where pressure changes were used as a variable in calorimetric studies. Randzio did not directly cite the published paper from the manufacturer [3] which provided more details on the PPC instrument, and which referenced his earlier calorimetric studies.

In the abstract of his paper, Randzio states "The design and operation of the commercial instrument used by Kujawa and Winnik is shown to be flawed". This conclusion was drawn as the result of several lines of criticism developed in the body of the paper which, in his opinion, lead to errors of appreciable but unknown magnitude. We feel that all the technical criticism offered by Randzio is unfounded, and disagree with his contention regarding the design and operation of the instrument. Below is included a point-by-point discussion of each of the negative technical points which he raised.

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doi:10.1016/j.tca.2003.11.013

2. Discussion

2.1. Validity of Eq. (4)

Randzio states "... Kujawa and Winnik do not define the limits of their thermal system. V in Eq. (4) in their paper is incorrectly defined as" the volume of the system". They do not distinguish between the molar (or specific) volume of the investigated sample and the volume in which it is confined".

This criticism is difficult to understand. Eq. (4) is: $Q_{rev} = -TV\alpha\,\Delta P$. This was derived as a general expression and is correct as described [2,3]. It applies to any liquid system (pure or multi-component) of total volume V. As the derivation continues in Eqs. (5)–(11), this general equation is applied directly to a two-component system and partial specific volumes are then introduced in order to focus on the properties of the solute and the active volume of the calorimeter cell.

2.2. Neglect of compressibility effects

In arriving at the final equations used to analyze PPC data, it was assumed [2,3] that compressibility effects on solutions can be neglected at the low pressures used (500 kPa). Randzio criticizes this approximation with the implication that pressure effects on molar volumes and thermal expansion coefficients are too large to be neglected, and that one must be concerned about changes in the mass of solution in the active volume of the calorimeter cells caused by compression of solutions. This criticism seems unfounded. The compressibilities of pure liquids and dissolved solutes are known to be small [5]. For example, water and aqueous solutions have isothermal compressibilities less than $\sim 50 \times 10^{-8}$ kPa^{-1} at one normal atmosphere. The maximum change in pressure of 500 kPa in PPC experiments thereby leads to a change in mass (at constant volume) of just ~2.5 parts in 10,000 which will have insignificant effect on experimentally derived parameters. Any differential effects from compression that might arise from cell asymmetry will also cancel from subtraction of the control heats (see below).

2.3. Mismatches between sample cell and reference cell

Randzio [4] points out that the active volumes of the sample cell and reference cell in the PPC instrument will not be exactly equal so that the effect of solvent will not completely cancel in the differential experiment involving sample solution in the sample cell versus buffer in the reference cell. This is certainly true, and is the reason why control experiments (buffer in both cells) are always run to provide companion data to the real experiment. Results from the control experiment under identical conditions are routinely subtracted point-by-point from the sample data, using an automated procedure [3] in the manufacturer's PPC software for data analysis. After subtraction of the control data, the results then correspond to the difference between sample solution and buffer solution when each is run *in the same sample cell*. Any small mismatch between the two cells is irrelevant and has no influence on the corrected data. This procedure of subtracting results from control experiments has been used in ultrasensitive DSC studies for several decades in order to correct for small differences in cell volumes.

Sharp peak seen in PPC raw data. When a pressure change is applied to sample and reference solution during a differential PPC experiment, it is commonly observed [2,3] that the resulting heat effect consists of two peaks; a fast sharp spike usually of small area, followed by a slow broader peak. Often, one of these peaks is exothermic while the other is endothermic. Randzio [4] argues that this bimodal configuration results from a mismatch (volume, response time, etc.) between sample and reference cell, which in turn causes the spike. This is probably the correct explanation [3], since any small differences between the two cells are accentuated because the absolute heat given off in each cell can be hundreds of time larger than the differential heat observed in the baseline trace. The behavior of the sharp peak is consistent with its being due to a small difference in the response time of the differential sensor to heat changes in the sample cell versus heat changes in the reference cell [3].

Randzio further concludes that the heat spike resulting from cell mismatch is artifactual and thereby introduces errors into the final data. His conclusion regarding errors in the final data resulting from cell mismatch seems incorrect, however, since (as discussed above) routine subtraction of data from control experiments permits comparison of sample solution and buffer solution *in the same cell*. It will also be shown below (cf. Fig. 1) that identical data are obtained in an experiment where the sample solution and buffer solution are switched in location between the two cells as are obtained with the two solutions in their normal location. These data provide empirical confirmation that there is no contribution from cell mismatch once control data have been subtracted.

2.4. Adiabatic or isothermal?

Randzio states "The compression and decompression in the experiments by Kujawa and Winnik was done rapidly compared to the time constant of the calorimeter which makes the process adiabatic and not isothermal as required by the Maxwell relation. Thus at least part of the recorded heat is described incorrectly by the equations used to analyze the data."

While part of this statement is accurate, the conclusion is not. Pressure changes are exerted quickly (1 s) relative to the time constant of the instrument (~10 s). Subsequent to the pressure change, however, data is collected for a long period of time (~2 min) during which the peaks are recorded and the baseline returns to the original equilibrium position which existed prior to the pressure change. The entire process from pre-pulse to post-equilibrium is isothermal and not adiabatic, and this is the process for which the heat is

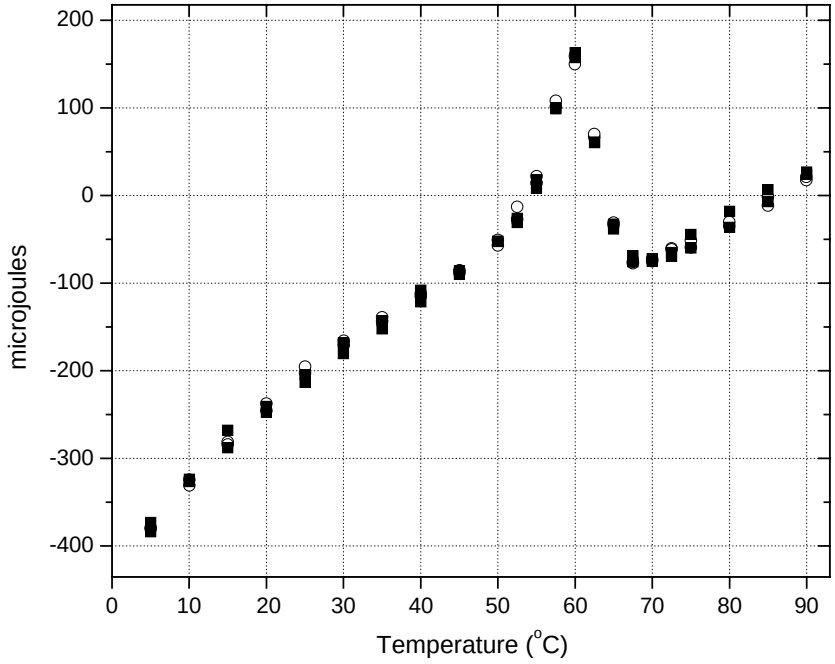

Fig. 1. Heats obtained from two compression and decompression experiments ($\Delta P = \pm 70.0$ psi) on a sample solution of 10.6 mg/ml ribonuclease A (50 mM KAc, pH 5.5) from 5 to 90 °C. Open circles refer to experiment where sample solution is in the usual sample cell, while filled squares correspond to experiment where sample solution is in the usual reference cell. Heats from a control experiment were subtracted from each data set prior to plotting.

obtained [2,3] by integration. Thermodynamic parameters, such as heat change for a reversible process, depend only on the initial and the final state and are independent of the path taken between these two states, so that even if there is an early adiabatic step it is of no consequence.

2.5. Measuring volume changes ΔV_P for thermal transitions

Randzio states "In first order transitions $(\partial V/\partial T)_P$ and α_P are discontinuous, and it is erroneous to write that one can derive the coefficient of thermal expansion at the transition from pressure-scanning calorimetric data."

First order transitions, as defined by Hill [6] for small systems in solution, are cooperative two-state transitions where only the initial state and final state are involved in equilibrium in the transition region. Thermal transitions of some proteins are highly cooperative and fall into this category [7,8]. Regardless of the degree of cooperativity, it is impossible [6] for a small system under thermodynamic control to display a discontinuous function of any kind. It is well known that even two-state transitions of proteins, for example, have finite values for derivative functions such as thermal expansion and heat capacity. Coefficients of thermal expansion can then be measured in the transition region and integrated to determine volume changes ΔV_P for the transition, just as heat capacity has for many years been measured throughout transitions and integrated to determine enthalpy changes ΔH for thermal transitions of small systems.

2.6. Reversal of sample solution and reference solution

Most of the criticism by Randzio focused on errors which might be introduced into the final results as a consequence of unavoidable differences between sample cell and reference cell. These problems should be eliminated by subtraction of control runs, as noted above, and this is easy to demonstrate empirically. Using a sample solution containing 10.6 mg/ml of ribonuclease A (50 mM potassium acetate buffer, pH 5.5), two otherwise-identical experiments ($\Delta P = \pm 70.0$ psi) were carried out in which the location of the sample solution and buffer solution were transposed between the "sample cell" and "reference cell". Results from these two experiments are compared in Fig. 1 where the open circles correspond to heats obtained with the sample solution in the usual sample cell and filled squares refer to results with the sample solution in the usual reference cell. Prior to plotting, both data sets were first corrected by subtracting heats obtained from the control run with buffer in each cell.

Compression and decompression experiments were carried out at 23 different temperatures from 5 to 90 °C. For both data sets, points in Fig. 1 are plotted corresponding to the compression direction ($\Delta P = +70$ psi) but the decompression heats ($\Delta P = -70$ psi) are also included with a sign change. Under these buffer conditions, ribonuclease undergoes a thermal unfolding transition exhibiting a very small decrease in molar partial volume ($\Delta V = -0.29\%$ of V [3]) and this is reflected in the peak centered at 60 °C. Including the compression and decompression data points at each of the different temperatures, the average deviation between

the corresponding points in the two data sets is 4.1 μJ. This is less than 1% of the total change in heats (∼550 μJ) over the temperature range examined.

It should be noted that the control heats (not shown) varied systematically from +75 μJ at 5 °C to −10 μJ at 90 °C. Without point-by-point subtraction of control heats, deviations between the two data sets would be more than ten times larger than found using the standard procedure involving subtraction. The conclusion to be drawn from these experiments is that PPC heats resulting from cell asymmetry are significant in size, but are satisfactorily eliminated by routine subtraction of control heats as recommended by the manufacturer.

2.7. Accuracy of PPC data

Randzio suggests that due to flaws in the commercial instrument and the recommended methodology, data obtained from the instrument will have appreciable but unknown error. The ultimate test of the accuracy of an instrument is the extent to which its data agree or disagree with prior results that have been accepted as accurate and non-controversial. It was reported earlier that the PPC method was used [3] to measure the coefficient of thermal expansion α of pure H_2O over the temperature range 0–70 °C, by pressurizing just the sample cell (H_2O) in the differential calorimeter and using the reference cell (H_2O) only as a thermal reference. These PPC-derived α values pass through zero at the appropriate temperature (4.0 °C) and show an average deviation of $0.025 \times 10^{-4}/^{\circ}$ from density-derived α values of Kell which themselves have estimated uncertainty of $\sim 0.02 \times 10^{-4}/^{\circ}$ [9]. The deviations between PPC-derived and density-derived α values correspond to a difference of less than 0.5% of the total α variation measured over the PPC temperature range 0–70 °C.

Shown in Fig. 2 are values of α for D_2O which were obtained in a differential PPC experiment with D_2O in the sample cell and H_2O in the reference cell. In cases such as this where the heat capacity of sample solution and reference solution are significantly different, the two solvents were independently calibrated in the calorimeter cell to attain highest accuracy. PPC results were obtained at temperature intervals of 5° over the range from 5 °C to nearly 100 °C. In Fig. 2, the circles indicate α values obtained following compression ($\Delta P = +300$ kPa) while the squares indicate those obtained following decompression ($\Delta P = -300$ kPa). The solid line represents density-derived α values for D_2O from the parametric equation of Kell [9] with estimated uncertainty of $\sim 0.04 \times 10^{-4}/^{\circ}$. The α values obtained by PPC agree nicely with those from the density measurements, both passing through zero at 11.2 °C and increasing to $7.5 \times 10^{-4}/^{\circ}$ at the highest temperature. The average deviation of all 40 experimental PPC points from the Kell line is $0.034 \times 10^{-4}/^{\circ}$, which again is less than 0.5% of the total measured α variation over the entire temperature range.

Since net PPC heats are quite large in each of the above two cases using pure solvents, these are not the best tests of the limiting sensitivity and accuracy of the PPC method. More critical assessment would come from comparison of PPC-derived and density-derived α_P values for solutes in

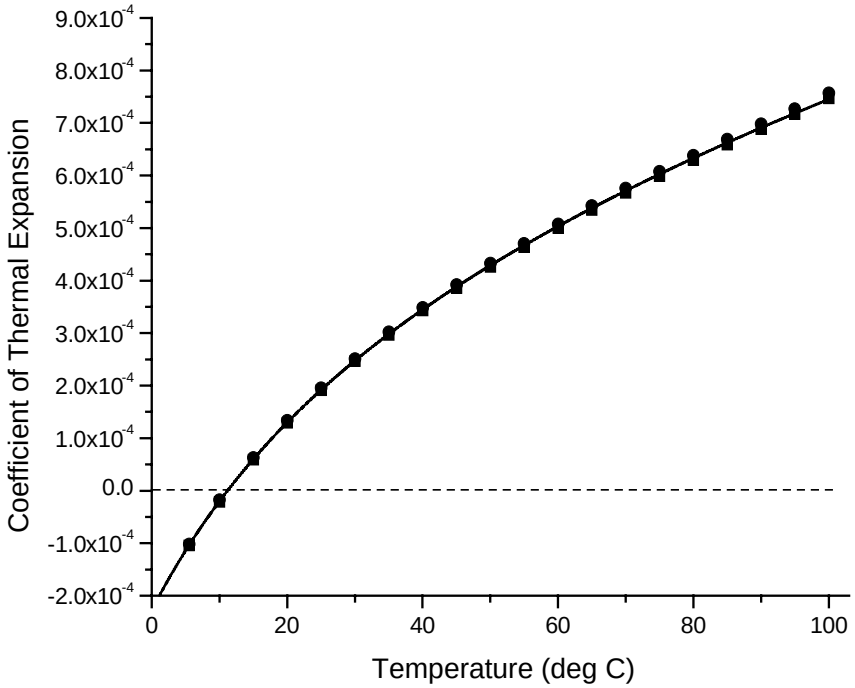

Fig. 2. Coefficient of thermal expansion of D_2O. Circles represent compression data and squares represent decompression data, obtained from pressure perturbation of 300 kPa. The solid line is from Kell's [9] equation based on density data.

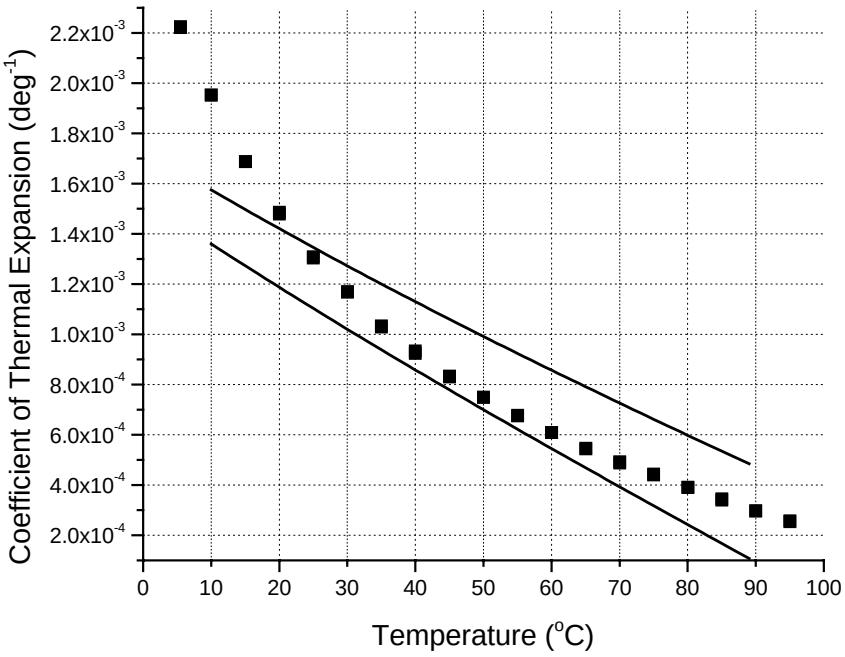

Fig. 3. Coefficient of thermal expansion of the partial volume of the tripeptide gly–gly–gly. Points are results from PPC compression experiment using pressure perturbation of 225 kPa. The solid lines are calculated values from density data reported in ref. [11] for the upper line and reported in ref. [12] for the lower line.

dilute solution where net heats are much smaller. The difficulty in making such high-resolution comparisons, however, is that precision achieved in differential density measurements is substantially lower than in PPC measurements at equivalent solute concentrations. Probably the most extensive density data obtained at low solute concentration comes from Hinz and co-workers [10–12]. They constructed a scanning differential density instrument using two matched Anton Paar DMA 602 HT cells in combination with a DMA 60 measuring unit. The instrument automatically makes differential density readings at 0.1–0.2° intervals over the range 10–90 °C. They used this device to study a number of tripeptides dissolved in water at concentrations near 1%. The relatively high precision of measurement allowed them to express partial molar volumes in a three-term power series in temperature t (°C)

$$V_{\mathrm{P}} = A + Bt + Ct^2 \tag{1}$$

From which α_{P} can be readily derived by differentiation

$$\alpha_{\mathrm{P}} = \frac{B + 2Ct}{A + Bt + Ct^2} \tag{2}$$

PPC results have previously been obtained [3] on two of the tripeptides examined by Hinz under similar solution conditions (solid tripeptides dissolved in distilled water at ~1% concentration, no added electrolytes). The PPC results for gly–gly–gly are shown as filled symbols in Fig. 3. Note that α_{P} shows a strong temperature dependence, decreasing 10-fold from 2.2×10^{-3} at 5 °C to 0.2×10^{-3} near 100 °C. Data are sufficiently precise to determine values of α_{P}, $(\partial\alpha_{\mathrm{P}}/\partial T)_{\mathrm{P}}$, and even $(\partial^2\alpha_{\mathrm{P}}/\partial T^2)_{\mathrm{P}}$. The large negative values for $(\partial\alpha_{\mathrm{P}}/\partial T)_{\mathrm{P}}$ and the large positive value for $(\partial^2\alpha_{\mathrm{P}}/\partial T^2)_{\mathrm{P}}$ were found to be typical of solutes which disrupt water structure [3]. Groups which increase water structure (e.g., aliphatic sidechains of proteins) show contrasting behavior with negative values of α_{P} at low temperature, large positive value of the first temperature derivative of α_{P} and large negative value of the second temperature derivative.

The Hinz group reported two slightly different sets of coefficients for Eq. (1) for the tripeptide gly–gly–gly, offering no explanation for the difference. The upper solid line in Fig. 3 is from their 1995 paper [11] while the lower solid line is from their 1999 paper [12]. Since instrumental precision limited them to the measurement of just three coefficients in Eq. (1), the lines representing α_{P} versus temperature in Fig. 3 can have no significant curvature. No error analysis of their parametric equations was provided. In the raw data shown in their Fig. 1 [12], it can be seen that discrepancies between identical scans and fluctuations within single scans are substantially larger at the lowest temperatures 10–35 °C in their measurement range and somewhat larger at the highest temperatures 80–90 °C. Overall agreement between their results and the PPC-derived results seems very satisfactory considering limitations in the density data, and particularly good in the center of their measuring range 40–60 °C where their parametric representation will be the most accurate.

We conclude that the agreement between these PPC-derived and density-derived α_{P} values is probably within errors of the density measurements over the 10–90 °C temperature range. Had it been possible to extend accurate density measurements down to 0 °C then the curvature seen prominently in the PPC data in Fig. 3 might have become

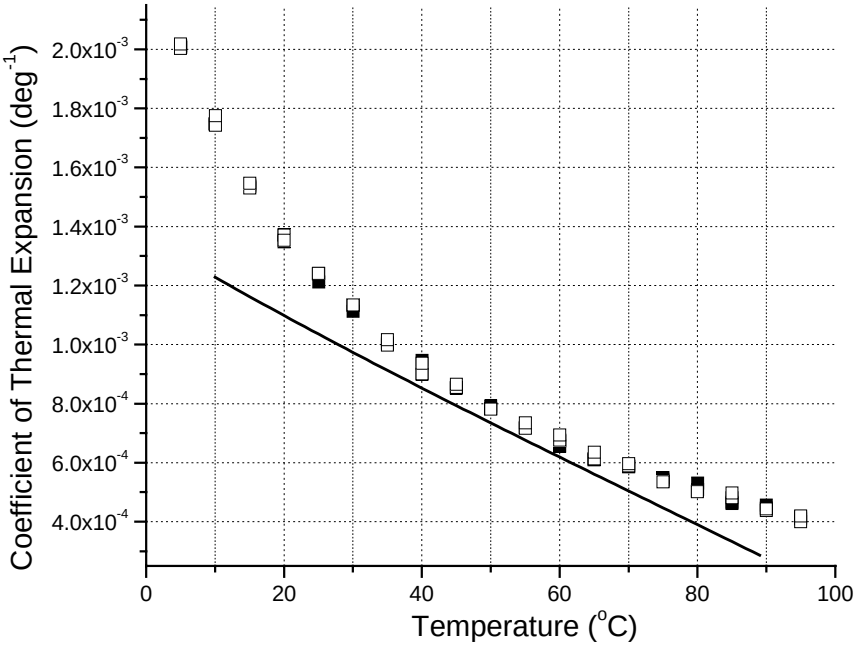

Fig. 4. Coefficient of thermal expansion of the partial volume of the tripeptide gly–his–gly. The experimental points (four overlapping points at each temperature) are PPC results from both compression (solid squares) and decompression cycles (open squares) for each of two separate experiments using pressure perturbations of 500 kPa. The solid line is calculated from density data reported in ref. [12].

evident in the density measurements. The apparent precision of the PPC values substantially exceeds that level of agreement between the two methods as seen from the smoothness of the PPC curve in Fig. 3. This is noteworthy when one considers that in this experiment, at 50 °C for example, absolute heats of ∼18,000 μJ are produced in both sample cell and reference cell while the net differential heat measured is only ∼120 μJ.

Similar representations of PPC data and density data for the tripeptide gly–his–gly are shown in Fig. 4. In contrast to gly–gly–gly, there was only one set of density results published for gly–his–gly [12]. Again, the agreement of PPC-derived values with density-derived values seems quite satisfactory, particularly over the mid-range temperatures from 40 to 60 °C where parametric representation of the density data will be most accurate.

3. Conclusions

The various technical points raised by Randzio [4] regarding the design and operation of the PPC instrument have been individually discussed above, and certain experimental data examined in detail. We find no evidence to support his contention that there are meaningful “flaws” in either the instrument itself or in the method of data analysis. Comparison of PPC data with existing density data shows excellent agreement within the limitations of the density data. It would seem that PPC is an accurate and convenient method for measuring the volumetric properties of solutes in dilute solution. While Randzio and others have previously utilized high pressure as a variable in calorimetric studies ([4,13,14] and references therein), the present PPC instrument is novel in being able to provide highly precise values of α_P for solutes in dilute solution. It can be particularly valuable for studying proteins and other biopolymers in aqueous solution, since it was shown that α_P provides information on important hydration properties [3]. Moreover, the high PPC sensitivity was shown to enable precise measurement of partial volume changes as small as 0.1% of the total volume [3] for thermal transitions of proteins, and such small changes cannot be accurately determined from density measurements [10].

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