THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

pubs.acs.org/JPCB

Calorimetric Determination of Rate Constants and Enthalpy Changes for Zero-Order Reactions

Luis Almeida e Sousa,[†] Anthony E. Beezer,[†] Lee D. Hansen,[‡] David Clapham,[§] Joseph A. Connor,[¶] and Simon Gaisford*,[†]

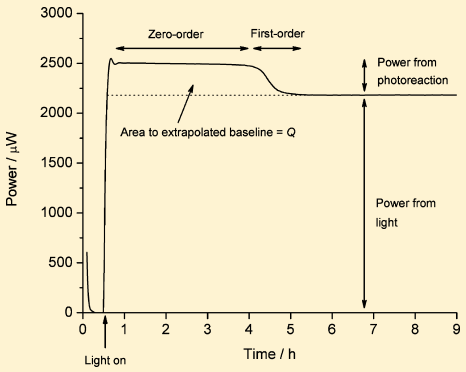
[†]UCL School of Pharmacy, University College London, 29-39 Brunswick Square, London, WC1N 1AX, U.K.

[‡]Department of Chemistry and Biochemistry, Brigham Young University, Provo, Utah 84602, United States

[§]GlaxoSmithKline, Gunnels Wood Road, Stevenage, Herts, SG1 2NY, U.K.

[¶]Medway Sciences, University of Greenwich, Medway Campus, Chatham Maritime, Kent, ME4 4TB, U.K.

ABSTRACT: Calorimetry is a general method for determination of the rates of zero-order processes, but analysis of the data for the rate constant and reaction enthalpy is difficult because these occur as a product in the rate equation so evaluation of one requires knowledge of the other. Three methods for evaluation of both parameters, without prior knowledge, are illustrated with examples and compared with literature data. Method 1 requires the reaction to be studied in two buffers with different enthalpies of ionization. Method 2 is based on calculation of reaction enthalpy from group additivity functions. Method 3 applies when reaction progresses to completion. The methods are applied to the enzymatic hydrolysis of urea, the hydrolysis of acetylsalicylic acid, and the photodegradation of nifedipine, respectively.



INTRODUCTION

The inherent beauty of heat as an analytical marker for the process and progress of reactions is its ubiquity, meaning that kinetics of reaction in almost any sample are amenable to calorimetric investigation. Isothermal calorimetric data (i.e., power versus time) can have complex forms representing the sum of many simultaneous or consecutive processes, and a series of methodologies for quantitative analysis of the data, including first-, second-, and *n*th-order solution phase kinetics,[1] consecutive kinetics,[2] direct calculation,[3] chemometric analysis,[4] solid-state kinetics,[5] and fitting to integrated rate laws,[6] have been described in the literature. However, quantitative analysis of isothermal calorimetric data for zero-order reactions has not been previously discussed.

Conceptually, zero-order reactions are the simplest to describe in quantitative terms but are difficult to interpret without ancillary information because all of the variables and parameters in the calorimetric rate expression occur as a single product (eq 1)

$$\frac{dq}{dt} = \Phi = -k\Delta HV \text{ or } -k\Delta Hm \quad (1)$$

Although the heat rate, dq/dt, or thermal power, Φ, is measured and the volume, V, or mass, m, of the sample is usually known, the rate constant, k, and reaction enthalpy, ΔH, occur as a product and so the value of either k or ΔH is required to evaluate the other.

Several examples of zero-order reactions that were studied calorimetrically can be found in the literature but no extensive quantitative analysis was made because of this analytical constraint. The reactions include autocatalytic oxidation of drugs,[7] polymer curing,[8] oxidation of lipids,[9] and enzymatic reactions.[10]

Three methods for quantitative evaluation of both k and ΔH are discussed here. The first method applies to reactions that involve proton exchange with a buffer. The second method uses tabulated data of enthalpies of formation to estimate enthalpies of reaction. The third method applies to reactions that progress to completion. Each method is illustrated with a practical example. Although each method is reasonably simple in construction and principle, we could find no discussion of their use or application to calorimetric data in the literature.

EXPERIMENTAL SECTION

Materials. Nifedipine (>98%) was purchased from TCI Europe. Ethanol (99.7−100%) was purchased from Hayman Ltd. (UK). Urease from Jack beans (0.98 U mg^{-1}), imidazole (ACS reagent), acetylsalicylic acid (>99%), and disodium hydrogen orthophosphate (ACS reagent) were purchased from Sigma-Aldrich Ltd. (UK). Potassium dihydrogen orthophosphate and hydrochloric acid solution (5 M) were purchased from Fisher Scientific Ltd. (UK). Urea was purchased from Fluka Ltd. (UK). Potassium chloride was purchased from VWR International Ltd. (UK). All reagents were used as received.

Received: March 27, 2012
Revised: May 15, 2012
Published: May 20, 2012

Solutions of nifedipine (1% w/v) were prepared in ethanol (25 mL). All solutions were prepared in a dark room under red light to minimize photodegradation prior to use.

Buffers for urea hydrolysis were prepared to a concentration of 0.75 M and pH 6.6. Phosphate buffer solutions were prepared by adding Na_2HPO_4 (2.662 g) and KH_2PO_4 (2.552 g) to water (50 mL). Imidazole buffer solutions were prepared by adding imidazole (2.553 g), KCl (2.252 g), and HCl solution (5.7 mL of 5 M solution) to water (50 mL). Solutions of urea (0.4 M) and urease in urea (0.5 U mL^{-1} in 0.4 M) were prepared in the buffers.

Saturated solutions of acetylsalicylic acid were prepared in 1 M phosphate buffer at pH 6.8. The buffer was prepared by dissolving Na_2HPO_4 (4.352 g) and KH_2PO_4 (2.632 g) in water (50 mL). An excess of acetylsalicylic acid (500 mg) was added to buffer (5 mL) and the mixture was stirred for 15 min. The mixture was filtered (0.45 μm, Millipore Ireland Ltd.) before an excess of acetylsalicylic acid (10 mg) was added to the solution.

Methods. *Photocalorimetry.* Experiments were performed with a modified MC-DSC (TA Instruments LLC, USA) operated at 25 °C. The instrument is of a heat-flux design with four ampules (three samples and a common reference). The ampules (1 mL volume and machined from Hastelloy) are of a two-piece design, comprising a lid that screws onto a base, a Viton O-ring ensuring a gastight seal. The ampules sit under two thermal shunts, designed to prevent exchange of heat with the surroundings. Modifications were made to both the ampules and the shunts. The lids of the ampules were machined to accept quartz discs (held in place with epoxy resin). In the lower shunt an LED (white light, RadioShack, USA) was mounted centrally and connected to a power supply operating at 5 V. Calorimetric data (1 point every 30 s) could be recorded for the sample while the LED was irradiating it. It is important to note that in this configuration there is no direct thermal contact between the ampule and the LED. Data were recorded with the dedicated software package MC-DSCrun 2.5 and analyzed with Nanoanalyze 1.2 (both TA Instruments LLC, USA). Data were plotted with Origin 8.5 (OriginLab Corp., USA). All experiments were performed in triplicate and all data are presented as a mean ± standard error. The calorimeter was calibrated prior to use by the electrical substitution method. There is no standard light source for photostability testing, so we note that the rate constant values used will vary with different light powers or wavelengths.

Isothermal Calorimetry. All experiments were performed in a 2277 thermal activity monitor (TAM, TA Instruments LLC, USA) operated at 25 °C (urea hydrolysis) or 37 °C (acetylsalicylic acid hydrolysis). Solutions (3 mL) were contained in glass ampules. A gastight seal was ensured with a crimped metal cap fitted with a rubber disk. Solutions were allowed to reach thermal equilibrium for 20 min prior to being lowered to the measurement position. Data (1 point every 30 s) were captured with the dedicated software package Digitam 4.1 (TA Instruments LLC, USA). Data were analyzed and plotted with Origin 8.5. All experiments were performed in triplicate and all data are presented as a mean ± standard error. The instrument was calibrated prior to use by the electrical substitution method.

Theory. *Analysis Method 1: Zero-Order Reactions Progressing in Different Buffers.* The concept applies to reactions that produce an acidic or basic species and is an approach often used in isothermal titration calorimetry (ITC) to determine the stoichiometry of reactions (e.g., refs 11 and 12). Every molecule of product produced by reaction results in ionization or neutralization of the buffer and the measured enthalpy is thus the sum of two components:

$$\Delta H_{\text{measured}} = \Delta_r H + n\Delta_i H \tag{2}$$

$\Delta_r H$ is the molar reaction enthalpy of the reaction under study, $\Delta_i H$ is the molar enthalpy of ionization of the buffer in which the reaction is progressing, and n is the number of moles of protons released or absorbed for each mole of product produced. If the experiment is performed in two buffers, at the same pH but with different molar enthalpies of ionization, then

$$\Delta H_{\text{measured1}} - \Delta H_{\text{measured2}} = n(\Delta_i H_1 - \Delta_i H_2) \tag{3}$$

where the subscripts 1 and 2 refer to the two buffers. Substitution into eq 1 and rearranging gives

$$k = \frac{-\left[\frac{\Phi_1}{V_1} - \frac{\Phi_2}{V_2}\right]}{n(\Delta_i H_1 - \Delta_i H_2)} \tag{4}$$

Note that there is no requirement for data collection to begin at time zero. If reaction starts during the time taken for sample preparation and loading, and so there is a portion of data not recorded by the calorimeter, there is no effect on the analysis.

Analysis Method 2: Calculation of the Enthalpy from Group Additivity Functions. Here, the enthalpy of reaction is estimated from group additivity functions taken from tabulated literature data. Knowledge of the reactant and product structures is required. Although many approaches and tables of data are available in the literature, the method shown here is based on the work of Salmon and Dalmazzone,[13,14] which allows prediction of enthalpies of formation for molecules in the solid state (at 298.15 K) containing atoms of carbon, oxygen, hydrogen, and nitrogen (the values are applied here to reaction in solution, the assumption being that there is no significant difference between formation in the solid state and formation in solution).

Analysis Method 3: Reactions That Progress to Completion. In this case, the total calorimetric curve of heat rate versus time and the duration of the process are known and so determination of the reaction enthalpy and rate constant is straightforward with eq 1. Equation 1 predicts that calorimetric data for a zero-order process will be a horizontal line, displaced from zero (unless $\Delta_r H$ is zero). Once all the reactants are exhausted, the power signal should decrease to zero. However, such behavior is rarely observed, usually because the system is following pseudo-zero-order kinetics. Pseudo-zero-order kinetics can result from at least one reactant being present in excess and so it is not depleted during the zero-order period or during the initiation phase of autocatalytic reactions. After the pseudo-zero-order period, the kinetics will change to another rate law and the power signal will fall to zero via an exponential or more complex decay. Integrating the total area under the curve (the total power change from reaction, Q) allows calculation of the reaction enthalpy, because the number of moles of limiting reactant initially present (A_0) is known. The fact that the reaction kinetics switch from zero order is irrelevant so long as the products formed are the same.

Alternatively, it is possible to calculate the rate constant from the time point at which the reaction changes from zero-order (t_1). This is easily achieved graphically by determining the point of deviation from a constant power signal. Integrating the area

dx.doi.org/10.1021/jp302933f | J. Phys. Chem. B 2012, 116, 6356−6360

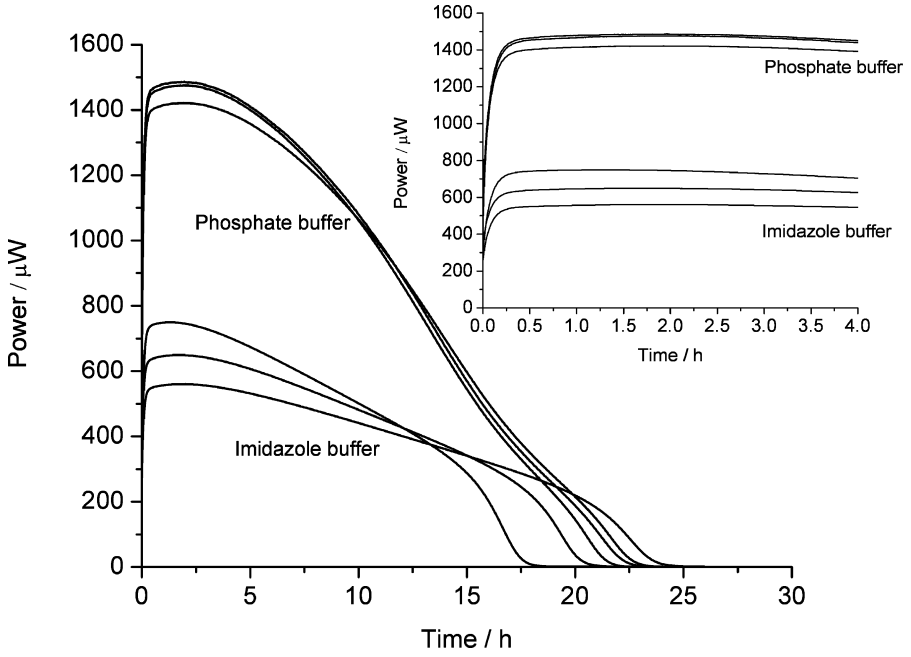


Figure 1. Calorimetric data for the enzymatic hydrolysis of urea in phosphate and imidazole buffers and (inset) an expanded view of the zero-order region.

under the curve to this time gives the zero-order heat change (q_{t_1}). It is then a simple matter to calculate the fraction of material that must have reacted under zero-order conditions:

$$\text{fraction reacted under zero-order conditions} = \frac{q_{t_1}}{Q} \tag{5}$$

From this calculation, and knowledge of the number of moles of reactant present initially, the rate constant is easily calculated:

$$k = \left(\frac{q_{t_1}}{Q} A_0\right)/t_1 \tag{6}$$

Finally, if the experiment is repeated at two different concentrations, then

$$\Delta H = \frac{Q_2 - Q_1}{(C_2 - C_1)V} \tag{7}$$

where Q_1 and Q_2 are the total areas under the curves from experiments conducted at concentrations C_1 and C_2, respectively. As in the case of method 1, a key advantage of eq 7 is that it does not matter if the initial portion of data is not captured, so long as the same portion of data is lost for both experiments.

RESULTS AND DISCUSSION

Method 1: Enzymatic Hydrolysis of Urea. The two buffers chosen were imidazole (pK_a 7.0, $\Delta_i H$ 36.64 kJ mol^{-1} [15]) and phosphate (pK_a 7.2, $\Delta_i H$ 3.6 kJ mol^{-1} [15]) because the enzymatic hydrolysis of urea is fastest around pH 7[16] and the large difference in ionization enthalpies maximizes data resolution. Typical calorimetric data are shown in Figure 1. Zero-order behavior was only observed during the first 3−5 h (inset graph in Figure 1), after which the rate decays to zero over several more hours because the pH changes significantly during reaction, increasing from 7 initially to ca. 8 at completion.

Despite this, analysis of the initial zero-order phase was still possible. The deflections were 1461 ± 28 μW in phosphate buffer and 653 ± 77 μW in imidazole buffer. Because buffer ionization is endothermic, the buffer with the largest ionization enthalpy (imidazole) produces the smaller deflection from baseline. In both cases, complete reaction occurred over ca. 22 h, demonstrating that the rate of reaction in the two buffers was the same. The parameter n was assumed to be 1, so the rate constant could be calculated with eq 4 (8.15×10^{-6} mol dm^{-3} s^{-1}). Once the value of k is known, it is possible to calculate the measured reaction enthalpy in each buffer with eq 1, leading to values of −59.8 kJ mol^{-1} in phosphate buffer and −26.7 kJ mol^{-1} in imidazole buffer. Equation 2 can then be used, with the enthalpies of ionization of the two buffers, to determine the uncoupled reaction enthalpy (−63.4 kJ mol^{-1}), in agreement with the value of −63.9 kJ mol^{-1} of Huttl[17] who assumed the following pathway

$$CO(NH_2)_2 + H_2O \rightarrow NH_2COO^- + NH_4^+$$
$$\Delta H = -24.27 \text{ kJ mol}^{-1}$$

$$NH_2COO^- + H_2O \rightarrow NH_{3(aq)} + HCO_3^-$$
$$\Delta H = 12.58 \text{ kJ mol}^{-1}$$

$$NH_{3(aq)} + H^+ \rightarrow NH_4^+ \qquad \Delta H = -52.22 \text{ kJ mol}^{-1}$$

which sums to

$$CO(NH_2)_2 + 2H_2O + H^+ \rightarrow 2NH_4^+ + HCO_3^-$$
$$\Delta H = -63.9 \text{ kJ mol}^{-1}$$

O'Neill[10] and Beezer[18] found the overall enthalpy to be −10.6 and −33 kJ mol^{-1}, respectively, in phosphate buffer at pH 7. The difference in these values was attributed to different values for the thermal volume of the flow calorimeter, but the larger

dx.doi.org/10.1021/jp302933f | J. Phys. Chem. B 2012, 116, 6356−6360

source of error appears to be their assumption of first-order kinetics in the decay period. Figure 1 shows that the decay period has two phases. As well as precluding a simple first-order analysis, this observation says that interpretation of calorimetric data must be undertaken with extreme care and with as much knowledge of the reaction processes occurring as possible.

Method 2: Hydrolysis of Acetylsalicylic Acid. Skaria[19] determined a reaction enthalpy of −23.6 ± 2.4 kJ mol^{-1} for hydrolysis of acetylsalicylic acid in pH 1 aqueous solution. Group additivity contributions for acetylsalicylic acid and its two hydrolysis products, salicylic acid and acetic acid, from Salmon and Dalmazzone[13,14] together with the enthalpy of formation of water, −285.8 kJ mol^{-1}, give an enthalpy change for reaction of −20.7 kJ mol^{-1} according to,

$$\Delta_r H = (\Delta_f H_{SA} + \Delta_f H_{AA}) - (\Delta_f H_{ASA} + \Delta_f H_{water}) \quad (8)$$

The rate constant determined from eq 1 is 5.2 × 10^{-6} mol dm^{-3} s^{-1}. The approach is less rigorous than methods 1 and 3. The reaction products must be known, and the method does not take into consideration the enthalpies of solution of the species. Despite this, the approach is useful where only an estimation of the reaction parameters is required (e.g., during preformulation characterization of a pharmaceutical substance).

Method 3: Photodegradation of Nifedipine. In ethanol, nifedipine decomposes into two photoproducts; a nitro-derivative under UV and a nitroso-derivative under daylight.[20] Figure 2 shows the reaction is pseudo-zero-order until the

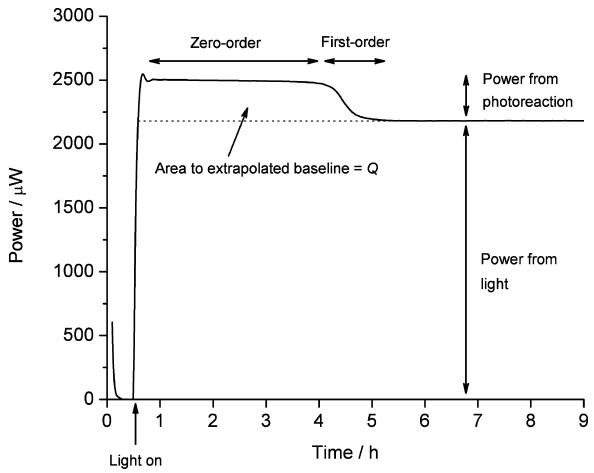


Figure 2. Calorimetric data for the photodegradation of nifedipine.

reactants are nearly exhausted, whereupon there is a first-order decay to baseline with the LED on. Initially, a zero power baseline is obtained with the LED off. Switching the LED on produces an exothermic deflection, caused by a combination of light power and photodegradation of nifedipine. As the nifedipine is exhausted, the power signal exponentially decreases to the power from the LED alone. Figure 3 shows how the calorimetric response varies with solution volume. Note that the zero baseline with the LED off is not shown in Figure 3.

The value of Q, the total heat from the photolysis reaction, is obtained from the area under the curve with respect to the baseline obtained with the LED on. Because the reaction goes to completion and no reaction occurs before the LED is switched on, the reaction enthalpy is calculated on dividing Q by the number of moles of nifedipine initially present. The

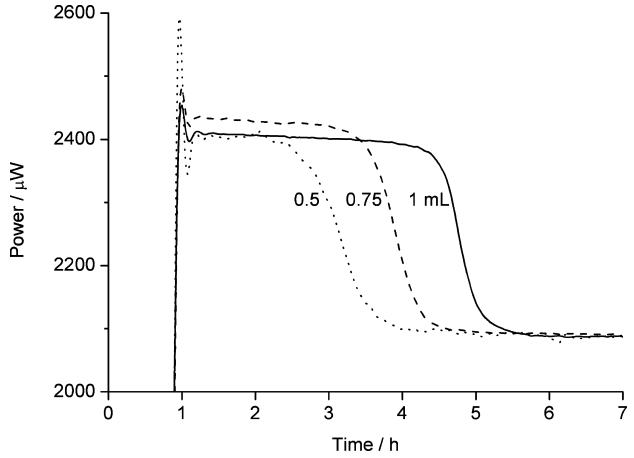


Figure 3. Calorimetric data for the photodegradation of nifedipine, shown for three volumes of ethanolic solution.

values obtained for ΔH are given in Table 1. No value for ΔH is available in the literature, but the enthalpy change based on the group additivity scheme discussed above is −145 kJ mol^{-1}, close to the values in Table 1.

Table 1. Thermodynamic and Kinetic Data for the Photodegradation of Nifedipine as a Function of Solution Concentration and Volume

concn (% w/v)	volume (mL)	Q (J)	$\Delta_r H$ (kJ mol^{-1})	k (×10^{-6} mol dm^{-3} s^{-1})
1	0.5	−2.45 ± 0.06	−167.0 ± 1.1	3.4 ± 0.3
1	0.75	−3.63 ± 0.06	−167.4 ± 2.6	2.1 ± 0.4
1	1.0	−4.38 ± 0.1	−151.7 ± 3.6	1.9 ± 0.3

To obtain the rate constant, eq 1 must be modified because the rate of reaction depends on the light power, not on the volume of solution. As demonstrated by the constant value of power during the zero-order phase, there is an excess of nifedipine molecules, relative to the number of photons supplied. Thus, under a constant light power (so constant number of photons) the number of molecules of nifedipine reacting per unit time must be the same, irrespective of the volume used. Once the concentration of nifedipine is reduced to such an extent that photons can pass through the solution without reacting, the reaction follows first-order kinetics. The rate constants given in Table 1 are thus proportional to the rate of photons entering the solution.

SUMMARY

Calorimetry is generally applicable to zero-order processes. Zero-order processes are deceptively easy to describe in terms of reaction kinetics but difficult to analyze quantitatively by calorimetry if neither the rate constant nor reaction enthalpy values are known in advance. Three methods have been presented that allow elucidation of the parameters. Method 1 applies to reactions that can be coupled to buffer ionization, method 2 is based on calculation of reaction enthalpy from group additivity functions, and method 3 is appropriate for reactions that progress to completion. Method 2 is probably the most generally applicable, since it can in principle be applied to any reaction assuming that the reactants and products are known. Method 3 is suited to those processes that can be

dx.doi.org/10.1021/jp302933f | J. Phys. Chem. B 2012, 116, 6356−6360

initiated within the calorimeter and that reach completion within an acceptably short time scale.

AUTHOR INFORMATION

Corresponding Author

*E-mail: s.gaisford@ucl.ac.uk. Tel.: +44(0) 207 753 5863. Fax: +44(0) 207 753 5942.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Willson, R. J.; Beezer, A. E.; Mitchell, J. C.; Loh, W. *J. Phys. Chem.* **1995**, *99*, 7108−7113.
(2) Gaisford, S.; Hills, A. K.; Beezer, A. E.; Mitchell, J. C. *Thermochim. Acta* **1999**, *328*, 39−45.
(3) Beezer, A. E.; Morris, A. C.; O'Neill, M. A. A.; Willson, R. J.; Hills, A. K.; Mitchell, J. C.; Connor, J. A. *J. Phys. Chem. B* **2001**, *105*, 1212−1215.
(4) O'Neill, M. A. A.; Beezer, A. E.; Tetteh, J.; Gaisford, S.; Dhuna, M. *J. Phys. Chem. B* **2007**, *111*, 8145−8149.
(5) Sousa, L. A.; Alem, N.; Beezer, A. E.; O'Neill, M. A. A.; Gaisford, S. *J. Phys. Chem. B* **2010**, *114*, 13173−13178.
(6) Hansen, C. W.; Hansen, L. D.; Nicholson, A. D.; Chilton, M. C.; Thomas, N.; Clark, J.; Hansen, J. C. *Int. J. Chem. Kinet.* **2011**, *43*, 53−61.
(7) Hansen, L. D.; Eatough, D. J.; Lewis, E. A.; Bergstrom, R. G.; Degraft-Johnson, D.; Cassidy-Thompson, K. *Can. J. Chem.* **1990**, *68*, 2111−2114.
(8) Lundgren, R.; Sanden, R.; Albertsson, A. C. *J. Appl. Polym. Sci.* **1989**, *37*, 1221−1231.
(9) Raemy, A.; Froelicher, I.; Loeliger, J. *Thermochim. Acta* **1987**, *114*, 159−164.
(10) O'Neill, M. A. A.; Beezer, A. E.; Mitchell, J. C.; Orchard, J.; Connor, J. A. *Thermochim. Acta* **2004**, *417*, 187−192.
(11) Li, X.; Chow, D.-C.; Tu, S.-C. *Biochemistry* **2006**, *45*, 14781−1478.
(12) Ortiz-Salmerón, E.; Yassin, Z.; Clemente-Jimenez, M. J.; Las Heras-Vazquez, F. J.; Rodriguez-Vico, F.; Barón, C.; García-Fuentes, L. *Protein Struct. Mol. Enzymol.* **2001**, *1548*, 106−113.
(13) Salmon, A.; Dalmazzone, D. *J. Phys. Chem. Ref. Data* **2006**, *35*, 1443−1457.
(14) Salmon, A.; Dalmazzone, D. *J. Phys. Chem. Ref. Data* **2007**, *36*, 19−58.
(15) Goldberg, R. N.; Kishore, N.; Lennen, R. M. *J. Phys. Chem. Ref. Data* **2002**, *31*, 231−370.
(16) Huang, T.-C.; Chen, D.-H. *J. Chem. Technol. Biotechnol.* **1991**, *52*, 433−444.
(17) Hüttl, R.; Bohmhammel, K.; Wolf, G.; Oehmgen, R. *Thermochim. Acta* **1995**, *250*, 1−12.
(18) Beezer, A. E.; Steenson, T. I.; Tyrrell, H. J. V. In *Protides of the biological fluids*, 20th ed.;Peeters, H., Ed.; Pergamon Press: New York, 1973.
(19) Skaria, C. V. Ph.D. thesis, University of London, 2007.
(20) Majeed, I. A.; Murray, W. J.; Newton, D. W.; Othman, S.; Al-turk, W. A. *J. Pharm. Pharmacol.* **1987**, *39*, 1044−1046.