

DSC XXVI – Instrumentation and Data Analysis

Boehm K., Guddorf J., and Hinz H. J. (2007) Application of pressure-modulated differential scanning calorimetry to the determination of relaxation kinetics of multilamellar lipid vesicles. *Biophys Chem* **126**, 4241-4249.

Abstract: We report an extension of the recently published PMDSC method that permitted synchronous determination of heat capacity and expansibility when using slow, defined pressure formats in a DSC scan. Here we applied continuously opposing pressure changes that are fast compared to the time constants of the DSC instrument to study relaxation kinetics of phospholipids. Investigations of multilamellar vesicles of DPPC or DSPC in water revealed for both lipids relaxation times of about 30 s at the maximum of the main transition peak and about 15 s at the maximum of the pretransition. The relaxation times in the transition range are proportional to heat capacity of main- and pretransition. The molecular origin of the relaxation processes appears to stem from pressure-induced water fluxes between the interbilayer region and the bulk water phase.

Boehm K., Rosgen J., and Hinz H. J. (2006) Pressure-modulated differential scanning calorimetry. An approach to the continuous, simultaneous determination of heat capacities and expansion coefficients. *Anal Chem* **78**, 984-990.

Abstract: A new method is described that permits the continuous and synchronous determination of heat capacity and expansibility data. We refer to it as pressure-modulated differential scanning calorimetry (PMDSC), as it involves a standard DSC temperature scan and superimposes on it a pressure modulation of preselected format. The power of the method is demonstrated using salt solutions for which the most accurate heat capacity and expansibility data exist in the literature. As the PMDSC measurements could reproduce the parameters with high accuracy and precision, we applied the method also to an aqueous suspension of multilamellar DSPC vesicles for which no expansibility data had been reported previously for the transition region. Excellent agreement was obtained between data from PMDSC and values from independent direct differential scanning densimetry measurements. The basic theoretical background of the method when using sawtooth-like pressure ramps is given under Supporting Information, and a complete statistical thermodynamic derivation of the general equations is presented in the accompanying paper.

Burgos I., Dassie S. A. and Fidelio G. D. (2008) Thermodynamic Model for the Analysis of Calorimetric Data of Oligomeric Proteins. *J Phys. Chem B* **112**, 14325-14333.

Abstract: The thermodynamic parameters for the process of protein unfolding can be obtained through differential scanning calorimetry. However, the unfolding process may not be a two-state one. Between the native and the unfolded state, there may be association or dissociation processes or the formation of an intermediate state. As a consequence of this, the precise interpretation of the calorimetric data should be done with a specific thermodynamic model. In this work, we present two general models for the unfolding process of an oligomeric protein: N n right harpoon over left harpoon nN right harpoon over left harpoon nD (model A) and N n right harpoon over left harpoon I n right harpoon over left harpoon nD (model B). In model A, the first step represents the dissociation of the oligomer into the monomeric native species, and the second step represents the denaturation process. In model B, the first step represents the conformational change of the oligomer, and the second step represents the dissociation of this species with the concomitant unfolding process. A canonical ensemble was employed to describe these systems, by considering that the total protein concentration remains constant. In the present work, we show and analyze the behavior of these systems in different conditions and how this analysis could help with the identification of the unfolding mechanism experimentally observed

Chu H. L. and Lin S. Y. (2001) Temperature-induced conformational changes in amyloid beta(1-40) peptide investigated by simultaneous FT-IR microspectroscopy with thermal system. *Biophys Chem* **89**, 173-180.

Abstract: Temperature-dependent secondary structures of the amyloid beta(1-40) peptide in the solid state were studied by simultaneous Fourier transform infrared/differential scanning calorimetry (FT-IR/DSC) microspectroscopic system with the heating-cooling-reheating cycle. The result indicates that a thermal transition temperature at 45 degrees C was easily obtained from the three-dimensional plot of the

transmission FT-IR spectra as a function of temperature. Furthermore, the thermal-dependent conformational transformations, due to denaturation and aggregation, of solid amyloid beta(1-40) were mainly evidenced by reducing the compositions from 37 to 20-24% for alpha-helical and random coil structures but increasing the components from 27 to 45% for intermolecular beta-sheet structures. Thermal-irreversible behavior and a poor thermal stability of solid amyloid beta(1-40) were also observed from the poor restoration of the secondary conformational changes in the heated sample.

Garbett N. C., Miller J. J., Jenson A. B., Miller D. M. and Chaires J. B. (2007) Interrogation of the plasma proteome with differential scanning calorimetry. *Clin. Chem* **53**, 2012-2014.

Garbett N. C., Miller J. J., Jenson A. B. and Chaires J. B. (2008) Calorimetry outside the box: a new window into the plasma proteome. *Biophys J.* **94**, 1377-1383.

Abstract: Differential scanning calorimetry provides a new window into the plasma proteome. Plasma from normal individuals yields a characteristic, reproducible thermogram that appears to represent the weighted sum of denaturation profiles of the most abundant constituent plasma proteins. Plasma from diseased individuals yields dramatically different signature thermograms. Thermograms from individuals suffering from Rheumatoid arthritis, systemic lupus and Lyme disease were measured. Each disease appears to have a distinctive and characteristic thermogram. The difference in thermograms between normal and diseased individuals is not due to radical changes in the concentrations of the most abundant plasma proteins, but rather appears to result from interaction of as yet unknown biomarkers with the major plasma proteins. These results signal a novel use for calorimetry as a diagnostic tool.

Garbett N. C., Miller J. J., Jenson A. B. and Chaires J. B. (2007) Calorimetric analysis of the plasma proteome. *Semin. Nephrol.* **27**, 621-626.

Abstract: The plasma proteome is a complex mixture of more than 3,000 proteins that has routinely been exploited by physicians for clinical diagnostic assays. More recently, the low-abundance region of the proteome has been examined for potential biomarkers of disease. A calorimetric assay has been developed that exploits a new physical basis with which to interrogate the plasma proteome. This article provides a brief overview of the use of the plasma proteome in clinical diagnosis and biomarker discovery and then introduces the new calorimetric assay. Some initial results are reported that indicate the potential clinical utility of the assay.

Langerman N. and Biltonen R. L. (1979) Microcalorimeters for biological chemistry: applications, instrumentation and experimental design. *Methods Enzymol* **61**, 261-286.

Mudd C. P., Gershfeld N. L., Berger R. L., and Tajima K. (1993) A differential heat-conduction microcalorimeter for heat-capacity measurements of fluids. *J Biochem Biophys Methods* **26**, 149-171.

Abstract: A heat-conduction calorimeter has been developed for measuring small changes in heat capacity of milligram samples of membrane lipid dispersed in water as a function of temperature. The operation of the instrument is based on the principle that the thermal response of the sample to a short (10 s), electrically generated heat burst is a function of the diffusivity of the sample. Modeling studies of the instrument's performance have revealed that the output response after the heat burst is a function of only the heat capacity, ρC_p . Calibration of the instrument experimentally confirmed this behavior. This feature obviated the need to measure the thermal conductivity in order to determine ρC_p from the diffusivity equation, $\eta = \lambda / \rho C_p$. The calorimeter has the following characteristics: reproducibility of loading: $\pm 400 \mu\text{J}/\text{C degrees.cm}^3$; baseline stability: $\pm 10 \mu\text{J}/\text{C degrees.cm}^3$ per 36 h; resolution ($\pm 1 \text{ S.D.}$): $\pm 50 \mu\text{J}/\text{C degrees.cm}^3$; sample size 600 microliters.

O'Neill M. A. (2005) Recent developments for the analysis of data obtained from isothermal calorimetry. *Curr Pharm Biotechnol* **6**, 205-214.

Abstract: Isothermal calorimetry is rapidly becoming an indispensable tool for the quantitative determination of a variety of kinetic and thermodynamic parameters for a wide range of systems. In particular calorimetry is finding increased application to the investigation of stability and incompatibility of pharmaceutical materials. In order to draw meaningful conclusions and to predict behaviour in related systems it is necessary to have the means to calculate accurately parameters such as the rate constant and enthalpy. To this end several groups have been developing equations which describe calorimetric output in

these terms. This paper will briefly outline some of these equations and discuss some of the limitations that currently exist in their application. A particular emphasis is placed on the recent developments relating to the application of these equations to flow calorimetric data. The main application of these equations is usually found in the pharmaceutical industry. Pharmaceutical formulations are usually extremely complex mixtures consisting of many different excipients as well as the active drug. Because of these large numbers of ingredients it is often observed that multiple chemical and physical processes occur over the lifetime of the study. This complexity is then reflected in the calorimetric data rendering the application of the simple equations useless. Dealing with this complexity is a major issue amongst the calorimetric community and some of the recent advances in this field are also discussed.

Plotnikov V. V., Brandts J. M., Lin L. N., and Brandts J. F. (1997) A new ultrasensitive scanning calorimeter. *Anal Biochem* **250**, 237-244.

Abstract: A new ultrasensitive differential scanning calorimeter is described, having a number of novel features arising from integration between hardware and software. It is capable of high performance in either a scanning or isothermal mode of operation. Upscanning is carried out adiabatically while downscanning is nonadiabatic. By using software-controlled signals sent continuously to appropriate hardware devices, it is possible to improve adiabaticity and constancy of scan rate through use of empirical prerun information stored in memory rather than by using feedback systems which respond in real time and generate thermal noise. Also, instrument response time is software-selectable, maximizing performance for both slow- and fast-transient systems. While these and other sophisticated functionalities have been introduced into the instrument to improve performance and data analysis, they are virtually invisible and add no additional complexities into operation of the instrument. Noise and baseline repeatability are an order of magnitude better than published raw data from other instruments so that high-quality results can be obtained on protein solutions, for example, using as little as 50 microg of protein in the sample cell.

Plotnikov, V., Rochalski, A., Brandts, M., Brandts, J. F., Williston, S., Frasca, V., and Lin, L.N. (2002) An Autosampling Differential Scanning Calorimeter Instrument for Studying Molecular Interactions. *ASSAY and Drug Development Technologies* **1**, 83-90.

Abstract: A new ultrasensitive differential scanning calorimeter (DSC) instrument is described, which utilizes autosampling for continuous operation. High scanning rates to 250 deg/h with rapid cooling and equilibration between scans facilitates higher sample throughput up to 50 samples during each 24 h of unattended operation. The instrument is suited for those pharmaceutical applications where higher throughput is important, such as screening drug candidates for binding constant or screening solution conditions for stability of liquid protein formulations. Results are presented on the binding of five different anionic inhibitors to ribonuclease A, which included cytidine 29-monophosphate (29CMP), 39CMP, uridine 39-monophosphate, pyrophosphate, and phosphate. Binding constants K_B (or dissociation constants K_d) are obtained from the shift in the transition temperature T_M for ribonuclease thermal unfolding in the presence of ligand relative to the transition temperature in the absence of ligand. Measured binding constants ranged from 155 M_{21} ($K_d = 6.45$ mM) for the weak-binding phosphate anion to 13,100 M_{21} ($K_d = 76.3$ mM) for the strongest binding ligand, 29CMP. The DSC method for measuring binding constants can also be extended to ultratight interactions involving either ligand-protein or protein-protein binding.

Privalov G., Kavina V., Freire E., and Privalov P. L. (1995) Precise scanning calorimeter for studying thermal properties of biological macromolecules in dilute solution. *Anal Biochem* **232**, 79-85.

Abstract: A precise scanning calorimeter for studying the heat capacity of liquids in a broad temperature range has been developed. By its design and capabilities this calorimeter is the first of a new generation for this type of instrument. This new scanning calorimeter operates differentially, is equipped with a pair of gold capillary cells and semiconductor sensors, and is able to scan up and down in temperature at user-selected rates. This instrument is completely operated by an integrated computer which also provides a full thermodynamic analysis of the results. Its construction does not involve the use of organic compounds, thus eliminating a source of baseline noise that has affected previous calorimeters. The operational temperature range of the instrument can be varied between 0 and 120 degrees C. The gold capillary cells (operational volume 0.8 ml) minimize temperature gradients in the heated/cooled liquid sample and permit easy washing and reloading without air bubbles. These features are crucial for the accuracy of difference heat

capacity measurements and determination of the absolute value of the partial heat capacity of solute molecules. The measurements can be performed under an excess constant pressure (up to 3 atm) to prevent formation of gas bubbles and boiling of aqueous solutions above 100 degrees C. The noise level of the recorded heating/cooling power difference is below 50×10^{-9} W (i.e., below 10 ncal/s) with a response half-time of 5 s. The reproducibility of the baseline without refilling the capillary cells is on the order of 0.5×10^{-6} W. Reproducibility of the baseline upon refilling the cell is of the same order of magnitude. This provides an accuracy in difference heat capacity determination on the order of 10 μ cal/degrees Kml at a heating rate of 1 degree K/min.

Rosgen J. and Hinz H. J. (2006) Pressure-modulated differential scanning calorimetry: theoretical background. *Anal Chem* **78**, 991-996.

Abstract: We demonstrate in this work and in the accompanying paper that it is possible to measure simultaneously heat capacity and expansibility of biomolecules in a single DSC experiment. In this study, we provide the theoretical basis for this new method based on rigorous statistical thermodynamics. The theoretical treatment presented here demonstrates that there are two additive contributions to the heat capacity at variable pressure, viz. (1) the isobaric heat capacity and (2) an expansibility term, and that these contributions can be experimentally separated to obtain simultaneously both heat capacity and expansibility in continuous DSC temperature scans performed under pressure modulation. Equations that describe the mixed heat capacity and expansibility signal are derived, and experimental strategies as well as data extraction procedures are discussed.

Rosgen J. and Hinz H. J. (2006) Pressure-modulated differential scanning calorimetry: theoretical background. *Anal Chem* **78**, 991-996.

Abstract: We demonstrate in this work and in the accompanying paper that it is possible to measure simultaneously heat capacity and expansibility of biomolecules in a single DSC experiment. In this study, we provide the theoretical basis for this new method based on rigorous statistical thermodynamics. The theoretical treatment presented here demonstrates that there are two additive contributions to the heat capacity at variable pressure, viz. (1) the isobaric heat capacity and (2) an expansibility term, and that these contributions can be experimentally separated to obtain simultaneously both heat capacity and expansibility in continuous DSC temperature scans performed under pressure modulation. Equations that describe the mixed heat capacity and expansibility signal are derived, and experimental strategies as well as data extraction procedures are discussed.

Talla-Singh D. and Stites W. E. (2008) Refinement of noncalorimetric determination of the change in heat capacity, $\Delta C(p)$, of protein unfolding and validation across a wide temperature range. *Proteins* **71**, 1607-1616.

Abstract: The change in heat capacity, $\Delta C(p)$, on protein unfolding has been usually determined by calorimetry. A noncalorimetric method which employs the Gibbs-Helmholtz relationship to determine $\Delta C(p)$ has seen some use. Generally, in this method the free energy change on unfolding of the protein is determined at a variety of temperatures and the temperature at which ΔG is zero, $T(m)$, and change in enthalpy at $T(m)$ are determined by thermal denaturation and $\Delta C(p)$ is then calculated using the Gibbs-Helmholtz equation. We show here that an abbreviated method with stability determinations at just two temperatures gives values of $\Delta C(p)$ consistent with values from free energy change on unfolding determination at a much wider range of temperatures. Further, even the free energy change on unfolding from a single solvent denaturation at the proper temperature, when coupled with the melting temperature, $T(m)$, and the van't Hoff enthalpy, $\Delta H(vH)$, from a thermal denaturation, gives a reasonable estimate of $\Delta C(p)$, albeit with greater uncertainty than solvent denaturations at two temperatures. We also find that nonlinear regression of the Gibbs-Helmholtz equation as a function of stability and temperature while simultaneously fitting $\Delta C(p)$, $T(m)$, and $\Delta H(vH)$ gives values for the last two parameters that are in excellent agreement with experimental values