VOLUMETRIC AND CALORIMETRIC TECHNIQUES

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Summary

A short description of traditional and modern methods for measuring volumes and/or densities of liquid and solids is presented. Criteria for the classification of the majority of commercial and homemade calorimeters are introduced and critically discussed. The heat conduction and heat flux principles and their advantages are compared with the power compensation method. Other kinds of calorimetric and thermoanalytical methods are also reported. Applications in life sciences, from basic biochemistry and biology to

pharmacology and biotechnology, from the food industry to ecology, are briefly presented

1. Introduction

In the classical and statistical formulations of thermodynamics, the equation of state strictly correlates pressure (p), temperature (T), volume (V), mass (m) and internal energy (see *Thermodynamic Systems and State Functions*). For the real gases, intrinsic volumes of molecules, intermolecular interactions, and rotational and vibrational degrees of freedom have been in some cases accounted for, modifying the simple equation of ideal gas, $pV = NkT$, where N is the number of material particles (molecules correlated with m by means of molecular weight and Avogadro number N_A) and k is the Boltzmann constant. For the condensed phases, the volume of a system cannot still be considered as an independently adjustable variable, like p and T, but rather a constitutive property mainly correlated to the intrinsic structure of the liquid or solid concerned. For this reason, volumetric and internal energy change determinations have become indispensable tools for correlating molecular properties, intermolecular interactions, intrinsic structure, and macroscopic properties of each specific condensed system. Such determination became possible as soon as precise and accurate balances, thermometers, and barometers were produced for giving absolute values of m, p, and T. As a consequence, densitometry, and in particular calorimetry, preceded and accompanied the birth and development of the thermodynamic theory. It is helpful to recall the studies of Carnot on thermal engines and those of Joule and Meyers on the equivalence between heat and work. At the same time the knowledge provided by calorimetric techniques proved to be of great practical interest for obtaining several quantitative inferences for energy studies and efficiency improvement of thermal engines. It was possible to determine quantitatively the heat friction evolved during the movements of mechanical parts of any device and apparatus during mechanical operations. The ancient idea about the delivering of a "fluid", the so called "caloric" during the transformation of a unique solid block into a set of small shavings (for the drilling of bronze cannon barrels, for instance) was shown to be completely wrong.

2. Volumetric Methods

Accurate determinations of gas volumes hold an important place in the history of chemistry. In fact it was recognized, by considering simply the stoichiometry of the volumes of reacting species and assuming the additivity of mixtures, that several pure gaseous substances are composed of biatomic molecules, not atoms, so allowing the determination of the value of the Avogadro number N_A. Moreover, one of the fundamental experimental tools for the foundation of thermodynamic principles was the gas thermometer, which was much more sensitive than the liquid ones. The theory of volume additivity of real gas was shown later to be true, except for reacting mixtures in chemical equilibrium (cfr. $2NO_2 \leftrightarrows N_2O_4$ etc).

In the following sections there will be a brief discussion of the experimental methods suitable for the determination of volumes or densities of liquids and solids. Volumetric and densimetric methods are complementary, as volume determinations can give precise density values and vice versa, but often it is easier to measure accurately only one of the

properties. Thermodynamic properties of pure substances (molal volumes) and substances in solution (partial molal volumes, see *Thermodynamic Systems and State Functions*) can be deduced by either of these methods. Other quantities, such as hydration or hydrodynamic volumes, are usually evaluated by non-thermodynamic properties and assumptions, or by calculations based on structural observations. For mesoscopic systems (for instance, globular proteins in aqueous solution) some interesting observations suggest that the specific volumes are the result of an average among domains whose local densities range from 0.6 kg L^{-1} to 3.0 or 3.5 kg L^{-1}. Further, the definition of domain volumes, as well as the definition of the whole volume of a macromolecule in intimate contact with solvent, suffers from a certain degree of arbitrariness.

2.1. Volumetry and Densitometry of Liquids and Solutions

A classical densitometric method is based on the determination of the mass of a defined liquid volume. Pycnometers are in fact glass bottles of a calibrated volume with subtle or capillary necks, which need to be filled up to a notch at a defined temperature. A compromise between the balance error and volume error restricts the method precision to $\pm 0.5 \times 10^{-4}$ kg L^{-1}. Displacement balances or indifferently suspended small floats represent other less precise methods. A series of small floats of different densities can, alternatively, be utilized for calibrating a gradient density (see Section 2.2). Nowadays, a more precise method based on a completely different principle is generally utilized. A quartz oscillating U-tube, filled with the liquid or solution to be studied, is rigidly supported at the ends and electromagnetically excited to vibrate at its natural frequency, which depends on the density of the liquid sample and temperature, not on other physical properties. By means of calibration with a highly stabilized quartz crystal frequency, the precision on the density can range from ± 1.5 to ± 3.5 x 10^{-6} kg L^{-1}. The density d of the liquid sample is obtained from the relationship: $\tau^2 = (M + dV) c^{-1}$, where τ is the absolute period of the filled oscillator, M its empty mass, V the volume to be filled, and c the spring constant of the oscillation. The spring constant depends strongly on temperature, and an ultrathermostat is therefore required for precise measurements. The measurements can also be carried on by using two cells: the sample and the reference cell, which is calibrated beforehand. Then the measurements give the relative density of the sample from the ratio of periods τ / τ_{ref}. In this manner, unwanted small temperature gradients are minimized, as the two cells are placed close together in the same ultrathermostat volume. With this arrangement of the instrument it is also possible to equip the ultrathermostat with a linearly programmed heating scanner, generating the new methodology called "Differential Temperature Scanning Densitometry" (DTSD), which is analogous to the "Differential Temperature Scanning Calorimetry" (see Section 3.4.5).

2.2. Densitometry of Solids

The measurement of the density of a solid is substantially based on the indifferent suspension of the sample (a crystal or a small piece of homogeneous glass or alloy of different solid substances) in a liquid of the same density. The solid must obviously be practically insoluble in this liquid. The best density value can be reached step by step, preparing a set of liquids of different densities (usually mixtures of two completely

miscible solvents) to evaluate, at first approximation, the limits of a small range of densities close to that of the sample. The procedure can be repeated a number of times, preparing mixtures of liquids closer in compositions and densities, up to the limits of sensitivity of the method ($\pm 10^{-3}$ kg L^{-1} in the best conditions). The use of a continuous and linear density gradient is in principle much faster, but the stability of the gradient can be easily perturbed. This methodology can be extended to mesoscopic systems, supplying a measure of the molecular weight of proteins or other natural or synthetic macromolecules, but the use of an ultracentrifuge is required to create the gradient. Nowadays, miniaturized systems can be used for accurate determination of the densities of very small crystals of biopolymers or other substances. However, these are not easily available and many authors prefer to estimate the density or the volume of a protein from structural data analysis using semi-empirical methods and additivity approximations.

3. Calorimetric Methods and Instruments

The advantage of calorimetric methods over the other physico-chemical and analytical tools is their applicability to any kind of process producing heat, even if the molecular structural changes undergone by the involved substances cannot be directly deduced. The process developing in a calorimeter can investigate not only homogeneous solutions, but also solids, suspensions, slurries, sewages, biological materials, where the opaqueness of medium prevents the use of spectroscopy. The calorimeters can be equipped with subsidiary devices, such as electrodes, for measuring pH, or analytical species concentrations, oxygen consumption, and so on. The main defect of calorimetric methods is represented by the non-specificity of the thermal signals resulting from the superposition of all the thermal events occurring simultaneously. Calorimetric methods make essential contributions to the building up of tables and databanks of thermochemical and thermophysical data and, together with other derived techniques (thermal analysis), in understanding the constitutive properties of any materials and thermal transformation features. Recently, the development of electronics has allowed the manufacture of instruments suitable for investigation of very small samples (a few mg). The same quantity can be studied as a solute dissolved in dilute solution (<1 ml) of water or other appropriate solvent. The need to study single processes, insulated from a complex network, involves using a few mg of sample per each experiment. The miniaturization of instruments has given a novel impetus to calorimetric researches in biological fields (from the metabolic activities to the thermal behavior of molecular systems such as proteins, nucleic acids, polysaccharides, their complexes, and cell membranes).

3.1. Units

The oldest unit of heat quantity was the calorie (cal), the quantity of heat necessary to heat 1 g of pure water from 14.5 °C to 15.5 °C, along with its multiple the kilocalorie (kcal). All the scientific international associations and organizations have for many years recommended that the same unit should be used to measure any kind of energy or work—the Joule unit (J) and its multiple the kilojoule (kJ). This unit is in fact immediately derived from other fundamental units of the International Standard System: $1 \text{ J} = 1 \text{ kg m}^2 \text{ s}^{-2} = 1 \text{ W s} = 1 \text{ N m}$ (W being the Watt and N the Newton units).

However, there is a strong resistance from many biologists and medicine doctors to abandoning the calorie because of the great popularity that nutritional and diet tables (among other things) have assumed in recent years. Another point is that the absolute value of the calorie has undergone slight changes. The following conversion factors are recommended: 1 J = 0.2388 cal or 1 cal = 4.1840 J, if the calorie is determined according to the procedure defined by the US Bureau of Standards. For older data the conversion factor is: 1 cal = 4.1868 J.

3.2. Characteristics of Calorimeters

Actually it is not possible to measure heat directly, but only the effects produced by its flux from one body to another or its accumulation in a defined system. The effects can may be thermal effects (temperature changes, phase transitions) or non-thermal effects (changes of volume, pressure, electrical resistance, and so on). The necessity (for different scientific or technological reasons) of measuring very large or very small quantities of released or absorbed heat, with largely differing time scales, resulted in the development of many methods and instruments, all based on ingenious practical solutions. A unitary kind of calorimeter classification, therefore, is not possible and probably not useful. The different criteria for classifying the commercial and homemade instruments used in research or control laboratories of academies, industries, or services are discussed in the following.

A calorimetric system is a measuring apparatus essentially constituted of:
- A calorimetric vessel (CV) containing the system or substance that produces or absorbs the heat evolved during a spontaneous or induced process, or the system or material exposed to a thermal treatment or stress or to absorption of any kind of energetical contribution promoting a heat release.
- A surrounding (S): a thermostat or a furnace or a heat sink; this part of the system can be a distinct apparatus, but it must be in intimate contact with the calorimetric vessel as it constitutes the nearest environment that is well separated from the outer world (laboratory room); (S) must provide or absorb heat from (CV) and transfer it to the outer environment (except in the case of adiabatic calorimeters, see below);
- The thermal resistance (R_H), composed by all the materials lying through (CV) and (S); (R_H) can be simply the contact interface between (CV) and (S) or vice versa the thermal shield inhibiting the heat flux.

The distinction among (CV), (S), and (R_H) is functional rather than material or geometrical because the connection cables, stirrers, and other components go through the overall calorimetric system, and thus form part of (CV), (S), and (R_H). Analogously the walls of (CV) and (S) are shared with (R_H). The outer walls of (S) can be in intimate contact with the laboratory environment or not, according to the operating mode. The insulation obviously prevents the disturbances and noises that can amplify errors, but it is often necessary to transfer great quantities of heat into the laboratory environment. It must be also considered that heat conduction (the main mechanism of conduction when the temperature of the source is not extremely high) is relatively slow with respect to the radiative mode and the thermal responses of sample materials are often also slow processes.

The operative characteristics of a calorimeter are:

- *Sensitivity*. The ratio between the change of the measured signal and that of the heat flux or the heat released.
- *Accuracy*. The degree of agreement between the mean value of measurements and the true value of the heat released. A measure of this is the absolute or relative error.
- *Precision*. The degree of agreement between the results of different measurements; this term includes repeatability or reproducibility, depending on the experimental procedure.
- *Uncertainty*. Relative (plus/minus) range around the measured mean value. In the absence of systematic errors the true value is expected to lie within this interval with a certain probability. A measure of the uncertainty is the standard deviation or a multiple of it.
- *Repeatability*. The degree of agreement between the results of successive measurements of the same quantity performed by the same method, same instrument, and same operator at different short intervals of time in the same laboratory. The repeatability of measurements relates to the random uncertainty, given as a plus/minus interval about the mean value.
- *Reproducibility*. The degree of agreement between the values of a parameter if the individual measurements are made using various methods or instruments, by different people, and at various laboratories. The reproducibility of a measured value (determined by means of a set of random investigations) may serve to estimate systematic errors of the calorimeter.
- *Stability*. This relates to the fluctuations of the instrument base line over a considerable period (hours or days) or during temperature scanning. Slow chemical processes or biological phenomena need stable instruments, otherwise the sensitivity will be reduced and uncertainty increased.
- *Linearity*. A quality characteristic that shows how the sensitivity (the calibration factor) can be accurately represented by a horizontal straight line. The corresponding error is called the "linearity error." It also indicates the dependence degree of the sensitivity or calibration factor on parameters concerning sample, instrument, and experimental procedure.

3.3. Operating Mode Classification Criterion

The first criterion for classifying calorimeters is based on the operating mode. Three main groups can be identified:
- adiabatic calorimeters
- isothermal calorimeters
- isoperibolic calorimeters

Some authors add scanning and differential scanning calorimeters as a fourth group. However, the scan represents a further operating procedure for each of the three previously characterized kinds of instruments (this will become clearer in Section 3.4). There is another distinction regarding the batch operating mode and the flow mode. In to the first mode, the solvent or a solution is initially introduced in the (CV), and the reactants are added in one or several steps. In the flow mode, both the reactants in solution or one reactant and the solvent are introduced continuously, the same total

volume of the final mixture flowing out simultaneously. In both cases a preconditioning procedure is indispensable.

3.3.1. Adiabatic Calorimeters

A calorimeter will be defined as "adiabatic" if there is no heat exchange between the vessel (CV) and the surrounding (S). This can be achieved statically by means of an extremely large (R_H) value (infinite, in principle), or dynamically by continuously equalizing the surrounding temperature T_S to that of the sample and its vessel T_{CV}. In the last case the temperature sensor is put in the vessel and T_{CV} is the temperature of the sample. Otherwise T_{CV} is the temperature of the outer wall of (CV) if the sensor is in contact with it, assuming that (CV) is formed by a highly conductive material and the resistance at the interface with the sample is nil.

The heat released by an exothermic process occurring in an ideally adiabatic calorimeter will produce an increase of temperature in (CV), ruled by the specific heat and mass of both the sample and vessel. The equation describing the thermal-dynamical behavior of the system is the following:

$$W(t) = dQ/dt = C_{CV}dT_{CV}(t)/dt \tag{1}$$

were $W(t)$ is the power supplied by the process at every time (t) and C_{CV} is the overall thermal capacity averaged on the mass of sample, vessel, and other involved parts as discussed above. The total heat quantity supplied by the process will be then given by:

$$Q = \int_0^{t'} W(t)dt = C_{CV}\int_{T(0)}^{T(t')} dT_{CV}(t) \tag{2}$$

where t' is the time after which no further increases of temperature are observed. Because it is not practically possible to define *a priori* the contribution to C_{CV} of thermistors, stirrers, and other parts of R_H subject to a temperature increase, it will necessary to make a preliminarily calibration of the system without the sample. The Joule effect, produced by electrical resistances incorporated in the body of (CV), will be useful in the case of exothermic processes. A thermopile of Peltier elements can be also used, but is less convenient for heating. On the other hand, it is useful for the calibration of endothermic processes.

The main difficulty in performing an adiabatic measurement however, is the non-infinite value of (R_H). Limited heat conduction along the connections of temperature sensors, calibration resistances, Peltier elements, or stirrers and other accessories cannot be eliminated. It will be necessary to introduce into Eqs. (1) and (2) empirical corrections depending on the actual specific experimental conditions and characteristics of the studied process. Nowadays, the adiabaticity is achieved dynamically by using a thermal shield whose temperature T_S is continuously equated to that of (CV) by means of a feedback regulation: in this case no heat will flux from (CV) to (S) because the thermal gradient is nil, whatever the value of (R_H). Here it is important to outline the concept that for adiabatic calorimeters the ideal behavior depends on the achievement of

this condition instantly every time, so it is possible to affirm that $T_S(t)$ is enslaved to $T_{CV}(t)$. Adiabatic calorimeters are often used for determining specific heats, especially at low temperatures.

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Bibliography

Beezer A.E. (ed.) (1980). *Biological Microcalorimetry*, 483 pp. London: Academic Press. [The first important assessment of the state of the art, with contributions from the majority of the pioneers in all the fields of biological applications of calorimetry.]

Della Gatta G. (ed.) (2000). *Thermochimica Acta: Special Issue 347*, 1–132. Amsterdam: Elsevier. [An up-to-date collection of articles on calibration and standardization methods in calorimetry.]

Garti N. (ed.) (2000). *Thermal Behavior of Dispersed Systems. Surfactant Science Series /93*, 540 pp. New York: Dekker. [Several chapters are devoted to the applications of calorimetric methods in the field of micelles, liposomes and microemulsions.]

Hemminger W. and Höhne G. (1984). *Calorimetry: Fundamentals and Practice*, 316 pp. (English edition translated by Y. Goldman). Weinheim: Werlag Chemie. [A detailed text on theory and practice of calorimetry with historical references and several illustrations.]

Hinz H.-J. (ed.) (1986). *Thermodynamic Data for Biochemistry and Biotechnology*, 456 pp. Berlin: Springer-Verlag. [A collection of volumetric and other thermodynamic properties on biological macromolecules and low molecular weight model molecules in solution, ranging from non-reacting to interacting system, from the solution processes to the conformational and phase transitions, and finally to enzyme catalysis.]

James A.M. (ed.) (1987). *Thermal and Energetic Studies of Cellular Systems*. Bristol: Wright. [Some chapters regard the action of antibiotics on bacteria and yeasts].

Jones M.N. (ed.) (1989). *Biochemical Thermodynamics: Studies in Modern Thermodynamics 1*. 2nd. edn, 403 pp. Amsterdam: Elsevier Scientific. [A wide-ranging text covering studies, mainly by means of calorimetry, on model molecules, macromolecules, membranes, and cellular systems.]

Ladbury J.E. and Chowdhry B Z. (eds.) (1998). *Biocalorimetry*, 346 pp. Chichester, UK: John Wiley . [A more up to date collection of contributions on the application of calorimetry to the study of thermodynamics of biological macromolecules in solution.]

Lamprecht I. and Schaarschmidt B. (eds.) (1977). *Application of Calorimetry in Life Science*, 344 pp. Berlin: W. De Gruyter. [Proceedings of the 1976 Conference in Berlin, dedicated to applications of calorimetry in Biochemistry, Microbiology, Medicine, and Ecology. The first appointment of European researchers in these fields.]

Marsh K.N. and O'Hare P.A.G. (eds.) (1994). *Solution Calorimetry: Experimental Thermodynamics*, 332 pp. IUPAC Chemical Data Series No.39, Vol. 4. Oxford: Blackwell Scientific. [Classical text ranging from the applications of calorimetry to solution thermodynamics of organic liquid mixtures and aqueous solutions of electrolytes or biological molecules, to those in the field of cryogenic systems or molten inorganic mixtures at high temperature and pressure.]

Millero F.J. (1972). The Partial Molal Volumes of Electrolytes in Aqueous Solutions. *Water and Aqueous Solutions. Chapt.13 and addendum.* (ed. R.A. Horne). 838 pp. New York: Wiley-Interscience. [A collection of data, critically revised, on volumetric and densimetric properties of electrolytes in water.]

Turi E.A. (1981). *Thermal Characterization of Polymer Materials.* New York: Academic Press. [A complete overview on the thermo-analytical methods applied to the study of synthetic polymers.]

Wendlandt W.W. (1964). *Thermal Methods of Analysis*. New York: Wiley-Interscience. [A benchmark among up-to-date papers on thermo-analytical methods in chemistry.]

Widmann G. and Riesen R. (1987). *Thermal Analysis Terms, Methods, Applications*. Heidelberg: A. Hunthing Verlag. [An up-to-date overview on thermo-analytical methods and applications in research laboratory and industry.]

Biographical Sketch

Guido Barone was born in Avellino in 1937. He was awarded a degree in Industrial Chemistry *cum Laude* in July 1961 at the Faculty of Science of the University "Federico II" of Naples, where he is now Full Professor of Physical Chemistry and Environmental Physical Chemistry. In the past he was responsible for other courses: Macromolecular Chemistry, Electrochemistry, and Biophysical Chemistry.The main research interests of Professor Guido Barone were developed in the fields of Biophysical Chemistry and Environmental Physical Chemistry, as is demonstrated by about 150 of his papers published in International Journals. His research was developed at first in the field of thermodynamics and electrochemistry of aqueous solutions of electrolytes and polyelectrolytes and uncharged organic molecules of biological interest. Afterwards his interest was devoted to biological macromolecules (proteins and nucleic acids), mainly studied by means of microcalorimetry. Thermodynamics of molecular recognition of ligands and conformational transitions were interpreted in the light of mechanical statistical models, molecular mechanical calculations, and spectroscopic results. More recently Professor Guido Barone joined the Center for Research on Parallel Computing and Supercomputers of the Italian National Council for Research (CPS-CNR) where he promoted a network of researchers (chemists and mathematicians) working in several institutes in Naples: the CPS-CNR, the Department of Chemistry and the Department of Mathematics of the University "Federico II" and the Institute of Mathematics of the Naval University. The aim was to promote studies on the modeling of air quality in urbanized coastal areas of the Mediterranean Sea.