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INTERUNION COMMISSION ON BIOThERMODYNAMICS*

CALORIMETRIC MEASUREMENTS ON CELLULAR SYSTEMS:
RECOMMENDATIONS FOR MEASUREMENTS AND
PRESENTATION OF RESULTS

Comments on these recommendations are welcome and should be sent within 8 months from March 1982 to:

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Comments from the viewpoint of languages other than English are especially encouraged. These may have
special significance regarding the publication in various countries of translations of the nomenclature eventually
approved by IUPAC.

*Membership of the Commission during the period (1977—80) in which the report was
prepared was as follows:

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CALORIMETRIC MEASUREMENTS ON CELLULAR SYSTEMS:
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INTRODUCTION

Calorimetric measurements are of basic importance in studies of the thermodynamic properties of living systems. However, calorimetric measurements on such systems are presently undertaken, to a large extent, as parts of diagnostic investigations without the objective of acquiring thermodynamic information. Examples of experiments of this kind are calorimetric monitoring of metabolic activity or identification of microorganisms by their calorimetric growth patterns. In such experiments heat quantities are determined, but, from a molecular thermodynamic point of view, the results are usually of marginal significance. This is because the systems investigated in general are poorly defined and the conditions under which the calorimetric measurements are made are frequently not reported in sufficient detail.

The Commission offers in this report some recommendations concerning experimental details and procedures for the reporting of results from calorimetric measurements on cellular systems. One aim has been to increase the usefulness of the thermodynamic quantities determined for such systems. In addition, we hope that the recommendations will assist in orienting some 'diagnostic' calorimetric studies towards procedures that will lead to results of thermodynamic significance. We believe that adherence to the recommendations should also be of value in purely 'diagnostic' calorimetric studies. It should facilitate reproduction of experiments within a laboratory, comparison of results obtained by different workers and comparison of results obtained for cells from different preparations.

Many types of calorimeters suitable for work on cellular systems are now available. The measured heats are often small and 'microcalorimeters' are normally employed. The term 'microcalorimeter' is not strictly defined but in current practice it usually implies (for reaction calorimeters) that the smallest measurable thermal power is of the order of 1 μW and that the reaction vessel is small, of the order of a few cm³ or less.

There is a general and lasting value in well documented energy data for biological systems. Thus, the use of arbitrary units like 'mm recorder deflection' should be avoided. Instead the calorimeter should be calibrated and the results reported in SI units, see e.g. (1). Calorimeters are normally calibrated electrically. The measurement of electrical energy is, today, a trivial procedure that can easily be made with an accuracy exceeding the needs of biological experiments. The problem is rather to make certain that the electrical energy is released in a manner closely comparable with that for the process studied. Microcalorimeters used in biological work are not always well suited for such strict comparisons. In particular for flow-through vessels* calibration procedures are often far from ideal, and it is currently rare that the results are checked by a suitable test reaction.

The response time of a calorimeter can be significant compared to the duration of the process being studied. Therefore, the calorimetric signal at any point in time may not have a simple relationship with the thermal power generated at that time within the reaction vessel (2). This must be taken into account when kinetic information is deduced from the results of calorimetric experiments.

It is necessary to specify carefully the physiological conditions for the cellular material during a calorimetric measurement. Special attention must be given to the practical design of the reaction vessel, the performance of the experiment and to the methods by which the physiological conditions in the reaction vessel are verified.

* We recommend the use of the term 'calorimetric vessel' in place of the often used term 'cell' to avoid confusion with 'biological cells' which may be the subject of the investigation.

PHYSICAL QUANTITIES. SI UNITS AND THEIR SYMBOLS

By international agreement a set of seven dimensionally independent units form the so-called SI * base units. The base physical quantities and units and their recommended symbols are summarized in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Base quantities</th>
<th>Symbol</th>
<th>Base units</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>length</td>
<td>l</td>
<td>metre</td>
<td>m</td>
</tr>
<tr>
<td>mass</td>
<td>m</td>
<td>kilogram</td>
<td>kg</td>
</tr>
<tr>
<td>time</td>
<td>t</td>
<td>second</td>
<td>s</td>
</tr>
<tr>
<td>electrical current</td>
<td>I</td>
<td>ampere</td>
<td>A</td>
</tr>
<tr>
<td>temperature †</td>
<td>T</td>
<td>kelvin</td>
<td>K</td>
</tr>
<tr>
<td>amount of substance</td>
<td>n</td>
<td>mole</td>
<td>mol</td>
</tr>
<tr>
<td>luminous intensity</td>
<td>I_v</td>
<td>candela</td>
<td>cd</td>
</tr>
</tbody>
</table>

All other physical quantities and units are regarded as being derived from the base quantities and units. Certain SI derived units have been given special names and symbols. Some of these are summarized in Table 2.

Table 2

<table>
<thead>
<tr>
<th>Physical quantity</th>
<th>SI unit</th>
<th>Definition of SI unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>force</td>
<td>newton</td>
<td>N</td>
</tr>
<tr>
<td>pressure, stress</td>
<td>pascal</td>
<td>Pa</td>
</tr>
<tr>
<td>energy</td>
<td>Joule</td>
<td>J</td>
</tr>
<tr>
<td>power</td>
<td>watt</td>
<td>W</td>
</tr>
</tbody>
</table>

In Table 3, SI symbols and units for some thermodynamic quantities (functions) are given. For an extensive tabulation of quantities and units recommended for chemistry and physics see (1).

* Le Système International d'Unités or The International System of Units; cf. (1).

† Thermodynamic ('absolute') temperature. Recommended symbol for Celsius temperature is \( t \) or \( \theta \). Where symbols are needed to represent both time and Celsius temperature, \( t \) is the preferred symbol for time and \( \theta \) for Celsius temperature (1).
Table 3

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>energy:</td>
<td></td>
</tr>
<tr>
<td>heat</td>
<td>q, Q*</td>
</tr>
<tr>
<td>work</td>
<td>w, W*</td>
</tr>
<tr>
<td>internal energy</td>
<td>U, (E)</td>
</tr>
<tr>
<td>enthalpy: $U + pV$</td>
<td>H</td>
</tr>
<tr>
<td>Gibbs energy: $H-TS$</td>
<td>G</td>
</tr>
<tr>
<td>Helmholtz energy: $U-TS$</td>
<td>A</td>
</tr>
<tr>
<td>entropy</td>
<td>S</td>
</tr>
<tr>
<td>power</td>
<td>P</td>
</tr>
<tr>
<td>electrical potential difference</td>
<td>$U, \Delta V, \Delta \phi$</td>
</tr>
<tr>
<td>volume</td>
<td>V</td>
</tr>
<tr>
<td>density</td>
<td>$\rho$</td>
</tr>
</tbody>
</table>

The SI base units are sometimes cumbersome to use in practical work and therefore the use of certain prefixes denoting multiples or submultiples are often found convenient. Recommended SI prefixes are summarized in Table 4.

Table 4

<table>
<thead>
<tr>
<th>Multiplier</th>
<th>Prefix</th>
<th>Symbol</th>
<th>Multiplier</th>
<th>Prefix</th>
<th>Symbol</th>
<th>Multiplier</th>
<th>Prefix</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-18}$</td>
<td>atto</td>
<td>a</td>
<td>$10^{-2}$</td>
<td>centi</td>
<td>c</td>
<td>$10^6$</td>
<td>mega</td>
<td>M</td>
</tr>
<tr>
<td>$10^{-15}$</td>
<td>femto</td>
<td>f</td>
<td>$10^{-1}$</td>
<td>deci</td>
<td>d</td>
<td>$10^9$</td>
<td>giga</td>
<td>G</td>
</tr>
<tr>
<td>$10^{-12}$</td>
<td>pico</td>
<td>p</td>
<td>10</td>
<td>deca</td>
<td>da</td>
<td>$10^{12}$</td>
<td>tera</td>
<td>T</td>
</tr>
<tr>
<td>$10^{-9}$</td>
<td>nano</td>
<td>n</td>
<td>$10^2$</td>
<td>hecto</td>
<td>h</td>
<td>$10^{15}$</td>
<td>peta</td>
<td>P</td>
</tr>
<tr>
<td>$10^{-6}$</td>
<td>micro</td>
<td>(\mu)</td>
<td>$10^3$</td>
<td>kilo</td>
<td>k</td>
<td>$10^{18}$</td>
<td>exa</td>
<td>E</td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>milli</td>
<td>m</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* It is recommended that $q > 0$ and $w > 0$ both indicate increase of energy of the system under discussion (I). Thus $\Delta U = q + w$.  


Presentation of experimental and derived results

The output of many calorimetric experiments is a plot of power (energy evolution per unit of time) as a function of time. The recommended unit for power (P) is watt (W) and for time the second (s) or some multiple thereof [e.g. kilosecond (ks)]. With the use of these units the time integral of the power-curve is given directly in joules (J) or some multiple thereof.

In graphs the axes should be labeled with both the name (or the symbol) of the quantity and the unit employed. Preferably the label should show the quantity divided by the unit in order to emphasize that the scale markers represent pure (dimensionless) numbers.

Some comments on terminology, symbols and units

To avoid confusion and to simplify communication we recommend standardization of terminology, symbols and units in accordance with the SI and with other rules accepted by the International Unions. Some physical quantities, SI units and their symbols are summarized in Tables 1-3.

In a report, values other than SI units may be included in parentheses when it is felt this will improve communication between the author and the readers. This may be considered desirable when there is still widespread current usage of a non SI unit for a certain quantity such as the calorie or the atmosphere.

The symbols for physical quantities should be printed in italic (sloping) type or underlined in typescript and the symbols for units should be printed in roman (upright) type.

No attempt will be made here to discuss all the names and symbols for physical quantities. Our attention will be directed toward those for which the international recommendations form a distinct variation from much established practice.

Energy

Results of energy measurements should be reported in joules (J, kJ, mJ, etc. as appropriate). In cases where energies are also expressed in calories (more properly called thermochemical calories, cal_{th}) or a multiple or submultiple thereof, the author should state the conversion factor used (1 cal_{th} = 4.184 J).

The 'nutritional' or 'large' calorie (sometimes abbreviated 'Cal' and equal to one kilocalorie) should not be used.

The author should realize that the use of calories is in decline because of the acceptance of the SI units, so that data reported in calories will probably require conversion relatively soon.

Temperature

Thermodynamic temperatures and temperature differences are expressed in kelvins, symbol K (e.g., not degree kelvin or °K). Celsius temperatures and temperature differences are expressed in degrees Celsius, symbol °C, (often improperly called the degree 'centigrade'). One degree Celsius is exactly equal to one kelvin.

Pressure

The unit of pressure is the pascal, which is one newton per square metre (1 Pa = 1 N m^{-2}). A convenient unit for many pressure measurements is the kilopascal (symbol kPa). The pressure unit commonly used until now, the atmosphere (atm), approximately equals 100 kPa.

One atmosphere, defined as 101 325 Pa, is presently the accepted standard pressure used in calculating equilibrium constants (cf. 3) and standard thermodynamic functions. *

* The IUPAC Commission on Thermodynamics has recently (4) proposed that the standard pressure shall be equal to 10^5 Pa (0.1 MPa) which is identical to 1 bar.
The biological thermodynamicist should recognize that the common thermodynamic term $pV$ is an energy term, and if $p$ is in pascals and $V$ is in cubic metres then the $p \cdot V$ product is obtained directly in joules. No combination of customary non-SI units avoids the use of a conversion factor; and such awkward energy units as litre-atmosphere are avoided by the usage recommended. The commonly found units—mm Hg, Torr, or their multiples—should be avoided.

Volume

The basic SI unit for volume, cubic metre ($m^3$) is often cumbersome for use in biology. More convenient units are its submultiples: cubic decimetre ($dm^3$), cubic centimetre ($cm^3$) and cubic millimetre ($mm^3$). The cubic decimetre is identical to the litre ($l$ or $L$). The use of the name litre and its submultiples millilitre ($ml \equiv cm^3$) and microlitre ($\mu l \equiv mm^3$) is not encouraged, but is likely to prevail for some time.

Composition of solutions

For thermodynamic applications the composition of a solution is commonly described in terms of concentration, molality, or mole fraction.

The molar concentration of a solute B is the amount of substance of B (expressed in moles) divided by the volume of the solution. Accepted symbols are $c_B$ and $[B]$. Concentration is sometimes called 'molarity.' A solution with a concentration of 0.1 mol $\cdot dm^{-3}$ is often called a 0.1 molar solution or a 0.1 M solution. Because the term molality and the symbol M are liable to be confused with molality, the term concentration and the symbol mol $\cdot dm^{-3}$ are preferred.

The mass concentration of a solute substance B is the mass of B divided by the volume of the solution. An accepted symbol is $\rho_B$ and an appropriate unit is kg $\cdot m^{-3}$ (g $\cdot dm^{-3}$).

The molality of solute B is the amount of substance B (moles) divided by the mass of solvent. A solution having a molality equal to 0.1 mol $\cdot kg^{-1}$ is sometimes called a 0.1 molal or a 0.1 m solution. An accepted symbol is $m_B$ and the appropriate unit is mol $\cdot kg^{-1}$.

SOME SPECIFIC RECOMMENDATIONS

The cell preparation

Where possible, experiments should employ material obtained from type collections with well defined organisms or cells. In other cases detailed information about the origin and method of preparation (isolation) should be reported. For cells which have been stored, maintenance conditions such as time, temperature and media should be reported. Details concerning passages of the organisms from the state of storage to experimental use should also be described (number of subcultures and details of the growth conditions). Counts of contaminating cells, e.g., for blood cell preparations, should be reported.

The calorimeter

Full details of the design of the calorimeter and its performance should be reported. Alternatively, reference can be given to other reports provided these are readily available. However, even minor changes in apparatus design and experimental procedure should be indicated. In each report the following details require attention:

1. The design of the calorimetric vessel, its volume and its construction material, gas phase, method for initiation of a process, method used for stirring or agitation of the liquid medium.

2. Time constants of the apparatus.

3. Results of calibration and test experiments including accuracy assignment.
(4) Base line stability (where relevant) over a period of time which normally should be greater than the measurement period.

(5) Amplification and recording equipment; pumps and other ancillary equipment.

(6) Method employed for cleaning and sterilization of the calorimetric vessel and flow lines where appropriate.

The calorimetric experiments

Experimentalists are reminded that all processes, both physical and chemical, will contribute to the observed heats. Thus, processes such as dilution, protonization, mixing, vaporization, etc. may give rise to significant systematic errors if not taken into account in the experimental design and in the evaluation of the results. In the special case of twin calorimeters, where two processes are compared, it is frequently useful to observe the separate output from each vessel to aid in the interpretation of the difference signal.

In each study the following details require attention:

(1) The measurement temperature must be reported.

(2) It is suggested that, where possible, chemically defined media should be used. Such media can be accurately reproduced and a detailed chemical analysis of reaction mixtures will be facilitated. In any case full details about the medium composition must be reported.

(3) If a gas or a liquid is perfused into, or through, the reaction vessel this must be reported in detail.

(4) Full details concerning inoculation of the medium must be reported (size, age and storage condition of inoculum, medium used and, where relevant, the growth phase). For preparations of, for instance, blood cells, counts of contaminating cells should be made and reported.

For growth experiments (microorganisms, cultured tissue cells) information about changes in the cell count is often essential. In any case initial and final cell concentrations (dry mass, etc.) should normally be reported, together with results of viability tests.

Details of the enumeration method followed, together with an estimate of the accuracy of the count, should be given. Relevant details of the extent of damaged cells should be given. (Note: intact enzyme substrate systems may contribute to the observed thermal power.)

(5) For the evaluation of results of calorimetric experiments, and to ensure their lasting value, heat measurements should ideally be accompanied by detailed analysis (pH, concentrations of $\text{O}_2$, $\text{CO}_2$, energy source(s), metabolites, etc.) performed continuously, or at intervals, on the reaction medium.

For almost all calorimetric experiments on cellular material it is essential that initial and final pH values be reported and that information be given concerning the concentration of oxygen. The problem with concentration gradients in experiments performed under static conditions should be noted, cf. effects of cell sedimentation.

Sometimes the necessary analytical information (including cell counts) must be obtained from parallel non-calorimetric experiments. It is important to perform such experiments under conditions which are as close as possible to those in the calorimetric experiment. They should be performed simultaneously, there should be no scale effect, vessels should be of the same material, medium and gassing rates, etc. should be identical. Even if great care is taken in
performing such parallel experiments the cellular processes may not proceed exactly as expected. This must be taken into account when uncertainty assignments are made.

(6) Experiments with cells are often carried out in suspensions. Depending on the type of organism, and on the calorimetric method, the cells may stay in a uniform suspension or they may partly or completely sediment before, or during, the calorimetric experiment. Cells originally in suspension may adhere to the walls of the calorimetric vessels. In flow calorimetric experiments, therefore, it is sometimes difficult to assess accurately the number of cells contained in the reaction vessel (during the experiment or at the end of the experiment) as they may have been partially trapped in the flow lines or they may have accumulated in the reaction vessel. Effects of this nature can be of crucial importance for the evaluation of the calorimetric results. It is, therefore, most important to consider these effects in the design of the calorimetric experiments and in the reporting of the results.

(7) When using differential scanning calorimeters for the study of intact cellular systems attention must be paid to the scanning rates employed. The scanning rates used should attempt to ensure equilibrium conditions throughout the temperature range of interest. For comparative purposes it is advisable to extrapolate the recorded data to zero heating velocities. Thus, experiments should be conducted using a minimum of three different heating rates.

The use of the term power-time-curve is recommended in place of the commonly used term 'thermogram' to avoid confusion with, for example, its use in differential thermal analysis.

It is often useful to derive values for the thermal power produced per cell, for a defined mass (dry weight) of cells or for a defined volume (wet, packed) of cells. Example: For freshly prepared human erythrocytes in plasma an approximate power per cell at 37°C and pH 7.40 is $P = 8 \text{ fW}$. For wet packed cells this value corresponds to about 80 mW dm$^{-3}$. Alternatively it may be of interest to report heat quantities associated with the consumption of specified substrates. When such values are reported they must be accompanied by relevant information about the experimental conditions since the power or heat quantities evolved can be very sensitive to variations in experimental conditions such as pH, medium composition (including concentrations of oxygen and carbon dioxide), temperature, storage conditions for the cells (time, temperature), cell concentration, etc.

The power-time-curve reported should take into account the dynamic characteristics of both the calorimeter and the process under study, see e.g. (5). Two situations can arise

1. where the dynamic parameters of the calorimeter do not affect the experimental power-time-curve (e.g. where steady state processes are observed or where the time constant for the process studied is long compared to that of the instrument),

2. where the experimental power-time-curve requires correction to take account of these instrumental parameters.

The corrections under (2) should be expressed in sufficient detail to permit regeneration, if required, or the experimental power-time-curve.

Where instrumental data handling systems take into account these dynamic characteristics prior to presentation of results, the appropriate references or descriptions of the method of correction employed and the values of the defined parameters should be reported.

**Interpretation of observed heat quantities associated with growth processes**

If possible, the enthalpy change associated with growth phenomena should be expressed in thermodynamic terms (6). It is recommended that the enthalpy change per unit of substrate metabolized be reported,
ΔΔH^\text{met}^\text{*}\) \* The recommended unit for \(ΔH^\text{met}\) is \(\text{J} \cdot \text{mol}^{-1}\). When the catabolic process is well known it is possible to calculate the enthalpy change corresponding to catabolism, \(ΔH^\text{cat}\). The enthalpy change corresponding to anabolism is similarly expressed as \(ΔH^\text{an}\). Thus, in well defined systems when an energy substrate is metabolized, the enthalpy balance can be written:

\[
ΔH^\text{met} = (1-α)ΔH^\text{cat} + αΔH^\text{an}
\]

\(α\) represents the fraction of the carbon source which is incorporated into the cellular material and can be calculated from the molecular growth yield.

When the nitrogen source is simple (e.g., \(\text{NH}_3, \text{NO}_2^-, \text{N}_2\)) it is possible to calculate the enthalpy change corresponding to anabolism from values for the elemental composition of the cellular material, enthalpies of combustion of the dried cells and the molecular growth yields. For this calculation it is simpler to express the results as the specific enthalpy change, \(\text{i.e., the enthalpy change per unit mass of dried cells, } \Delta h^\text{an}\),

\[
\Delta h^\text{an} = \frac{αΔH^\text{an}}{Y^\text{s}}
\]

where \(Y^\text{s}\) is the molecular growth yield. Recommended units are for \(Δh^\text{an}\) J g\(^{-1}\) and for \(Y^\text{s} \text{ g mol}^{-1}\). Equation (1) then becomes

\[
ΔH^\text{met} = (1-α)ΔH^\text{cat} + Y^\text{s} Δh^\text{an}.
\]

References


3. Interunion Commission on Biothermodynamics (IUPAC, IUB, IUPAB): Recommendations for Measurement and Presentation of Biochemical Equilibrium Data, see e.g., J. Biol. Chem. 251, 6879- (1976).


\* Reference (7) proposes that such symbols be written \(Δ^\text{met}_H\) rather than \(Δ^\text{met}\). At present we leave the choice open.

\* There is in the literature some confusion over the definition of the physical state of microorganism subjected to combustion calorimetry. Attention has to be directed in the future to obtaining agreement on the conditions under which such experiments should be conducted.

\* When an extensive quantity is represented by a capital letter, the corresponding specific quantity may be denoted by the corresponding lower case letter (1).