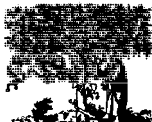

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Isothermal microcalorimetry for the characterization of interactions between drugs and biological materials[1]

Ingemar Wadsö

Thermochemistry, Chemical Center, Lund University, P.O. Box 124, S-221 00 Lund, Sweden

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Abstract

Studies of interactions between drugs and biological material form a broad application area for isothermal microcalorimetry. Characterization of binding of drugs and related compounds to well-defined biological substances by use of titration microcalorimetry is a very important area. This technique has now been taken into use in practical work ("rational drug design") by several laboratories in the pharmaceutical industry. More fundamental studies of solute–solvent interactions for drugs and for simple model systems should be encouraged. Microcalorimetric techniques suitable for investigations of the effect of drugs on microorganisms and animal cellular systems have been much developed during recent years. However, few scientific groups are presently active in this field and it seems as if the technique has not yet been adopted by R&D laboratories in the pharmaceutical industry or in applied clinical work.

Keywords: Isothermal microcalorimetry; Drug; Biological material; Interaction

1. Introduction

Isothermal microcalorimetry conducted at ambient temperatures has developed significantly during the past decade [1,2]. In several areas such techniques are now of practical importance, for example in the characterization of physical and chemical stabilities of solid materials used in the pharmaceutical industry (see e.g. [3–5]). However, recent method development work in isothermal microcalorimetry has mainly been concerned with applications in solution chemistry, biochemistry and cell biology, areas which also are of direct interest for the characterization of drugs.

This paper focuses on the use of isothermal microcalorimetry for

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- studies of the binding of drugs and related substances to carrier and target molecules
- model investigations for the support of the analysis of results from the drug binding experiments
- studies of the effects of drugs on living cellular systems

When a drug has been administrated to a patient, orally, by injection or by some other technique, it normally finds its way to the blood stream where a large fraction of the drug molecules are bound to plasma albumin. A smaller fraction is usually bound to the blood cells, in particular to the membrane of red cells. Phospholipid vesicles (liposomes) and macrocyclic compounds, such as cyclodextrins, are in this connection of potential interest as artificial drug carriers.

Bound and free drug molecules are transported by the blood stream to different parts of the body where a certain fraction is bound to a primary drug target: an enzyme, a carrier molecule, an ion channel or a "receptor" molecule (see e.g. [6]). For most drugs these molecular targets are proteins or protein complexes, whereas anticancer and antimicrobial drugs are often bound to DNA or RNA. Non-specific depressant substances, for example anesthetics are usually believed to interact with lipids in the cell membrane. Other important drug-binding processes are those where some kind of drug conjugate is formed, for example between an anticancer drug and a tumor specific antigen.

The thermodynamic characterization of drug binding processes today form a central part of "rational" or "structure based" drug design work in many pharmaceutical laboratories. Microcalorimetric techniques for studies of ligand binding processes, leading to values for ΔG°, ΔH°, ΔS° and $\Delta C_P{}^\circ$ have now been available for about 25 years [7], but have since been much improved with respect to sensitivity, speed and ease of measurement, and the derivation of thermodynamic data [1,8,9]. Recently, advances have also been made in dissolution microcalorimetry, of particular interest for studies of drugs and simple model systems for drug binding processes. When the drug molecule has been bound to its biological target, there are some physiological consequences, for example: inhibition of an enzyme or a transmembrane carrier process, an ion channel can be blocked, a receptor molecule might not regulate a certain process or the genetic machinery might be effected (see e.g. [6]). Many drugs or drug metabolites are toxic and will, in addition to the intended pharmacological action, interact with the cellular material in an adverse way. All such physiological effects will give rise to changes in the metabolic rate or metabolic pattern, or both, and will thus cause a change in the heat production rate of the cellular system. Extensive microcalorimetric method work has now been conducted on microorganisms and on cellular material from humans and animals [1,2,10].

2. Isothermal microcalorimetry

In this paper, the term "isothermal microcalorimeter" is used for instruments for which the sensitivity is better than 1 μW, and which, in contrast to differential scanning calorimetry (DSC), are designed for use at isothermal, or nearly isothermal, conditions. The principles and practice of isothermal microcalorimetry used in solution chemistry and in biology have been discussed in some detail elsewhere [1,2,7]. Here some basics from this area are summarized briefly.

2.2. Principles and properties

Many different microcalorimeters have found use in solution chemistry and in biology. A rather confusing terminology exists, but from the point of view of heat measurement principles, there are mainly three types in use: heat conduction (heat flow), adiabatic and power compensation calorimeters.

2.3. Heat conduction calorimeters

The heat evolved or absorbed in the reaction vessel of a heat conduction calorimeter is allowed to flow to or from a heat sink, usually a metal body, surrounding the vessel. The heat flow is recorded by letting it pass through a thermopile wall. In modern instruments used at ambient temperatures, the thermopile wall consists of one or more semiconducting thermocouple plates placed between the vessel and the heat sink. At steady-state the heat flow is directly proportional to the thermopile potential, U. The heat production rate or the thermal power, P, is thus

$$P = \varepsilon U \tag{1}$$

where ε is a calibration constant. Ideally ε is equal to the quotient between the average heat conductance of the thermocouples forming the thermopile and the Seebeck coefficient for the thermocouple material (see e.g. [12]).

The heat quantity, $\mathrm{d}Q$, evolved during a time $\mathrm{d}t$ is thus

$$\mathrm{d}Q = \varepsilon U \,\mathrm{d}t \tag{2}$$

For a non-steady-state process, the relationship between the thermopile potential and the thermal power of the process is, ideally, given by Tian's equation,

$$P = \varepsilon(U + \tau \,\mathrm{d}U/\mathrm{d}t) \tag{3}$$

The time constant, τ, is given by

$$\tau = C/G \tag{4}$$

where C is the heat capacity of the vessel and its content plus the heat capacity of half of the thermopile. G is the thermal conductance of the thermopile plus that of the leads, mechanical supports and the surrounding air. The time constant for a heat conduction microcalorimeter fitted with a 1 ml vessel is often about 1–3 min. In practice it is sometimes necessary to use an expression more complex than Eq. (3) [13]. Integration of Eq. (2) gives

$$Q = \varepsilon \int_{t_1}^{t_2} U \,\mathrm{d}t \tag{5}$$

provided that the initial and the final potential are identical (ideally = 0).

Most isothermal microcalorimeters currently used in solution chemistry and in biology are of the heat conduction type.

2.4. Adiabatic calorimeters

In an adiabatic calorimeter there is no heat exchange between the calorimetric vessel and the surroundings. The heat quantity evolved during an experiment is equal to the product between the measured temperature change and the heat capacity of the vessel and its content. With an adiabatic calorimeter it is a property proportional to the temperature change which is measured and the value for the calorimetric signal, which is proportional to Q, thus depends on the heat capacity value for the vessel and its content. This is in contrast to the case for heat conduction calorimeters (Eq. 5). However, for both types of instrument the heat capacity value must be taken into account when a value for the heat production rate is derived. For an adiabatic calorimeter a "practical" heat capacity value (a calibration constant) is determined experimentally. Semi-adiabatic instruments, usually called "isoperibolic" calorimeters, are often used. For such instruments the heat exchange with the surroundings is significant and a corrected value for the temperature change should be used.

In a strict sense, adiabatic calorimeters recording temperature changes are not isothermal calorimeters. However, for an adiabatic microcalorimeter, which not is used as a DSC, the temperature change accompanying a measured process is usually small.

2.5. Power compensation calorimeters

When an adiabatic calorimeter is used to measure an exothermic process, the temperature increase of the reaction vessel will not change when the process has gone to completion. In a heat conduction calorimeter a corresponding process will cause a temporary temperature increase, which soon will return very close to its initial value. In both cases it is possible to compensate for the temperature increase by applying a cooling power to the reaction vessel. This is most conveniently achieved by use of Peltier effect cooling: when an electrical current, I, is allowed to pass through a thermopile plate, there will be a cooling effect on one side of the plate, $-P$, and a corresponding heating effect on the other side.

$$-P = \pi I - rI^2 \tag{6}$$

where π is a material constant and r is an "effective" resistance value for the thermopile. The thermal power of the process is, ideally, equal to P provided that the temperature of the reaction is kept constant.

2.6. Some comments

Practically all microcalorimeters used in solution chemistry/biology are designed as twin instruments, i.e. it is the differential signal between a reaction vessel and a nearly identical reference vessel that is recorded. The twin design is important as disturbances

from the surroundings will be canceled or at least be much reduced. In particular twin calorimeters of the heat conduction type have proved to be suitable for very long reaction periods. For some designs, the baseline stability is better than 1 μW during several days. This property can be important in experiments with living materials and in dissolution microcalorimetry involving slightly soluble compounds [1]. In some cases a twin design is a consequence of the measurement technique (see e.g. [8,11]), where a thermopile is employed as a differential thermometer between two reaction vessels.

For some microcalorimeters the vessels are not permanently mounted in the instruments. Instead "insertion vessels" are used which greatly facilitates the design of multifunctional calorimeters. Such instrument systems normally are of the thermopile heat conduction type. The time required for a stirred adiabatic reaction vessel to reach equilibrium following the initiation of a fast process is usually about 1–2 min. Depending on design features, this will often apply to power compensation calorimeters as well. However, a heat conduction calorimeter is inherently slow and it often takes about 20 min before the heat pulse from a fast process has been conducted out to the heat sink (99.99% of the heat is transported to the heat sink during the time span of about 9τ (cf. Eqs. (3),(4)) [12]. The large thermal inertia for heat conduction calorimeters can be of significant disadvantage, in particular when used in multistep titration experiments. However, this problem has now largely been overcome (see below).

3. Ligand binding studies

Currently most microcalorimetric studies of ligand binding processes are performed as stepwise batch titration experiments but flow mixing calorimeters are also used. In a stepwise batch titration experiment, the titrant usually is added by injection. Typically the solution volume of the reaction vessel is 0.5–3 ml and 1–10 μl is injected at each titration step. At the present time, most batch titration experiments reported in the literature are conducted by use of the instruments produced by MicroCal Inc., Northampton, MA, USA; Thermometric, Järfalla, Sweden; and CSC (Hart Scientific), Pleasant Grove, USA. The instrument from MicroCal ("Omega") [8] can be characterized as a hybrid between an adiabatic instrument and a power compensation calorimeter. A thermocouple plate surrounded by two reaction vessels (volume 1.4 ml) is used as a differential thermometer. The assembly is separated from the surroundings by an adiabatic shield. During an experiment, a constant electric power ($\leq$1 mW) is supplied to one of the vessels serving as reference. The thermopile signal activates a feedback circuit supplying heat to the reaction vessel which drives the temperature difference between the vessels back to zero. An exothermic process in the reaction vessel will cause a decrease and an endothermic reaction leads to an increase of the feedback power. The temperature of the calorimetric assembly will thus slowly increase during a measurement.

Thermometric's titration calorimeter, being part of a modular system (Thermal Activity Monitor, "TAM") (cf. [1,2,14]), is a typical heat conduction calorimeter employing insertion reaction vessels (1–5 ml). The CSC instrument is also a heat conduction calorimeter; its vessel design is very similar to Thermometric's titration vessel.

As pointed out above, the large thermal inertia of heat conduction calorimeters can be

of significant disadvantage when used in multi-step titration experiments. When the calorimetric titration consists of about 15 injection steps, the total time for heat conduction and baseline periods between the injections often amounts to about 10 h. This should be compared to the time typically required in a corresponding experiment with an adiabatic instrument, about 1 h. However, it was recently shown [12,15] that the experimental time for titration experiments with heat conduction calorimeters can be reduced by one order of magnitude, without loss of accuracy. The time interval between the injections can be made as short as 1–2τ, i.e. an injection is made long before heat from the proceeding reaction step has been conducted to the heat sink. The heat quantities evolved following each injection is then evaluated by use of a "dynamic correction" procedure based on the Tian equation (Eq. 3).

In a (continuous) flow mixing experiment, the two streams of solution, flow rates typically 5–20 ml h^{-1}, are brought together in a mixing chamber or at a mixing point in the flow vessel. Usually the concentration of one of the solutions is kept constant whereas the concentration of the other solution is changed stepwise. With continuous flow mixing calorimetry, it is essential that the reaction mixture does not leave the heat sensitive area of the flow vessel before the process is completed [1,2].

Commercial flow microcalorimeters from Thermometric (part of their TAM system) and from Sodev, Sherbrook, Canada (a semi-adiabatic instrument, the "Picker" flow calorimeter [16] have been used in ligand binding experiments. The flow microcalorimeter described by Yamamoto and Aki [11] is of particular interest in the present connection as it has been used in several studies of binding of drugs to biological material. This twin instrument is of the semi-adiabatic type employing a thermopile as temperature sensor between the flow vessels.

The interpretation of results from a calorimetric titration experiment is based on the assumption that heat quantities evolved, corrected for dilution effects, are proportional to the amount of titrant reacted. If the equilibrium constant is very large, the titrant will be almost quantitatively consumed until the equivalent point has been reached (batch experiment). Such measurements can lead directly to a value for the molar enthalpy change and will also provide information about the stoichiometry for the process. However, the results do not provide any information about the (concentration) equilibrium constant, K_c except that it is high. For processes with moderately high K_c values, a significant and increasingly large fraction of the titrant will not be consumed as the stepwise injections continue. The fraction of non-reacted titrant obviously depends on the binding constant for the process.

In order to derive values for the thermodynamic quantities it is first necessary to predict a molecular model for the reaction measured, in most cases a simple 1:1 binding reaction:

$$A + B = AB \tag{7}$$

However, for an unknown process the possibility of more complex reaction schemes like

$$A + B = AB, \quad A + AB = A_2B \tag{8}$$

or

$$A + B = AB, \quad AB + B = AB_2 \tag{9}$$

should not be neglected.

The calorimetric results are fitted to the assumed model using K_c and the enthalpy change, ΔH°, as the fitting parameters (see e.g. [7–9,17]). Small and random least square residuals are obtained as a result of the minimization calculations when a correct model has been used. Such results will support but not prove a certain model. Commercial instruments are delivered with computer programs for the calculation of K_c and ΔH° values.

Values for the standard Gibbs energy change, ΔG°, and the entropy change, ΔS°, are calculated from Eqs. (10) and (11). From experiments conducted at different temperatures the change in heat capacity, $\Delta C_p{}^\circ$, can be derived (Eq. 12).

$$\Delta G^\circ = RT \ln K_c \tag{10}$$

where ΔG° is the standard Gibbs energy change.

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \tag{11}$$

and

$$\Delta C_p{}^\circ = \mathrm{d}(\Delta H)/\mathrm{d}T \tag{12}$$

For a 1:1 binding reaction it is in practice not possible to determine by calorimetry K_c values higher than about 10^7 mol^{-1} (cf. the discussion by Hallén [18]). However, using the technique of "displacement titration", it is possible to arrange for experimental conditions where the fraction of non-reacted titrant is significant even when very strong complexes are investigated. Assume that $K_{c,1}$ (Eq. (13) is too high to be measured directly by titration calorimetry. One may then titrate compound B with a complex AC (Eq. 14) for which the binding constant is known or can be determined (Eq. 15).

$$A + B = AB, \quad K_{c,1}, \quad \Delta H_1{}^\circ \tag{13}$$

$$AB + C = AC + B, \quad K_{c,2}, \quad \Delta H_2{}^\circ \tag{14}$$

$$A + C = AC, \quad K_{c,3}, \quad \Delta H_3{}^\circ \tag{15}$$

$$K_{c,1} = K_{c,2} \cdot K_{c,3} \tag{16}$$

$$\Delta H_1{}^\circ = \Delta H_2{}^\circ + \Delta H_3{}^\circ \tag{17}$$

Experimentally, the determination of the very large standard Gibbs energy change for Eq. (13) is thus divided into two steps, Eqs. (14) and (15). For a recent example where this technique was applied, see e.g. [19].

One of the great features with titration microcalorimetry is that it can be applied on all kinds of processes; for many equilibria there is no suitable specific analytical method readily available. However, particularly for binding reactions more complex than 1:1 equilibria, it is desirable to support the assumed binding model and the derived K_c values by more specific measurements than calorimetry. Even for the determination of K_c for a simple 1:1 binding reaction a non-calorimetric analytical technique will sometimes give a more accurate equilibrium constant, for example if the ΔH value is close to zero. Why then use calorimetry at all in a thermodynamic investigation of a binding process for which suitable specific analytical methods are available? Why not determine K_c at different temperatures and evaluate ΔH by the van't Hoff equation? The answer is that with this approach extreme accuracy is needed in the determination of K_c over a wide temperature range. Otherwise the propagation of error in K_c to those in ΔH and ΔS and, in particular, in ΔC_p will make these latter values almost useless. This is illustrated by examples from an error analysis made by King [20] (Table 1).

Besides the determination of the thermodynamic properties, information about the stoichiometry and the possible presence of different classes of binding sites can be important results of a microcalorimetric investigation of a drug binding process (see e.g. [21]). The number of protons released or taken up during a ligand binding process can be determined calorimetrically by conducting the process with two different buffer substances with different enthalpy of protonation (see e.g. [22]). It must then be assumed that the buffer substances do not significantly interfere with the reaction components, except for the reaction with protons. Therefore it can be desirable to confirm the result by running the experiment in an additional buffer.

The thermodynamic consequences of structural differences between a series of related ligands (drugs) and a common binding partner is often much more pronounced in the values for ΔH° and ΔS° than in the ΔG° values. That kind of enthalpy–entropy compensation is illustrated by the results from a microcalorimetric study by Aki and Yamamoto [23] (Table 2) on the binding of a series of anionic antiinflammatory drugs to intact erythrocyte ghost membranes. A one-class binding was assumed. Can such data be interpreted at the present time? No, at least not on a quantitative molecular level, but other types of useful information can be derived. For example, the thermodynamic description in terms of ΔG°, ΔH° and ΔS° values for binding of the drugs to ghost membranes is

Table 1

Determination of values for ΔH° and $\Delta C_p{}^\circ$ from equilibrium measurements at different temperatures: propagation of errors in log K for a 1:1 binding reaction to errors in ΔH° and $\Delta C_p{}^\circ$ at 25°C (±SD) (from King [22])

±SD in log K	Experimental temperatures/°C	±SD in ΔH°/kJ mol^{-1}	±SD in $\Delta C_p{}^\circ$/J K^{-1} mol^{-1}
0.02	20, 22, 24, 26, 28, 30	4	2800
0.02	5, 10, 15, 20, 25, 30, 35, 40, 45, 50	0.8	120
0.001		0.04	6

Table 2

Thermodynamic properties for the binding of a series of non-steroidal anionic antiinflammatory drugs to human erythrocyte ghost membranes (from Aki and Yamamoto [23])

Drug	$-\Delta G°$/ kJ mol^{-1}	$-\Delta H°$/ kJ mol^{-1}	$\Delta S°$/ J K^{-1} mol^{-1}
Flufenamic acid	20.7	20.9	−0.4
Mefenamic acid	21.0	18.3	9
Ibuprofen	22.3	2.8	63
Flurbiprofen	20.4	7.7	41
Indomethacin	19.6	6.3	43

similar to corresponding values for the binding of the same drugs to intact erythrocytes. This suggests that these drugs do not strongly interact with substances in the interior of the erythrocytes (mainly hemoglobin). However, for a series of cationic drugs (phenothiazines) a remarkable difference was noted between results of their binding to ghosts and to the intact cells, from which it was concluded that these drugs to a large extent bind to the hemoglobin of the erythrocytes.

3.1. Rational drug design

The starting point for a "rational" or structure based drug design is a detailed knowledge of the structure of the binding site for the drug, obtained by X-ray or NMR [24]. The task is then to find a substance which has a strong affinity for the binding site and which by other criteria is a suitable candidate for a drug. In order to minimize the number of substances that must be synthesized, it is then of crucial importance to be able to correlate structural information of the binding site and of the drug candidates, with their binding constant in the actual cellular medium. This is far from a trivial task, but important progress in that direction is being made (see e.g. [24–27]). However, it seems as if analyses based on experimental calorimetric data for the binding processes, and for model systems, will lead to more generally applicable solutions to the problem than correlations between the structural parameters and the equilibrium constant. As mentioned above, enthalpy–entropy compensations are commonly observed for ligand binding reactions suggesting that the enthalpy and the entropy terms form a better base for such correlations than the K_c or the $\Delta G°$ value. Further, for some time it has been recognized that heat capacity values for solutes in dilute aqueous solution are highly additive [28], in particular when they not are influenced by neighboring charges. Experimental $\Delta C_p°$ values can therefore lead directly to information, on the empirical level, of changes in exposure of hydrophobic or hydrophilic molecular surfaces. Recently, more fundamental thermodynamic analyses, based on calorimetric model compound data and on results from high sensitivity DSC measurements of protein unfolding processes, have been reported; see [22,29–34] and references given there.

An example of that thermodynamic approach is presented in a paper by Murphy et al. [22] on the structural recognition between a peptide hormone and its protein receptor

molecule. The binding reaction was investigated by titration microcalorimetry conducted at different temperatures leading to values for $\Delta G°$, $\Delta H°$, $\Delta S°$ and $\Delta C_p°$. The heat capacity and the enthalpy terms were both taken to be directly related to the change in solvent contacts for apolar and polar surfaces, whereas the entropy change was rationalized as a solvent contact depending term and a statistical term. In the recent reports by Connelly et al. [35] and Weber et al. [36], both from industrial pharmaceutical laboratories, related thermodynamic analyses were also applied on results of X-ray and titration microcalorimetric measurements.

3.2. *Investigations of solute–solvent investigations*

Calorimetric results from investigations of properties of simple organic solutes, hydrocarbons alcohols, carboxylic acids, amines, amides, etc., are essential for our present understanding of the thermodynamics of biopolymer–ligand binding processes. The most important type of measurement in this connection is dissolution calorimetry conducted on the macro or the micro scale. From dissolution of a pure compound into water, and into other solvents, enthalpy and heat capacity values ($\Delta_{sol}H$, $\Delta_{sol}C_p$) are determined. When microcalorimetric techniques are used, it is frequently possible to obtain directly values which are close to those for infinite dilution, $\Delta_{sol}H^\infty$, $\Delta_{sol}C_p^\infty$, i.e. the state at which there is no interaction between the solute molecules. In other cases it can be necessary to complement the dissolution experiments with dilution measurements, which can also be of interest per se [1]. When a molecular model for the association equilibrium can be formulated, the enthalpy of dilution values can be used to calculate the corresponding equilibrium constant and the molar enthalpy value (see e.g. [37]). From determination of $\Delta_{sol}H$ ($\Delta_{sol}C_p$) for two solvents the transfer value is obtained

$$\Delta_{trans}H = \Delta_{sol}H(1) - \Delta_{sol}H(2)$$

where (1) and (2) indicate, for example, water and an organic solvent, respectively. Such transfer processes may serve as models for the transfer of the solute (the drug) between an aqueous solution and the environment provided by a binding site or a lipid phase of a membrane.

From a fundamental point of view particularly important quantities are the enthalpy and the heat capacity of transfer from the (ideal) gaseous phase to infinite dilute solutions (the ideal solvation process) $\Delta_{solv}H^\infty$ and $\Delta_{solv}C_p^\infty$. These quantities expressing properties solely related to interactions between solute and solvent are derived from the differences between dissolution and vaporization data

$$\Delta_{solv}H^\infty = \Delta_{sol}H^\infty - \Delta_{vap}H° \quad (18)$$

$$\Delta_{solv}C_p^\infty = \Delta_{sol}C_p^\infty - \Delta_{vap}C_p° \quad (19)$$

Similarly, the very useful partial molar heat capacity value for a solute (A) at infinite dilution, $C_{p,A}^\infty$, is free from contributions due to intermolecular forces between the solute

molecules. $C_{p,A}^{\infty}$ values can be obtained from the sum of $\Delta_{solv}C_{p,A}^{\infty}$ and the heat capacity for the pure solute, $C_{p,A}^{*}$

$$C_{p,A}^{\infty} = \Delta_{sol}C_{p,A}^{\infty} + C_{p,A}^{*} \tag{20}$$

Values for $C_{p,A}^{\infty}$ can also be derived from direct heat capacity measurements performed on dilute solutions.

Micro dissolution calorimetry is now well developed [1,38]. Instruments and working procedures have been reported for dissolution of easily or slightly soluble liquids [39], for slightly soluble gases [40,41] and for solids [42–44]. The instruments developed in our laboratory [39–42,44] form part of a modular system of titration–perfusion instruments where the solvent flows through the vessel. In one of the instruments for solid solutes [42], the (slightly soluble) solute initially is in contact with a saturated solution, whereas in the other instrument [44] the solid material (easily or slightly soluble) initially is dry. The enthalpies of dissolution measured by these two instruments can thus be significantly different. Murphy and Gill have reported an interesting dissolution method for slightly soluble (wet) solids using a titration microcalorimeter [43].

For easily soluble liquids and solids it can sometimes be advantageous to use a "classical" macrocalorimetric dissolution calorimeter. New developments in "isoperibol" macro solution calorimetry has significantly narrowed the sensitivity difference between macro and micro techniques (Thermometric's 2225 Precision Solution Calorimeter).

Accurate heat capacity data ($\Delta_{sol}C_{p,}$, $C_{p,A}^{\infty}$) have in most cases been determined by use of the solutes in their pure state. Many heat capacity measurements have also been made on dilute solutions by use of Picker's flow Cp microcalorimeter [45] (Sodev) and by a drop Cp microcalorimeter developed in our laboratory [46]. This latter instrument can be used for measurements on liquids or solids (cf. Eq. (20)).

Sensitive vaporization calorimeters have been reported and many calorimetric measurements of $\Delta_{vap}H$ have been carried out in the past, mainly for liquids [47]. However, at present there are very few laboratories in the world active in this field.

During the past two decades a large collection of high quality calorimetric dissolution and heat capacity data were produced. Today, that type of work is not very common. It is felt, however, that more $\Delta_{sol}H^{\infty}$ and $C_{p,A}^{\infty}$ data are needed for aqueous solutions of several groups of simple organic compounds. For example, data for hydrocarbons are judged to be very uncertain in the higher temperature range (>40°C). They are of great importance for the theoretical treatment of protein binding data [29]. Sulfur (–S– and –S–S–) and nitrogen compounds need more attention. More sensitive calorimeters for enthalpies of vaporization need to be developed and should be applied to, in particular, solid substances for which few reliable $\Delta_{vap}H$ data are available.

For the interpretation of thermodynamic data of drug binding processes it is necessary to have available thermodynamic data for the drugs in aqueous solution (and, ultimately, in the aqueous environments of the cellular systems). It can be concluded that very few such data exist today, despite the fact that good macro- and micro-calorimetric techniques are available (the same as those used for model compounds). Do the thermodynamic researchers in the drug field presently concentrate too much on the properties of

the host molecules in drug binding reactions and neglect the important thermodynamic changes for the drug itself?

4. Drug–cell interactions

In microcalorimetric measurements on living cellular material it is normally the rate of heat production as a function of time which is measured. In some cases such experiments are performed as strict thermochemical experiments where the main aim is to determine material–energy balances or to obtain other fundamental physiological information. However, in most microcalorimetric work conducted on cellular material the instruments have merely been used as general "process monitors" giving records of the biological activity in terms of the heat production rate.

4.1. Microorganisms

To a large extent microcalorimetric measurements on microbial systems – bacteria, yeast and mycoplasma – have been conducted with flow instruments [1,2]. The cell suspension is then pumped from a storage (growth) vessel to which the drug can be added. The cell suspension leaving the calorimetric vessel can be analyzed continuously or stepwise for metabolites. Flow calorimetric experiments with microorganisms are in most cases easy to perform as the heat production rate is high and, except for yeast cells, there is usually no problem with the flow of the cell suspension. The experimenter must take care to avoid oxygen starvation in the calorimetric vessel when the experiment is intended to take place under aerobic conditions [2,48].

Many microcalorimetric investigations have been reported on the growth and metabolism of microorganisms (see e.g. [49–52]) and a significant part of them have been concerned with the effect of drugs; see e.g. [3,53,54]. Investigations have largely been on a phenomenological and qualitative level, but in some investigations calorimetric data for drug action on microorganisms have been expressed in quantitative terms; see in particular the work by Takahashi [54], Beezer [3] and their coworkers. For example, the Beezer group has described relationships between drug structure and drug activity on microorganisms.

4.2. Animal (human) cells

Microorganisms are easier to handle in a calorimeter than animal cells which are more fragile and generally stickier. However, microcalorimetric techniques for work with animal cells and tissue pieces are now well developed. Cells can be in suspension or be adhered to a surface. A stirred batch vessel of the insertion type, equipped with an injection device for reagents (drugs) should preferably be used [1,2]. Such vessels can also be operated as perfusion vessels and may be equipped with electrodes (pH, oxygen) [55]. Tissue pieces can be well exposed to a medium perfusing through the vessel by placing them in, for example, a rotating cage [1,2]. However, most reports on animal cells and tissue pieces have so far been conducted by use of static vessels where the cellular material

usually will sediment during the experiment. The physiological conditions are then poorly defined. Most microcalorimetric work on animal cellular systems have been conducted on human cells, largely on blood cells. As may be expected many investigations in this field have had a direction towards applications in medicine, including pharmacology. Many studies have been made on cellular material from patients.

Experiments are usually not conducted under strictly sterile conditions (except for measurements with cultured tissue cells) and media are therefore often supplied with antibiotics in order to suppress microbial growth during the measurements. Calorimetric control experiments with some antibiotics show marginal or no effect on the metabolism of a few cell types of human origin [56,57].

There are many reports on investigations of the action of drugs and related substances on cells of human origin, for example the effect of xanthine derivatives on thrombocytes [58], phorbol-12-myristate-13-acetate on granulocytes [59], anthraline and related compounds on skin cells [60], insulin and norepinephrine on adipocytes [61], β-blockers on fiber bundles from muscle fibers [62] and monoclonal antibodies on cultured melanoma cells [63].

Chemotherapeutic treatment of tumors is usually not preceded by any predictive tests of the drugs despite the fact that it is well known that tumor cells may be resistant to certain antitumor drugs. Isothermal microcalorimetry has been thought of as a potential analytical tool for such (in vitro) tests and model experiments using cultured T-lymphoma cells (CCRF-SEM) have been conducted with interesting results [64–67]. Work with a drug resistant subline of CCRF-SEM has also been performed [67].

It is concluded that much microcalorimetric development work and applications have been made in areas of interest for the characterization of the effects of drugs on animal cells. However, it is surprising to note that at the present time only a handful of groups in the world seem to be actively engaged in this field. The pharmaceutical industry has as yet not shown any interest in this area.

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