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Review

Pharmaceutical Applications of Microcalorimetry

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General principles and applications of microcalorimetry are reviewed. Microcalorimetry is useful in the study of physical, chemical, and biological drug interactions. The sensitivity of the present instrumentation is approximately 0.1 μW. With this high sensitivity, additional applications have been developed, including the interactions of drugs with food, lymphoma cells, microorganisms, blood, excipients, and cyclodextrin. A recent application of microcalorimetry is the measurement of degradation rates of drugs.

KEY WORDS: drug stability; degradation rate; calorimetry; drug interactions; excipient interactions; microcalorimetry.

I. INTRODUCTION

Calorimetry or microcalorimetry is the measurement of the heat evolved or adsorbed by a chemical or physical process. It serves to study the rate and extent of reactions, since the heat evolved or adsorbed is proportional to the rate of the process. Biological processes that involve physical and/or chemical reactions can also be studied by microcalorimetry.

Microcalorimetry, as a non-specific technique, has both limitations and advantages. Non-specificity can result in the measurement of the wrong physical/chemical interaction, but it can also be used to study many different interactions that may be too complex to be studied by a single specific technique.

Microcalorimetry has been used to study the interactions of drugs with food, lymphoma cells, microorganisms, blood, excipients, and cyclodextrin. One of the earliest uses of microcalorimetry was for the study of the biological interactions of drugs. The effect of drugs on the growth rate of microorganisms can be determined by measuring the heat output before and after the addition of the drug.

More recently, microcalorimetry has been used to measure degradation rates of drugs as solids or in solution. Such applications were initiated by Suurkuusk and Wadso in 1982 outlining a new system of microcalorimeters (1). The system is based on the use of semiconducting "Peltier elements", through which heat flows to a surrounding heatsink. This newest generation of microcalorimeters can measure heat flows as low as 0.1 μW.

II. INSTRUMENTATION

Several types of calorimeters have been used to study drug stability, vis. Tronac Model 350 RA, Hart Scientific Model 7708, and the 2277 Thermal Activity Monitor (TAM) (Thermometric AB, Sweden) (formerly distributed by LKB). Frequently, these calorimeters are referred to as microcalorimeters because of their high sensitivity (0.1 μW). The calorimeter that is probably best suited for studying drug stability is the 2277 Thermal Activity Monitor. It has the greatest sensitivity and in this author's opinion the best design. The 2277 Thermal Activity Monitor has been previously described in detail by Suurkuusk and Wadso (1).

For static experiments, in which no mixing of reactants or drugs is required, most workers used either flame sealed glass ampoules or stainless steel containers with a screw cap and Teflon seal. Glass ampoules with rubber stoppers could lead to spurious heat outputs caused by relaxation of the rubber stoppers. Matching of the sample and standard containers (type and age) and letting the stoppers relax for a period of at least 24 hrs prior to calorimetric measurement eliminated the spurious heat outputs. The quantity of solid drug or solutions in the sample ampoules was generally 1–3 g. Angberg et al. have evaluated the applicability of the 2277 Thermal Activity Monitor to pharmaceutical stability studies (2,3,4), with testing of thermal stability and noise characteristics.

For biological applications involving the mixing of drugs with microorganisms or blood cells, flow microcalorimeters are typically used. Flow microcalorimeters allow for the stirring of the measuring cell and addition of reactants or gasses from outside the cell.

III. APPLICATIONS

A. Drug stability studies

Measuring the degradation rate of a drug candidate at an early stage is important for its further development. For rapid determination of drug shelf-life, accelerated stability testing is usually required. The degradation rate of a drug

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and the formulated product can be significantly different. The addition of excipients to a drug to produce the formulated product may significantly increase the degradation rate. Thus, stability needs to be determined for the drug as well as the formulated product. Excipient compatibility studies are performed to help formulate the most stable dosage form. Liquid dosage forms generally have much greater degradation rates than solid dosage forms due to their increased rate of hydrolysis.

Bulk drug degradation rates >0.5%/yr at 25°C may have a detrimental impact upon the development of the drug candidate. Chromatographic determination of the degradation rate of a relatively stable drug candidate would be too costly and time consuming, since it may take one or more years for enough degradation to occur at 25°C to be accurately quantified by a stability indicating assay. Conventionally, the degradation rate of a drug candidate is determined by storage at several elevated temperatures (to accelerate the degradation rate) and measurement of the degradation products or non-degraded fraction at selected temperatures and time intervals. The Arrhenius equation, which assumes that the activation energy is independent of temperature, is used to calculate the degradation rate at 25°C by extrapolation from high temperature data.

For techniques which use elevated temperatures, the degradation mechanisms(s) must be similar over the temperature range of the study and at 25°C. A non-linear Arrhenius plot may indicate a change in mechanism(s), making the extrapolation to a 25°C degradation rate inaccurate. Hydrates or drugs having unbound water are more prone to water loss at elevated temperatures. Thus, the degradation mechanism at 25°C and elevated temperatures may be different. These limitations necessitate long-term stability tests at 25°C to confirm the the rate predicted from elevated temperature data (5,6).

Previous workers have used isothermal differential scanning calorimetry (DSC) rate-time curves to determine Arrhenius parameters and kinetics of chemical reactions (7,8). This requires time-rate data to be collected at several temperatures and over several reaction half-lives to predict the kinetic model. In 1983, Pical first described the use of microcalorimetry for the stability testing of pharmaceutical systems (9). In this short application note on the 2277 Thermal Activity Monitor, Pical showed the correlation between the heat output (exothermic) of some pharmaceutical systems with a degradation rate previously determined by another method. In several cases the degradation rate was not proportional to the heat output. The pharmaceutical systems studied included, 10% aspirin in $NaHCO_3$, solid drugs formulated with dextran or mannitol, and Cephalothin with 0.3 – 2.0% H_2O. The two types of applications of microcalorimetry to pharmaceuticals he thought to be particularly promising were:

1) Preliminary screening of formulations for a potential product to select the most stable formulation(s) for further study by conventional techniques;

2) Preliminary evaluation of the relative stability of raw material as a function of polymorphic form or method of manufacture.

Other workers have studied autocatalytic and first order reactions using microcalorimetry (10–12). Autocatalytic reactions generally have an induction period which lasts from minutes to years depending on the material and the concentration of reactants which induce degradation. After the induction period, the rate rapidly increases to its maximum. It then decreases until the reaction is complete. For drugs that degrade by an autocatalytic mechanism, the useful shelf-life is usually equal to the length of the induction period (12).

Theoretical treatment of drug stability data

The theoretical treatment of kinetic data is generally much easier for degradation reactions in solutions than in solids. Reactions in solution are usually governed by zero, first, or second order rate equations with straightforward data analysis. Conversely, solid-state reactions can be very complex with the kinetics being governed by fractional or autocatalytic rate laws. Several reviews have been written on the complex kinetics and mechanisms governing solid-state degradation reactions (13–15).

Ng (16) has shown that in general the rate of reaction may be expressed by Eq. 1

$$d\alpha/dt = k\alpha^{1-x}(1-\alpha_t)^{1-y} \quad (1)$$

where α is the fraction of the reaction which has occurred at time t, x and y are constants characteristic of the reaction mechanism, and k is the rate constant. A zero order rate law is obtained when $x = y = 1$ and a first order rate law is obtained when $x = 1$ and $y = 0$. Fractional values of x and y are obtained for autocatalytic reactions (11). For a zero order reaction

$$d\alpha/dt = k \quad (2)$$

The rate of heat production, q, can be directly measured using microcalorimetry.

$$q = -\Delta H\beta D_0(d\alpha/dt) \quad (3)$$

where q is the rate of heat output, D_0 is the initial amount of drug present, ΔH is the enthalpy change for the reaction, and β is the reactive portion of the sample. Substituting Eq. 2 into Eq. 3 we obtain

$$q = -\Delta H\beta D_0 k \quad (4)$$

If $-\Delta H$ and β are not a function of temperature over the range of the experiment, then the rate constant, k, is directly proportional to the initial rate of heat output, q_0. Thus,

$$q_0 = Ck \quad (5)$$

The relationship between the rate constant and temperature, is given by the Arrhenius equation.

$$k = Ae^{-Ea/RT} \quad (6)$$

Where, A is the pre-exponential factor, E_a is the activation energy, R is the gas constant, and T is the absolute temperature. Substituting Eq. (5) into Eq. (6) and expressing the result in logarithmic form results in Eq. (7).

$$\ln q_0 = \ln C - E_a/(RT) \quad (7)$$

If q_0 is measured at several temperatures, the slope of a plot of $\ln q_0$ vs. 1/T will be equal to $-E_a/R$; assuming that a zero-order rate law adequately describes the kinetics during

the initial portion of the reaction. Koenigbauer et al. used HPLC in combination with microcalorimetry to predict the degradation rate at 25°C using Eq. (8).

$$k_1 = k_2/\{\exp\{E_a(T_2 - T_1)/[(T_2T_1)R]\}\} \quad (8)$$

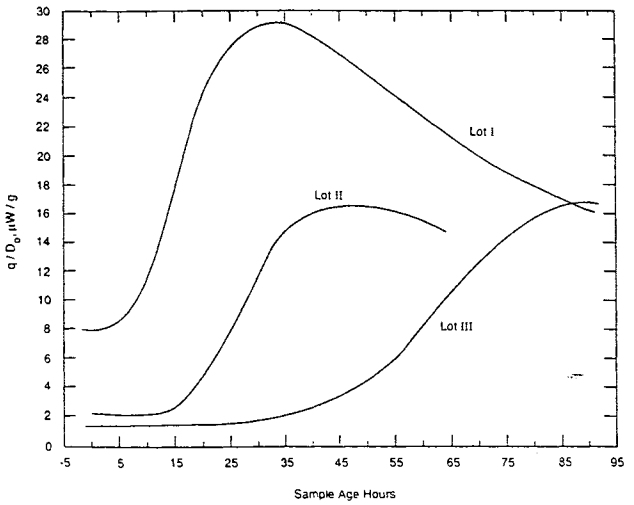
Where E_a is the activation energy determined by isothermal calorimetry, k_2 is the degradation rate determined by HPLC of a sample that has been stored at temperature T_2, and k_1 is the degradation rate at 25°C (T_1) (6). Koenigbauer stated that the estimation of the value of E_a is the most critical parameter when extrapolating to the degradation rate at 25°C from elevated temperature data. The E_a determined from elevated temperature data may actually be the weighted mean of several activation energies from simultaneously occurring reactions in the solid state.

Experimental results

Three research groups were simultaneously using microcalorimetry to evaluate drug stability (2,10,17). Angberg was first to publish in 1988 (2). Angberg studied the degradation kinetics of acetylsalicylic acid in aqueous solutions at pH 1.1 by measuring the heat flow from 30 to 50°C. The degradation reaction obeyed a pseudo-first order rate law. They calculated the value of the activation energy to be 71.9 kJ/mol by plotting the rate constants obtained at each temperature in Arrhenius fashion. The apparent rate constant at 25°C was 8.4×10^{-3} h^{-1}, obtained by extrapolation from higher temperature data. The activation energy obtained by the microcalorimetric method agreed with the literature values determined by isothermal and non-isothermal spectroscopy.

Hansen et al. used isothermal calorimetry to determine the decomposition mechanism of Lovastatin, a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol lowering agent (10,12). They measured the rate of heat production for several different lots of Lovastatin as a function of temperature and time. Different lots of Lovastatin were found to degrade at different rates and the lengths of their induction periods varied (figure 1). Hansen et al. showed Lovastatin degraded by an autocatalytic mechanism in the presence of O_2. The concentration of the induction reaction products increased linearly with time, conforming to pseudo-zero order kinetics. By comparing different lots of Lovastatin, they concluded that a single measurement of q/D_0 at a known sample age can be used to predict the relative total degradation different lots of the same material will ultimately undergo. Thus, if age, temperature history, kinetics, and the degradation reaction of two lots are the same, then q/D_0 will be directly proportional to the amount of drug that will degrade. They noted the caveat that different lots of the same material may undergo different degradation reactions or have different rate laws governing their degradation, thus invalidating the predicted amount of degradation.

Pikal and Dellerman have used isothermal calorimetry to correlate the decomposition rates of several cephalosporins in solid and aqueous solution states (17). They found that for crystalline solids and amorphous samples of low and moderate moisture content, the mean amount of heat evolved per unit of decomposition is relatively independent



1. Calorimetric data on three different lots of Lovastatin derivative at 50°C (reference 10).

of temperature, water content, and polymorphic form. They concluded the decomposition rates of amorphous cephalosporins increase with increasing water content in a highly non-linear fashion and the stability of a series of crystalline pseudo-polymorphs is quantitatively related to their heat of crystallization.

Angberg et al. later extended their work on the hydrolysis of acetylsalicylic acid in aqueous solutions and determined the rate constants at 40°C and 50°C over the pH range of 1.9–7.5. The heat flow at 4 hrs and the total heat produced between 2–4 hrs were found to give a more precise and accurate estimate of the degradation rate than experimentally determined rate constants. The time necessary to measure the degradation rate was also reduced.

Oliyai and Lindenbaum used microcalorimetry to study the stability of ampicillin in aqueous solutions as a function of concentration of ampicillin, pH, and temperature (5). Decomposition by hydrolysis was pseudo-first order. The observed rate constants were found to agree with published values. The mechanism for degradation was pH dependent (with maximum stability at pH 4) and the molal enthalpy change of the reaction varied with pH.

Koenigbauer et al. used microcalorimetry and HPLC at several elevated temperatures to calculate the activation energy of decomposition reactions of several relatively stable pharmaceutical compounds in the solid state (6). The compounds studied were phenytoin, digoxin, tetracycline, diltiazem, and several proprietary ZENECA compounds (Table I). The activation energy, determined by microcalorimetry, and a degradation rate, determined at a single elevated temperature by HPLC, were used to calculate the degradation rate at 25°C. The degradation reactions were found to obey a zero order rate law. The activation energies and degradation rates at 25°C determined by HPLC and isothermal calorimetry were compared and found to agree in most cases. Disagreement was caused by inaccuracies in the HPLC data. Activation energies determined by microcalorimetry were more precise than those determined by HPLC assay of samples stored at elevated temperatures.

Tan et al. also used microcalorimetry and HPLC to

TABLE I
Activation energies and degradation rates determined by isothermal calorimetry and HPLC for the compounds used in this study (reference 6)

COMPOUND	ACTIVATION ENERGY (E_a), Kcal/mole		DEGRADATION RATE (k_1) at 25°C, %/year	
TECHNIQUE	Calorimetry	HPLC	Calorimetry[a]	HPLC
ZENECA #1 HCl	30.0 ± 1.6[b]	19.9 ± 2.9	0.012	0.088
ZENECA #1 MESYLATE	23.6 ± 3.0	12.4 ± 3.3	0.022	0.17
ZENECA #2	21.9 ± 0.7	21.4 ± 1.7	0.21	0.20[c]
ZENECA #3	28.1 ± 1.2	34.4 ± 10.0	0.0022	0.00044
ZENECA #4 ACETONE RECRYSTALLIZED	18.2 ± 1.2	ND[d]	0.0006	ND
ZENECA #4 THF RECRYSTALLIZED	23.6 ± 1.2	ND	0.0006	ND
ZENECA #4 FORMULATED WITH Na_2CO_3	29.5 ± 0.7	ND	0.0006	ND
TETRACYCLINE	29.4 ± 2.7	25.7 ± 2.8	0.0011	0.0024
DIGOXIN	23.2 ± 0.8	33.3 ± 4.2	0.040	0.00068
PHENYTOIN	19.0 ± 1.4	ND	0.0006	ND
TRIAMTERENE	20.6 ± 1.3	ND	0.0006	ND
DILTIAZEM	24.8 ± 0.8	ND	0.0006	ND
THEOPHYLLINE	17.9 ± 1.0	ND	0.0006	ND

a. Calculated using Eq. (8).
b. The ± values represent 1 SD.
c. The determination of the HPLC degradation rate at 25°C for compound #2 utilized samples stored at 25°C and assayed over a 6-month period.
d. Not determined. The degradation rate was too slow to measure by HPLC.

study the solid-state stability of 13-*cis*-retinoic acid and all-*trans*-retinoic acid (11). In air, the decomposition of 13-*cis*-retinoic acid is autocatalytic, while all-*trans*-retinoic acid was found to be zero order. The retinoic acids were stable in a nitrogen atmosphere and only underwent a physical change.

In summary, the applicability of microcalorimetry to the determination of room-temperature degradation rates and activation energies for zero-order, pseudo-first order, and autocatalytic reactions in both solutions and solids has been demonstrated. Microcalorimetry has been used to study the kinetics and degradation rates of a variety of compounds. The results obtained by microcalorimetry were found to agree with literature values and in several cases were found to be more precise. Microcalorimetry in conjunction HPLC can reduce the time and errors in predicting solid-state degradation rates. Although microcalorimetry is not presently used extensively by the pharmaceutical industry to determine stability, its use will likely increase as additional researchers realize its utility.

B. Microorganism—drug interactions

Since growing microorganisms produce heat, microcalorimetry can be useful for characterizing the effect of drugs on their growth. The effects of various drugs on the growth rate may be determined by comparing the power-time curves of bacteria before and after addition of drugs. Several reviews on the use of microcalorimetry to study the interaction of various drugs with microorganisms have been written (18–21). Rodriguez-Tebar et al. used a microcalorimetric titration technique to study the effects of vancomycin and ristocetin (glycopeptide antibiotics) against Gram-positive bacteria (22). They determined the changes in Gibbs energies, enthalpies, entropies and heat capacities for the antibiotic-bacteria binding reactions.

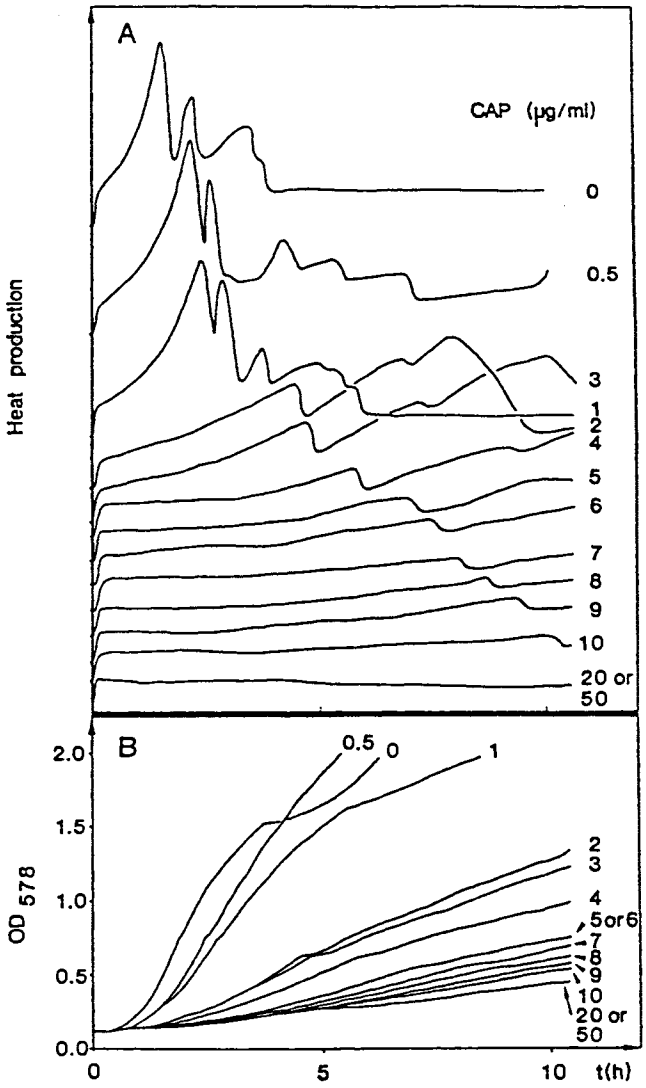
Allerberger and Dierich used microcalorimetry to study the effects of disinfectants on bacterial metabolism by measuring the thermal output of the bacteria, which corresponds to metabolism and bacterial growth (23). The results obtained by microcalorimetry agreed with those of traditional turbidity measurements.

Kruger and Giesbrecht used flow microcalorimetry and photometric mass determination of staphylococci in suspension to access the effects of chloramphenicol on metabolic activity (figure 2) (24). The intensity, extent, and efficiency of bacterial metabolism were distinctly affected by chloramphenicol. Microcalorimetry was an expedient means of determining the effectiveness of drugs.

Bunker and James studied the effects of platinum group metal complexes on the growth of Klensiella aerogenes and staphylococcus aureus using microcalorimetry (25). Several other groups have also used microcalorimetry to study the effects of drugs on bacterial growth (26,27).

C. Food—drug interactions

The effect of food on the dissolution rate of tetracycline hydrochloride was studied using microcalorimetry and compared with the rate obtained by the USP dissolution method (28,29). The influence of calcium on the dissolution rate was found to be negligible using the USP dissolution method. An interaction was observed using microcalorimetry between tetracycline and calcium, milk, and Ensure during the dissolution process. The first-order release processes was dependent upon the type of dissolution fluid. Fluids with the

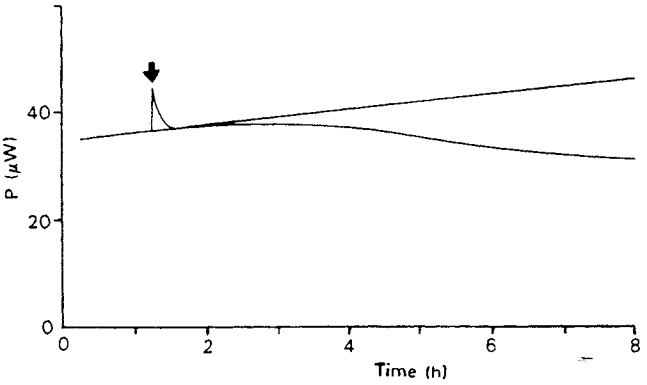


2. Effect of different doses of chloramphenicol on heat production (arbitrary units, panel A) and on optical density (panel B) of S. aureus SG 511 Berlin growing in bactopeptone broth. The heat production curves have separate origins on the Y axis and are drawn one beneath the other, to improve the clarity of the illustration. The antibiotic was added at t = 0 just before inoculation of the staphylococci (reference 24).

highest lipid content had the greatest effect on dissolution rates.

D. Lymphoma cell—drug interactions

Microcalorimetry has been used to determine the effect of drugs on the growth of T-lymphoma cells. Wadso et al. studied the effect of methotrexate, in the concentration range of 0.02–2.00 μM, on cultured T-lymphoma cells using a stirred microcalorimetric vessel. Calorimetric results obtained over a period of 8 hours (figure 3) showed the utility of the technique for determining the growth inhibitory effects of drugs (30). Wadso et al. studied the effects of the drugs Ara-C, cisplatin, vinblastine, chlorambucil, prednimustine, dimethyl sulfoxide, penicillin/streptomycin, gentamicin, and amphotericin B and pH on the growth of T-lymphoma cells



3. Power-time curve for a sample of T-lymphoma cells where MTX (methotrexate) is injected (P_{MTX}) and for a reference sample (P_{ref}) run in parallel. The addition of MTX to a final concentration of 0.18 μM was made at the time indicated by the arrow (reference 30).

using microcalorimetry (31,32,33). Microcalorimetry in contrast to conventional techniques, was found to be particularly valuable in detecting unknown or unexpected phenomena occurring in the cells.

E. Blood—drug interactions

The antibiotic gentamicin is widely used in the treatment of gram-negative infections, with the most frequent side-effects being nephrotoxicity and ototoxicity. Nassberger and Monti used microcalorimetry to measure the increase in heat production of red blood cells (granulocytes) when incubated with gentamicin at concentrations above the therapeutic level (34). There was no increase in the heat production rate of lymphocytes at any gentamicin concentration.

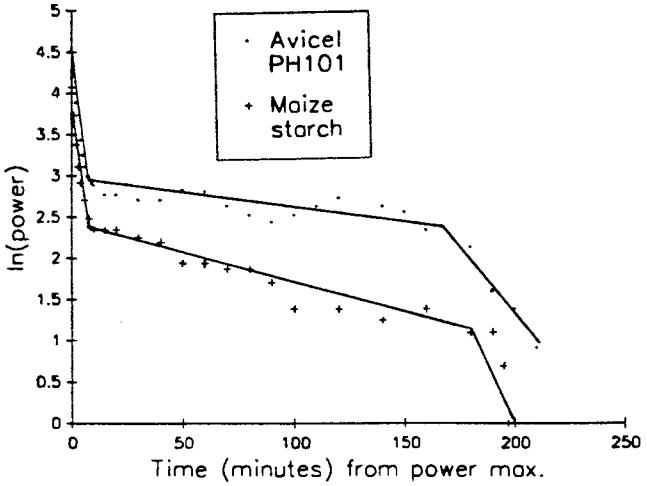
The interactions of drugs with albumin have been shown to affect their distribution, metabolism, elimination, and pharmacological action. Hardee et al. used flow microcalorimetry to predict the competition of two drugs for a single binding site (35). Their technique had significant advantages over spectroscopic techniques which directly determine the thermodynamic parameters at physiological concentrations of albumin.

Aki and Yamamoto designed and built a differential flow microcalorimeter, having resolution and sensitivity similar to that of the 2277 TAM, to study the hemolytic effect and binding of several drugs to human erythrocytes (36,37). Binding was found to be an important factor in hemolysis. The different hemolytic actions of cationic and anionic drugs was related to their binding mechanism and the degree to which they bound.

F. Water—excipient interactions

Microcalorimetry has been used to study the interaction of water vapor with starch, microcrystalline cellulose, and lactose powder. Beezer et al. studied the kinetics of the water uptake by maize and potato starch, and Avicel (PH-101, CL-611, and RC-581) (figure 4) (38,39). The sorption process was triphasic in nature, with the potato starch sorbing more than the other two starches.

Angberg et al. used microcalorimetry to study the crys-



4. Ln displacement (power) as a function of time, showing three sequential apparent first-order processes. Stage 1 from 0 to 10 min, stage 2 from 10 to about 160 min, and stage 3 from about 160 min until the end (reference 38).

tallographic changes of anhydrous lactose powder after water uptake (4). They developed a new approach for continuous microcalorimetric measurements in abundant water vapor and concluded the uptake of water vapor by powders is a very complicated process (40). The enthalpy change due to the bonding of the water of hydration to roller-dried β-lactose was calculated to be approximately 16 kJ/mol at 25°C. The microcalorimeter detected the uptake of water after 1 day of storage at 58% RH and after 112 days of storage at 94% RH. A standard DSC instrument could not discriminate a change in the heat of dehydration and thus could not detect the process.

G. Excipient—drug interactions

Fubini et al. used microcalorimetry to study the effect of menadione and prednisone on the stability of the microemulsions water/isopropylmyristate/2(ethyl-hexyl)sulphosuccinate sodium salt and water/lecithin/isopropylmyristate/butanol. The stability of the microemulsions was not changed by the presence of drugs. Microcalorimetry was effective in detecting weak interactions not easily detectable by other techniques (41).

The interaction of dopexamine with several drugs and excipients was studied by Pereira-Rosario et al. using flow and batch microcalorimetry (42,43). Dopexamine had a significant interaction with heparin, with the interaction being the strongest in parenterals which contained glucose. The interaction did not occur in normal saline, indicating the reaction was ionic in nature and was reduced or eliminated by cations in the solutions.

H. Cyclodextrin—drug interactions

Cyclodextrin-drug interactions can be studied by microcalorimetry because the chemical or physical processes accompanying these interactions are usually endothermic or exothermic in nature. Buckton and Beezer have written a recent review on the applications of microcalorimetry in physical pharmacy (44).

Ueda et al. used flow microcalorimetry to study the β-cyclodextrin complexes of cinnarizine, a poorly soluble drug which increases cerebral blood flow, and determined stability constant values for their interaction (45). The values agreed very well with those obtained by the solubility method.

Tong et al. examined the interaction of α, β, or γ-cyclodextrin with 13 amine drugs having diphenylmethyl functionalities using titration microcalorimetry (46). The standard free energy (ΔG°) decreased upon formation of the inclusion complex. The standard enthalpy of change (ΔH°) and the standard entropy of change (ΔS°) also decreased, except for cases in which the stoichiometry of the inclusion complex was 1:2 (drug:β-cyclodextrin). Van der Waals interactions dominated the stabilization of the α- and β-cyclodextrin complexes. The correlation between drug structure and the stability constants, thermodynamics, and inclusion geometries were also examined.

I. Conclusion

Microcalorimetry has been used to study the interactions of drugs with food, lymphoma cells, microorganisms, blood, excipients, and cyclodextrins. Although its utility has been well established, there are many applications that have not been explored. The recent advances in the technique are partly due to improvements in sensitivity.

Microcalorimetry has not received widespread usage by the pharmaceutical industry; possibly due to the nonspecificity of the technique, instrument costs, advances in other techniques (spectroscopy and chromatography), and regulatory considerations. The greatest promise of microcalorimetry lies in measurements of very complex systems where a single specific technique would not be applicable.

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