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Review Commentary

Microcalorimetry as a tool in mechanistic studies of organic reactions

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ABSTRACT: Isothermal microcalorimetry is a powerful tool in studies of organic reactions in solution. Both reaction kinetics and thermodynamic parameters at a single temperature are conveniently derived by this technique. Some applications of batch microcalorimetry in reaction mechanistic studies are reviewed. These include kinetic and thermodynamic studies of aromatization reactions of biologically interesting oxides, hydrates of aromatic hydrocarbons and studies of complex reactions and equilibria. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: microcalorimetry; kinetics; reaction mechanism

INTRODUCTION

Microcalorimetry of isothermal and non-isothermal types are sensitive and non-destructive techniques for measuring heats of reaction. Both techniques can in principle be employed also for determining reaction rate constants and equilibrium constants. However, isothermal microcalorimetry is generally preferred for kinetic studies. Certainly, there are methods for determining reaction kinetic constants for non-isothermal systems but they require rather complex calculations to account for the temperature dependence of these parameters.

The detection limit of modern isothermal microcalorimeters approaches 1 nW. Most of them are of the heat conduction type, but calorimeters of the power compensation type are also commercially available. In a power compensation microcalorimeter, the heat effect of the reaction is balanced by a cooling power (usually Peltier effect cooling). Some properties and current uses of isothermal microcalorimetry in science and industry have recently been discussed.[1] The design and properties of isothermal microcalorimeters have been reviewed.[2–4]

A disadvantage of microcalorimetry in chemistry applications is the inherent sensitivity to systematic errors. Not only competing reactions forming by-products with large reaction enthalpies but also non-chemical processes such as vaporization, for example, may seriously disturb the reaction study. However, the non-specificity can be an advantage in other applications. Thus, microcalorimetry can be used as a general 'process monitor.[1] For example, it is employed for studies of the slow decomposition of pharmaceutical products under normal storage conditions.

Microcalorimetric methods are used in only a few physical organic chemistry laboratories, but are employed more frequently in biochemistry and cell biology laboratories where the major application is measuring binding constants. A few kinetic studies of enzyme-catalyzed reactions have been reported.[5–7] An advantage of employing microcalorimetry in such applications is that native, unmodified substrates can be used. This paper will briefly discuss some applications of isothermal microcalorimetry as a tool in elucidation of reaction mechanisms of organic reactions in solution.

THE BASIC PRINCIPLES OF HEAT CONDUCTION CALORIMETRY

Principle

Most chemical processes involve heat production or absorbtion. This heat is in the calorimeter (completely) exchanged with a surrounding heat sink, usually an aluminum block maintained at constant temperature. The sensor for the heat flow (thermal power) is generally a set of thermopiles positioned between the sample and the heat sink (Fig. 1). The voltage from the detector thermopiles is proportional to the temperature difference between the reaction vessel and the heat sink. Thus, the detector response is directly proportional to the heat flow.

The temperature difference between the reaction

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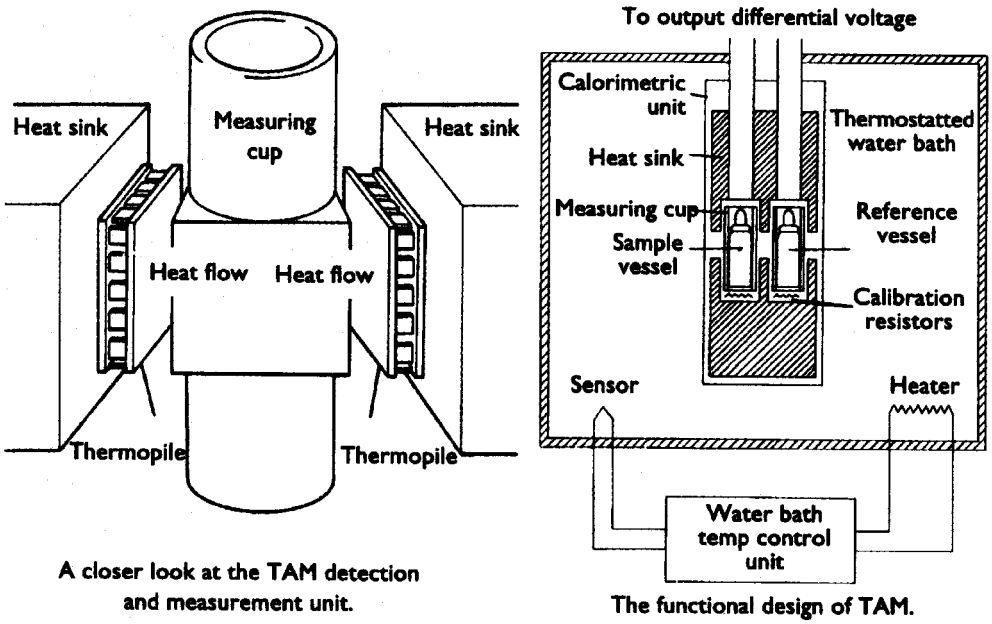


Figure 1. An example of the functional design of an isothermal heat-flow microcalorimeter (courtesy of Thermometric AB)

vessel and the heat sink is small owing to a relatively large thermal conductance. Accordingly, this type of instrument can be considered as *isothermal*. This review will discuss *batch* microcalorimetry in which a closed vessel is charged with the reaction solution. However, an application of flow microcalorimetry will be discussed briefly at the end of the paper.

Kinetic and thermodynamic expressions

The heat output of a reaction (dQ_R/dt, defined as a negative quantity for an exothermic reaction) is a function of the standard reaction enthalpy (ΔH°) and the kinetics of the reaction. For a simple first-order process with rate constant k, the heat flow of the reaction is described by

$$dQ_R/dt = \Delta H^\circ kn \quad (1)$$

where n is the amount (mol) of substrate undergoing reaction at time t. For a first-order reaction we have $n - n_\infty = (n_0 - n_\infty)\exp(-kt)$, where n_0 and n_∞ denote the amount of substrate at the start of the reaction and at time infinity, respectively. If $n_\infty = 0$, substitution into Eqn. (1) yields

$$dQ_R/dt = \Delta H^\circ kn_0 \exp(-kt) \quad (2)$$

The rate of the heat conduction from the reaction cell to the detector, where magnitude is dependent on the construction of the instrument, is usually expressed as a first-order cooling constant κ (s^{-1}; cf. Newton's law of cooling) or, alternatively, as the corresponding half time ($t_{1/2}$) or as a time constant ($\tau = 1/\kappa$). The measured heat flow at time t ($dQ/dt = P$) is a function of κ and the heat flow of the reaction:

$$dQ_R/dt = P + (1/\kappa)dP/dt \quad (3)$$

The solution of this differential equation, after substitution with Eqn. (2), is

$$P = \Delta H^\circ kn_0\kappa\{1/(\kappa - k)[\exp(-kt) - \exp(-\kappa t)]\} \quad (4)$$

$$P \approx \Delta H^\circ kn_0\kappa/(\kappa - k)\exp(-kt) \quad (4a)$$

$$P \approx \Delta H^\circ kn_0 \exp(-kt) \quad (4b)$$

To facilitate the study of fast reactions, the cooling constant should be as large as possible. Thus, Eqns ((4a)) and ((4b)) are good approximations of Eqn. (4) for $t \gg \kappa^{-1}$ and if $\kappa \gg k$.

It is sometimes possible to obtain useful kinetic data even for relatively fast reactions by correcting for the inherent slow heat flow of the calorimeter by use of Eqn. (4). The heat flow of mixing is not negligible in this case and should be included as an extra term in Eqn. (4). Thus, the time dependence of the measured heat flow caused by mixing (P_M) can be simulated as a first-order reaction with a very large rate constant (k_M), where M is the heat of mixing (in J):

$$P_M = Mk_M\kappa\{1/(\kappa - k_M)[\exp(-k_M t) - \exp(-\kappa t)]\} \quad (5)$$

The complete expression for the measured heat flow is

then

$$P = \Delta H^{\circ} k n_0 \kappa \{1/(\kappa - k)[\exp(-kt) - \exp(-\kappa t)]\} + M k_{\mathrm{M}} \kappa \{1/(\kappa - k_{\mathrm{M}})[\exp(-k_{\mathrm{M}} t) - \exp(-\kappa t)]\} \quad (6)$$

The cooling constant of the calorimeter is easily determined with high accuracy by heating the reaction cell of the calorimeter with a calibration current for a short period, and then following the decay in heat flow as a function of time.

Calibration of the detector response with a calibration current is a very convenient method. However, the most accurate method for calibrating the response from the detector is to run a standard reaction in the reaction vessel. Such a standard reaction is the hydrolysis of triacetin in pyrimidine–acetic acid buffer.

Some applications of microcalorimetry for measuring rate constants, equilibrium constants and thermodynamic parameters will be discussed in the following sections.

APPLICATION: AROMATIZATION OF NAPHTHALENE HYDRATES

Recently, this laboratory reported on a mechanistic study of the acid-catalyzed solvolysis of naphthalene hydrates and the corresponding ethers in highly aqueous media (Scheme 1).[8] It was of interest to study these reactions since they show very high elimination-to-substitution ratios, in contrast to most other solvolytic reactions through relatively stable, solvent-equilibrated carbocations. Such carbocations generally yield mainly substitution products in nucleophilic solvents.[9–12] The report clearly showed that the predominant elimination is attributable to the large thermodynamic stability of the naphthalene product and not to an unusually slow reaction of the carbocation with nucleophiles.[8]

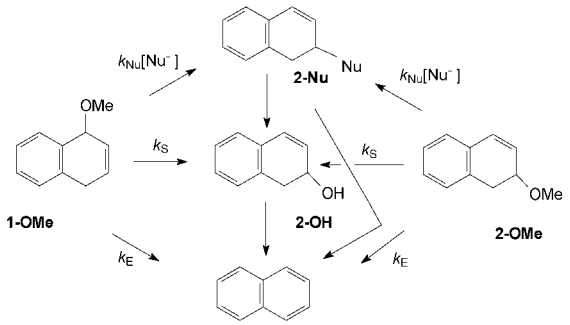
The studied reactions are also of biochemical interest since aromatic hydrates such as naphthalene hydrates are thought to be intermediates in the mammalian metabolism of aromatic hydrocarbons, along with arene oxides and dihydrodiols.[13]

As part of this study, microcalorimetry was used to measure the reaction enthalpies and kinetics of the aromatization of the three isomeric naphthalene hydrates **1-OH, 2-OH** and **3-OH** (Scheme 2). The reactions were run at 25 °C in highly aqueous media with acetonitrile (50 vol.%) or glycerol (25 vol.%) as the cosolvent. The reactions are specific acid catalyzed with a second-order rate constant k_{H}:

$$\mathbf{R-OH} + \mathrm{H^+} \rightleftharpoons \mathbf{R-OH_2}^+ \longrightarrow \text{naphthalene}$$

$$k_{\mathrm{obs}} = k_{\mathrm{H}}[\mathrm{H^+}] \quad (7)$$

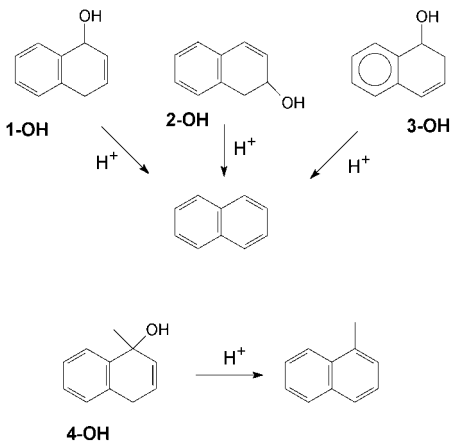
Thus, it was easy to adjust the reaction rate by adjusting



Scheme 1

the pH of the medium so that the decay in heat flow of the reaction could be conveniently studied with the microcalorimetric equipment. The apparatus (Thermometric Thermal Activity Monitor) was equipped with two calorimetric units, each one of twin type. The right part of Fig. 1 shows such a calorimetric unit having one measuring cell and one reference cell. One of the purposes of the reference vessel is to cancel out possible heat flows which do not come from the reaction. The vessels were glass vials (3 ml) equipped with gas-tight TFE-coated septa.

In a typical experiment, the reaction and reference vials were filled with 2.5 ml of a premixed reaction solution of aqueous buffer and organic cosolvent. After this step, the reaction vial and the reference vial were charged with 20 µl of substrate in acetonitrile and pure acetonitrile, respectively. After sealing with the septa, the vials were slowly introduced into the compartments of the instrument for about 15 min of prethermostating. They were then lowered further down into the detection chambers. The recording of the first-order heat decay was started after a total thermal equilibration time of 30–60 min. The reactions were followed for at least 10 half-lives. Most of the runs in aqueous acetonitrile gave good rate constants and enthalpies, but the results of a few were discarded. The bad quality of these experiments was



Scheme 2

Table 1. Second-order rate constants and reaction enthalpies for the acid-catalyzed dehydration reactions of the naphthalene hydrates **1-OH, 2-OH** and **3-OH** and the substituted naphthalene hydrate **4-OH** at 25 °C

Substrate[a]	k_H (l mol^{-1} s^{-1})	P_0[b] (μW)	$\Delta H°$ (kcal mol^{-1})
1-OH (0.8 mM)	258 (254)[c]	20	−23.7 ± 0.4 (12 runs)
2-OH (0.6 mM)	1.22 (1.22)[c]	12	−18.4 ± 0.2 (6 runs)
3-OH (0.4 mM)	0.30	22	−22.7 ± 0.7 (9 runs)
4-OH (0.4 mM)	3192	28	−21.7 ± 0.9 (8 runs)

[a] Solvent: 25 vol.% glycerol in water.
[b] Heat effect at time zero (extrapolated).
[c] Rate constants measured by UV spectrophotometry.

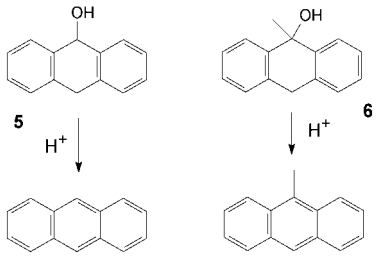
presumably caused by loose septa. Even better reproducibility was obtained with aqueous glycerol, probably owing to the low vapor pressure of this solvent.

The microcalorimeter was statically calibrated after a kinetic run with an electrical heat pulse with the vials left in the instrument. The half-time ($\ln 2/\kappa$) of the instrument was found to be 140 s, i.e. no correction was necessary for calculation of the rate constants of the heat decays. The rate constants were obtained from data of heat flow versus time [Eqn. (4b)] by means of a non-linear regression program. Very good first-order rate constants (k_{obs}) were measured. These agree very well with those measured by the UV spectrophotometric technique (Table 1).

The extrapolated heat flow at time zero (P_0) was used for calculation of the reaction enthalpy ($\Delta H°$) according to the following equation [derived from Eqn. ((4b))]:

$$P_0 \approx \Delta H° k_{obs} n_0 \quad (8)$$

where n_0 is the amount of substrate (mol) in the reaction vial. Alternatively, the reaction enthalpy can be derived by employing the integrated expression of Eqn. ((4b)), i.e. by measuring the area under the heat flow versus time graph.



Scheme 3

The alcohol **4-OH** is even more reactive and the reaction enthalpy was found to be 2 kcal mol^{-1} (1 kcal = 4.184 kJ) smaller than that of **1-OH** (Scheme 2 and Table 1).[14] The carbocation intermediate formed in the solvolysis of the corresponding methyl ether does not give any water adduct as was observed with **1-OMe**. However, trapping by azide ion was observed.

The aromatization reactions of the alcohols **5** and **6** (Scheme 3) have also been studied by the same microcalorimetric technique.[15] The results are shown in Table 2. One of the runs with substrate **5** is shown in Fig. 2. These alcohols are less soluble in aqueous glycerol and were therefore studied at low concentrations in acetonitrile–water mixtures.

The reactions of the substrates **5** and **6** have also been run on another type of isothermal microcalorimeter, a Microcal VP ITC, which is of the power compensation type primarily designed for titration applications. The cooling constant (κ) is much larger for this instrument and the specified response half-time is as short as 10 s. Hence much faster reactions can be studied. The calorimeter has a fixed reaction cell of 1.4 ml, and a reference cell of the same size (Fig. 3). The experimental procedure was as follows. The two cells were filled with aqueous buffer–acetonitrile mixture (1.4 ml in each). After prethermostating for 30 min, the substrate solution was added (40 μl). Owing to the large cooling constant, it was possible to start collecting data of the reaction after a relatively short period (9 min). A representative run with

Table 2. Second-order rate constants and reaction enthalpies for the acid-catalyzed dehydration reactions of the hydrates **5** and **6** in 50 vol.% acetonitrile in water at 25 °C

Substrate	Instrument	k_H (l mol^{-1} s^{-1})	P_0[a] (μW)	$\Delta H°$ (kcal mol^{-1})
5 (1.0 mM)	Thermometric	0.316[b]	11	−12.3 ± 0.8 (9 runs)
5 (0.7 mM)	Microcal	0.535[c]	82	−12.5 (1 run)
6[c] (1.0 mM)	Thermometric	0.181[b] (0.185)[b,d]	1.4	−10.2 ± 1.0 (4 runs)

[a] Heat effect at time zero (extrapolated).
[b] 2-methoxyacetate buffer (0.1 M), pH 3.58 (before mixing).
[c] Phthalate buffer (0.1 M), pH 2.53 (before mixing).
[d] Rate constant measured by UV spectrophotometry.

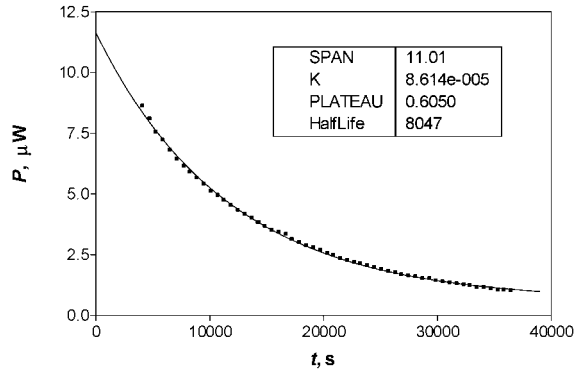


Figure 2. Heat flow (squares) of the acid-catalyzed aromatization reaction of **5** as a function of time followed with the Thermometric instrument (Table 2). The line is computer simulated

the alcohol **5** is shown in Fig. 4 and Table 2, which could be compared with the microcalorimeter experiment using the Thermometric instrument (Fig. 2). The reproducibility has not been tested with the Microcal instrument.

A problem with microcalorimetric studies of reactions in mixtures of water and an organic solvent is the huge heat of mixing. The addition of the substrate dissolved in acetonitrile cools the reaction solution significantly and it takes a long time to reach thermal equilibrium. A large cooling constant κ shortens the equilibration time.

APPLICATION: HOMOAROMATICITY OF BENZENE OXIDE

We have recently addressed the theoretically interesting question of the possible homoaromaticity of benzene

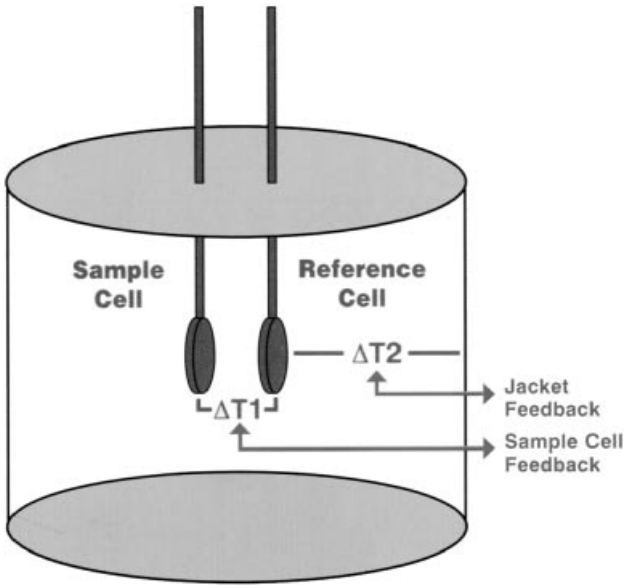


Figure 3. An example of the functional design of an isothermal power compensation microcalorimeter (courtesy of Microcal Inc.)

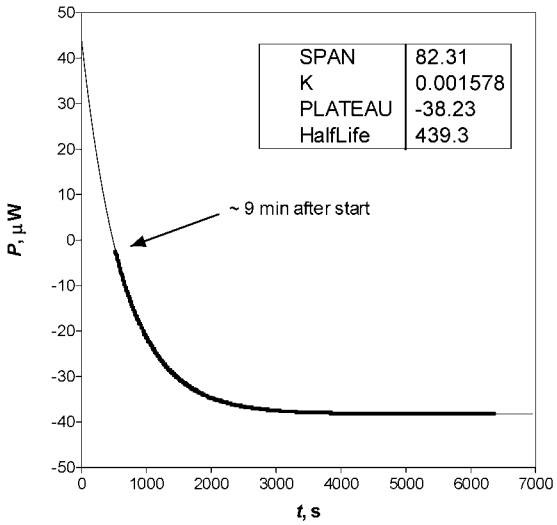


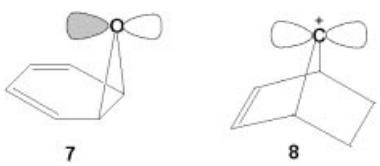
Figure 4. Heat flow (thick line) of the acid-catalyzed aromatization reaction of **5** as a function of time followed with the Microcal instrument (Table 2). The thin line is computer simulated

oxide **7** (Scheme 4).[16] More O'Ferrall and coworkers have suggested that the low reactivity of benzene oxide, which is about 10^7 times less reactive than expected (corresponding to $\sim$10 kcal mol^{-1}), is due to an unusual stability caused by homoaromatization.[17] Such stabilization has generally been considered unimportant for neutral molecules but there is a consensus about the importance of homoaromaticity in stabilizing carbocations, e.g. 7-norbornenyl cation **8** (Scheme 4). Moreover, arene oxides are, like arene hydrates, reactive compounds of considerable biochemical interest.[13,18–20] For example, the oxidation of aromatic hydrocarbons to phenols in biological systems has been proposed to occur via arene oxides.

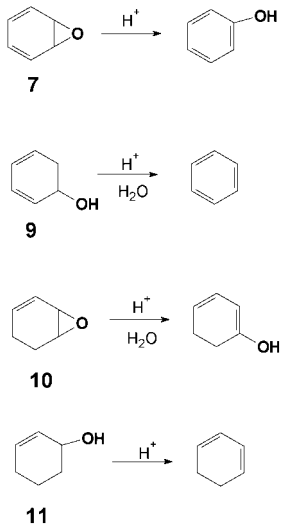
Reaction heats and rate constants for the reactions of **7**, **9** and **10** (Scheme 5) were measured with the Thermometric instrument. The enthalpy of the reaction of **11** has been reported previously, $\Delta H^\circ \approx 0$.[21] The reactions are all specific acid catalyzed but the reactions of the oxides also proceed by a non-catalyzed pathway:

$$k_{obs} = k_0 + k_H[H^+] \quad (9)$$

Large amounts of heat were produced and the heat decay was easy to follow by heat-flow microcalorimetry. Figure 5 shows a typical run with benzene oxide (**7**). The



Scheme 4



Scheme 5

Table 3. Rate constants and heats of reaction for the dehydration of **7**, **9** and **10** at 25 °C

Substrate[a]	$10^6\ k_{obs}$ (s^{-1})	P_0[b] (μW)	ΔH^0 (kcal mol^{-1})	ΔH_{calc}[h] (kcal mol^{-1})
7	535 ± 15[c]	169	-57.0 ± 1.9	−49.8
9	155 ± 2[d]	44	-38.7 ± 0.8	−32.1
10	173 ± 4[e]	13	-23.7 ± 0.6 (-24.9)[f]	−19.7
11			∼0[g]	+3.4

[a] Substrate concentration ca 0.5 mM.
[b] Heat flow at time zero (extrapolated).
[c] 25 vol.% glycerol in water, 0.1 M phosphate buffer, pH 7.00 (before mixing).
[d] 50 vol.% glycerol in water, 0.1 M acetate buffer, pH 5.57 (before mixing).
[e] 50 vol.% acetonitrile in water, 0.1 M phosphate buffer, pH 5.99 (before mixing).
[f] After correction for dehydration.[16]
[g] Ref. 21.
[h] Calculated enthalpies using B3LYP/6–311 + G**//B3LYP/6–31G* and AM1/SM2 for solvation.

instrument used was the heat-flow microcalorimeter (Thermometric) described above using a 3 ml glass vial as the reaction vessel. The reaction heat of benzene oxide is as large as -57.0 kcal mol^{-1} (Table 3). The other reaction heats were used as references. Thus, the aromatization energies of benzene oxide (**7**) and benzene hydrate (**9**) were estimated as $\Delta\Delta H^\circ = -57.0 - (-24.9) = -32.1$ and $\Delta\Delta H^\circ = -38.7 - 0 = -38.7$ kcal mol^{-1}, respectively. Thus, benzene oxide seems to be particular stable, and the homoaromaticity could be estimated as $\Delta\Delta\Delta H^\circ = -32.1 - (-38.7) = 6.6$ kcal mol^{-1}. This strongly supports the proposal that benzene oxide is homoaromatic. However, the stabilization of 6.6 kcal mol^{-1} does not fully account for the low reactivity of benzene oxide. The remainder (∼4 kcal mol^{-1}) was suggested to originate from the unusually high energy of the carbocation-forming transition state. The proposed mechanisms of the two aromatization reactions are shown in Scheme 6.

Calculated enthalpies using the B3LYP hybrid functional agree with those measured (Table 3). The difference in reaction enthalpies between the aromatization reactions of **7** and **9** was measured as $\Delta\Delta H^\circ = -18.3$ kcal mol^{-1} and calculated as -17.7 kcal mol^{-1} (in water, Table 3). Similarly, the differences for the other two reactions, those of **10** and **11** were found to be $\Delta\Delta H^\circ = -24.9$ and -23.1 kcal mol^{-1}, respectively.

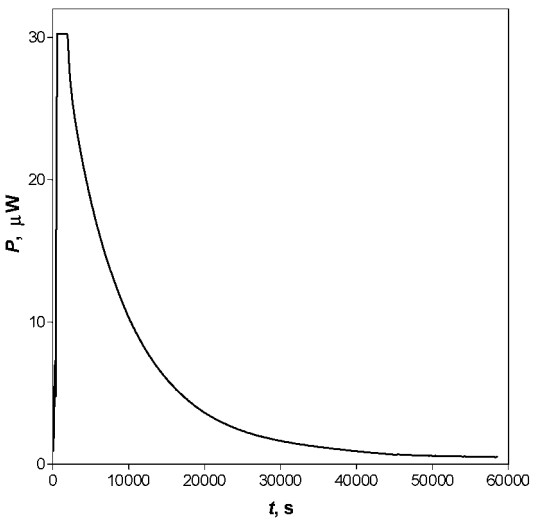
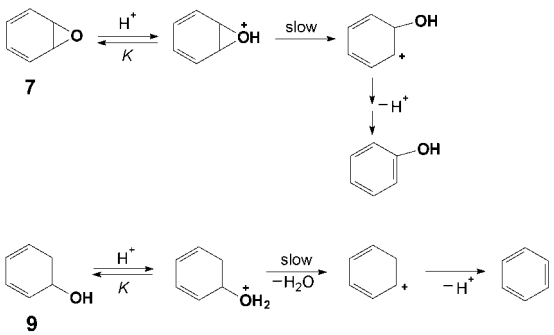


Figure 5. Heat flow of the aromatization reaction of **7** as a function of time followed with the Thermometric instrument (Table 2)

APPLICATION: A KINETIC STUDY OF A COMPLEX REACTION SYSTEM

Microcalorimetry has also been used in the study of a complex reaction system. This study was a part of an investigation of the role of tightly hydrogen-bonded carbanions in reactions in solution, e.g. base-promoted elimination reactions. The reaction conditions for these base-promoted elimination reactions, which compete with rearrangement reactions (Scheme 7),[22] were metha-



Scheme 6

nol as solvent at 20 °C with the tertiary amine quinuclidine as base. The reactions were run with a large excess of base, i.e. under pseudo-first-order conditions.

For example, when starting from pure **12** the concentrations (in mole fraction) vary as[23]

$$x_{12} = a\exp(-m_1 t) + (1-a)\exp(-m_2 t) \quad (10)$$

$$x_{13} = b\exp(-m_1 t) - b\exp(-m_2 t) \quad (11)$$

where

$$a = (k_{12} + k_{13} - m_2)/(m_1 - m_2)$$

$$b = k_{12}/(m_2 - m_1)$$

$$m_1 = [(k_{12} + k_{13} + k_{21} + k_{23})^2/4 - k_{12}k_{23} - (k_{21} + k_{23})k_{13}]^{1/2} + 1/2(k_{12} + k_{13} + k_{21} + k_{23})$$

$$m_2 = -[(k_{12} + k_{13} + k_{21} + k_{23})^2/4 - k_{12}k_{23} - (k_{21} + k_{23})k_{13}]^{1/2} + 1/2(k_{12} + k_{13} + k_{21} + k_{23})$$

When several reactions produce heat in the reaction cell, the measured heat flow P is the sum of the heat flows from all reactions. The contribution from reaction i can be written as [cf. Eqn. (4)]

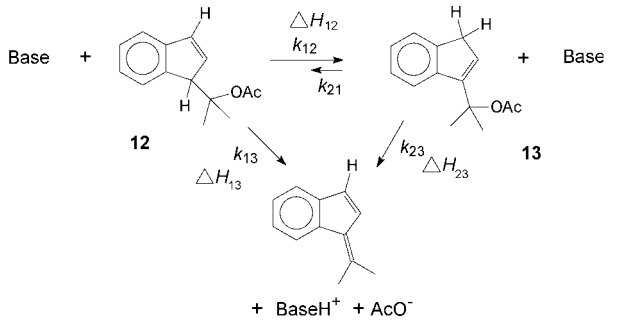
$$P_i = \Delta H_i^\circ k_i n_{i,0}\kappa\{1/(\kappa - k_i)[\exp(-k_i t) - \exp(-\kappa_i t)]\} \quad (12)$$

For example, the expression for the total heat flow when starting from pure substrate **12** is described by Eqn. (13),[23] which is obtained by combining Eqns (10) and (11) with Eqn. (12):

$$\begin{aligned} P = {} & (k_{12}\Delta H_{12}^\circ + k_{13}\Delta H_{13}^\circ)n_{12,0}\kappa\{(a/(\kappa - m_1))[\exp(-m_1 t) - \exp(-\kappa t)] \\ & + (1-a)/(\kappa - m_2)[\exp(-m_2 t) - \exp(-\kappa t)]\} \\ & + (-k_{21}\Delta H_{12}^\circ + k_{23}\Delta H_{23}^\circ)n_0\kappa \\ & \{b/(\kappa - m_1)[\exp(-m_1 t) - \exp(-\kappa t)] \\ & - b/(\kappa - m_2)[\exp(-m_2 t) - \exp(-\kappa t)]\} \end{aligned} \quad (13)$$

where a, b, m_1 and m_2 are functions of the four separate rate constants (Scheme 7).

The isothermal calorimeter was an older one, a batch instrument of twin type (LKB 2107-111) equipped with two two-compartment (2.5 + 4.5 ml) gold cells. The experimental procedure was as follows. Prethermostated solutions of base (4 ml) and substrate (2 ml) in methanol were introduced with syringes into the two compartments of the reaction cell. The exact volumes were determined by weighing the syringes before and after delivering the solutions. One of the two compartments of the reference cell was filled with base solution and the other with pure solvent. After thermal equilibration for 30 min, the solutions were mixed by rotation of the calorimeter, which was placed in a large air thermostat bath. The heat flow was registered as a function of time with a recorder. The cooling constant of the calorimeter was measured after the reactions were finished with the cells still filled with the solutions by using a small calibration current.



Scheme 7

The rate constants and reaction heats were derived by a simple computer simulation technique based upon the thermokinetic data obtained from runs starting with pure **12** and **13**, respectively. The results are shown in Table 4. The kinetic data agreed very well with those obtained with a sampling–quench high-performance liquid chromatographic procedure.

APPLICATION: MEASURING THE THERMODYNAMIC PARAMETERS OF A COMPLEXATION EQUILIBRIUM

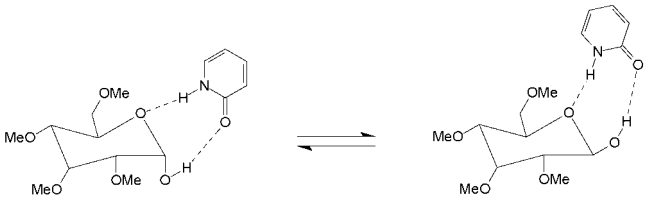
Microcalorimetry is a valuable tool for measuring equilibrium constants over a wide range. An advantage with this method is that only measurements at one temperature are required for determination of ΔG°, ΔH°, and ΔS°. This technique was used by Engdahl *et al.* for studies of the dimerization equilibrium of 2-pyridinone and of the complexation of the two isomeric forms of 2,3,4,6-tetra-*O*-methyl-D-glucopyranose (α-TMG and β-TMG) by 2-pyridinone in benzene at 25 °C.[24] These complexation equilibria were of interest since they provide important information concerning the bifunctional catalysis of 2-pyridinone on the epimerization reaction (Scheme 8).

The apparatus was the same as used for the study of the base-promoted elimination described above. Figure 6 shows four experiments which were run with different concentrations of the reactants. The intersection of the plotted lines gives ΔH° and the equilibrium constant of the complexation.

Table 4. Rate constants and heats of reaction for the reactions of **12** and **13** at 20 °C[a]

$10^4(k_{12}+k_{13})$[a] (l mol^{-1} s^{-1})	$10^4\,k_{12}$ (l mol^{-1} s^{-1})	$10^4\,k_{13}$ (l mol^{-1} s^{-1})	$10^4\,k_{23}$ (l mol^{-1} s^{-1})	ΔH^0_{12} (kcal mol^{-1})	ΔH^0_{13} (kcal mol^{-1})	ΔH^0_{23} (kcal mol^{-1})
26.4 ± 1.3	14.67 ± 0.82	11.73 ± 0.82	5.92 ± 0.12	-1.9 ± 0.6	-12.1 ± 0.4	-10.2 ± 0.3
(25.16 ± 0.55)[b]	(14.22 ± 0.55)[b]	(10.94 ± 0.55)[b]	(5.72 ± 0.11)[b]			

[a] Substrate concentration ca 3 mM.
[b] Obtained by another technique, viz. sampling–quench HPLC procedure.



Scheme 8

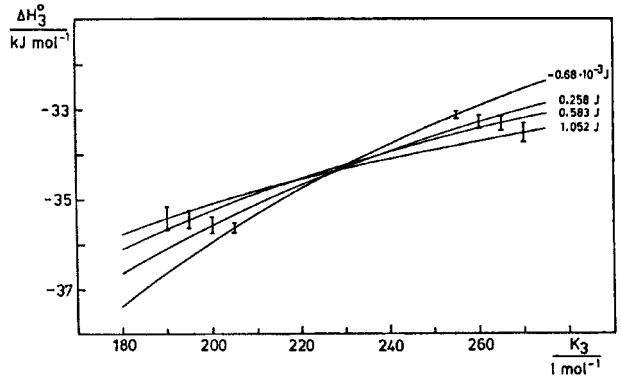


Figure 6. Reaction heat–equilibrium constant relations of four microcalorimetric runs (reproduced with permission of the authors).[24]

FLOW MICROCALORIMETRY

The kinetics of relatively fast reactions can be studied by isothermal flow microcalorimetry. Using the alkaline hydrolysis of ethyl acetate as a model reaction, the applicability of this technique to measure bimolecular reaction rates with half-lives down to about 5 s has been demonstrated.[25] The instrument was run in flow-mix mode, i.e. the two flows of reagents were mixed within the calorimeter cell.

SUMMARY

This review has briefly discussed the use of microcalorimetry as a tool in mechanistic studies of organic reactions. Microcalorimetry was shown to be a sensitive tool for measuring rate constants and reaction heats and also the thermodynamic parameters for equilibrium reactions. Only small amounts of pure material are required. The kinetics of fast reactions can be studied by flow methods, but the sensitivity of these methods is generally lower.

This review has pointed out the potential of microcalorimetry for accurate measurements of kinetic data, enthalpies and other thermodynamic properties of chemical systems. Combined with conventional experimental methods used by the physical organic chemist, high-quality data could be obtained. Such data could also be utilized, for example, for calibrating *ab initio* and semiempirical calculation methods.

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