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(28) There are some indications that the quaternary ammonium ion effect in the generation of dichlorocarbene does not fit the pattern. First, vigorous stirring has been reported to be helpful[29] and interface effects have been suggested.[18] Second, benzyltriethylammonium chloride is an effective catalyst. And most significantly, tributylamine and other large tertiary amines have been found to work just as well as ammonium salts.[30] All these are in contrast to the results presented herein. We found no catalysis in our system by tributylamine, nor can permanganate be solubilized in benzene by tertiary amines.[21]

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Determination of Reaction Rate Parameters by Flow Microcalorimetry

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Abstract: Using the alkaline hydrolysis of ethyl acetate as a model reaction, the applicability of a flow microcalorimeter to measure bimolecular (or higher molecularity) reaction rates with half-times in the range of at least 5 sec to several hours is demonstrated. In addition, an experimental scheme is developed which allows the calorimetric determination of the enthalpy change of slow reactions. The precision and accuracy of both ΔH values and apparent rate constants is estimated to be better than ±2%.

The determination of reaction rate parameters is a cornerstone of chemistry and provides invaluable information about reaction mechanisms. In this communication it will be demonstrated that a flow microcalorimeter, whose usefulness for obtaining thermodynamic data is well established,[2-5] can be used to determine rates of reactions in solution that have half-lives between a few seconds and several hours. Since the production or absorption of heat is a ubiquitous property of chemical reactions, this instrument can be used to obtain both thermodynamic and kinetic information about a wide variety of chemical reactions.

Most thermal techniques previously used to determine reaction rates monitor the change in temperature[6-10] of the system as a function of time in an apparatus similar to the classical "adiabatic" calorimeter. A few methods which directly measure the rate of heat production have also been reported.[11-16] Methods of the former type require estimation of the time derivative of experimental data in order to obtain a value for the reaction velocity. Such an analytical process can significantly increase the error of the estimated reaction rate. Methods of the latter type have the advantage that the measured signal is time invariant and directly proportional to the average velocity of the reaction. The flow microcalorimetric technique to be described provides data of the second type and is similar, in principle, to the thermal method of Roughton and coworkers[17] and the stirred flow reactor described by Hammett and colleagues.[18]

The flow microcalorimeter used in this work is manufactured by LKB Instruments, Inc., based on an original design by Monk and Wadsö.[19] It can operate in two modes, one in which the rate of heat generation in a solution flowing through the calorimeter is measured (the flow-through mode) and one in which the rate of heat generation in a liquid system mixed within the calorimetric cell is measured (the mixing mode). Wadsö and coworkers[15,20] and Beezer and Tyrrell[14] have used the flow-through mode to monitor the velocity of slow reactions ($\tau_{1/2} > 10^3$ sec) initiated externally to the calorimeter. Beezer and Tyrrell[21] have also developed the theoretical relationships applicable to the special case of zero-order and pseudo-first-order reactions in both modes. However, the general characteristics of the calorimeter are such that, in principle, the reaction rate parameters can be determined for reactions of order ≥ 2 when this instrument is used in the mixing mode. Using the alkaline hydrolysis of ethyl acetate as a model reaction it will be demonstrated that the reaction rate, the reaction order, and the enthalpy change for liquid phase reactions having a half-life of 5 sec to 50 hr can be precisely determined with this technique.

Theoretical Section

Fast Reactions. The LKB flow microcalorimeter was originally designed to measure heats of essentially instantaneous reactions. Such reactions generate heat at the point of mixing which is dissipated throughout the calorimetric cell. Under constant flow conditions, a steady state temperature difference between the cell and the heat sink is established (ΔT_{hs}). This temperature difference generates the voltage signal of the calorimeter (V).

Under steady state conditions the rate of heat production within the calorimetric cell (W) is equal to the heat flux out of the cell. The total heat flux is composed of three components: that heat conducted to the heat sink via the thermal elements (W_t), that transferred via the air gap and tubing at the edges of the gold calorimetric cell (W_a), and that transported out of the cell by the flowing solution (W_s). From Newton's law of heat transfer it follows that

$$W_t = \alpha \Delta T_{\mathrm{hs}} \text{ and } W_a = \beta \Delta T_{\mathrm{hs}}$$

where α and β are proportionality constants. Assuming that the exiting solution is in thermal equilibrium with the body of the calorimetric cell it follows that

$$W_s = C_p \Delta T_{\mathrm{hs}} f$$

where C_p is the heat capacity of the solution per unit volume and f is the total flow rate through the calorimetric cell. Thus

$$W = (\alpha + \beta + C_p f)\Delta T_{\mathrm{hs}} = \epsilon V \qquad (1)$$

where ϵ is the proportionality factor, or calibration constant, relating the observed voltage to the total heat flux under steady state conditions. The calibration constant determined by introducing a known amount of electrical power into the calorimetric cell under constant flow conditions using a heater located near the point of mixing and measuring the steady state voltage is identical to that obtained by measuring the signal generated upon initiating a fast reaction of known enthalpy change (e.g., the dilution of sucrose). This demonstrates that thermal equilibration between the flowing solution and the body of the calorimetric cell is established for fast reactions.

Because of frictional heating, pressure effects, and the absence of *true* thermal equilibration between the heat sink and the solutions flowing *into* the calorimetric cell a small voltage is observed even when identical solutions are mixed in the calorimetric cell. Signals are therefore measured relative to a "solvent–solvent base line" in order to calculate the heat effect due to mixing of the reactants (W_m).

For fast reactions W_m is composed of the heats of dilution of the components of the two solutions (W_d) and the heat of reaction (W_r). Thus

$$W_r = W_m - W_d = f\Delta H \Delta[\mathrm{R}]$$

where f is the flow rate of the resulting solution, ΔH is the molar enthalpy change for the reaction as written, and $\Delta[\mathrm{R}]$ is the change in concentration of the limiting reactant R. The reaction velocity of extremely fast reactions cannot be determined, but ΔH or the change in concentration of reactants can be calculated if the other is known.

Slow Reactions. In contrast to fast reactions, a significant amount of reactants may remain unreacted when the solution undergoing a slow reaction exits the mixing cell. If thermal equilibration between the solution and the body of the calorimetric cell is achieved, the heat of reaction is proportional to the amount of product formed in the time the solution resides within the calorimeter cell. Assuming this to be the case

$$W_r = f\Delta H[\mathrm{P}]_f$$

It is this proportionality which makes the flow microcalorimeter potentially capable of measuring reaction rates.

The amount of product formed within the calorimetric cell, $[\mathrm{P}]_f$, is related to the residence time of the solution within the cell, τ. The residence time is proportional to the "effective volume", v, such that

$$v = \tau f$$

where τ is the residence time of a volume element in the calorimeter cell from the point of mixing to the point of exit.

Knowing the flow rate, the effective volume, and ΔH the average velocity of any reaction, $\bar{r}$, over the time interval τ can be calculated

$$\bar{r}\tau = W_r / f\Delta H$$

$\bar{r}$ can then be used to calculate the rate constant by solving the integral equation

$$\bar{r}\tau = k\int_0^{\tau}\left(\prod_i [\mathrm{R}_i]^{n_i}\right)\mathrm{d}t \qquad (2)$$

where R_i is the time-dependent concentration of reactant i and n_i is the order of the reaction with respect to that component. Thus it is, in principle, possible to determine the rate constant for any bimolecular or higher molecularity reaction using this technique.

Results

The potassium hydroxide catalyzed hydrolysis of ethyl acetate was chosen as a model reaction to demonstrate the applicability of a flow microcalorimeter to accurately determine reaction rates. This reaction

$$\mathrm{H_2O} + \mathrm{CH_3COOCH_2CH_3} + \mathrm{OH^-} \longrightarrow$$
$$\mathrm{CH_3COOH} + \mathrm{CH_3CH_2OH} + \mathrm{OH^-} \longrightarrow$$
$$\mathrm{CH_3CH_2OH} + \mathrm{CH_3COO^-} + \mathrm{H_2O}$$

is formally bimolecular in dilute aqueous solution such that

$$r = k_2[\mathrm{CH_3COOCH_2CH_3}][\mathrm{OH^-}]$$

It has previously been demonstrated that this reaction is well described as being first order with respect to each component over limited concentration ranges, although variation in the apparent second-order rate constant over wide ranges of reactant concentration has been observed.[22–26]

Under our reaction conditions the final products are the alcohol and ionized acid. Since the enthalpy changes for the first step of the reaction and subsequent ionization of acetic acid are small, the major portion of the observed heat change used to monitor the reaction is the result of the neutralization reaction $\mathrm{H^+} + \mathrm{OH^-} \rightarrow \mathrm{H_2O}$.

Demonstration of Kinetic Response and Determination of Reaction Order. For a first-order or pseudo-first-order reaction the integrated rate expression is

$$W_r = \Delta H f(1 - e^{-k_1\tau})[\mathrm{R}]_0$$

where $[\mathrm{R}]_0$ is the initial concentration of the reactant R and k_1 is the first-order rate constant. Thus the average rate and hence the signal for first-order reactions under constant flow rate conditions is a linear function of the variable reactant. Pseudo-first-order conditions for the hydrolysis of ethyl acetate were established by using a constant amount of excess KOH and varying the initial concentration of ethyl acetate in the reaction mixture. The results of one such experiment in which the degree of reaction during time τ was ~80% are shown in Figure 1. Least-squares analysis of the $\ln W_r$ versus $\ln [\mathrm{R}]_0$ representation of these data yielded a value of 1.002 ± 0.003 for the order of the reaction.

For higher order reaction schemes the relationship between the average rate and the initial concentration of reactants is more complex. However, if conditions can be established such that the rate of the reaction is essentially constant over the time τ (i.e., initial velocity conditions), then

$$W_r \simeq \Delta H v k\left(\prod_i [\mathrm{R}_i]_0^{\,n_i}\right)$$

and the average rate is equal to the initial rate of the reaction. Such conditions were established for the ethyl acetate

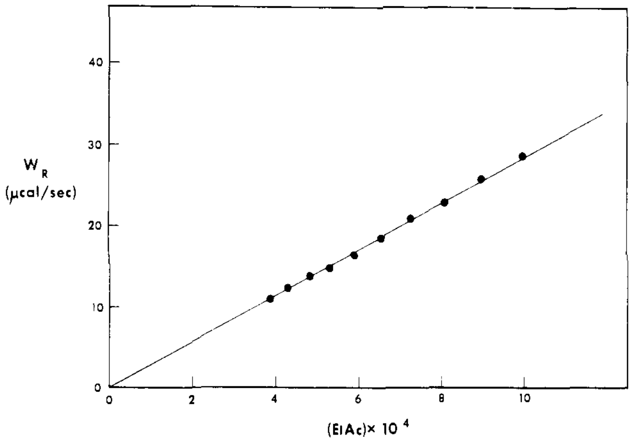

Figure 1. Heat effect, W_r, produced by the alkaline hydrolysis of ethyl acetate under pseudo-first-order conditions: [KOH] = 0.1 M, f = 2.73 μl/sec, the line is the calculated best least-squares fit to the data. See text for details.

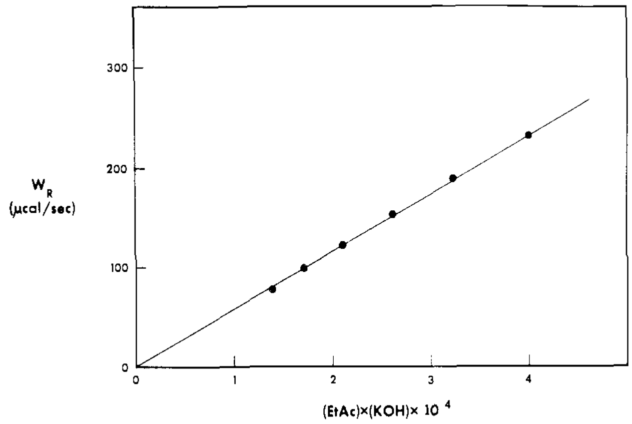

Figure 2. Heat effect, W_r, produced by the alkaline hydrolysis of ethyl acetate under conditions where the reaction rate remains constant during the calorimeter transit time, τ: [KOH] = [EtAc], f = 21.5 μl/sec, extent of reaction = 2-4%, the line is the calculated best least-squares fit to the data. See text for details.

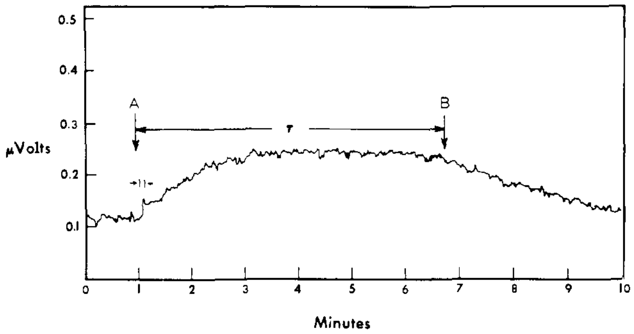

Figure 3. Signal produced by a small volume of ethyl acetate undergoing alkaline hydrolysis as it travels through the calorimeter mixing cell; small arrows indicate the size of the volume element (see text for details), A indicates mixing point, B indicates exit point, τ is the residence time of the volume element, [EtAc] = [KOH] = 0.005 M, f = 1.18 μl/sec. The signal-to-noise ratio is relatively large in this experiment because the size of the volume element undergoing the reaction (~15 μl) represents only 4% of the effective volume of the calorimetric cell. In all other experiments reported here the signal-to-noise ratio was at least 20 times greater than this.

reaction by maintaining $[\mathrm{OH}^-] = [\mathrm{EtAc}] < 0.03\ M$ and $f > 20$ μl/sec. Under these conditions the extent of reaction in the calorimetric cell was always less than 4%[27] and

$$W_r = \Delta H v k_2[\mathrm{EtAc}]_0[\mathrm{OH}^-]_0$$

The results of such an experiment are shown in Figure 2. Least-squares analysis of the ln W_r versus ln ($[\mathrm{OH}^-]$ [EtAc]) representation of these data yielded a value of 2.062 ± 0.003 for the apparent order of the reaction.

The results summarized in Figures 1 and 2 clearly demonstrate that the response of the flow microcalorimeter is directly proportional to the average rate of the reaction within the calorimetric cell, that the alkaline hydrolysis of ethyl acetate is well approximated as first order with respect to each of the components over a limited concentration range, and that the order of a reaction of any component can be determined if initial velocity conditions are maintained within the calorimetric cell for the time interval τ.

Although determination of reaction order requires only that the observed signal is proportional to the rate of the reaction, estimation of rate constants from flow microcalorimetric data requires knowledge of the flow rate, the effective volume or residence time, and the enthalpy change for the reaction. The flow rate is simply a function of the two pumps and was determined as described in the experimental section. The effective volume and the enthalpy change for reaction were determined in the following ways.

Effective Volume. The most direct method to determine the effective volume is to measure the time required for a small volume element to travel from the point of mixing to the point of exit at constant flow rate. This was accomplished by pumping together two reactants which react sufficiently slowly to satisfy initial velocity conditions. Initially only one of the reactants is pumped through the calorimeter. After a base-line signal has been established the second pump is turned on for a short period of time and then shut off. This procedure creates a small volume element of reacting solution within the calorimeter cell which during its travel through the cell generates heat at a constant rate and produces a measurable voltage. When this volume element exits the cell, the observed signal begins to return to its base-line value.

An example of performing this procedure with KOH and ethyl acetate is shown in Figure 3. At time zero only the KOH solution is flowing through the cell. At the point marked A the ethyl acetate pump was turned on for a small period of time indicated by the two vertical bars. The calorimetric signal began to increase until attaining a steady state value which was maintained until the small reacting volume element exited the cell at point B. The distance $\overline{AB}$ on the time axis is the residence time, τ, from which the effective volume of the calorimetric cell can be calculated. Several such experiments yielded an average value of v = 396 ± 4 μl. This estimate of v is consistent with a nominal value for the volume of the cell of 0.44 ml reported in the LKB application notes. The fact that the time constants for the rise of the signal at point A and the decay of the signal at point B are identical to the response time (~75 sec) of the instrument[28] using electrical power input indicates that effective mixing has been achieved and that the solution exits the cell at a well defined point in time.

Determination of the Reaction Enthalpy. The flow microcalorimeter was initially designed to measure enthalpy changes of very fast reactions. However, it is also possible to determine enthalpy changes for slow reactions by appropriate experimental design. A particularly convenient scheme is available for second-order reactions for which the integrated rate expression is

$$\frac{1}{W_r} = \frac{1}{\Delta H f[\mathrm{R}]_0} + \frac{1}{\Delta H f k_2 \tau [\mathrm{R}]_0^2} \qquad (3)$$

If the reaction is run at several different initial concentra-

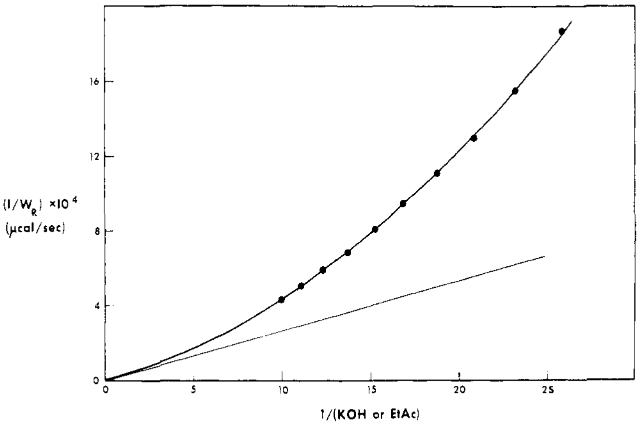

Figure 4. Heat effect, W_r, produced by the alkaline hydrolysis of ethyl acetate as a function of initial concentration when $[KOH]_0 = [EtAc]_0$ plotted in the form of eq 3: $f = 2.73$ µl/sec, the curved line is the calculated best least-squares fit to the data, and the straight line is the calculated linear term of eq 3.

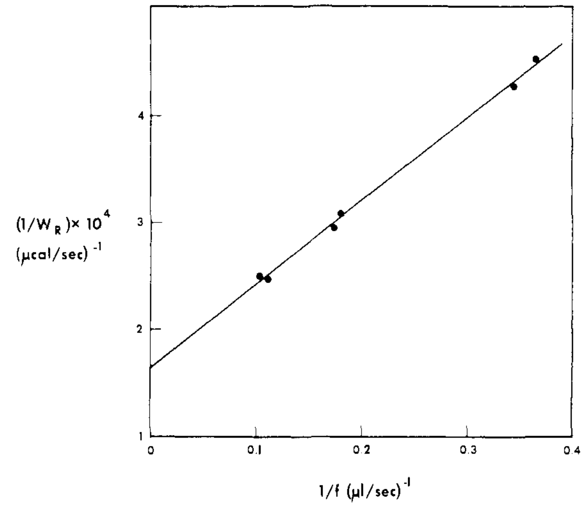

Figure 5. Heat effect, W_r, produced by the alkaline hydrolysis of ethyl acetate when $[KOH]_0 = [EtAc]_0 = 0.1\ M$ as a function of flow rate plotted in the form of eq 4; the line is the calculated best least-squares fit to the data.

tions of reactant at constant flow rate the data can be analyzed according to eq 3 to yield estimates of ΔH and k_2. Data obtained for the alkaline hydrolysis of ethyl acetate under constant flow rate conditions where $[EtAc]_0 = [OH^-]_0 = [R]_0$ are shown in Figure 4. Least-squares analysis of these data yielded values of $\Delta H = -13.37 \pm 0.30$ kcal/mol and $k_2 = 6.35 \pm 0.16\ \text{min}^{-1}$.

ΔH values can also be determined by measuring W_r under second-order conditions as a function of the flow rate. Substitution of f/v for τ^{-1} in eq 3 gives

$$\frac{1}{W_r} = \frac{1}{\Delta H[R]_0}(f^{-1}) + \frac{1}{\Delta H v k_2 [R]_0^2} \tag{4}$$

Data obtained under constant initial concentration of reactants as a function of flow rate are shown in Figure 5. Least-squares analysis of these data yielded values of $\Delta H = -12.95 \pm 0.20$ kcal/mol and $k_2 = 7.11 \pm 0.15\ \text{min}^{-1}$.

The most accurate way to determine ΔH is to force the reaction to completion within the calorimetric cell. This can be accomplished by having an excess of all reactants except one and performing the experiment at a sufficiently slow flow rate. This procedure under conditions where $[OH^-] = 0.44\ M$ and $[EtAc] = 0.011\ M$ yielded a value of $\Delta H = -12.95$ kcal/mol.

All three determinations of ΔH agree well with the values of −13,100 cal/mol found by Papoff and Zambonin[10] and −13,080 cal/mol by Becker and Spalink.[29] The value of −13,000 cal/mol has been used in all subsequent calculations.

Determination of Apparent Second-Order Rate Constant for the Alkaline Hydrolysis of Ethyl Acetate. It was demonstrated in the previous section that both the enthalpy change and rate constant for a second-order reaction can be determined from the variation in heat effect as a function of the initial concentration of reactants at constant flow rate or from the variation in the heat effect as a function of the flow rate at constant initial concentration of reactants.

In the case of the alkaline hydrolysis of ethyl acetate, however, it has been previously demonstrated that the apparent second-order rate constant increases with concentration.[22,23] Therefore, in order to assess the precision and accuracy of this method for determining reaction rates, the apparent second-order rate constant for the alkaline hydrolysis of ethyl acetate has been calculated for each experiment using an appropriate integral form of eq 2 for a second-order reaction. In the case where the acetate and hydroxide concentrations were equal, eq 5 was used to calculate k_2

$$k_2\tau = \frac{1}{[R]} - \frac{1}{[R]_0} \tag{5}$$

where $[R] = [R]_0 - W_r/\Delta H f$, the concentration of each reactant at time τ. In cases where $[OH^-] \neq [EtAc]$, k_2 was estimated by iterative stepwise numerical integration of eq 2 over the time interval $t = 0$ to $t = \tau$ assuming that the reaction was first order with respect to each reactant. This procedure was repeated until a value of k_2 was obtained which provided a calculated W_r in agreement with the experimental W_r. The accuracy of the integration procedure was determined to be better than 0.1%.

Estimates of the apparent second-order rate constant for the alkaline hydrolysis are listed in Table I over a limited concentration range at different flow rates. These results demonstrate that the calculated value of the rate constant is independent of the flow rate within the range of current experimental error. Similar estimates for k_2 obtained at several concentration ranges are summarized in Table II and compared with values of k_2 reported previously under similar experimental conditions. Good agreement of these values with those previously reported is observed. In addition, these results are consistent with previous observations that k_2 increases with reactant concentration.

Discussion

Using the alkaline hydrolysis of ethyl acetate as a model reaction it has been demonstrated that a flow microcalorimeter, based on the heat-leakage principle, can be used to measure reaction velocities which in turn can provide reliable values for reaction order, rate constant, and enthalpy change for the reaction. This application of the instrument required that thermal equilibrium between the reacting solution and the body of the calorimeter cell be at least approximately attained, and that the residence time of the solution in the calorimeter cell be well defined. The reported results establish that both requirements had been satisfied.

Thermal Equilibration. If temperature equilibration between the reactant solution and the calorimetric cell is not achieved

$$W = (\alpha + \beta + C_p f)\Delta T_{hs} + C_p f \delta T = \epsilon V + C_p f \delta T$$

where δT is the temperature difference between the exiting solution and the body of the calorimetric cell. If this situation existed for slow reactions, the observed signal would

Table I. The Calculated Second-Order Rate Constant for the Alkaline Hydrolysis of Ethyl Acetate in Aqueous Solution at 25° as a Function of Flow Rate When $[\mathrm{KOH}]_0 \cong [\mathrm{EtAc}]_0$

[EtAc or KOH],[a] M	f, µl/sec	k, min^{-1}[b]	N[c]
0.012–0.02	21.5	6.78 ± 0.19	6
0.017–0.05	9.07	6.92 ± 0.22	4
0.017–0.05	5.48	6.63 ± 0.21	4
0.038–0.06	2.73	6.67 ± 0.17	5

[a] Range of initial concentrations. [b] Second order rate constant ± range. [c] N = number of experiments.

not be directly proportional to the velocity of the reaction since δT would be expected to be a function of the reaction-rate profile within the calorimetric cell.

Wadsö and Monk have demonstrated that $C_p f \Delta T_{\mathrm{hs}}/W \sim 0.03$ for instantaneous reactions at a flow rate of 0.17 ml/min.[19] The variation of the electrical calibration constant, ϵ, over the range of flow rates used in this study was less than 10% relative to ϵ determined at zero flow rate. Thus a maximum systematic error of only 1% in the calculated average velocity of the reaction would be produced if $\delta T \leq 0.1\ \Delta T_{\mathrm{hs}}$. The following argument establishes that δT is in fact much smaller than ΔT_{hs}.

Under steady state conditions the rate of heat transfer from the flowing solution to the calorimetric cell, Q, will be approximately equal to the heat flux between the cell and the heat sink

$$Q = k_{\mathrm{hs}}\frac{A_{\mathrm{hs}}}{X_{\mathrm{hs}}}\Delta T_{\mathrm{hs}} = k_{\mathrm{s}}\frac{A_{\mathrm{s}}}{X_{\mathrm{s}}}\overline{\delta T} \geq k_{\mathrm{s}}\frac{A_{\mathrm{s}}}{X_{\mathrm{s}}}\delta T$$

and

$$\frac{\delta T}{\Delta T_{\mathrm{hs}}} \leq \left(\frac{k_{\mathrm{hs}}}{k_{\mathrm{s}}}\right)\left(\frac{A_{\mathrm{hs}}}{X_{\mathrm{hs}}}\right)\left(\frac{X_{\mathrm{s}}}{A_{\mathrm{s}}}\right)$$

δT is the average temperature difference between the calorimetric cell body and the flowing solution, A_{s} and A_{hs} are the contact surface areas between the calorimetric cell body and the solution and heat sink, respectively, X_{s} and X_{hs} are the thicknesses of the two contact interfaces, and k_{s} and k_{hs} are their respective thermal conductivities. Based upon the physical size of the tubing of the calorimetric cell and the thermal elements[30] used in the construction of this calorimeter it is estimated that

$$X_{\mathrm{s}}/A_{\mathrm{s}} < 10^{-3}\ \mathrm{cm}^{-1}$$

and

$$A_{\mathrm{hs}}/X_{\mathrm{hs}} \cong 80\ \mathrm{cm}$$

Since the thermal conductivity at a liquid–solid interface is substantially greater than that at a solid–solid interface it follows that

$$\delta T/\Delta T_{\mathrm{hs}} < k_{\mathrm{hs}}/10k_{\mathrm{s}} < 0.1 \qquad (6)$$

Several results establish that the inequality given in eq 6 is correct. First, the observed signal exhibits either first- or second-order behavior under the appropriate conditions. Second, the extrapolated value for ΔH is correct whether the reactant concentration or the flow rate is used as the independent variable. Third, the measured heat effect obtained in a "stopped flow" experiment was identical to within 5% of that obtained in a normal, continuous flow experiment.[31] The accuracy of this experiment is ±5% at best because of necessary extrapolation of the observed signal to zero time. Since the deviation between W_r and ϵV will be flow-rate dependent if $\delta T \neq 0$, the most convincing evidence that thermal equilibration was achieved is that the calculated rate constant for the alkaline hydrolysis is, within experimental error (±2%), independent of flow rate (see Table I). We thus conclude that thermal equilibration between the reactant solution and the body of the calorimetric cell is achieved and that *the measured heat effect is directly proportional to the extent of reaction within the calorimetric cell.*

Table II. The Calculated Second-Order Rate Constant for the Alkaline Hydrolysis of Ethyl Acetate in Aqueous Solution at 25° as a Function of Initial Concentration of Reactants When $[\mathrm{KOH}]_0 \cong [\mathrm{EtAc}]_0$

[EtAc or KOH],[a] M	k, min^{-1}[b]	N[c]	Lit value	Ref
0.01–0.03	6.74 ± 0.07	10	6.86	22
			6.69	25
			6.5	23
0.03–0.06	6.75 ± 0.05	9	6.76	23
			6.66	24
0.06–0.13	6.95 ± 0.06	14	7.22	22
			6.96	29
0.13–0.20	6.94 ± 0.05	8		

[a] Range of initial concentrations. [b] Second order rate constant ± standard error of mean. [c] N = number of experiments.

Accuracy. The inherent accuracy of the flow microcalorimeter is best demonstrated by the ΔH value obtained for the reaction which agrees to within ±1% with previous estimates. It is to be noted that data in Figures 4 and 5 were extrapolated to either infinite concentration of reactant or zero flow rate to yield values of ΔH. Under such conditions the reaction becomes instantaneous and hence independent of the residence time in the calorimeter.

Although ΔH values determined by the procedures described herein are accurate to at least ±1%, the accuracy of the estimates of k_2 may not be this good. At the flow rates used in this work the major inaccuracy is the result of error in the measurement of the effective volume. Insofar as the calculated second-order rate constants are in very good agreement with previously reported values, it does not appear that a significant error in v exists. We thus estimate that the reaction velocities reported are as accurate as they are precise (±2%).

It is to be noted that k_2 determined from analysis of the data in Figure 4 is significantly lower than that obtained from calculations based on each datum. The probable reason for this discrepancy (~5%) is that the alkaline hydrolysis of ethyl acetate is not a true second-order reaction and the assumed functional representation of the data (eq 3) is not strictly correct. Our results as well as those of others[22,23,26] suggest that this is, in fact, the case. Therefore, values of k_2 obtained from such an analysis are not a realistic indication of the accuracy of the method.

The keys to maintaining a high degree of accuracy in rate determinations using a flow microcalorimeter are the attainment of rapid thermal equilibration between the flowing solution and the body of the calorimetric cell and efficient mixing of the two solutions. The former problem may become serious at high flow rates, but is not serious at the flow rates employed in this work. The latter problem will become serious when the mixing time is a significant fraction of the residence time. The mixing time is probably the limiting factor which will determine the ultimate sensitivity of this instrument in measuring fast reactions. Under the conditions employed in this work, however, it does not appear to be an important factor.

Sensitivity and Precision. The calorimeter described in this work can measure a signal of 0.1 µV (~0.4 µcal/sec) with about 10% precision. Assuming a nominal effective volume of 0.4 ml, this sensitivity is equivalent to a reaction rate of 10^{-7} mol l.$^{-1}$ sec^{-1} for a reaction whose enthalpy change is 10 kcal/mol.

The ultimate precision of the measurement of the heat effect with this calorimeter as presently assembled is about ±0.1% at signal levels greater than 10 µV. This limiting value is primarily due to variation in flow rates and baseline stability. For the experiments reported the precision is about ±1% due to errors in the determination of the concentration of reactants and errors in data acquisition which were obtained from strip-chart recordings. Digital data acquisition would eliminate the latter problem.

The fastest reaction rate measured in this calorimeter corresponds to a half-time of approximately 50 sec. However, it is estimated that reaction half-times as small as 5 sec can be determined with the calorimeter in its present configuration. Improvements in base-line stability and reduction of the residence time would reduce this lower limit. It may be possible that reactions with half-times as small as 100 msec can be accurately studied using this instrument with appropriate modification if mixing is sufficiently rapid.

Applications. The use of flow microcalorimetry to measure reaction rates in the liquid phase has wide potential in all areas of organic, inorganic, and biological chemistry because it measures a ubiquitous property of chemical reactions. In principle this method could also be used for gas phase reactions. Its particular advantages include the high degree of attainable precision and accuracy, its high sensitivity, the wide range of reaction times which can be resolved, and the fact that it can provide both thermodynamic and kinetic information. Another aspect of its remarkable versatility is that it can be easily adapted for automatic data acquisition.

There are two particular areas of application that deserve special note. The first of these are for reactions of organic compounds which have high vapor pressures. Since the flow microcalorimeter lacks any vapor space, reaction rates of volative materials can be conveniently measured.

The second area of special significance is that involving enzyme-catalyzed reactions. Since this technique relies only on heat generation by the reaction, it is thus not necessary to develop complicated assay procedures to monitor the reaction. In addition full recovery of the enzyme after reaction is feasible. Assuming a useful sensitivity of 0.1 μcal/sec and a reaction enthalpy of 10 kcal/mol, it is possible to detect enzymatic activity at concentrations as low as 10^{-15} M for those enzymes which exhibit the highest turnover numbers (e.g., carbonic anhydrase). The application of flow microcalorimetry to determine kinetic parameters for enzyme reactions will be discussed in a future publication.

Experimental Section

Calorimeter. The flow microcalorimeter used in these studies is made by LKB Instruments, Inc., and based on the design of Monk and Wadsö.[19] The major change made by LKB was to redesign the flow-through and mixing cells so that the solutions follow a spiral path about 60 cm × 1 mm through the gold cell rather than the zig-zag channel described in ref 19. At least two other commercially available flow microcalorimeters presumably possess a similar capability to measure reaction rates.[32,33]

The calorimeter was submerged in a water bath maintained to ±0.001° with a proportional controller (Tronac, Inc.). All experiments were performed at 25°.

Procedure. An experiment is performed by pumping the two reactants into the calorimeter through Teflon tubing with separate peristaltic pumps. The steady state calorimetric signal was measured relative to the signal observed when solvent was pumped through both sides. The heats of dilution of the components were subtracted from the heat of mixing to obtain the heat of reaction. Concentrations of reactants in the calorimeter after mixing were determined by multiplying their initial concentrations by the dilution factor, the ratio of the flow rate of the solution before mixing to the total flow rate.

Ethyl acetate was found to strongly interact with the silicon tubing used in the heads of the peristaltic pumps. This problem was circumvented by pulling both reactants through the calorimeter with one pump and pushing the KOH solution through the calorimeter with the other. This procedure allowed the ethyl acetate solution to enter the mixing cell without making contact with silicon tubing.

The choice of flow rate depended upon optimizing two mutually exclusive requirements. The flow rate had to be sufficiently rapid to ensure that the reaction was less than 95% complete during the residence time but slower flow rates produce more stable base lines. The flow rate was determined by weighing the amount of water pumped through the calorimeter over a defined time interval.

The calibration constant (ϵ) was determined by introducing a known quantity of electrical power into a small heater located at the mixing point and measuring the steady state voltage produced. ϵ is a function of the flow rate but varies less than 9% over the range of flow rates used in this study. This variation was also observed by Monk and Wadsö.[19]

Materials. Ethyl acetate was obtained from Matheson Coleman and Bell. Its purity was found to be 98.5% by reaction with an equivalent amount of standard base and back titrating with standard acid. The KOH, obtained from Baker Chemical Co. as a concentrated, CO_2 free, standardized solution, was always used within 3 days of opening. Deionized water was used to prepare all solutions.

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channel inside. The thermal elements are 4 cm × 4 cm × 4 mm with a honeycomb arrangement of 98 individual elements which decreases its effective cross-sectional area to 2 cm × 2 cm.

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Chain-Length Effects upon the Interaction of Remote Functional Groups. The Low Energy Electron Impact Mass Spectra of α,ω-Dialkoxyalkanes Examined by Ion Cyclotron Resonance Spectroscopy[1a]

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Abstract: The techniques of ion cyclotron resonance spectroscopy have been used to investigate the analogy between bimolecular ion–molecule reactions and intramolecular mass spectral rearrangements of a series of bifunctional ethers, $CH_3O(CH_2)_nOR$, $n = 1$-6 and R = H, CH_3, or C_2H_5. The first step in exploring this analogy has been the examination of the ion–molecule reactions of simple ethers. The second step has been the investigation of the rearrangement ions produced from bifunctional ethers by low energy electron impact. The third step has been a probe of selectivity using deuterium labels. It is found that intramolecular hydrogen transfer is highly regioselective, while the bimolecular analog is not. More pronounced deviations from the analogy arise with variation of the length of the methylene chain separating the functional groups. This indicates a selectivity in the rearrangement process which has no analog in ion–molecule reactions, and this chain-length selectivity has been probed by deuterium labeling and by examination of the ion–molecule reactions of the rearrangement ions. The term *vinculoselection* is proposed to designate a reaction of a polyfunctional molecule in which a specific chain length or spatial separation between functional groups is requisite for the reaction to occur. Thermodynamic and structural factors affecting vinculoselective reactions of cations from bifunctional ethers are discussed.

Interaction of functional groups within organic molecules is a well recognized aspect of chemical reactivity. Intramolecular reactions of bifunctional compounds are generally described by analogy to bimolecular reactions of the same functional groups, and this analogy is often useful in predicting the behavior of polyfunctional molecules. Such an approach has led, for example, to the development of stereoselective synthetic transformations in rigid cyclic systems,[3] the characterization of intramolecular strong hydrogen bonding in bifunctional molecules,[4] and the suggestion that mass spectral rearrangements in long chain bifunctional molecules resemble ion–molecule reactions,[5] as represented in reactions 1a and 1b.

$$RX^{+} + RY \longrightarrow \text{ion–molecule reaction products} \qquad (1a)$$

$$\underbrace{X^{+}\qquad Y}\ \longrightarrow \text{mass spectral rearrangement ions} \qquad (1b)$$

The present study is an investigation of this analogy in the mass spectral rearrangements of bifunctional ethers of the general formula $CH_3O(CH_2)_nOR$, where R may be hydrogen, methyl, or ethyl, and n is varied from 1 to 6. Ethers have been chosen for examination because of the simplicity of their ion chemistry, their volatility, and the ease with which deuterium labels can be placed at specific positions within a molecule. The techniques of ion cyclotron resonance spectroscopy (ICR) have been used in this study for three reasons: (1) the relatively low spread in energy of the ionizing electron beam at energies near ionization thresholds;[6] (2) the ability to vary ion residence time from 10^{-3} to 1 sec and the pressure of neutrals from 10^{-7} to 10^{-3} Torr, which permits the observation of ion–molecule reactions; and (3) the capability of identifying reaction partners through ICR double resonance techniques.[7] As part of these studies, we have already described the examination of clustering reactions by ICR to detect intramolecular strong hydrogen bonding in bifunctional molecules.[4] More recent ICR studies have measured the strength of this form of interaction of remote functional groups.[8] In addition, ICR has been widely utilized for the identification of the structures of fragment ions arising from electron impact[7,9] and has revealed many details regarding the bimolecular reactions of organic ions with neutral molecules.[7,10]

The first step in exploring the analogy between reactions 1a and 1b has been the observation of the ion–molecule reactions of molecular ions from simple ethers. The second step has been the examination of the rearrangement ions produced from bifunctional ethers by low energy electron impact, with the result that the most prevalent rearrangement process corresponds to the intramolecular analog of a bimolecular ion–molecule reaction. The final step has been the realization that specific instances of major deviations from this analogy arise with variation of the methylene chain separating the functional groups. This indicates a selectivity not found in ion–molecule reactions, and this selectivity has been probed using deuterium labeling techniques and the examination of ion–molecule reactions of the rearrangement ions.

Selectivity is a prominent feature of many mass spectral