Absorbance-Stat Method for the Study of Reaction Kinetics in Solution

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A method is described for the kinetic study of reactions in which the concentration of one reactant is held essentially constant by addition of that reactant throughout the course of the reaction; this condition of constancy is attained by maintaining the solution absorbance, which is made a function of reactant concentration, at a constant value. This technique, the absorbance-stat method, is exemplified by alkaline ester hydrolysis, an acid-base indicator being added to the solution. By titrating with alkali to keep the absorbance of the indicator constant, the hydroxide-ion concentration is held constant. The principles of this technique are developed, and the method is compared with the conventional pH-stat procedure. The second-order rate constants of alkaline hydrolysis of methyl and ethyl acetates have been determined. The method is satisfactorily accurate and precise.

In the "continuous-titration" method of studying reactions that produce or consume hydrogen or hydroxide ion, a poorly buffered solution of the reacting substrate is titrated with acid or alkali to keep the pH at an essentially constant value. The rate of addition of titrant is related to the rate of the chemical reaction. As commonly carried out, the procedure utilizes potentiometric detection of the solution pH by means of a glass electrode–saturated calomel electrode system. The titrant can be added manually while its volume and the corresponding time are recorded. During the past decade, however, instruments have been designed to carry out the pH measurement, titration, and recording steps automatically; several of these instruments, called pH-stats, are available commercially. The pH-stat method for studying reaction rates has been reviewed by Jacobsen *et al.* (1). Throughout the present paper the term "pH-stat" will be applied to the continuous-titration method with potentiometric pH detection, whether carried out manually or instrumentally.

The continuous-titration technique appears to have been introduced in 1922 by Knaffl-Lenz (2), who studied rates of enzyme-catalyzed ester hydrolyses. Knaffl-Lenz added an acid-base indicator to the reaction solution to function as a visual pH detector, by titrating to keep the solution a definite color, the pH was maintained constant. Later development of this technique utilized electrometric pH determination, as described above. With the present availability of highly sensitive and stable spectrophotometers, the original colorimetric procedure regains its attractiveness, and in this paper the theory and practice of this technique, which will be called the "absorbance-stat" method, are explored.

Consider the reaction between a substrate S and a reagent R, in which reagent is consumed on a stoichiometric basis.

$$S + R \rightarrow \text{products}$$

The velocity of this reaction is given by the rate equation $v = k\,[S][R]$, where k is the second-order rate constant. When [R] is maintained constant, this takes the first-order form $v = k_{app.}\,[S]$, where $k_{app.}$, the apparent first-order rate constant, is equal to $k[R]$. The absorbance-stat method permits the evaluation of $k_{app.}$ for reactions of two general types. (*a*) Reactions in which R possesses an absorption spectrum such that at a selected wavelength R is the only reaction component that absorbs radiation. Then the absorbance due to R is maintained constant by continuous titration with a solution of R. (*b*) Reactions in which an indicator is added whose absorption intensity is a function of the R concentration; the reaction mixture is titrated with a solution of R to keep the absorbance constant.

The first of these possibilities might be exemplified by oxidations with permanganate or by bromine-addition reactions. The second class includes, as its most important examples, some reactions in which R is hydrogen ion or hydroxide ion. Thus acid-catalyzed amide hydrolysis or alkali-catalyzed ester hydrolysis might be studied by the absorbance-stat method.

In this paper the potentialities of this technique have been investigated by applying it to hydroxide ion-catalyzed ester hydrolyses. The theoretical treatment is also based upon this reaction. With suitable modification the theory can be adapted to other reactions.

PRINCIPLES

Dependence of Titrant Volume on Time—The relationship between the rate of addition of titrant and the rate of chemical reaction must be established to permit evaluation of the reaction rate constant. The reaction is written

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$$E + OH^- \xrightarrow{k_{OH}} \text{products}$$

with 1 equivalent of hydroxide being consumed per mole of ester hydrolyzed. The solution buffer capacity is assumed to be negligible and the volume change of mixing upon the addition of titrant is neglected. The volume change resulting from conversion of E and OH^- to reaction products also is neglected. The temperature is assumed constant. Then the ester concentration can change through chemical reaction and by dilution with titrant.

$$d[E] = (\delta[E]/\delta t)_V dt + (\delta[E]/\delta V)_t dV \quad \text{(Eq. 1)}$$

The first of these derivatives is given by the rate equation:

$$(\delta[E]/\delta t)_V = -k_{OH}[E][OH] \quad \text{(Eq. 2)}$$

The dilution effect is found by differentiating the definition of concentration, $[E] = q_E/V$, where q_E is the milliequivalents of ester contained in V milliliters of solution. The result is

$$(\delta[E]/\delta V)_t = -[E]/V \quad \text{(Eq. 3)}$$

The differential dV in Eq. 1 can be evaluated from a consideration of the effects of the reaction and the titration on the hydroxide-ion concentration.

$$d[OH] = (\delta[OH]/\delta t)_V dt + (\delta[OH]/\delta V)_t dV = 0 \quad \text{(Eq. 4)}$$

In Eq. 4 the differential $d[OH]$ is set equal to zero, expressing the experimental objective of the continuous-titration method. From the rate equation, the time effect can be found:

$$(\delta[OH]/\delta t)_V = -k_{OH}[E][OH] \quad \text{(Eq. 5)}$$

To find the titration effect, the hydroxide-ion concentration is written

$$[OH] = (\text{meq. of } OH^- \text{ present initially} + \text{meq. of } OH^- \text{ added})/V$$

$$[OH] = (q_{OH} + NV_t)/V \quad \text{(Eq. 6)}$$

where q_{OH} represents the milliequivalents of hydroxide present in the initial sample solution, N is the titrant normality, V_t is the volume of titrant added, and V is the total volume of reaction solution. Therefore, $V = V_0 + V_t$, if V_0 is the volume of sample solution before any titrant is added. Equation 6 is differentiated to give

$$(\delta[OH]/\delta V)_t = 1/k'V \quad \text{(Eq. 7)}$$

where $k' = 1/(N - [OH])$. Equations 4, 5, and 7 can be combined, yielding Eq. 8.

$$dV = k'k_{OH}V[E][OH]dt \quad \text{(Eq. 8)}$$

Substitution of Eqs. 2, 3, and 8 into Eq. 1 gives

$$\frac{d[E]}{[E](1 + k'[E])} = -k_{obs.}dt \quad \text{(Eq. 9)}$$

where a later result has been anticipated by defining $k_{obs.} = k_{OH}[OH]$. Equation 9 gives, on integration,

$$\ln \frac{[E](1 + k'[E]_0)}{[E]_0(1 + k'[E])} = -k_{obs.}t \quad \text{(Eq. 10)}$$

in which $[E]_0$ represents the ester concentration at time zero, and $[E]$ is its concentration at time t. An equation similar to Eq. 10 has been presented by Breuer and Jenkins (3), who, however, give $1/N$ as the value of k'.

The number of milliequivalents of ester initially present is equal to $V_0[E]_0$, which is equal to the number of milliequivalents of hydroxide consumed in the entire reaction, or $V_\infty N$.

$$[E]_0 = V_\infty N/V_0 \quad \text{(Eq. 11)}$$

V_∞ is the volume of titrant added at $t = \infty$. The number of milliequivalents of ester unreacted at time t is equal to the total meq. added minus the meq. hydrolyzed, leading to Eq. 12 for the concentration $[E]$.

$$[E] = N(V_\infty - V_t)/V \quad \text{(Eq. 12)}$$

With Eqs. 11 and 12, Eq. 10 can be expressed in terms of titrant volumes:

$$\ln \left[\frac{V_\infty - V_t}{V_\infty}\right] \left[\frac{V_0 + k'NV_\infty}{(V_0 + k'NV_\infty) + V_t(1 - k'N)}\right] = -k_{obs.}t \quad \text{(Eq. 13)}$$

When $V_t(1 - k'N) <<< (V_0 + k'NV_\infty)$, Eq. 13 becomes

$$\log \left[\frac{V_\infty - V_t}{V_\infty}\right] = -\frac{k_{obs.}t}{2.303} \quad \text{(Eq. 14)}$$

Equation 14 is therefore a satisfactory approximation when $(N - [OH]) \cong N$ or when $V_t <<< V_0$. Though these are separate conditions, they are usually both satisfied by employing a very concentrated titrant solution.

The above considerations apply to both the pH-stat and the absorbance-stat methods. According to Eq. 14, a plot of $\log(V_\infty - V_t)$ against time should be linear with slope equal to $-k_{obs.}/2.303$.

Effect of Volume Change on Indicator Concentration—During the course of the reaction a volume change in the range 1–5% will usually result from the addition of titrant. The case for keeping this change as small as possible was presented in the preceding paragraphs. In the absorbance-stat method this volume change can exert a secondary effect by reducing the total indicator concentration in the reaction solution. This reduction in indicator concentration will lead to an absorbance decrease. Since the absorbance is the quantity that is maintained constant in this technique, the reduction in indicator concentration will be compensated for by a further addition of titrant, which will convert some of the indicator to the more highly absorbing form and thus restore the absorbance to its initial value. (This description supposes that the basic form of the indicator is responsible for the light absorption.) But this means that the pH has changed, because an alteration in the ratio of acid to base forms of the indicator is a reflection of a pH change. Thus, the hydroxide-ion concentration will undergo a small but possibly significant shift during the reaction because of this dilution effect on the indicator.

The possible error from this source has not been analyzed quantitatively because it is easily eliminated. The remedy is simply to add to the titrant the same concentration of indicator that is present in the initial sample solution. Then no matter how much titrant is added, the total indicator concentration will remain constant. By holding the absorbance at a constant value, the pH will

remain unchanged. The experimental problem is not difficult. Proportional amounts of an indicator stock solution are added to the titrant and to the sample solution. The new titrant normality can be calculated from the initial normality and volumes.

Calculation of Second-Order Rate Constants—The preceding considerations provide a basis for the evaluation of the first-order rate constant, $k_{obs.}$ The goal of the kinetic measurements usually is the second-order constant, k_{OH}, which is given by $k_{OH} = k_{obs.}/[OH]$. An estimate must therefore be made of [OH] in the reaction solution. This can be made in two general ways. (*a*) The *concentration* of hydroxide ion, which will be symbolized as c_{OH}, can be obtained; this is based ultimately upon titration data. (*b*) The conventional *activity* of hydroxide ion, a_{OH}, may be found; this is ultimately based upon a potentiometric determination of pH. Writing for the experimental first-order constant the two equivalent expressions

$$k_{obs.} = (k_{OH})^c c_{OH}$$

$$k_{obs.} = (k_{OH})^a a_{OH}$$

shows that the second-order rate constants expressed on the concentration and activity scales are related by Eq. 15,

$$(k_{OH})^c/(k_{OH})^a = a_{OH}/c_{OH} = y_{OH} \quad \text{(Eq. 15)}$$

where y_{OH} is the apparent molar activity coefficient, which in its present use simply has the significance that it is a factor relating the rate constants calculated on these two bases. y_{OH} depends upon the ionic strength of the solution. In most practical situations y_{OH} is appreciably different from unity, and it is essential to distinguish between the rate constants $(k_{OH})^c$ and $(k_{OH})^a$.

pH can be measured potentiometrically at any time during the reaction; then the quantity a_{OH} can be calculated with the use of pKw at the appropriate temperature. (It is assumed here that the reaction mixture is a fully aqueous solution.) In the calculation of second-order hydrolytic rate constants based on pH measurements, the pH determination usually contributes a greater share of error than does the measurement of $k_{obs.}$ The accuracy inherent in the kinetic measurements may be preserved by basing the second-order constant on the concentration, c_{OH}, which can often be established with greater accuracy than can a_{OH}.

If c_{OH} is not to be calculated for the absorbance-stat reaction solution, the exact value of absorbance selected as the static value is not of great importance, and immediately after the reaction is initiated a suitable value can be adopted. But if c_{OH} is to be estimated, the absorbance becomes critical. Suppose the sample solution is prepared with V_S ml. of solvent, V_I ml. of indicator stock solution, and V_A ml. of a standard solution of alkali of normality N_A. At the cell path length and wavelength selected for the kinetic experiment, this solution possesses an absorbance, A'. The reaction is next initiated by adding V_E ml. of ester (either neat or in a stock solution). The absorbance immediately changes because of three effects: (*a*) dilution of the indicator; (*b*) conversion of some indicator from the base to the acid form because of the dilution effect on the hydroxide ion; (*c*) hydrolysis of the ester to reduce the hydroxide-ion concentration. The third effect does not enter the argument because the present concern is to select the proper absorbance value at $t = 0$ before any ester has hydrolyzed, this then being the absorbance value at which the solution will be maintained throughout the reaction. The correct absorbance is obtained by taking the dilution of the indicator into account (Eq. 16),

$$A = A'(V_S + V_I + V_A)/V_0 \quad \text{(Eq. 16)}$$

where $V_0 = V_S + V_I + V_A + V_E$. Even in the absence of the ester hydrolysis, however, the observed absorbance would be less than A because of the dilution effect on hydroxide ion, so the solution is immediately titrated to achieve the absorbance, A, and this value is maintained through the reaction. In effect, this procedure restores c_{OH} to the value it had before addition of the ester. Then the quantity, c_{OH}, to be used in converting $k_{obs.}$ to $(k_{OH})^c$, is obtained with Eq. 17.

$$c_{OH} = V_A N_A/(V_S + V_I + V_A) \quad \text{(Eq. 17)}$$

Sensitivity of the Absorbance-Stat Method—The acid-base indicator equilibrium is

$$HI \rightleftarrows H^+ + I^-$$

and the acid dissociation constant for the indicator may be written $K_I = a_H[I]/[HI]$. The indicator ratio is given by

$$[I]/[HI] = (A - A_{HI})/(A_I - A)$$

where A_{HI} is the absorbance of a solution containing indicator transformed totally to its acid form, A_I is the absorbance when the indicator is in its base form, and A is the absorbance when it is present in any mixture of the two forms; all of these quantities refer to the same total concentration of indicator. The hydrogen-ion activity can be expressed in terms of these absorbances as in Eq. 18.

$$a_H = K_I(A_I - A)/(A - A_{HI}) \quad \text{(Eq. 18)}$$

From Eq. 18 the derivative da_H/dA can be found, and this can be transformed with the relation $d\text{pH} = -da_H/2.3a_H$ to give (in incremental form) Eq. 19.

$$\Delta\text{pH} = \frac{(A_I - A_{HI})\Delta A}{2.3(A_I - A)(A - A_{HI})} \quad \text{(Eq. 19)}$$

This equation provides a basis for evaluating the relative sensitivities of the pH-stat and absorbance-stat methods. Sensitivity in these continuous-titration procedures may be expressed as the smallest change in detector signal that will elicit a response from the titrator mechanism. It will be clear from Eq. 19 that the sensitivity in the absorbance-stat method depends upon the solution absorbance, increasing with concentration of the indicator; moreover, sensitivity is maximal when $\text{pH} = \text{pK}_I$, which corresponds to the condition $A = (A_I - A_{HI})/2$. The relationship of A to pH for any indicator suggests, however, that good sensitivity can be achieved in the approximate range $\text{pH} = \text{pK}_I \pm 1$.

The comparative sensitivities of potentiometric and spectrophotometric control of pH can be demonstrated with Eq. 19 and some hypothetical but reasonable values for the experimental quantities. Suppose $A_I = 2.00$, $A_{HI} = 0.00$, $A = 0.90$, and

ΔA = 0.005. Then Eq. 19 yields ΔpH = 0.0044. Since ΔA = 0.005 seems to be a conservative estimate of attainable spectrophotometric sensitivity, while ΔpH = 0.0044 probably is an optimal sensitivity for the pH-stat, it appears that the absorbance-stat should be capable of more sensitive response to the changing solution conditions than is the pH-stat.

EXPERIMENTAL

Materials—Ethyl acetate (Baker and Adamson reagent grade) was distilled, b.p. 76–77°. Methyl acetate (Matheson Coleman & Bell) was distilled, b.p. 55–56°. Alizarin yellow R (Coleman & Bell) was used directly. Other chemicals were of reagent or primary standard grade. All water was redistilled from alkaline permanganate.

Indicator stock solution was prepared by dissolving 25 mg. of alizarin yellow R in 0.5 ml. of 1.0 *N* sodium hydroxide; this was diluted to 50 ml. with water, the solution was allowed to stand overnight in a refrigerator, it was filtered, and 25 ml. of the filtrate was diluted to 50 ml. with water.

Apparatus—Reactions were carried out in a flask originally designed for photometric titrations (4). The flask (capacity about 120 ml.) was constructed with a jacket through which water was circulated from a constant-temperature water bath. This apparatus was mounted directly over an air-driven magnetic stirrer, the entire assembly being placed near the thermostated cell compartment of a Cary model 14 recording spectrophotometer. Short lengths of rubber tubing connected the inlet and outlet of the reaction flask to a flow-through spectrophotometer cell that was firmly positioned in a cell holder in the sample cell compartment. A Teflon-covered stirring bar provided the stirring and pumping action. The reaction flask was tightly closed with a rubber stopper, through which passed the tip of a 2-ml. micrometer buret (Roger Gilmont Instruments); the buret tip extended below the surface of the reaction solution.

pH measurements were made with a Sargent model DR pH meter equipped with a wide-range glass electrode.

Procedure—A typical experiment was conducted as follows. To the reaction flask 75.0 ml. of freshly boiled water, 3.0 ml. of indicator stock solution, and 5.0 ml. of standard 0.1 *N* sodium hydroxide were added. This solution was allowed to reach temperature equilibrium. The spectrophotometer wavelength selector was set at 495 mμ (the absorption maximum of the base form of the indicator), and the recorder was activated. About 2 meq. of ester was added to initiate the reaction. From the buret 1 *N* NaOH was added to keep the absorbance as constant as possible; the chart tracing is a helpful aid in minimizing the fluctuations about the mean absorbance value. The titrant volume was recorded as a function of time; reactions were followed until no further change in absorbance was observed. All reactions were carried out at 25.0°.

RESULTS AND DISCUSSION

Ethyl acetate was selected as the ester for the developmental stage of this investigation because its hydrolysis is uncomplicated, it is water soluble, and many other studies of its alkaline hydrolysis have been made; its volatility is a disadvantage. Alizarin yellow R was found to be a suitable indicator in the pH range required to give a convenient rate of reaction. This indicator possesses good sensitivity from pH 9.8 to 11.8, and it is adequately stable under these conditions.

The volatility of ethyl acetate prevented the use of a nitrogen stream as protection against atmospheric contamination, and it was necessary to stopper tightly the reaction flask to prevent absorption of carbon dioxide from the atmosphere as well as loss of ethyl acetate to the atmosphere. After such sources of error were eliminated, very well-behaved kinetic data were obtained. Figure 1 shows a characteristic plot of titrant volume as a function of time. The first-order plot according to Eq. 14 is shown in Fig. 2; the line is straight over about 4 half-lives, and this behavior could be reproduced at will. The V_∞ value tended to rise slightly with unrealistically long periods of waiting, probably because of a slight decomposition of the indicator, so the V_∞ value used in the calculations was taken after the expiration of 7 to 9 half-lives. With the conditions described here, it was found unnecessary to incorporate indicator in the titrant.

The mean value of $(k_{OH})^c$ for ethyl acetate at 25.0° was found, from such measurements, to be 0.103 L./mole-sec., with a standard deviation of 0.0013 L./mole-sec. This must be considered

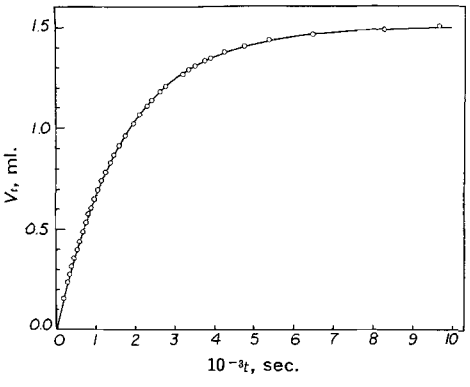

Fig. 1—Plot of titrant volume against time for the absorbance-stat study of the alkaline hydrolysis of ethyl acetate at 25°; c_{OH} = *0.00569* N, *absorbance = 0.64.*

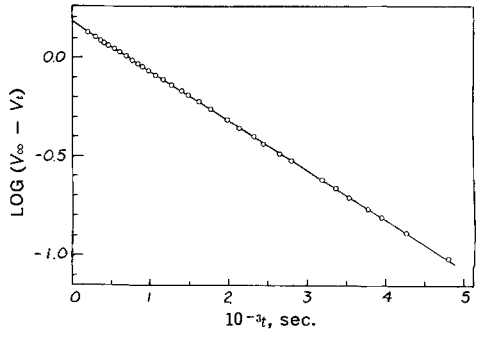

Fig. 2—First-order plot of the data shown in Fig. 1.

excellent precision, and it reflects the very good reproducibility of the observed first-order rate constants. The mean value of $(k_{OH})^a$, from these same determinations, was 0.126 L./mole-sec., with a standard deviation of 0.012 L./mole-sec., and this poorer precision is a result of the difficulty in obtaining precise pH measurements. In these studies the pH was in the range 11.6–11.8.

Literature values of $(k_{OH})^c$ for ethyl acetate at 25° vary widely. Terry and Stieglitz (5) give the value 0.113 L./mole-sec. with a claimed accuracy of 0.75%; they also review earlier reports, with the mean value of six workers being 0.110 L./mole-sec. (ranging from 0.106 to 0.114). Salmi and Kantola (6) found 0.109 L./mole-sec., Shrivastava (7) reported 0.091 L./mole-sec., Myers *et al.* (8) gave 0.111 L./mole-sec., and Flom and Elving (9) found 0.110 L./mole-sec. (standard deviation 0.010). Wilson and Terry (10) observed a progressive decrease of the rate constant with increase in ionic strength (NaCl), with the constant dropping from 0.113 with no NaCl present (initial NaOH 0.01 molal) to 0.102 L./mole-sec. at 0.51 *M* NaCl. Bell and Waind (11), however, found that increasing concentrations of NaCl up to 0.5 *M* had no significant effect on the rate constant; they reported values in the range 0.099–0.101 L./mole-sec. The value of 0.103 L./mole-sec. obtained in the present study, therefore, lies close to many reliable literature values, though it may be slightly lower than the best value.

Methyl acetate was also studied by the absorbance-stat method. The mean $(k_{OH})^c$ for this ester at 25° was 0.186 L./mole-sec., with a standard deviation of 0.0075 L./mole-sec. [Gooch and Terry (12) give 0.195 L./mole-sec. for this constant.] The same data yielded, for $(k_{OH})^a$, the mean value 0.226 L./mole-sec. (standard deviation 0.067).

Guggenheim's method (13) of plotting first-order rate data was also applied to all experiments, and the rate constants obtained from these plots agreed closely with those from the conventional graphs. The Guggenheim method obviates the necessity of obtaining a value for V_∞, which may be subject to small errors caused by indicator instability, carbon dioxide absorption, or spectrophotometer base line drift.

Other indicators could doubtless be used, especially in the less alkaline pH ranges. Attempts to locate suitable indicators for use above pH 12 were unsuccessful, the substances studied being too unstable for practical use.

The absorbance-stat method for studying reaction rates appears, on the basis of the analysis and results reported in this paper, to be a useful technique for the precise and accurate determination of rate constants in suitable systems. The procedure could easily be placed on an automatic basis by substituting a photometer for the pH meter as the detector in a conventional pH-stat. The absorbance-stat is potentially more sensitive than is the pH-stat. These two techniques will probably complement each other, for the absorbance-stat method will not be successful in heterogeneous systems where the pH-stat might perform satisfactorily, while the absorbance-stat will function in systems (such as low dielectric constant solutions) that might vitiate measurements with the glass electrode; moreover, the absorbance-stat is not limited to reactions involving solvent ions.

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