tween salt and metal in the solution. We may then write

$$t_e(c,m) = \frac{t_e(c,O)}{\left(1 + R\,\frac{\Lambda_{NaCl}(m)}{\Lambda_{Na}(c)}\right)} \qquad (2'')$$

where

c = concentration of metal
m = concentration of salt
R = salt/metal ratio = m/c

The Λ's are the equivalent conductances of the salt and metal at the concentrations m and c, respectively, and the t_e's are the transference numbers of electrons in the pure metal and mixed solutions.

The transference numbers shown in Fig. 2 of this paper were calculated using equation 2″ with $t_e(c, 0)$ calculated from the ratio of ion conductances at infinite dilution. Other methods of approximating t_e yield comparable values.

MURRAY HILL, N. J.

[CONTRIBUTION FROM THE DEPARTMENT OF INDUSTRIAL CHEMISTRY, THE FACULTY OF ENGINEERING, KYOTO UNIVERSITY]

Kinetics of the Formation of the Formal of Polyvinyl Alcohol

BY YOSHIRO OGATA, MASAYA OKANO AND TAKASHI GANKE

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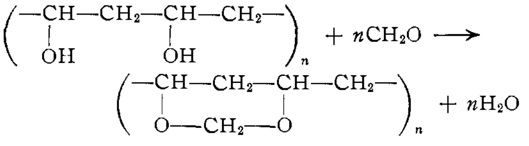
The sulfuric, perchloric or hydrochloric acid-catalyzed formation of the formal of polyvinyl alcohol in aqueous media has been investigated kinetically at 70° by estimating iodometrically the formaldehyde consumed. It was found that the rate was proportional to the product of the stoichiometric concentrations of formaldehyde and the hydroxyl group of polyvinyl alcohol. A linear relationship with almost unit slope (−1.07) was obtained between log k and the acidity function, H_0, of the solution. A mechanism is postulated which involves the rate-determining attack of protonated formaldehyde on the hydroxyl group of polyvinyl alcohol followed by the rapid cyclization with the neighboring hydroxyl group.

Several authors have reported kinetic studies of the formation of acetals from aliphatic alcohols.[1] A mechanism which involves the rapid reversible formation of hemiacetal,[2] followed by the rate-determining condensation of it with another alcohol molecule, appears to gain general acceptance.[3] However, the analysis of the kinetic data does not seem satisfactory. Polyvinyl alcohol is different from ordinary alcohols in that it forms a cyclic acetal with a six-membered ring.



$$\left(\begin{array}{c}-\underset{\displaystyle OH}{\underset{|}{CH}}-CH_2-\underset{\displaystyle OH}{\underset{|}{CH}}-CH_2-\end{array}\right)_n + nCH_2O \longrightarrow \left(\begin{array}{c}-CH-CH_2-CH-CH_2-\\ |\qquad\quad\;\; |\qquad\quad\\ O-CH_2-O\qquad\quad\end{array}\right)_n + nH_2O \qquad (1)$$

Previous velocity measurements for this reaction have only qualitative meaning.[4] The present paper summarizes the results of kinetic studies of the formal formation of polyvinyl alcohol.

Experimental

Materials.—Nippon Gosei Chemical Co. polyvinyl alcohol (the average degree of polymerization, 1930) was used. Aqueous formaldehyde and sulfuric, perchloric and hydrochloric acids of the best grade were used.

A Typical Procedure for the Rate Measurements.—A mixture of 50 cc. of 0.60 *M* aqueous polyvinyl alcohol solution,[5] 80 cc. of 2.50 *N* sulfuric acid and 60 cc. of distilled water was placed in a flask dipped in a thermostat (70 ± 0.2°). After the temperature equilibrium was established, 10 cc. of 1.50 *M* aqueous formaldehyde solution, previously allowed to attain the same temperature, was added with stirring to the flask from a calibrated pipet. A 10-cc. aliquot was pipetted out at regular time intervals and added to an aqueous solution of *ca.* 50 cc. of 0.5 *N* sodium hydroxide. To this solution was added 30 cc. of 0.1 *N* iodine solution, then *ca.* 25 cc. of 1 *N* sulfuric acid; after standing *ca.* 20 minutes, the solution was titrated with 0.05 *N* sodium thiosulfate.[6,7] Since the reaction system usually became turbid after 30–40% of the equivalent amount of formaldehyde was consumed, the estimation was limited to this range.

Experimental Results and Calculations.—Apparent second-order rate constants, k, were calculated by means of the equations: if $p \neq 2f$

$$k = \frac{1}{t(p - 2f)} \ln \frac{f(p - 2x)}{p(f - x)} \qquad (2)$$

and if $p = 2f$

$$k = \frac{1}{2t}\left(\frac{1}{f - x} - \frac{1}{f}\right) \qquad (3)$$

Here, p and f are the initial concentrations of polyvinyl alcohol[8] and formaldehyde, respectively, and x is the consumed formaldehyde after t seconds. Table Ia shows the effect of the molar ratio and the initial concentration of reactants on the rate. The effect of the concentration of catalytic acids is given in Table Ib. Values of pH's were determined at 30°.

Some Complementary Experiments. (a) **Reversibility of the Reaction.**—In order to check the reversibility of this reaction[9] the amount of formaldehyde produced from the product was estimated as follows. Partially (*ca.* 40%) reacted polyvinyl alcohol which deposited during the rate measurement was collected, washed thoroughly with water, and dried. About 1.3 g. (0.03 mole) of it was heated for two hours in 100 cc. of 0.25 *M* aqueous sulfuric acid at 70°,

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(1) H. Adkins and E. W. Adams, THIS JOURNAL, **47**, 1368 (1925); H. Adkins and A. E. Broderick, *ibid.*, **50**, 178 (1928).

(2) *Cf.* H. L. de Leeuw, *Z. physik. Chem.*, **77**, 284 (1911); H. Adkins and A. E. Broderick, THIS JOURNAL, **50**, 499 (1928); W. Herold and K. L. Wolf, *Z. physik. Chem.*, **B12**, 165 (1931); I. Lauder, *Trans. Faraday Soc.*, **48**, 1015 (1952).

(3) For example, L. P. Hammett, "Physical Organic Chemistry," McGraw–Hill Book Co., Inc., New York, N. Y., 1940, p. 304.

(4) N. Fujimoto, T. Osugi and I. Sakurada, *Chem. High Polymers Japan*, **7**, 14 (1950) [*C. A.*, **46**, 883 (1952)].

(5) The concentration of polyvinyl alcohol is represented by that of its hydroxyl group.

(6) *Cf.* J. F. Walker, "Formaldehyde," 2nd ed., Reinhold Publishing Corp., New York, N. Y., 1953, p. 385.

(7) Since polyvinyl alcohol forms an addition compound with iodine [*cf.* H. Staudinger, K. Frey and W. Starck, *Ber.*, **60**, 1791 (1927); W. Gallay, *Can. J. Res.*, **14**, B 105 (1936)], it is necessary to correct the titer from the value of the blank test in the same condition, and to use a large excess of the iodine solution.

(8) Strictly speaking, in the initial concentration of hydroxyl group, a maximum reaction percentage (86.5%) should be taken into consideration [*cf.* P. J. Flory, THIS JOURNAL, **61**, 1518 (1939)]; but such accurate treatment seems unnecessary, because kinetics in this case were restricted only to the initial stage of reaction.

(9) I. Sakurada and N. Nakamura, *Bull. Inst. Chem. Research Kyoto Univ.*, **28**, 78 (1952) [*C. A.*, **46**, 8861 (1952)].

TABLE I

THE RATE CONSTANTS OF FORMAL FORMATION OF POLYVINYL ALCOHOL AT 70.0 ± 0.2°

(a) Effect of molar ratio and concentration of the reactants

Initial concn., M Polyvinyl alc.	Formaldehyde	Concn. H_2SO_4, M	pH	$k \times 10^{4a}$ (l./mole. sec.)
0.300	0.150	0.50	0.5	6.8 ± 0.1
.150	.075	.50	.5	6.7 ± .1
.075	.0375	.50	.5	6.7 ± .1
.300	.075	.50	.5	6.7 ± .1
.300	.075	.50^b	.5	6.8 ± .1
.300	.0375	.50	.5	6.6 ± .1
.150	.150	.50	.5	6.7 ± .1
.150	.300	.50	.5	6.7 ± .1

(b) Effect of catalytic acids and their concentration. Initial concns.: polyvinyl alc., 0.300 M; formald., 0.075 M

Catalytic acid and concn., M	pH	$H_0{}^c$	$k \times 10^{4a}$ (l./mole sec.)
H_2SO_4, 0.75	0.4	0.06	10.7 ± 0.1
.50	.5	.26	6.7 ± .1
.375	.65	.39	4.33 ± .05
.25	.75	.57	2.74 ± .03
.125	1.05	.90	1.25 ± .02
$HClO_4$, 0.75	0.3	0.06	10.9 ± 0.1
.50	.4	.27	7.1 ± .1
.375	.6	.41	4.18 ± .04
.125	1.05	..	1.22 ± .02
HCl, 0.75	0.3	0.07	11.3 ± 0.1
.125	1.05	.94	1.29 ± .02

[a] The probable error for each constant is given. [b] 0.1 M Na_2SO_4 was added in order to check the salt effect. [c] Interpolated from data by L. P. Hammett and J. Deyrup, THIS JOURNAL, **54**, 2721 (1932); L. P. Hammett and M. A. Paul, *ibid.*, **56**, 827 (1934).

but only 1–2% of the formaldehyde linked in the formal was liberated. Hence the rate of the reverse reaction may be negligible.

(b) Comparison of the Reactivity of Polyvinyl Alcohol to Formaldehyde with that of its Sulfate.—Polyvinyl sulfate was synthesized by the condensation of polyvinyl alcohol with chlorosulfonic acid in the presence of pyridine.[10] The pyridinium salt thus obtained was converted to the potassium salt. The crude salt of the sulfate was purified by adding excess methanol to its concentrated aqueous solution. The degree of esterification was 80.8%.

Anal. Calcd. for 100% esterification ($C_2H_3KO_4S$): S, 19.76. Found: S, 15.97.

The reactivity of the sulfate to formaldehyde was compared with that of the free alcohol; this comparison is shown in Table II. It is to be concluded that there is no possibility of reaction between polyvinyl sulfate and formaldehyde.

TABLE II

COMPARISON OF THE FORMALDEHYDE CONSUMPTION RATES OF POLYVINYL ALCOHOL WITH THOSE OF ITS SULFATE

Initial concns.: polyvinyl alcohol or its sulfate, 0.15 M; formaldehyde, 0.075 M; catalytic acid and its concn.: H_2SO_4, 0.50 M; temp. 70°.

Time, hr.	Conversion, %^{a} For polyvinyl alc.	For polyvinyl sulfate^b
0.5	14.9	..
1	20.5	2.2
2	42.3	3.7

[a] Calculated by assuming that one mole of formaldehyde reacts with two moles of OH or OSO_3H group in polyvinyl alcohol or its sulfate, respectively. [b] *Ca.* 20% of whole OH groups remained not being esterified.

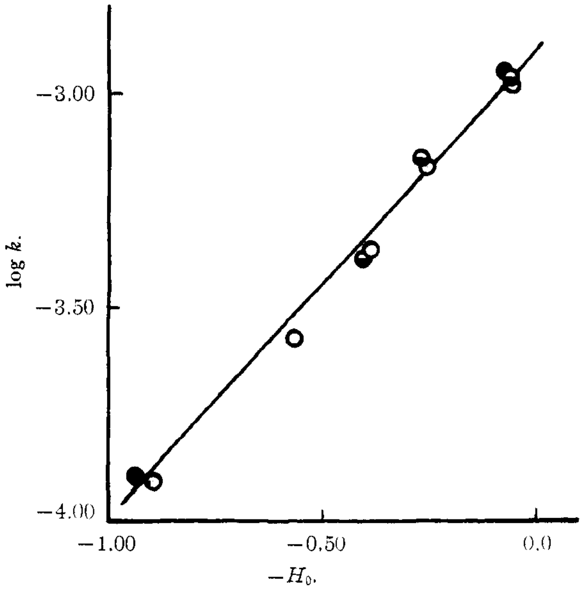
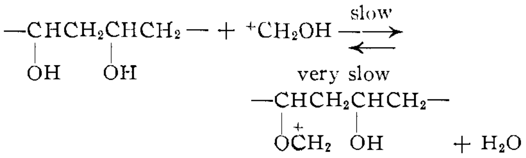


Fig. 1.—Relationship between log k and $-H_0$ (slope, 1.07): catalytic acid, ○ H_2SO_4, ◒ $HClO_4$, ● HCl.

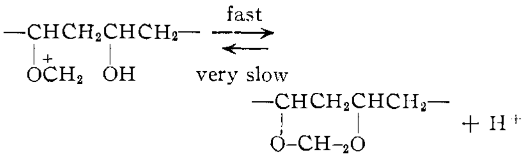
Discussion

On the basis of the experimentally obtained rate equation, the following mechanism is suggested for the reaction

$$CH_2O + H^+ \rightleftarrows \overset{+}{C}H_2OH \text{ (mobile)} \qquad (4)$$



$$\begin{array}{l}-CHCH_2CHCH_2-\\ \ \ \ |\qquad\ \ \ |\\ \ \ OH\quad\ OH\end{array} + {}^+CH_2OH \underset{\text{very slow}}{\overset{\text{slow}}{\rightleftarrows}} \begin{array}{l}-CHCH_2CHCH_2-\\ \ \ \ |\ {}^+\qquad |\\ \ \ OCH_2\ \ OH\end{array} + H_2O \qquad (5)$$



$$\begin{array}{l}-CHCH_2CHCH_2-\\ \ \ \ |\ {}^+\qquad |\\ \ \ OCH_2\ \ OH\end{array} \underset{\text{very slow}}{\overset{\text{fast}}{\rightleftarrows}} \begin{array}{l}-CHCH_2CHCH_2-\\ \ \ \ |\qquad\quad\ |\\ \ \ O{-}CH{-}_2O\end{array} + H^+ \qquad (6)$$

where H^+ comes from all proton donors, *i.e.*, H_3O^+ and undissociated acids, etc., K_4 is the equilibrium constant of step 4, and k_5 the rate constant of step 5. The rate equation may be given as

$$dx/dt = k_5\,[\text{OH in polyvinyl alc.}]\,[{}^+CH_2OH] = K_4k_5[\text{OH in polyvinlyl alc.}]\,[CH_2O]\,[H^+] \equiv k(p - 2x)(f - x) \qquad (7)$$

Therefore

$$k = K_4k_5[H^+] \qquad (8)$$

If oxonium ion were the only available source of proton, k would hold constant at the same pH range, regardless of the sort of anion of catalyzing acid, but this effect is not observed, as shown in Table Ib. On the contrary, if all acids are available as proton donors (*i.e.*, general acid catalysis), k should be constant at the same H_0. Equation 9 is then expressed as

$$\log k = -H_0 + \text{const.} \qquad (9)$$

This linear relationship with unit slope seems fulfilled, as shown in Fig. 1. Recently it has been observed with methyl alcohol that the formation of

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[10] P. Karrer, E. Usteri and B. Camerino, *Helv. Chim. Acta*, **27**, 1427 (1944).

the hemiacetal exhibits general acid and base catalysis.[11] It is of interest to note that step 5 is in this case faster than step 6, which is considered in general to be the slowest one in acetal formation, as described above. This fact may be accounted for by considering step 6 as involving an intramolecular methylene bridge formation between neighboring hydroxyl groups, affording a stable six-membered ring.[12]

Other explanations for the accelerative influence of sulfuric acid, for instance the contribution of the simultaneous reaction of polyvinyl sulfate with formaldehyde or the influence of ionic strength, were eliminated, as shown in the Complementary Experiment and the salt effect in Table Ia.

Acknowledgments.—The authors wish to express their gratitude to Professors R. Oda and I. Sakurada for their aid in performing these experiments.

(11) G. W. Meadows and B. B. Darwent, *Trans. Faraday Soc.*, **48**, 1015 (1952).

(12) The formation of the acetal ring, step 6, is not conceivable as rate-determining, since then rate equation 7′ ($dx/dt = K_4K_5k_6$[OH in polyvinyl alc.]^2[CH_2O][H^+]/[H_2O]) is derived and it is not attainable that the rate is proportional to the square of the concentration of the hydroxyl group. Perhaps this mechanism is pertinent to the formation of the non-cyclic acetal. In the case of polyvinyl alcohol, the evidence for the presence of intermolecular crosslinking has scarcely been obtained.

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[Contribution from Gibbs Chemical Laboratory, Harvard University]

The Kinetics of Ester Hydrolysis by Liver Esterase

By G. B. Kistiakowsky and Paul C. Mangelsdorf, Jr.[1]

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The kinetics of hydrolysis of ethyl *n*-butyrate and methyl *n*-butyrate esters by purified horse liver esterase were studied in unbuffered solutions by a conductometric method over a range of *p*H at 25°. The enzyme was found to be reversibly inactivated at measurable rates in solutions of *p*H higher than about 10, a slower irreversible denaturation being also observed. After correction for this inactivation was made, it was found that the enzymatic activity is independent of *p*H from about 9.2 to higher than 10.6. The dependence of the rate on substrate concentration was studied in detail at *p*H 10.18. The data show significant deviations from the Michaelis–Menten mechanism and two more parameters suffice to describe the data satisfactorily, except for the fall-off of the rate at the highest substrate concentrations. The additional parameters may be interpreted as indicating the existence of two sites on an enzyme molecule (or of two distinct enzymes); the relative numerical magnitude of the kinetic parameters observed is such as to suggest this hypothesis. The relation of the present data, in particular of a lower value of the average K_m observed, to previous work is discussed.

Previous work from this Laboratory on urease[2] and the studies of fumarase by Alberty[3] and coworkers have demonstrated that the Michaelis–Menten mechanism is but a first approximation, accurate rate measurements revealing finer details of the kinetic behavior of enzymes. The present paper deals with some aspects of the kinetics of liver esterase, chosen because of its broad activity for the ester linkage, in contrast to the complete specificity of urease for the urea molecule. The particular enzyme used was horse liver esterase prepared according to the method of Connors, Pihl, Dounce and Stotz,[4] which method yielded them a liver esterase of the highest purity ever achieved. The kinetics of ester hydrolysis by horse liver esterase, which had previously been studied by Bamann and Schmeller[5] over a wide range of methyl butyrate concentrations, were reinvestigated by Connors, *et al.*, using their purified enzyme, but over a narrower range of ester concentrations and only in buffered solutions. It has been demonstrated that buffers and, in fact, any neutral salts may exert profound influence on enzyme kinetics.[6] The present experiments, therefore, were carried out in unbuffered solutions of low ionic strength, using essentially a conductometric titration method to follow the liberation of acid from the ester by the enzyme.

Experimental Details

In order to avoid complicating effects of carbonic acid, all the experimental equipment and procedures were designed to exclude CO_2 from the solutions used.

Glass distilled water was used for all solutions and was freed from CO_2 by prolonged bubbling of nitrogen at reduced pressure. All operations preparatory to the rate measurements were carried out in a CO_2-free "dry box" made from a fume hood which had been made air-tight and was provided with snug-fitting flexible armholes, hatches and a fan with suitable ducting for removing CO_2 by continuous circulation of air over moist sodium hydroxide sticks. All stock solutions were made and stored in this box in sealed vessels.

The reactions were carried out in a flanged Pyrex vessel 4 inches deep and 2 inches in diameter with rounded bottom. A lid made of 1/4 inch aluminum was clamped tightly to the lip of the vessel by means of a rubber gasket. It is interesting to note that when originally a transparent polyester plastic sheet was used for the lid, the enzymatic activity was reduced by a factor of 20, presumably due to inhibition by the volatile plasticizer, since the lid came into no contact with the liquid.

The electrode system was supported by two narrow glass tubes which were sealed through the lid and carried electrical leads. The electrodes were two parallel multiperforate bright platinum sheets, 2 × 2 cm., held 1 to 2 mm. apart by a surrounding horizontal open-ended Pyrex cylinder to which the edges of the electrodes were sealed by gently melting the glass along the lines of contact. With the lid in place the electrodes hung well below the level of the liquid, which was stirred by a small magnet sealed in a polyethylene tube. The whole was submerged in a water thermostat, the temperature being held constant to 0.01° at 25°.

The resistance of solutions was measured on a Leeds and

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(1) Junior Fellow, Society of Fellows, Harvard University.

(2) G. B. Kistiakowsky and A. J. Rosenberg, This Journal, **74**, 5020 (1952).

(3) R. A. Alberty, V. Massey, C. Frieden and A. R. Fuhlbrigge, *ibid.*, **76**, 2485 (1954).

(4) W. M. Connors, A. Pihl, A. L. Dounce and E. Stotz, *J. Biol. Chem.*, **184**, 29 (1950).

(5) E. Bamann and M. Schmeller, *Z. physiol. Chem.*, **194**, 1 (1931).

(6) G. B. Kistiakowsky, P. C. Mangeldorf, Jr., A. J. Rosenberg and W. H. R. Shaw, This Journal, **74**, 5015 (1952); G. B. Kistiakowsky and W. H. R. Shaw, *ibid.*, **75**, 2751 (1953).