laboratory, we recommend that they be stored in the appropriate solvent medium.

**Cell Techniques.**—Part of the success of the present work is attributed to the cell design. A special type of dilution cell was constructed and is shown in Fig. 3. The cell consists of a small electrode chamber A, and a larger dilution chamber B. With a clamp attached to the connecting arm D, the cell was mounted in a manner such that a free rocking motion about an axis perpendicular to the plane of (a) in Fig. 3 could be obtained when desired. The electrodes were mounted in C and C′ with the aid of tightly fitted cork stoppers. Dilution of the original sample, placed in compartment A, was accomplished by adding solvent from a buret to compartment B *via* the port E, without removing the cell from the thermostat. After solvent was added, the cell was rocked about its axis until complete mixing was attained. Return of the cell to its horizontal position left a sufficient quantity of solution in the electrode compartment and an e.m.f. reading could be obtained. Electrode equilibration was obtained in 10–15 min. in all experiments and e.m.f. values were found to remain constant for as long as eight hours with solutions $5 \times 10^{-4}$ *M* in HCl.

The dilution cell techniques eliminate the necessity for pre-rinsing the electrodes carefully between measurements on individually prepared solutions, decrease the possibility of solution contamination and increase appreciably the mechanical efficiency of measurements. Careful checks showed that e.m.f. values obtained by the dilution cell techniques were virtually indistinguishable from those obtained with individually prepared solutions. The reproducibility of measurements was found to be ±0.05 mv. over the entire concentration range studied.

Surface effects were studied by varying the surface area exposed to the solutions in the cell as well as the volume of solution. Three types of cells were employed: (a) the dilution cell, (b) a cell similar to the dilution cell, but consisting of a single compartment, and (c) a beaker cell consisting of a 180-ml. electrolytic beaker equipped with a tightly fitted cork stopper supporting the electrode. E.m.f.'s measured in solutions as dilute as $1.2 \times 10^{-4}$ *M* HCl at widely varying surface/volume ratios differed at most by 0.1 mv. (see text, E.M.F. Measurements and Electrode Behavior). Surface effects are thus considered to be negligible.

E.m.f. measurements in the HCl-NaCl and HCl-KCl mixtures at varying acid/salt ratios were obtained as follows. Approximately 100 ml. of *ca.* 0.001 *M* HCl was prepared in the dilution cell. Aliquots of *ca.* 0.01 *M* salt solution were successively added to the cell from a semi-micro buret. After the addition of each aliquot of salt solution, the solution was thoroughly mixed and an e.m.f. measurement made. The procedure was then reversed and e.m.f. measurements obtained after successive additions of HCl aliquots to *ca.* 0.001 *M* salt solutions. This method resulted in wide variation of the individual electrolyte concentrations as well as the acid/salt ratios (*cf.* Table VI and VII).

**Experiments with Cell 8.**—The electrodes were a lightly platinized Beckman No. 1281 platinum electrode and a Beckman No. 1190-42 glass electrode. Matheson reagent grade hydrogen gas was passed first through a cold trap at −78°, then through a Greiner–Friedrichs gas washing bottle filled with 70.00 wt. % dioxane–water, and finally through a sintered glass disk into the solution to be measured. Electrode equilibrium appeared to be established in 2 to 3 hours, but all solutions were observed periodically over a 24-hour period. The hydrogen was allowed to pass over the platinized platinum electrode at a rate of *ca.* 0.2 ml./sec. for 30 minutes before, and during, each measurement, but was turned off between measurements. The e.m.f. values increased rapidly to a maximum, which remained constant for many hours, but eventually decreased slowly. The maximum e.m.f. was therefore accepted as the equilibrium value. All readings were corrected to a hydrogen pressure of 1 atm.

TALLAHASSEE, FLORIDA

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[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF FLORIDA STATE UNIVERSITY]

# The Measurement of Acid Dissociation Constants and Ion-pair Dissociation Constants in Solvents of Intermediate Dielectric Constant. Carboxylic Acids and their Sodium and Potassium Salts in 70% Dioxane–30% Water[1]

BY E. LEE PURLEE AND ERNEST GRUNWALD

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The e.m.f. of cells of the type: glass electrode/MA($c$), MCl($c$), HA($c_{\mathrm{HA}}$)/AgCl–Ag, has been measured at 25° using 70% dioxane as the solvent and formic acid, acetic acid and propionic acid and their sodium or potassium salts as solutes. The data obtained at ionic strengths below 0.0025 *M* have been extrapolated to infinite dilution according to the equation $K_{\mathrm{A}}' = K_{\mathrm{A}}[1 + 2c(y^*)^2(K_{\mathrm{MA}}^{-1} - K_{\mathrm{MCl}}^{-1})]$, where $K_{\mathrm{A}}$ is the thermodynamic acid dissociation constant, $K_{\mathrm{MA}}$ the ion-pair dissociation constant of the sodium or potassium salt of the acid and $K_{\mathrm{MCl}}$ that of NaCl or KCl, and $y^*$ is the known molar activity coefficient of HCl at the concentration $2c$. The validity of the extrapolations has been established by obtaining good agreement between the $K_{\mathrm{A}}$ values based on the data for the sodium salts and those based on the data for the potassium salts. From the slopes of the extrapolation plots, the ion-pair dissociation constants of the sodium and potassium salts of the acids have been deduced.

In solvents of intermediate dielectric constant, the measurement of acid dissociation constants is complicated by the formation of ion pairs.[2–4] In the preceding paper,[5] we described a method for treating ion-pair dissociation, which is based on equations 1 and 2. In these equations, $a_i$ is the activity of a given ionic species, $c_i$ is the actual molar concentration of *free ions* of the type i, and $y'$ is their molar activity coefficient; $S$ is the Debye–Hückel limiting slope, and $\mu$ the free-ion ionic strength.

$$a_i = c_i y' \tag{1}$$

$$-\log y' = 0.8686\left[1 - \frac{\log(1 + 2.303S\sqrt{\mu})}{S\sqrt{\mu}}\right] \tag{2}$$

This approach will now be applied to the measurement of $K_{\mathrm{A}}$ for formic acid, acetic acid and propionic acid, and to that of the dissociation constants of the sodium and potassium salts of these acids. All measurements were made in 70.00 wt.% dioxane–30.00 wt.% water at 25.00°, at ionic strengths ranging from 0.0004 to 0.002 *M*, under which conditions equations 1 and 2 have been shown to apply.[5]

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(1) This work has been supported by a grant from the National Science Foundation.

(2) I. M. Kolthoff and S. Bruckenstein, THIS JOURNAL, **78**, 1 (1956).

(3) E. Grunwald, *Anal. Chem.*, **26**, 1696 (1954).

(4) E. J. Corey, THIS JOURNAL, **75**, 1172 (1953).

(5) E. L. Purlee and E. Grunwald, *ibid.*, **79**, 1366 (1957).

The most relevant previous work on $K_A$ determinations for these acids in 70% dioxane is that of Harned and co-workers.[6] In their work $K_A$ was obtained by a rather long extrapolation to infinite dilution, from potentiometric data with the cell 3 at salt concentrations above 0.01 $M$

$$\text{Pt-H}_2\,(1\text{ atm.})/\text{HA}(c),\ \text{NaA}(c),\ \text{NaCl}(c)/\text{AgCl-Ag} \quad (3)$$

Our previous experience[5] with hydrochloric acid in 70% dioxane indicates that this concentration might be too high for accurate extrapolation; moreover, their value of $E^0$, 0.0658 v. (on the $c$-scale), has been shown to be in error, the best estimate from their data for hydrochloric acid being 0.0670 v.[5]

**Principle of the Method.**—The cell used in this work was 4, where M is either sodium or potassium.

$$\text{glass electrode}/\text{MA}(c),\ \text{MCl}(c),\ \text{HA}(c_{\text{HA}})/\text{AgCl-Ag} \quad (4)$$

This cell accurately measures $a_{\text{H}}a_{\text{Cl}}$ according to equation 5, as described in the preceding paper.[5]

$$a_{\text{H}}a_{\text{Cl}} = \text{antilog}_{10}\,(E^* - E)/(2.303RT/F) \quad (5)$$

We shall define a function $K_A'$ by equation 6a. $K_A'$ may be written in the form 6b, where $c_{A-}$

$$K_A' = a_{\text{H}}a_{\text{Cl}}/c_{\text{HA}} \quad (6a)$$

$$K_A' = (a_{\text{H}}a_{\text{A}}/c_{\text{HA}})(c_{\text{Cl}^-}/c_{\text{A}^-}) \quad (6b)$$

and $c_{\text{Cl}-}$ are the respective free-ion concentrations since the values of $y'$ for the two ions are equal (see equation 2). It will be shown that in our experiments $y_{\text{HA}} = 1$; hence equation 7 is obtained from 6b.

$$K_A' = K_A(c_{\text{Cl}^-}/c_{\text{A}^-}) \quad (7)$$

We shall now seek an approximate relationship among $c_{\text{Cl}-}/c_{\text{A}-}$ and $c$, $K_{\text{MA}}$ and $K_{\text{MX}}$ which becomes correct in the limit at low concentrations. Let $w$ be the concentration of $\text{M}^+\text{Cl}^-$ ion pairs, and $v$ be that of $\text{M}^+\text{A}^-$ ion pairs. Then the concentrations of the free ions $\text{Na}^+$, $\text{Cl}^-$ and $\text{A}^-$ will be, respectively, $2c - v - w$, $c - w$, and $c - v$. (The concentrations of free $\text{H}^+$ and of $\text{H}^+\text{Cl}^-$ are very small compared to $c$, $w$ and $v$ in these experiments.) Thus the ion-pair dissociation constants are given by equations 8.

$$K_{\text{MCl}} = (2c - w - v)(c - w)(y')^2/w \quad (8a)$$

$$K_{\text{MA}} = (2c - w - v)(c - v)(y')^2/v \quad (8b)$$

In deriving the approximate relationship, we shall suppose that: (i) $w < c$, $v < c$; hence $(2c - v - w) \approx 2(c - w) \approx 2(c - v)$; and $(c - w)/c = \alpha_{\text{Cl}}$ is approximately equal to $(c - v)/c = \alpha_{\text{A}}$. (ii) $\alpha_{\text{Cl}}y' = y_{\text{Cl}} \approx y_{\text{A}} \approx y^*$, where $y^*$ is the molar activity coefficient of hydrochloric acid at the same total electrolyte concentration, namely, $2c$.

The ratio $c_{\text{Cl}-}/c_{\text{A}-}$ is equal to $(1 - w/c)/(1 - v/c)$. Upon expanding $(1 - v/c)^{-1}$ in power series, we obtained equation 9. In order to solve this

$$c_{\text{Cl}^-}/c_{\text{A}} = 1 + \frac{v}{c} - \frac{w}{c} + \frac{v}{c}\left(\frac{v}{c} - \frac{w}{c}\right) + \ldots\ldots \quad (9)$$

equation, the expressions 10 were derived from equations 8 and from the approximations (i) and (ii).

$$w = 2c^2(y^*)^2/K_{\text{MCl}} \quad (10a)$$

$$v = 2c^2(y^*)^2/K_{\text{MA}} \quad (10b)$$

Upon substitution of these in equation 9, and retaining only the linear terms, we obtained equation 11. Finally upon substitution of 11 in 7, we obtained equation 12.

$$c_{\text{Cl}^-}/c_{\text{A}^-} = 1 + 2c(y^*)^2[K_{\text{MA}}^{-1} - K_{\text{MCl}}^{-1}] \quad (11)$$

$$K_A' = K_A + 2c(y^*)^2\left[\frac{K_A}{K_{\text{MA}}} - \frac{K_A}{K_{\text{MCl}}}\right] \quad (12)$$

Equation 12 predicts that the function $K_A'$ varies linearly with $2c(y^*)^2$ at low concentrations, the intercept being $K_A$ and the slope $(K_A/K_{\text{MA}} - K_A/K_{\text{MCl}})$. Thus $K_A$ may be obtained by this method without explicit knowledge of $K_{\text{MA}}$ and $K_{\text{MCl}}$. $K_{\text{MA}}$ or $K_{\text{MCl}}$ may be obtained from the slope if one of these constants is already known. In the present case, values of $K_{\text{MCl}}$ are available for sodium chloride and potassium chloride.[5]

**Activity of Acetic Acid.**—In view of recent evidence that carboxylic acids in aqueous solution are measurably dimerized,[7,8] we felt it was necessary to measure the activity coefficient $y_{\text{HA}}$ for at least one of the acids in 70% dioxane under the conditions of the $K_A$ determinations. The following experiment was devised: Using cell 3 with acetic acid, sodium acetate and sodium chloride, $c$ was kept constant at $0.001064_1$ $M$, and $c_{\text{HA}}$ was varied from 0.002043 to $0.01277_0$ $M$. Since acetic acid is very weak in this solvent, the concentration of hydrogen ion is so small that, even though it is changing in these experiments, its contribution to the total ionic strength is negligible. Hence, the activities $a_{\text{Na}}$, $a_{\text{Ac}}$ and $a_{\text{Cl}}$ are constant, and by virtue of equation 5 and the defining equation, $K_A = a_{\text{H}}a_{\text{Ac}}/a_{\text{HAc}}$, we obtain the result 13. If $y_{\text{HAc}} = 1$, that is, if dimerization is negligible, the left-hand side of equation 13 will be directly proportional to

$$\text{antilog}_{10}[(E^* - E)/(2.303RT/F)] = \text{constant} \times a_{\text{HAc}} \quad (13)$$

$c_{\text{HAc}}$. Within the experimental error of *ca.* 0.3%, this was indeed the case. Since in water, the dimerization constants for formic, acetic and propionic acid are of the same order of magnitude,[7] we have set $y_{\text{HA}}$ equal to unity in all our measurements.

**Application of Equation 12.**—The experimental data for formic, acetic and propionic acid are plotted according to equation 12 in Figs. 1, 2 and 3. The validity of the equation was tested by the following criteria: (i) linearity of the plots within the replicability of the data; (ii) equality of the intercepts, that is, of the calculated $K_A$ values, for different cations. It is apparent from the figures that both criteria are satisfied; a detailed analysis is given only of the data for acetic acid.

The complete set of data for solutions containing acetic acid, sodium acetate and sodium chloride is given in Table I. The standard error of single $10^9K_A'$ values is estimated as 0.015 from their replicability. (This corresponds to 0.08 mv. error in e.m.f.) The equation

$$10^9K_A' = 4.584 + 502.4 \times 2c(y^*)^2$$

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[6] (a) H. S. Harned and G. L. Kazanjian, THIS JOURNAL, **58**, 1912 (1936); (b) H. S. Harned and L. D. Fallon, *ibid.*, **61**, 2377 (1939); (c) H. S. Harned and R. S. Done, *ibid.*, **63**, 2579 (1941); (d) H. S. Harned and T. R. Dedell, *ibid.*, **63**, 3308 (1941).

[7] A. Katchalsky, H. Eisenberg and S. Lifson, *ibid.*, **73**, 5889 (1951).

[8] M. Davies and D. M. L. Griffiths, *Z. physik. Chem., Neue Folge*, **2**, 353 (1954).

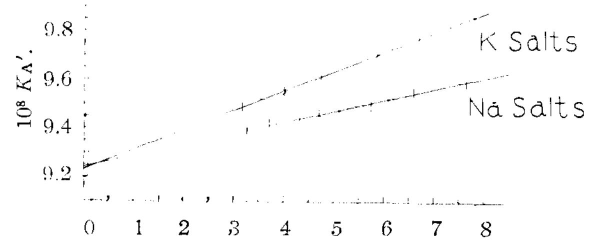

Fig. 1.—Data for formic acid in 70.00 wt. % dioxane-water, 25.00°.

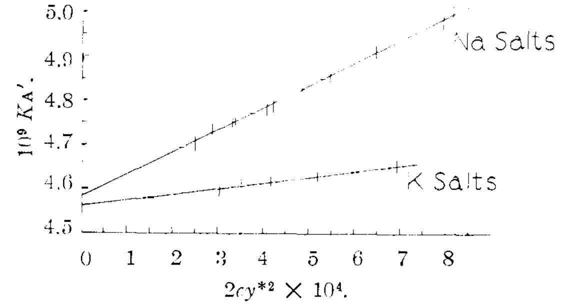

Fig. 2.—Data for acetic acid in 70.00 wt. % dioxane-water, 25.00°.

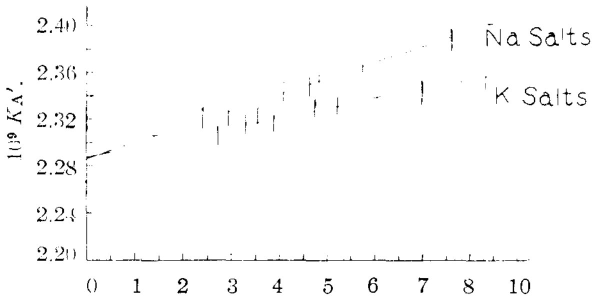

Fig. 3.—Data for propionic acid in 70.00 wt. % dioxane-water, 25.00°.

was obtained from the data by the method of least squares, and the standard error of fit to the $10^9 K_A'$ values was found to be 0.011. This is consistent with the replicability of these values (see Table I). The standard error of the intercept is estimated as 0.007, and of the slope as 13.

The least-squares fits of all our data to equation 12 are summarized in Table II. In every case the standard error of fit is consistent with the replicability of the data, and can be accounted for by errors in e.m.f. of 0.05 mv. or less. For all the acids, the intercepts obtained with the sodium salts are consistent with those obtained with the potassium salts even though the slopes differ by large factors. The deviations are, 0.1% for formic acid, 0.5% for acetic acid, and 0.04% for propionic acid. We therefore feel justified in identifying the intercepts with $K_A$.

TABLE I

DATA FOR SOLUTIONS CONTAINING ACETIC ACID, SODIUM ACETATE AND SODIUM CHLORIDE, 70.00 WT. % DIOXANE, 25.00°

| $10^4 c$ | $10^4 c_{HA}$ | $2c(y^*)^2 \times 10^4$ | $10^9 K_A'$ Obsd. | $10^9 K_A'$ Calcd. |
|---|---|---|---|---|
| 9.778 | 79.61 | 7.960 | 4.973 | 4.984 |
| 9.778 | 79.61 | 7.960 | 4.967 | 4.984 |
| 6.977 | 56.81 | 6.472 | 4.910 | 4.909 |
| 5.422 | 44.14 | 5.458 | 4.868 | 4.858 |
| 5.422 | 44.14 | 5.458 | 4.887 | 4.858 |
| 3.749 | 30.54 | 4.217 | 4.786 | 4.796 |
| 2.867 | 23.34 | 3.390 | 4.750 | 4.754 |
| 2.867 | 23.34 | 3.390 | 4.731 | 4.754 |
| 2.320 | 18.89 | 2.948 | 4.739 | 4.732 |
| 10.178 | 85.13 | 8.156 | 4.988 | 4.994 |
| 10.178 | 85.13 | 8.156 | 5.009 | 4.994 |
| 5.421 | 45.34 | 5.458 | 4.851 | 4.858 |
| 3.694 | 30.90 | 4.068 | 4.786 | 4.788 |
| 3.694 | 30.90 | 4.068 | 4.804 | 4.788 |
| 2.802 | 23.44 | 3.331 | 4.749 | 4.751 |
| 2.248 | 18.80 | 2.877 | 4.723 | 4.729 |
| 2.248 | 18.80 | 2.877 | 4.741 | 4.729 |
| 1.883 | 15.75 | 2.501 | 4.714 | 4.710 |
| 1.883 | 15.75 | 2.501 | 4.696 | 4.710 |

TABLE II

FIT OF EQUATION 12 TO THE DATA FOR CARBOXYLIC ACIDS, 70.00 WT. % DIOXANE, 25.00°

| Acid | $10^9 \times$ slope | $10^9 K_A$ | $10^9 \times$ stand. error of Slope | $K_A$ | $K_A'$[a] |
|---|---|---|---|---|---|
| Sodium salts | | | | | |
| Formic | 4578 | 92.41 | 365 | 0.19 | 0.14 |
| Acetic | 502.4 | 4.584 | 13 | .007 | .011 |
| Propionic | 139.5 | 2.287 | 7 | .004 | .003 |
| Potassium salts | | | | | |
| Formic | 8087 | 92.31 | 230 | .13 | .10 |
| Acetic | 133.5 | 4.561 | 23 | .011 | .007 |
| Propionic | 86.2 | 2.288 | 6 | .003 | .003 |

[a] Standard error of fit.

**Ion-pair Dissociation Constants.**—According to equation 12, the slopes listed in Table II are equal to $K_A [K_{MA}^{-1} - K_{MCl}^{-1}]$. Using the $K_A$ values given in Table II and the $K_{MCl}$ values reported previously,[5] $K_{MA}$ values have thus been calculated. The results are shown in Table III; the errors of the $K_{MA}$ values are conservative estimates, based on the *maximum* errors in $K_{MCl}$. Since a number of approximations were made in the derivation of equation 12, the accuracy of the $K_{MA}$ values was checked by exact calculation, and the results for sodium acetate, which are typical, are shown in Table IV. The table also lists the results of five separate experiments in which the concentrations of sodium acetate and sodium chloride were not equal but differed by factors ranging from 0.5 to 8.

TABLE III

SUMMARY OF ION-PAIR DISSOCIATION CONSTANTS IN 70.00 WT. % DIOXANE, 25.00°

| Salt[a] | $10^3 K$ | Salt[a] | $10^3 K$ |
|---|---|---|---|
| NaCl | 5.35 ± 0.27 | KCl | 2.35 ± 0.16 |
| NaFo | 4.23 ± .18 | KFo | 1.95 ± .11 |
| NaAc | 3.37 ± .12 | KAc | 2.20 ± .14 |
| NaPr | 4.03 ± .17 | KPr | 2.16 ± .13 |

[a] Fo, formate; Ac, acetate; Pr, propionate.

The method of calculation was as follows: first, the ratio of free-ion concentrations $c_{Cl^-}/c_{Ac^-}$

TABLE IV

EXACT CALCULATION OF $K_{NaAc}$

| $10^4c_{HAc}$ | $10^4c_{NaAc}$ | $10^4c_{NaCl}$ | $10^3K_{NaAc}$ |
|---|---|---|---|
| 79.61 | 9.778 | 9.778 | 3.31 |
| 56.81 | 6.977 | 6.977 | 3.27 |
| 44.14 | 5.422 | 5.422 | 3.24 |
| 30.54 | 3.749 | 3.749 | 3.35 |
| 23.34 | 2.867 | 2.867 | 3.36 |
| 18.89 | 2.320 | 2.320 | 3.24 |
| 85.13 | 10.178 | 10.178 | 3.29 |
| 45.34 | 5.421 | 5.421 | 3.32 |
| 30.90 | 3.694 | 3.694 | 3.35 |
| 23.44 | 2.802 | 2.802 | 3.34 |
| 18.80 | 2.248 | 2.248 | 3.74 |
| 18.80 | 2.248 | 2.248 | 3.25 |
| 15.75 | 1.883 | 1.883 | 3.25 |
| 17.77 | 16.98 | 2.001 | 3.15 |
| 30.65 | 10.194 | 5.184 | 3.24 |
| 29.16 | 4.955 | 9.852 | 3.00 |
| 24.64 | 10.037 | 5.004 | 3.02 |
| 24.82 | 9.858 | 10.003 | 3.41 |

was calculated from the experimental data *via* equation 7. Next, using an assumed value for the ionic strength $\mu$ ($= c_{Cl^-} + c_{Ac^-}$), a corresponding value of $y'$ was obtained from equation 2, and of $c_{Cl^-}$ from equation 14.

$$c_{Cl^-} = \mu/(1 + c_{Ac^-}/c_{Cl^-}) \qquad (14)$$

From these and known formal concentrations, a corresponding value of $K_{NaCl}$ was obtained. This value was, of course, not equal to the experimental value of $K_{NaCl}$, but by assuming different values for $\mu$, the correct value of $\mu$ could be found for which the calculated $K_{NaCl}$ was equal to the experimental $K_{NaCl}$. Using this correct value of $\mu$, $K_{NaAc}$ was calculated.

It is seen that the calculated values of $K_{NaAc}$ are reasonably precise; the standard deviation of the individual values corresponds to a standard error in e.m.f. of approximately 0.05 mv. This is further proof of the validity of equations 1 and 2 upon which this treatment of the data has been based. The value and the standard deviation of the mean of the $K_{NaAc}$ values is $(3.29 \pm 0.04) \times 10^{-3}$. This is to be compared with the value, $3.37 \times 10^{-3}$, obtained by the use of equation 12 (see Table III). The difference between the two values is small, and statistical analysis shows that it is probably not significant. We conclude that equation 12 provides a simple and accurate means for obtaining both $K_A$ and $K_{MA}$.

Regarding the magnitude of the $K$ values, it appears that the sodium salts are consistently more dissociated in 70% dioxane than the corresponding potassium salts. This fact suggests that the greater solvation energy of sodium ion compared to potassium ion is a more important factor than the interaction energy in the ion pairs, which is probably also greater for the sodium salts. In solvents which are less good at solvating the free ions, the reverse order is sometimes observed.[9]

**Data at Higher Concentrations.**—Some measurements were made for acetic acid and propionic acid at ionic strengths up to 0.16 $M$ in order to find out at what concentration the $K_A'$ values deviate from the straight line obtained at the low concentrations. The results for the sodium salts are plotted in Fig. 4. In both cases the deviations became significant at ionic strengths greater than 0.005 $M$. However, the directions of the deviations are different for the two acids.

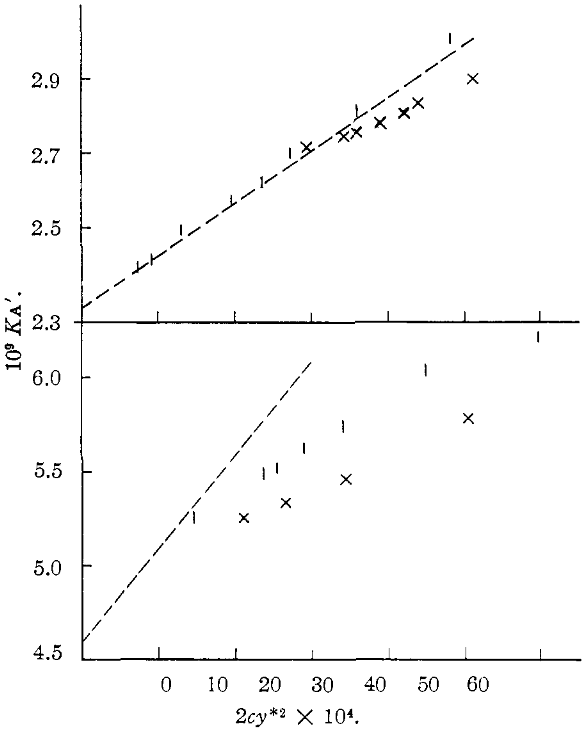

Fig. 4.—$K_A'$ values at higher ionic strengths. The dotted lines represent the data at low ionic strengths: |, this work; X, ref. 6; top, propionic acid; bottom, acetic acid.

Figure 4 also shows the previous data of Harned and co-workers,[6] recalculated with $E^0 = 0.0670$ v. The data for acetic acid which were reported in 1936[6a] differ appreciably from our results, whereas the more recent data for propionic acid[6d] are in fair agreement. However, the extrapolated value which one would obtain from the previous data for propionic acid differs appreciably from the $K_A$ value reported by us. In the case of acetic acid, the extrapolated value from the previous data by coincidence agrees fairly well with our value.

## Experimental

**Materials.**—70 wt. % dioxane–water mixtures and hydrochloric acid solutions were prepared as previously described.[5] Sodium and potassium hydroxide solutions (*ca.* 0.03 $M$) were prepared by treating the pure metals with a known weight of pure dioxane containing a small quantity of 70 wt. % dioxane–water solution. During the reaction process the solutions were kept under an atmosphere of nitrogen. The required weight of water was added after reaction was completed. Hydrochloric acid solutions (prepared from constant boiling HCl) were used as acidimetric standards in determining the hydroxide titers.

Acetic and propionic acid solutions (*ca.* 0.1 $M$) were prepared gravimetrically from reagent grade carboxylic acids further purified by distillation. The titers of hydroxide solutions obtained by titration with these acetic and propionic acid solutions agreed within 0.2% of those obtained by titration with the standard hydrochloric acid solutions. Formic acid solutions (*ca.* 0.1 $M$) were prepared by adding reagent grade, aqueous formic acid (85–90%) to 70% dioxane. The required amount of pure dioxane was added to

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[9] C. A. Kraus, *J. Phys. Chem.*, **60**, 129 (1956).

compensate for the water content of the formic acid solution. The titers of the formic acid solutions were obtained by titration with the hydroxide solutions. Only *freshly* prepared solutions were utilized. All volumetric ware was calibrated. Titrations were carried out in an atmosphere of nitrogen.

In the experiments with concentrated solutions, the above method of preparing MOH solutions resulted in phase splitting. Therefore, the procedure was as follows. Saturated aqueous MOH was added to a known amount of HA in 70% dioxane, pure dioxane was added to compensate for the water content of the MOH, and the resulting solution, which contained a slight excess of HA, made up to volume. Since the amount of MOH was not measured accurately, the resultant mixture was standardized by titration, the HA with standard base to brom thymol blue, and the MA with standard HCl in 70% dioxane to methyl orange. To our knowledge, the latter titration has not been reported previously. The analytical determinations for MA plus HA were within 0.08% of the synthetic value, based on the amount of HA taken.

**E.M.F. Measurements.**—E.m.f. measurements were made with the equipment as described earlier,[5,10] but with a modification in technique which eliminated the $V_p$ measurement as described in procedure II of the experimental part of ref. 10. Equilibrium e.m.f. readings were obtained in the normal manner by adjustment of the "helipot" of the Model GS *p*H meter. The "helipot" was then locked at its position and the output terminals of the Rubicon potentiometer connected across the input terminals of the *p*H meter. The actual e.m.f. was obtained by balancing the output of the Rubicon potentiometer against the potentiometer circuit of the *p*H meter. In this connection the galvanometer of the *p*H meter served as the null indicator. The accuracy of e.m.f. measurements obtained by this technique is estimated to be ±0.02 mv. Glass electrode "C" of ref. 5 was used in all measurements.

**Cell Techniques.**—In all experiments at the lower concentrations, mixtures of MCl, MA and HA were prepared by adding the required amount of MOH to an appropriate mixture of HCl and HA. In the experiments at the higher concentrations, the required amount of HCl was added to a solution containing a known amount of HA and MA. In the experiments devised to measure the acetic acid activity and in those devised to measure $K_{NaAc}$ with $c_{NaAc}/c_{NaCl}$ ratios not equal to one (see Table IV), the e.m.f. measurements were made on individually prepared solutions in beaker-type cells.[5]

Dilution cell techniques[5] were employed in all the other measurements. In the $K_{HA} - K_{MA}$ determinations, the initial carboxylic acid concentration was varied from *ca.* 0.005 to 0.01 $M$. The initial electrolyte concentration was *ca.* 0.002 $M$.

Throughout the course of the experiments $E^*$ for cell (4) was checked frequently by obtaining e.m.f. measurements in the HCl–HA mixtures prior to the addition of MOH and by e.m.f. measurements in HCl solution. In all measurements $E^*$ was found to be $-0.63180 \pm 0.00005$ v.

All e.m.f. measurements for duplicate solutions were found to be reproducible to within at least ±0.1 mv.

TALLAHASSEE, FLORIDA

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF WISCONSIN]

# The Conversion of Fibrinogen to Fibrin. XXI. Polymer Structure and Thermodynamic Interactions in Alkaline Clotting Systems Inhibited by Hexamethylene Glycol[1]

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Ultracentrifugation and light scattering studies have been made on solutions of partially polymerized fibrinogen in a sodium chloride–glycinate buffer at *p*H 9.5 and ionic strength 0.45 with added hexamethylene glycol at 0.5 $M$ to suppress the formation of fibrin gel. Sedimentation shows that in 12 hours at 25°, the enzymatic action of thrombin at a concentration of 1 unit/cc. results in the conversion of about 80% of the fibrinogen in solutions originally containing about 4 g./l. to polymeric form. When corrected for the effect of thermodynamic non-ideality, the angular distribution of 4358 Å. light scattered by the polymeric fraction through angles from 25 to 140° agrees quite closely with that theoretically required for a system of cylindrical rods of length indeterminate, but at least of the order of 5000 Å., and mass/length ratio 2.3 times that for monomeric fibrinogen, also presumed to be rod-like. Thermodynamic interactions between solute components—ordinarily obtained from the variation of the second virial coefficient with composition—are here deduced from the change in concentration dependence of scattered intensity with scattering angle. The net interaction between polymer molecules is one of repulsion, quantitatively somewhat greater than is accounted for by the mutually excluded volume for two impenetrable cylinders. The effective monomer–polymer intermolecular force is, however, one of attraction; *i.e.*, the presence of monomeric particles is favored in the statistical environment of a polymer molecule.

## Introduction

Paper XIX[4] in this series concerned a study by light scattering of the soluble polymer (or polymers) of bovine fibrinogen formed by the enzymatic action of thrombin at *p*H 9.5. That investigation involved only polymer formed at very low protein concentration, less than 0.5 g./l., since at higher concentrations further reaction occurs and this intermediate polymer is converted to fibrin gel. In this paper we discuss polymers prepared in the same buffer system but stabilized at much higher protein concentration by the addition of hexamethylene glycol, one of a number of substances capable of inhibiting gelation,[5] at least within certain ranges of ionic strength, *p*H and concentration of protein and inhibitor.

In the earlier work the task of interpreting light scattering data from the very dilute solutions was simplified by two factors: the conversion of fibrinogen to polymer apparently was substantially complete—and was assumed to be so—and the concentration was sufficiently low for the solute to be considered thermodynamically ideal (*i.e.*, the scattered intensity due to solute was directly proportional to concentration). In the present study of inhibited clotting systems neither of these simplifications holds. Thus we use the velocity ultracentrifuge to obtain an estimate of the extent of conversion of fibrinogen to polymer and evaluate

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