

Thermodynamic Quantities for the Dissociation Equilibria of Biologically Important Compounds

1. THEORY AND EXPERIMENTAL METHOD

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A study of the thermodynamics of acid-base dissociations of substances of biological importance is necessary for many reasons. Most of the substances taking part in the energy-exchange mechanisms of living cells carry groups which interact with hydrogen ions. As one substance is converted into another, variations in the degree of association of the hydrogen ions will contribute to the overall energy changes. It is well known, for example, that ester phosphates are stronger acids than is phosphoric acid itself (Kumler & Eiler, 1943). Their hydrolysis therefore results in the production of a weaker acid from a stronger. In a medium where the pH is maintained sensibly constant, this results in a diminution of the proportion of the ionized form, the energy of the association consequently appearing as part of the overall free-energy change of the reaction (cf. Dixon, 1949). The evaluation of the importance of ionization changes in these reactions demands a knowledge of the free energy and other thermodynamic functions of the dissociation equilibria.

Further, the setting of these and related processes in the perspective of cellular activity as a whole requires a more detailed knowledge of cellular buffer systems than is currently available. The day will soon be past when sufficient information is given about a biological buffer system by determination of the apparent pK at a random concentration by titration with a glass electrode and application of the Henderson-Hasselbalch equation. An excellent example of the improvement in the interpretation of cellular buffer systems to be gained by the use of activity coefficients will be found in the work of Trevelyan, Mann & Harrison (1952) on phosphorylase.

In addition to their importance in the cell many of the buffer systems are potentially useful in biological or chemical investigation. In any event, their study is intrinsically useful as a contribution to the physical chemistry of solutions since most of the compounds involved differ radically from those usually studied by the physical chemist.

We have made a beginning with such an investigation by measuring the thermodynamic constants for several phosphate esters and other, non-phosphorylated, compounds. In this paper are set

out the theory and experimental procedure for the determinations. Subsequent papers will contain the results.

THEORY

List of symbols

Symbols are used according to the recommendations of the Report of the Symbols Committee of the Royal Society (1951). Those which require definition are listed below. The Report also recommends that among the conventions for the representation of thermodynamic data for chemical reactions:— 'It is preferred that quantities of heat, as of other forms of energy, be expressed in joules; where data are given in calories, the corresponding value in joules should be added, with the conversion factor used.' That recommendation has been followed here. The conversion factor used for converting International joules to International calories was $4.1833 \text{ joules. cal.}^{-1}$:

- A** = constant of Debye-Hückel theory.
- A** = acid anion, or constant in $\log K$ - temperature equation.
- a'** = closest distance of approach of oppositely charged ions.
- a_x** = activity of species **x**.
- a** = subscript to indicate acidic form of dissociating species.
- B** = constant of Debye-Hückel theory.
- b** = subscript to indicate basic form of dissociating species.
- β** = variable parameter of activity coefficient equations.
- C** = constant in $\log K$ - temperature equation.
- c_x** = molarity of species **x**.
- D** = constant in $\log K$ - temperature equation.
- d₀** = density of solvent (usually water).
- d** = density of solution.
- γ_x** = activity coefficient of species **x** on molal scale.
- I** = ionic strength, $= \frac{1}{2} \sum Z_x^2 m_x$.
- ln** = logarithm to base **e**.
- log** = logarithm to base 10.
- m₁** = molality of HCl.
- m₂** = molality of salt.
- m_x** = molality of species **x**.
- M** = gram-molecular weight.
- Z_x** = valence (charge) of ionic species **x**.

Concentrations are expressed on the molal scale; moles of solute/kg. of solvent. It is related to the more familiar molar scale (moles of solute/l. of solution) by the following reciprocal relations:

$$c_x = \frac{1000m_x d}{1000 + \Sigma m M} \quad \text{and} \quad m_x = \frac{1000c_x}{1000d - \Sigma c M},$$

which at great dilution simplify to

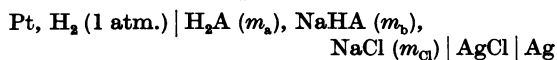
$$c_x = m_x d_0,$$

where the Σ 's indicate that the summation is to be taken over all molecules in the solution.

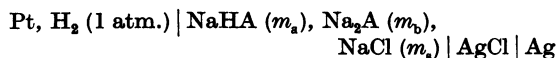
Cells

The dissociation constants were evaluated from electromotive force measurements of galvanic cells without liquid junctions composed of hydrogen and silver-silver chloride electrodes in solutions containing mixtures of the acids and their salts with chloride. The method is essentially the same as that used by Harned & Ehlers (1932) for acetic acid, and by Nims (1933, 1934) and Bates & Acree (1943) for the dissociation constants of phosphoric acid.

The cells may be represented:



for first dissociation constants, and



for second dissociation constants. The acid anion is here shown as dibasic and the sodium salt has been written for convenience, although other alkali metal salts can and have been used. Metals of higher valencies have been avoided because of the greater activity-coefficient corrections and because many of their salts are incompletely dissociated.

The electromotive force of these cells is given by the Nernst equation:

$$E = E_0 - \frac{RT}{F} \ln (a_{\text{H}} \cdot a_{\text{Cl}}) \\ = E_0 - \frac{2 \cdot 3026 RT}{F} \log (\gamma_{\text{H}} \cdot \gamma_{\text{Cl}} \cdot m_{\text{H}} \cdot m_{\text{Cl}}). \quad (1)$$

The dissociation constants of an acid are given by expressions of the type

$$K = \frac{a_{\text{H}} \cdot a_{\text{b}}}{a_{\text{a}}} = \frac{m_{\text{H}} \cdot m_{\text{b}}}{m_{\text{a}}} \cdot \frac{\gamma_{\text{H}} \cdot \gamma_{\text{b}}}{\gamma_{\text{a}}}. \quad (2)$$

Eliminating m_{H} from equations (1) and (2) we obtain

$$-\log K = \frac{(E - E_0) F}{2 \cdot 3026 RT} + \log \frac{m_{\text{Cl}} \cdot m_{\text{a}}}{m_{\text{b}}} + \log \frac{\gamma_{\text{Cl}} \cdot \gamma_{\text{a}}}{\gamma_{\text{b}}}. \quad (3)$$

Since $\gamma \rightarrow 1$ as the solution becomes infinitely dilute, a series of measurements of E at decreasing concentrations and extrapolation of the first two terms on the right-hand side of equation (3) to zero concentration would give $-\log K$. However, the extrapola-

tion would not be linear, and extrapolation of curves is an unsatisfactory procedure giving results of low accuracy.

Expressions for the activity-coefficient

In order to obtain linear extrapolations it is necessary to find a means of estimating the activity coefficients with sufficient accuracy to cause the right-hand side of equation (3) either to become constant or to vary linearly with the concentration. Such an estimate should contain the minimum number of arbitrary parameters consistent with adequate representation of the data. Further, it should be possible to apply it consistently and without ambiguity to as wide a range of data as possible so that all calculations rest upon the same assumptions.

Two such forms have been used by different workers. Harned and his co-workers (Harned & Owen, 1950) and Nims (1933, 1934) have used the limiting form of the Debye-Hückel law (1923) with the addition of a term linear in the ionic strength, i.e. they write

$$\log \gamma_x = -Z_x^2 A I^{\frac{1}{2}} + \beta I, \quad (4)$$

where A is the universal constant of the Debye-Hückel theory dependent only on the temperature and the dielectric constant and density of the solvent, and β is an empirical constant whose value is determined by the best straight line to fit the experimental points.

Bates and his co-workers (e.g. Bates & Acree, 1943) have used the full Debye-Hückel expression with the addition of a linear term as suggested by Hückel (1925)

$$\log \gamma_x = \frac{-Z_x^2 A I^{\frac{1}{2}}}{1 + B a' I^{\frac{1}{2}}} + \beta I, \quad (5)$$

where B is another universal constant and a' is the nearest distance of approach of ions of opposite sign expressed in Ångström units. Although a' should strictly take a different value for each ion pair, it is generally assumed to have some average value in a given solution, this same value being used to calculate the activity coefficient of each ion.

The Debye-Hückel expression. The effect of substituting either of the expressions (4) or (5) for the activity coefficient term of equation (3) varies according to whether it applies to a first or to subsequent dissociation steps. For a first dissociation, the acidic form is uncharged and $\gamma_{\text{a}} = 1$ to a close approximation. The remainder of the term consists of the quotient of two univalent ion activities so that, using either (4) or (5), the terms in $I^{\frac{1}{2}}$ cancel and only a linear function of I is left.

The residual function for second dissociations is more complex. Here γ_{a} will be the activity coefficient of an univalent ion ($Z = 1$) and γ_{b} that of

a divalent ion ($Z=2$). The $I^{\frac{1}{2}}$ terms do not then cancel, and we have

$$\log \frac{\gamma_{\text{Cl}} \cdot \gamma_{\text{a}}}{\gamma_{\text{b}}} = \frac{2AI^{\frac{1}{2}}}{1 + Ba' I^{\frac{1}{2}}} + \beta I \quad (6)$$

from expression (5), or

$$\log \frac{\gamma_{\text{Cl}} \cdot \gamma_{\text{a}}}{\gamma_{\text{b}}} = 2AI^{\frac{1}{2}} + \beta I \quad (6a)$$

from expression (4). Substituting these values in equation (3) and rearranging we get

$$-\log K - \beta I = \frac{(E - E_0) F}{2 \cdot 3026 RT} + \log \frac{m_{\text{Cl}} \cdot m_{\text{a}}}{m_{\text{b}}} + \frac{2AI^{\frac{1}{2}}}{1 + Ba' I^{\frac{1}{2}}} \quad (7)$$

from (5), and

$$-\log K - \beta I = \frac{(E - E_0) F}{2 \cdot 3026 RT} + \log \frac{m_{\text{Cl}} \cdot m_{\text{a}}}{m_{\text{b}}} + 2AI^{\frac{1}{2}} \quad (7a)$$

from (4). If equation (7a) is used, its right-hand side contains only quantities which are known or can be determined and is plotted against ionic strength. Almost straight lines are obtained, but there is some residual curvature which makes extrapolation rather unsatisfactory.

The procedure is more complex with equation (7) because of the extra variable parameter a' whose value can only be obtained from the experimental data. This is done by replotting the right-hand side of equation (7) with a series of values of a' until one is found which gives the best straight line. Most values appear to lie in the region 3–6 Å. This equation with the correct value of a' gives good straight lines up to ionic strengths of 0.8 (Pinching & Bates, 1948).

Criticisms of the Debye-Hückel expression. In the work to be described neither of these expressions has been used. The first (eqn. 4) was discarded because of the residual curvature and because it gives plots with a large slope. Variations in the slope of the lines due to random experimental errors cause smaller variations in the extrapolated values when the lines are more nearly horizontal since the variation of the position of the intercept is proportional to the secant of the angle from the horizontal.

The complete Debye-Hückel law was not used for the following reasons. The constant a' , which is introduced during the derivation of the law as the distance of closest approach of ions of opposite sign, might appear at first sight to have a definite physical meaning. However, careful consideration will show that this is not so. The only way of evaluating it is by continued replotting of the data until the best straight lines are obtained, i.e. by a purely empirical process. This is not surprising since the constant as it appears in equations such as (7) is a lumped constant of the three negative ions in the preceding logarithmic term, together with whatever variety of

positive ions there may also be in the solution. Its value must therefore vary with the relative proportions of all the components of the solution. Accurate work also shows that it varies according to the temperature and concentration range over which the data extends (Harned & Owen, 1950, p. 380).

The 'constant' is thus in reality a purely empirical parameter and can be considered, together with B as a single empirical multiplier for $I^{\frac{1}{2}}$. In other words, equation (7) is an empirical equation containing two arbitrary parameters (a' and β) expressing the activity coefficient term in terms of I , and as such is superior to other empirical equations only in so far as it gives better linear extrapolations.

If data of the highest precision are available up to ionic strength of 0.5 and over, equation (7) undoubtedly yields excellent results. However, in many investigations, especially those involving complex organic acids, the data are not of the same accuracy as those obtainable with materials such as phosphoric or acetic acids. Moreover, many biological materials are scarce and difficult to obtain in a state of high purity. It is thus often not possible to measure points much above $I = 0.1$. In this range of I and with such data it is impossible to make a final decision about the best value of a' . This is unfortunate since the choice influences appreciably the slope of the line, and thus of the extrapolated value at infinite dilution (Bates & Pinching, 1949).

Alternative expression. We have accordingly chosen to apply to our data the empirical equation of Güntelberg (1926) modified by the addition of a linear term in I , thus

$$\log \gamma_{\text{x}} = \frac{Z_{\text{x}}^2 AI^{\frac{1}{2}}}{1 + I^{\frac{1}{2}}} + \beta I. \quad (8)$$

It thus resembles closely the equation suggested by Guggenheim (1935) and is identical with the Debye-Hückel equation (5) with $Ba' = 1$, i.e. it contains only the one adjustable parameter (β). It nevertheless represents activity coefficients up to an ionic strength of approximately 0.1 with an accuracy of the order of 0.5%. This equation will undoubtedly give different slopes from the equation with $Ba' \neq 1$ and hence slightly different values of the extrapolation. However, these differences will generally be within the error of the experimental results.

There is a further reason for not using the Debye-Hückel equation. As has already been pointed out, fairly concentrated solutions must be used to obtain reasonably certain values of a' . The extrapolation values thus depend almost entirely upon measurements at values of the ionic strength at which the approximations of the simple Debye-Hückel theory of electrolytes are certainly no longer valid. Particularly with the complex molecules with which the biochemist has to deal, unexpected deviations may

occur in these regions. Suggestions that this may be so can be found in the data of Bates & Pinching (1949) for citric acid pK_3 . If the extrapolation were confined to the region below $I = 0.15$, the pK_3 at 25° might well be raised by 0.01, an amount ten times greater than the estimated error of the extrapolation. We have therefore thought it preferable to confine ourselves to ionic strengths not much greater than 0.1 and to using the simplest extrapolation function that would adequately represent the data in this region.

Procedure for weak dissociations. Important examples are the second dissociations of phosphate esters. The solutions are prepared by adding the requisite amount of hydrochloric acid (final molality, m_1) to Na_2A solutions (final molality m_2). Then

$$\left. \begin{aligned} m_a &= m_1 - m_H, \\ m_b &= m_2 - m_1 + m_H. \end{aligned} \right\} \quad (9)$$

For weak dissociations ($K < 10^{-4}$) m_H is negligible compared with either m_1 or $m_2 - m_1$. In these circumstances

$$m_a = m_1, \quad m_b = m_2 - m_1, \quad m_{Cl} = m_1.$$

Inserting into equation (3) these values and the value of the activity coefficient expression derived from equation (8), we have

$$-\log K - \beta I = \frac{(E - E_0)_F}{2.3026 RT} + \log \frac{m_1^2}{m_2 - m_1} + \frac{2AI^{\frac{1}{2}}}{1 + I^{\frac{1}{2}}}. \quad (10)$$

The right-hand side of equation (10) contains only terms which are known or are determinable and is plotted against ionic strength. The line through the points is straight for any one temperature and can be fitted by the method of least squares. Its slope is β and its intercept on the ordinate (zero ionic strength) gives the thermodynamic value of $-\log K$.

Procedure for strong dissociations. The above procedure is not possible with strong dissociations such as the first dissociation of phosphate esters. Here m_H is not negligible and, as it occurs in both the logarithmic and ionic strength terms, some method must be found for estimating it. The most convenient procedure is to calculate an 'apparent hydrogen-ion concentration' from the measured electromotive force of the cell (Harned & Owen, 1930), by the use of equation (1). The activity coefficient term, which consists of two univalent ion coefficients can be estimated by the use of the Güntelberg equation. Rearranging and remembering that $m_{Cl} = m_1$, we get

$$\log m_H = -\frac{(E - E_0)_F}{2.3026 RT} - \log m_1 + \frac{2AI^{\frac{1}{2}}}{1 + I^{\frac{1}{2}}}. \quad (11)$$

The value of m_H thus calculated has no thermodynamic significance since it depends upon the

method used for estimating the activity coefficients. Nevertheless, the final extrapolated value of $-\log K$ obtained by using this estimate of m_H is a good approximation to the thermodynamic pK . The error in the estimate of m_H continually decreases as the ionic strength decreases and vanishes at infinite dilution where the activity coefficients become unity. Thus the method of approximating m_H affects the result only in so far as bad estimates may give rise to residual curvatures which make the extrapolation difficult.

The ionic strength of these solutions is given by

$$I = 2m_2 + m_H. \quad (12)$$

There is thus no simple way of solving equation (11), and this must be done by a process of successive approximation, beginning with a value of m_H obtained by first ignoring m_H in equation (12). In the mixtures of HCl and Na_2A used for first dissociation constants

$$m_a = m_1 - m_2 - m_H \quad \text{and} \quad m_b = 2m_2 - m_1 + m_H.$$

Inserting these values into equation (2) we get

$$K = \frac{m_H(2m_2 - m_1 + m_H)}{m_1 - m_2 - m_H} \frac{\gamma_H \cdot \gamma_b}{\gamma_a}. \quad (13)$$

Now, since the acidic form is here uncharged, $\gamma_a = 1$ to a close approximation, and γ_H and γ_b can be approximated by equation (8). Then

$$\log \gamma_H \cdot \gamma_b = \frac{-2AI^{\frac{1}{2}}}{1 + I^{\frac{1}{2}}} + \beta I. \quad (14)$$

Therefore, taking logarithms of equation (13), inserting the value of the activity coefficient term from (14) and rearranging, we have

$$-\log K - \beta I = -\log m_H - \log \left(\frac{m_2}{m_1 - m_2 - m_H} - 1 \right) + \frac{2AI^{\frac{1}{2}}}{1 + I^{\frac{1}{2}}}. \quad (15)$$

Equation (15) resembles equation (10) in containing on the right-hand side only quantities which are known or determinable, and in giving at any one temperature straight lines when the right-hand side is plotted against ionic strength (I). The slopes of these lines are the values of β and their intercepts at $I = 0$ are the thermodynamic values of $-\log K$.

The determination of apparent hydrogen-ion concentrations by means of equation (11) illustrates a further advantage of the Güntelberg equation over the Debye-Hückel equation. There is no means of deciding what value of a' is to be used in equation (11) if the Debye-Hückel equation is used to replace the activity coefficient term. It certainly will not be the same as that suitable for equation (15). Nevertheless, the final extrapolated value of $\log K$ derived from equation (15) depends upon the value of m_H obtained from equation (11), the dependence becoming more sensitive as the pK decreases. Thus Bates (1949) has shown that a variation of a' from

2 to 6 Å causes the first pK of phosphoric acid determined from Nim's (1934) data to vary by nearly 0.03. No such ambiguity arises from the use of the Güntelberg equation. The extrapolated values will certainly differ a little from those obtained by the use of the Debye-Hückel equation. However, it seems preferable to accept these rather than values obtained by the introduction of a parameter whose value cannot be determined.

Calculation of thermodynamic quantities

The variation of the thermodynamic pK with temperature is accurately represented by an equation of the form

$$\log K = -A/T + D - CT, \quad (16)$$

where A , D and C are constants (Harned & Robinson, 1940; Harned & Owen, 1950, pp. 490, 512).

Thermodynamic quantities for the dissociation equilibria can be computed from the constants A , D and C .

The standard change in free energy

$$\Delta G_i^0 = -RT \ln K = 2.3026R(A - DT + CT^2). \quad (17)$$

The standard change in entropy

$$\Delta S_i^0 = -(\Delta G_i^0/dT) = 2.3026R(D - 2CT). \quad (18)$$

The standard change in heat content

$$\Delta H_i^0 = \Delta G_i^0 + T\Delta S_i^0 = 2.3026R(A - CT^2). \quad (19)$$

The change in heat capacity

$$\Delta C_{p,i}^0 = d\Delta H_i^0/dT = 2.3026R(-2CT). \quad (20)$$

In addition, the temperature at which maximum ionization occurs is given by:

$$T_{\max} = \left(\frac{A}{C}\right)^{\frac{1}{2}}, \quad (21)$$

and the value of $\log K$ at this temperature by

$$\log K_{\max} = 2(AC)^{\frac{1}{2}} - D. \quad (22)$$

EXPERIMENTAL

Materials. Water was all distilled twice, the second distillation being carried out in a Bourdillon still with a quartz condenser (Reilly & Rae, 1940). The water was collected so as to exclude CO_2 and ammonia. Its conductivity was less than 10^{-6} mho.cm.⁻³. Before use, air was displaced from the water by bubbling with H_2 . Constant-boiling HCl was prepared from A.R. acid by the method of Shaw (1926). Its composition was checked by estimating chloride gravimetrically as AgCl. The theoretical and estimated strengths agreed to better than 0.1%. Salts were purified by recrystallizing several times, varying the solvent when possible. Their purity was checked by m.p. and analysis.

Cells and electrodes. The cells were of the usual H-type (Noyes & Ellis, 1917) made of Pyrex glass. Two hydrogen saturators were made integral with the main cell after the manner of Harned & Morrison (1937). The cell was fitted with standard ground-glass sockets, the electrodes being supported in standard cones. There were two Ag-AgCl reference electrodes and two hydrogen electrodes in each

cell. The Ag-AgCl electrodes were those designated by Harned (1929) as type 2. Spectroscopically pure AgO (Johnson-Matthey and Co. 73-83 Hatton Garden, London, E.C. 1) was used to prepare the electrodes. It was found that better agreement between pairs of electrodes and more rapid equilibration was obtained if the electrodes were heated in water at 50° for 2 hr. after the chloride had been deposited. The hydrogen electrodes consisted of stout platinum foil on which a thin coat of platinum black was deposited by electrolysis in a lead-free solution containing 2.5% (w/v) H_2PtCl_6 , $6\text{H}_2\text{O}$ in $n\text{-HCl}$ for approximately 1 min. at 0.3A/cm^2 . The coating of platinum black thus deposited was not sufficiently thick to obscure completely the reflectivity of the underlying metal. 'Velvety' coatings of platinum black were avoided.

Solutions. These were all made by weighing, weights being corrected to vacuum. Stock solutions were made approximately 0.2 molal with respect to salt, and suitably diluted to obtain the experimental solutions. Sufficient experimental solution was prepared to fill two cells so that the temperature range could be covered in two halves. All solutions were kept in the refrigerator in Pyrex flasks fitted with ground-glass stoppers. Before use the solutions were bubbled with H_2 saturated with water vapour for at least 1 hr. at room temperature in order to remove dissolved O_2 . The electrodes were washed four times with the experimental solution in a special vessel fitted with two ground-glass sockets and adapted so that H_2 could be bubbled through the contents. The cell in the meantime was blown through with H_2 and filled with fresh experimental solution by transferring it through ground-glass connexions by means of hydrogen pressure. After washing, the electrodes were transferred to the cell with as little exposure to air as possible. The cell was then placed in a constant-temperature bath, connected to the H_2 supply and allowed to equilibrate until the e.m.f. was constant (2-3 hr.). H_2 was obtained from a cylinder, freed from O_2 by passing it through a platinum purifier ('Deoxo', Baker Platinum Co. 52 High Holborn, London, W.C. 1). It was then saturated by bubbling through water at room temperature.

Measurements. The cell was kept in a kerosene bath whose temperature could be kept constant to $\pm 0.01^\circ$ or raised 5° in 5 min. when required. The temperature of the bath was measured with a platinum resistance thermometer (Cambridge Instrument Co., Calorimetric Resistance Thermometer) using the maker's calibrations.

The e.m.f. of the cells was measured with a Tinsley Vernier potentiometer in the range which can be read to $10\ \mu\text{V}$. The standard cell was kept in a constant temperature bath at 25° . Its e.m.f. was checked by the National Physical Laboratory.

The e.m.f. of the cells was considered to be steady if there was a difference of not more than $20\ \mu\text{V}$ over a period of 20 min., and if the two electrode pairs did not differ by more than $20\ \mu\text{V}$. When the steady state was reached, the e.m.f. was recorded, the bath temperature was read with the platinum resistance thermometer and the barometric pressure was determined. Corrections to the barometer for temperature and latitude were applied.

The temperature of the bath was then raised 5° the thermoregulator being set to within 0.1° of the required temperature using a mercury-in-glass thermometer. The e.m.f. of one cell was measured from 5 to 25° . Another portion of the same dilution was measured in a second cell from 25 to 50° . In a few experiments the full range of temperature was covered with one filling of the cell.

CALCULATIONS

Electromotive force. Each value was corrected to 1 atmosphere pressure of hydrogen and to a round temperature. In making the pressure correction the effect of bubbler depth noted by Hills & Ives (1949) was ignored. No information about the bubbling depth employed by Harned & Ehlers (1932) during their determination of E_0 for the Ag-AgCl electrode is available. However, as our cells are similar to theirs and of approximately the same dimensions, it is unlikely that the bubbling depth employed by us differed from theirs by more than 5 cm. According to Hills & Ives (1949) this would result in an error of less than 20 μ v, which is within the experimental error.

Extrapolation functions. From the measured e.m.f.'s and the known molalities for the solutions, the ionic strengths and the extrapolation functions (equations 10 or 15) were calculated. In making these calculations the values for the various constants listed in Table 1 were used. The best straight lines were fitted to these points by the method of

Table 1. *Values of fundamental constants used in this work*

Temp. (°)	E_0^\ddagger (International volt)	A \ddagger
5	0.23392	0.4921
10	0.23126	0.4960
15	0.22847	0.5002
20	0.22551	0.5046
25	0.22239	0.5091
30	0.21912	0.5139
35	0.21563	0.5189
40	0.21200	0.5241
45	0.20821	0.5295
50	0.20437	0.5351

$$R = 1.9869 \text{ cal.}_{15} \text{ deg.}^{-1} \text{ mol.}^{-1}$$

$$T = 273.1 + t^\circ \text{C}$$

$$F = 9.6500 \times 10^4 \text{ coulombs g.equiv.}^{-1}$$

$$J = 4.1833 \text{ cal.}_{15} \text{ deg.}^{-1}$$

* Harned & Ehlers (1933).

† Constant of Debye-Hückel theory. Based on values of physical constants given by Birge (1941).

least squares and the extrapolations to zero ionic strength calculated.

Thermodynamic quantities. The values of the thermodynamic pK's thus obtained at different temperatures were fitted to an equation of the type of (16) by writing it in the form

$$T \log K = -A + DT - CT^2,$$

and fitting the points to the quadratic by means of orthogonal polynomials (Fisher, 1946). The values of the constants of equation (16) may be used to calculate pK values at temperatures other than those given: e.g. at 37°. They may certainly be used for interpolation and probably allow extrapolation down to 0° and up to 60° with little loss in accuracy. Outside these limits they must be used with caution as there is little in the literature to indicate what trends in pK are to be expected outside this temperature range, although there are indications that the dissociation constant of water is approximately represented up to temperatures of 500° by an equation of this type (Harned & Owen, 1950, p. 492). The thermodynamic quantities were calculated from the constants of equation (16) by the use of equations (17) to (22).

Conversion to the molar scale. If pK_c is the thermodynamic pK on the molar scale and pK_m that on the molal scale, the two are related by

$$pK_c = pK_m - \log d_0,$$

where d_0 is the density of water. The corrections are listed in Table 2. The thermodynamic quantities will also be affected to a small extent corresponding to the variation in the value of pK. The expressions relating the two scales are:

$$\Delta G_c^0 = \Delta G_m^0 - 2.3026RT \log d_0,$$

$$\Delta S_c^0 = \Delta S_m^0 + 2.3026R \left(\log d_0 + T \frac{d \log d_0}{dT} \right),$$

$$\Delta H_c^0 = \Delta H_m^0 + 2.3026RT^2 \frac{d \log d_0}{dT},$$

$$\Delta C_{p,c}^0 = \Delta C_{p,m}^0 + 2.3026RT \left(2 \frac{d \log d_0}{dT} + T \frac{d^2 \log d_0}{dT^2} \right),$$

Table 2. *Corrections to be added to molal values to convert to molar*

Temp. (°)	pK	ΔG^0		ΔH^0		ΔC_p^0		ΔS^0	
		Cal.	Joule	Cal.	Joule	Cal. deg. ⁻¹ mol. ⁻¹	Joule deg. ⁻¹ mol. ⁻¹	Cal. deg. ⁻¹ mol. ⁻¹	Joule deg. ⁻¹ mol. ⁻¹
0	0.00006	-0.1	-0.3	+10.2	+42.8	-13.2	-55.7	+0.04	+0.15
5	0.00000	-0.0	-0.0	-2.1	-8.9	-11.8	-49.5	-0.01	-0.03
10	0.00012	-0.2	-0.6	-13.9	-58.3	-10.9	-45.5	-0.05	-0.21
15	0.00038	-0.5	-2.1	-24.7	-103.3	-10.0	-41.7	-0.09	-0.36
20	0.00077	-1.0	-4.3	-35.4	-148.0	-9.3	-38.8	-0.12	-0.52
25	0.00127	-1.7	-7.3	-45.1	-188.9	-8.8	-36.6	-0.16	-0.66
30	0.00188	-2.6	-10.9	-55.1	-230.4	-8.4	-34.9	-0.19	-0.79
35	0.00259	-3.6	-15.3	-65.2	-272.6	-8.1	-33.8	-0.22	-0.93
40	0.00338	-4.8	-20.3	-74.9	-313.5	-7.9	-33.2	-0.26	-1.07
45	0.00426	-6.2	-25.9	-84.7	-354.5	-7.8	-32.8	-0.29	-1.20
50	0.00521	-7.7	-32.2	-95.1	-397.7	-7.8	-32.5	-0.32	-1.33
55	0.00624	-9.4	-39.2	-104.9	-439.0	-7.7	-32.4	-0.35	-1.46
60	0.00734	-11.2	-46.8	-115.3	-482.2	-7.7	-32.2	-0.38	-1.59

where the subscripts 'c' and 'm' refer to the molar and molal scales, respectively. The calculated corrections for aqueous solutions in terms of calories and joules are listed in Table 2. It will be seen that for most work the differences can be neglected for aqueous solutions. However, in other solvents, the corrections may be large.

SUMMARY

1. The theory of the e.m.f. method for measuring accurate dissociation constants is described, together with the calculation from such data of the thermodynamic quantities associated with the dissociation.

2. Suitable functions for representing activity coefficients are discussed.

3. The experimental apparatus and procedure, and the calculations of results, are described.

4. Corrections are presented for correcting values of thermodynamic quantities on the molal scale to values on the molar scale.

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APPENDIX

Estimation of the Variance of the Data used in the Calculation of Dissociation Constants

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Estimation of log K

At each temperature values of y were obtained for each of n different values of I . Denote the values of I by I_1, I_2, \dots, I_n and the corresponding values of y , at the t th temperature, by $y_{t_1}, y_{t_2}, \dots, y_{t_n}$. A straight line $y = a_t + b_t(I - \bar{I})$ was fitted by least squares and the intercept of this line at $I = 0$, (y_{t_0}) was taken as the estimate of log K at that temperature.

Thus
$$y_{t_0} = a_t - b_t \bar{I}, \quad (1)$$
 where \bar{I} is the mean value of I .

Relation between log K and T

To estimate the constants A, D and C we see that equation (16) of Ashby, Crook & Datta (1954) may be written in the form

$$T \log K = -A + DT - CT^2, \quad (2)$$

and, replacing log K by the intercept above, the required estimates can be obtained by fitting, by least squares, a quadratic in T to the m values of $T_t y_{t_0}$. This may most easily be done by the use of orthogonal polynomials (Fisher, 1946) since the