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 $y_{t1}, y_{t2}, \dots, y_{tn}$. 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_{t0}) was taken as the estimate of log K at that temperature.

Thus $y_{t0} = a_t - b_t \bar{I}$, (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 $T_t y_{t0}$. This may most easily be done by the use of orthogonal polynomials (Fisher, 1946) since the

temperatures are at equal intervals of 5° . It will be noted that this method of estimating the constants is approximate as it assumes that the variance of $T \log K$ is constant over the range of T , which, in the experiments considered, was from 273.1 to 323.1. The approximation is, however, extremely good.

It is convenient to change the notation here and write

$$x = \frac{1}{h} (T - \bar{T}),$$

where h = interval between successive values of T (5°); $\bar{T}$ = mean value of T .

The values of x , corresponding to ten observed temperatures, increase by steps of unity from $-9/2$ to $+9/2$ and the equation which is actually fitted is

$$T_i y_{t_i} = \gamma_0 + \gamma_1 P_1(x) + \gamma_2 P_2(x), \quad (3)$$

where the orthogonal polynomials are

$$P_1(x) = x, \quad P_2(x) = x^2 - \frac{1}{m} \sum_{t=1}^m x_t^2,$$

and $\gamma_0, \gamma_1, \gamma_2$ are estimated constants.

To simplify the formulae we shall write

$$S_1 = \sum_{t=1}^m P_1^2(x),$$

$$S_2 = \sum_{t=1}^m P_2^2(x),$$

$$k = 2.3026R.$$

Thus $P_2(x) = x^2 - S_1/m$.

By comparison of (2) and (3) it is seen that

$$\left. \begin{aligned} A &= -\gamma_0 + \gamma_1 \frac{\bar{T}}{h} - \gamma_2 \left(\frac{\bar{T}^2}{h^2} - \frac{S_1}{m} \right), \\ D &= \frac{\gamma_1}{h} - 2\gamma_2 \frac{\bar{T}}{h^2}, \\ C &= -\frac{\gamma_2}{h^2}. \end{aligned} \right\} \quad (4)$$

Substituting (4) in relations (17) to (20) of Ashby, Crook & Datta (1954), we find that

$$\left. \begin{aligned} \Delta G_i^0 &= -k\{\gamma_0 + \gamma_1 x + \gamma_2 P_2(x)\}, \\ \Delta S_i^0 &= \frac{k}{h} (\gamma_1 + 2\gamma_2 x), \\ \Delta H_i^0 &= k \left\{ -\gamma_0 + \gamma_1 \frac{\bar{T}}{h} + \gamma_2 \left(x^2 + \frac{2\bar{T}}{h} x + \frac{S_1}{m} \right) \right\}, \\ \Delta C_{p,i}^0 &= k \frac{2\gamma_2}{h^2} (hx + \bar{T}). \end{aligned} \right\} \quad (5)$$

The main advantages of using orthogonal polynomials are that the computation is simplified and, since the γ 's are statistically independent, the variances of linear functions of these quantities can be obtained.

Estimation of variances

Denote the variance of the points y_{t_i} about the fitted line for the t th temperature by σ_t^2 . An estimate of this quantity is

$$\sigma_t^2 = \frac{1}{n-2} \sum_{i=1}^n d_{t_i}^2,$$

where d_{t_i} = the deviation of observed y about the line. Since $y_{t_i} = a_i - b_i \bar{T}$, the variance of y_{t_i} , due to the errors in the points y_{t_i} is

$$V(y_{t_i}) = \left\{ \frac{1}{n} + \frac{\bar{T}^2}{\sum_{i=1}^n (I_i - \bar{I})^2} \right\} \sigma_t^2. \quad (6)$$

Let $V(y_0)$ denote the mean of $V(y_{t_i})$ for all temperatures.

Now, to examine the fit of the points y_{t_i} to the relation (16) denote by $V(\log K)$ the variance of those values about that relation. An estimate is

$$V(\log K) = \frac{1}{\bar{T}^2 m - 3} \left[\sum_{t=1}^m y_t^2 T_t^2 - m \gamma_0^2 - \gamma_1^2 S_1 - \gamma_2^2 S_2 \right].$$

For the experiments in the following paper the values obtained were:

	pK_1	Set I, pK_2	Set II, pK_2
$V(y_0) \times 10^8$	2104	474	151
$V(\log K) \times 10^8$	2052	15	155

In the experiments for pK_1 , in set II of pK_2 , and in several other experiments on other compounds to be reported later, the agreement between $V(y_0)$ and $V(\log K)$ was good, suggesting that the errors in the estimates of $\log K$ were due to random errors in the value of y_{t_i} . In order to obtain an idea of the magnitude of the variances of the various estimates of thermodynamic quantities from these experiments the value of $V(y_0)$ was used. The discrepancy for set I is probably due to systematic errors affecting y at all temperatures and is discussed later.

Now, approximate formulae for the variances of the γ 's are

$$\left. \begin{aligned} V(\gamma_0) &= \bar{T}^2/m V(y_0), \\ V(\gamma_1) &= \bar{T}^2/S_1 V(y_0), \\ V(\gamma_2) &= \bar{T}^2/S_2 V(y_0), \end{aligned} \right\} \quad (7)$$

whence we may obtain the variances of A, D, C using (4), e.g.

$$\begin{aligned} V(D) &= \frac{1}{h^2} V(\gamma_1) + \frac{4\bar{T}^2}{h^4} V(\gamma_2) \\ &= \left(\frac{1}{S_1} + \frac{4\bar{T}^2}{h^2 S_2} \right) \frac{\bar{T}^2}{h^2} V(y_0). \end{aligned}$$

Also, from (5) we have

$$V(\Delta G) = k^2 [V(\gamma_0) + x^2 V(\gamma_1) + P_2^2(x) V(\gamma_2)],$$

which reduces to

$$\left. \begin{aligned} V(\Delta G) &= k^2 \left[\frac{1}{m} + \frac{x^2}{S_1} + \frac{F_2^2(x)}{S_2} \right] \bar{T}^2 V(y_0), \\ \text{and similarly} \\ V(\Delta S) &= k^2 \left[\frac{1}{S_1} + \frac{4x^2}{S_2} \right] \frac{\bar{T}^2}{h^2} V(y_0), \\ V(\Delta H) &= k^2 \left[\frac{1}{m} + \frac{\bar{T}^2}{h^2 S_1} + \right. \\ &\quad \left. \frac{1}{S_2} \left(x^2 + \frac{2\bar{T}}{h} x + \frac{S_1}{m} \right)^2 \right] \bar{T}^2 V(y_0), \\ V(\Delta C_p) &= k^2 \frac{4}{h^4 S_2} (hx + \bar{T})^2 \bar{T}^2 V(y_0). \end{aligned} \right\} \quad (8)$$

It will be noted that the variances (8) depend on the temperature being used.

The discrepancy between $V(y_0)$ and $V(\log K)$ for set I of pK_a was found to be due to errors which, at given values of I , were correlated for the various

temperatures. Detailed analysis of the experimental records suggests that these correlated variations are due to small persistent differences in the silver-silver chloride electrodes. These errors account for part of the large value of $V(y_0)$ and, since they affect all temperature lines, would not greatly affect the values of y_i relative to one another. Hence, in these cases the fit of the quadratic equation (2) is better than would be expected.

It will be noted, therefore, that if $V(y_0)$ is inflated by correlated errors the various formulae for variances will give considerable overestimates. It was found, however, that such situations rarely occurred.

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Thermodynamic Quantities for the Dissociation Equilibria of Biologically Important Compounds

2. THE ACID DISSOCIATIONS OF GLYCEROL 2-PHOSPHORIC ACID

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Glycerol 2-phosphate was chosen for first investigation because it is obtainable commercially and is readily purified. It is thus a convenient type substance for the important group of carbohydrate phosphate esters and a convenient compound for use in working out methods to be used with the scarcer materials.

EXPERIMENTAL

Purity of glycerol 2-phosphate. Although most commercial samples of disodium glycerol 2-phosphate are mixtures of the 1- and 2-isomers, the two can easily be separated. Thus Toal & Phillips (1949) showed that the first crop of crystals obtained from a 70% aqueous solution of a salt containing approximately equal proportions of the two isomers was predominantly the 2-form. It has been found in the present studies that preliminary crystallization from aqueous ethanol followed by crystallization from water, taking the first crop each time, gives substantially pure 2-isomer. It is essential to maintain the pH above 10 both to prevent the formation of acid salt and to obviate isomerization to the 1-ester which is catalysed by acid.

Disodium glycerol 2-phosphate (British Drug Houses Ltd.) was recrystallized three times from water:ethanol (75:25, v/v) and once from water. The crystals were washed with ice-cold water and dried over CaCl_2 . M.p. 100–102° (corr.). King & Pyman (1914) give m.p. 98–100°.

The product thus obtained analysed correctly for $\text{C}_3\text{H}_7\text{O}_6\text{Na}_2 \cdot 5\text{H}_2\text{O}$ by the following methods:

(i) Dry weight, obtained by heating to constant weight in an oven at 120°: calc., 70.57; found, 70.57, 70.58, 70.53%. (ii) Ignition to pyrophosphate of the dried salt in a platinum dish over a gas burner at bright-red heat until no further loss in weight occurred. Pyrophosphate as percentage of hydrated salt; calc., 43.43; found, 43.48, 43.38%. (iii) Paper chromatography of the ester (Hanes & Isherwood, 1949) was carried out using the modifications of Walker & Warren (1951). The papers were developed 28 hr. in *n*-propanol:ammonia:water containing ethylenediaminetetraacetic acid, which has been found to separate glycerol 1- and 2-phosphate. However, no orthophosphate, or glycerol 1-phosphate or other esters could be detected. Control experiments indicated that less than 1% of any could be detected by this method. Inorganic P was estimated by the method of Martin & Doty (1949) and was found to be 0.014% of the total P. Estimation of glycerol 1-phosphate by the method of Burmaster (1946) indicated the presence of 0.50% and this probably must be regarded as a maximum, since the acid present during the periodate oxidation may cause a small amount of isomerization.

The results of these experiments are in accord with the salt being highly purified disodium glycerol 2-phosphate. Its general properties agree in every way with descriptions in the literature of this salt (King & Pyman, 1914; Paolini, 1911, 1912; Bailly, 1916; Karrer & Salomon, 1926).