bound to an amide by the free electron pair on the nitrogen through a H-bond[17] it is possible to account for the change of absorption spectra without requiring a value of "i" greater than 2. The amount of solvent bound would not be sufficiently large to be measurable by cryoscopic methods. The marked effect of nitrogen substituents on pK_2 might be theoretical support for assuming that the effect is on the electron pair on the nitrogen. The enormous effect of the ammonium group in glycinamide is not as clear.

The pK_a for acetic acid was determined since acetylglycine has a carboxylic acid group. The value of -6.1 is much too negative compared to the pK_2 of acetylglycine (-4.33) and it is not reasonable to correlate the pK_2 of acetylglycine with the proton binding by the COOH. We are continuing this study for simple and complex peptides and proteins and will report the results of these studies in the not too distant future.

(17) The authors feel that the comments of referee I should be incorporated here since they are of interest. With his permission we are reporting them without further comment.

"There is some question in my mind whether the hydrogen bond is discrete enough to be treated as a thermodynamic equilibrium. At least to my knowledge no one has yet shown that treating hydrogen bonding as a thermodynamic equilibrium correlates some physical property with concentration. A similar situation arose in the failure of the Arrhenius theory of ionization to treat association of solvated ions as equilibria."

"The fact that the C=O group exists primarily as $C{=}OH^+$ is no reason that it cannot be also in equilibrium with $C\begin{smallmatrix}OH\\OSO_3H\end{smallmatrix}$. A similar situation exists in the conversion of alcohols to alkyl hydrogen sulfates (THIS JOURNAL, **72**, 3852 (1950)) where the reaction does not become appreciable until concentrations of H_2SO_4 are reached at which the alcohols are primarily in the protonated form (private communication from Dr. Bartlett). Lest the authors feel that I am "pushing" this alternative explanation, let me hasten to emphasize that to me the data do not seem to favor either interpretation."

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The Estimation of the Validity of Stepwise Association Constants[1]

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Several methods have been reported in the literature for the estimation of successive association constants of mononuclear complexes.[2] All of the methods involve successive approximations, graphical solution, or both. Frequently, less rigorous methods have also been employed and the set of constants obtained over-emphasize certain experimental points. The result is that the constants are not the best choices to fit all of the available data. The procedure discussed below is a quick and reliable method for determining the validity of constants derived by any procedure.

The following symbols, with parentheses indicating concentration, will be employed

A = ligand, M = central atom or ion
MA_i = formula for a complex species containing one M and i A's
γ_i = activity coefficient of complex MA_i
γ_a = activity coefficient of complex A
γ_M = activity coefficient of complex M
$\beta_i = \dfrac{\gamma_i(MA_i)}{\gamma_M\gamma_a{}^i(M)(A)^i}$ = association constant for species MA_i

If C_A is the total concentration of ligand and C_M is the total concentration of cation, we obtain the conservation equations

$$C_M = (M) + \sum_{i=1}^{n} \frac{\beta_i\gamma_M\gamma_a{}^i}{\gamma_i}(M)(A)^i \qquad (1)$$

$$C_A = (A) + \sum_{i=1}^{n} \frac{i\beta_i\gamma_M\gamma_a{}^i}{\gamma_i}(M)(A)^i \qquad (2)$$

Rearranging equations 1 and 2

$$0 = -\frac{(M) - C_M}{(M)} + \sum_{i=1}^{n} \frac{\beta_i\gamma_M\gamma_a{}^i}{\gamma_i}(A)^i \qquad (3)$$

$$0 = -\frac{C_A}{(M)} + \left[\frac{1}{(M)} + \frac{\beta_1\gamma_M\gamma_a}{\gamma_1}\right](A) + \sum_{i=2}^{n} \frac{i\beta_i\gamma_M\gamma_a{}^i}{\gamma_i}(A)^i \qquad (4)$$

It is assumed that C_A, C_M and (M) are the measured quantities and that the activity coefficients and the β's have been derived from these quantities. Equations 3 and 4 are seen to be power series in the concentration of free ligand, with the coefficients being functions of the experimental quantities, the equilibrium constants and the activity coefficients. The variable can be eliminated from two power series in the same variable by Sylvester's method of dialytic elimination.[3] This involves treating each power of the variable as an independent unknown and multiplying the equations through by the variable until there are the same number of equations as there are unknowns. Multiplying equations 3 and 4 by (A), n times we obtain the set of $2n$ equations 5.

$$\begin{aligned}
0 &= \frac{(M) - C_M}{(M)}(A) + \sum_{i=1}^{n} \frac{\beta_i\gamma_M\gamma_a{}^i}{\gamma_i}(A)^{i+1} \\
0 &= -\frac{C_A}{(M)}(A) + \left[\frac{1}{(M)} + \frac{\beta_1\gamma_M\gamma_a}{\gamma_1}\right](A)^2 + \sum_{i=2}^{n} \frac{i\beta_i\gamma_M\gamma_a{}^i}{\gamma_i}(A)^{i+1} \\
&\vdots \qquad\qquad \vdots \qquad\qquad \vdots \qquad\qquad \vdots \\
0 &= \frac{(M) - C_M}{(M)}(A)^n + \sum_{i=1}^{n} \frac{\beta_i\gamma_M\gamma_a{}^i}{\gamma_i}(A)^{i+n} \\
0 &= -\frac{C_A}{(M)}(A)^n + \left[\frac{1}{(M)} + \frac{\beta_1\gamma_M\gamma_a}{\gamma_1}\right](A)^{n+1} + \sum_{i=2}^{n} \frac{i\beta_i\gamma_M\gamma_a{}^i}{\gamma_i}(A)^{i+n}
\end{aligned} \qquad (5)$$

In order to have a solution the determinant of the coefficients must vanish, *i.e.*,

(1) This work was supported by Grant G 3604(C2) from the National Institutes of Health, Department of Health, Education, and Welfare.

(2) These methods have been discussed critically and extended by J. C. Sullivan and J. C. Hindman, THIS JOURNAL, **74**, 6091 (1952), and by J. Z. Hearon and J. B. Gilbert, *ibid.*, **77**, 2594 (1955).

(3) See, for example, J. M. Thomas, "Theory of Equations," McGraw-Hill Book Co., New York, N. Y., 1938, pp. 171–175.

TABLE I

VALUES OF THE DETERMINANT FOR VARIOUS CONSTANTS REPORTED FOR URANIUM SULFATE COMPLEXES

$(HSO_4^-) \times 10^3$ \ β_1 / β_2	131[a] 1380	131[a] 1320	128[a] 1370	130[a] 1330	128[b] 1480	126[c] 1210	126[c] 1160	168[d] 62.2
1.83	+0.0073	+0.0074	+0.0108	+0.0085	+0.0107	+0.0135	+0.0136	−0.0311
4.59	− .0585	− .0577	− .0474	− .0541	− .0489	− .0364	− .0371	− .2306
9.18	− .0144	− .0117	+ .0066	− .0053	+ .0017	+ .0298	+ .0370	− .2205
18.35	− .0570	− .0400	− .0087	− .0277	− .0394	+ .0670	+ .0811	− .2273
45.9	+ .4119	+ .5107	+ .5555	+ .5366	+ .3745	+ .9037	+ .9859	+1.0329
45.9	− .3018	− .1892	− .1509	− .1639	− .3572	+ .2376	+ .3314	+0.5502
92.5	− .3979	+ .0685	− .0491	+ .0813	−1.0332	+1.3751	+1.7637	+6.5200

Methods employed: [a] Leden, [b] Bjerrum, [c] Fronaeus, [d] Betts and Leigh.

$$\begin{vmatrix} \frac{(M)-C_M}{(M)} & \frac{\beta_1\gamma_M\gamma_a}{\gamma_1} & \cdots & \frac{\beta_n\gamma_M\gamma_a^n}{\gamma_n} & 0 & \cdots \\ -\frac{C_A}{(M)} & \frac{1}{(M)}+\frac{\beta_1\gamma_M\gamma_a}{\gamma_1} & \cdots & \frac{n\beta_n\gamma_M\gamma_a^n}{\gamma_n} & 0 & \cdots \\ \vdots & \vdots & & \vdots & \vdots & \\ 0 & 0 & \cdots & \frac{(M)-C_M}{(M)} & \frac{\beta_1\gamma_M\gamma_a}{\gamma_1} & \cdots \\ 0 & 0 & \cdots & -\frac{C_A}{(M)}\frac{1}{(M)} & +\frac{\beta_1\gamma_M\gamma_a}{\gamma_1} & \cdots \end{vmatrix} = 0$$

Now if the values for the constants, the activity coefficient and the experimental quantities are put into the determinant for each experimental point, the values for the determinant should, in the ideal case, all be zero. Since, however, experimental errors are present always, the criterion of validity of the constants is the oscillation of the sign of the expanded determinant as the experimental conditions are changed.

As an example we shall investigate the validity of the constants for uranium sulfate complexes obtained by Sullivan and Hindman[2] from the data of Betts and Leigh.[4] Since the activity coefficients are not known and were not used in the calculation of the constants, the values are for concentration constants and the activity coefficients are included in the calculated association constants. In order to make the terms in the expanded determinant have the same order of magnitude for the various experimental points, we multiply the determinant by $(M)^2$. For the case of Betts and Leigh's data with only two constants, the determinant then becomes

$$(M)^2\begin{vmatrix} \frac{(M)-C_M}{(M)} & \beta_1 & \beta_2 & 0 \\ -\frac{C_A}{(M)} & \frac{1}{(M)}+\beta_1 & 2\beta_2 & 0 \\ 0 & \frac{(M)-C_M}{(M)} & \beta_1 & \beta_2 \\ 0 & -\frac{C_A}{(M)} & \frac{1}{(M)}+\beta_1 & 2\beta_2 \end{vmatrix}$$

Table I lists the values of the determinant obtained using the various values of constants calculated for Betts and Leigh's data as reported by Sullivan and Hindman.[2] It is evident that the values $\beta_1 = 128$ and $\beta_2 = 1370$ give the best fit of those listed. It is also apparent that the constants as obtained by Betts and Leigh's and Fronaeus' procedures do not fit the data. The experimental points represented by row 2 and row 5 must have relatively large experimental errors because in the first case the determinant is always negative and in the second case it is always positive.

The method developed is also applicable to polynuclear species except that the coefficients of the power series will contain (M) explicitly in all coefficients corresponding to complex species which contain more than one M. In principle it would be possible to obtain the constants by a least-squares treatment of the expanded determinant using all of the data at once with the elimination of approximation and graphical methods. This would be a reasonable approach for the uranium sulfate complexes but for cases involving more than two or three constants the method becomes much too tedious, and therefore its most useful feature is in testing the validity of constants derived by other procedures.

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(4) R. H. Betts and R. Leigh, *Can. J. Research*, **B28**, 374 (1949).

The Heat of Solution of Sodium Borohydride and the Entropy of Borohydride Ion[1]

BY STUART R. GUNN AND LEROY G. GREEN

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The heat of formation of crystalline sodium borohydride has been determined by Davis, Mason and Stegeman,[2] and the free energy of aqueous borohydride ion has recently been measured by Stockmayer, Rice and Stephenson.[3] In the present work the heat of solution of sodium borohydride has been measured and the heat of formation and entropy of the ion have been derived.

Experimental

Sodium borohydride (Metal Hydrides, Inc., Beverly, Mass.) was purified by two successive recrystallizations from M2M (diethylene glycol dimethyl ether), the solution being saturated at 40° and heated to 100° in order to precipitate the unsolvated crystals.[4] All operations were performed in a closed system or under an argon atmosphere. The material was filtered and dried under vacuum for three days at 80–90°. The product was analyzed gasometrically

(1) This work was performed under the auspices of the U. S. Atomic Energy Commission.

(2) W. D. Davis, L. S. Mason and G. Stegeman, THIS JOURNAL, **71**, 2775 (1949).

(3) W. H. Stockmayer, D. W. Rice and C. C. Stephenson, *ibid.*, **77**, 1980 (1955).

(4) H. C. Brown, "The Chemistry of Trialkoxyborohydrides," American Chemical Society, Cincinnati, 1955.