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CONDUCTANCE OF IONOPHORES

Sir:

Recently,[1-3] equations were presented which permit calculation of conductance for 1–1 electrolytes in terms of three arbitrary parameters: Λ_0, the limiting equivalent conductance, a, the center-to-center distance at contact of cation and anion and K_A, the association constant. These equations have since been revised; the final results, given below, are much more convenient for practical computations. The algebraic form of the equations has been rearranged. The dilemma regarding the Stokes radius in the velocity term of the relaxation field has been resolved. A virtual force arising from asymmetry of osmotic pressure has been included. When association is slight, the $c^{3/2}$ terms are negligible for $\kappa a < 0.2$; when association is not negligible, the $c^{3/2}$ term in activity completely swamps the J_2 term; hence the latter has been dropped. In order to save space, all symbols not explicitly defined here will have the meanings given in refs. 1–3.

Define $\Lambda_\eta \equiv (1 + Fc)$ where $Fc = 5\phi/2$. This is the observed conductance, corrected for *volume* viscosity (see first column, p. 3309[3]). This becomes the dependent variable if viscosity data are available; if not, the viscosity effect is approximated by moving $(-F\Lambda_0 c)$ to the right as before. Then for negligible association, $\Lambda_\eta = (\Lambda_0 - Sc^{1/2} + Ec \log c + Jc)$, where $J = (\sigma_1 \Lambda_0 + \sigma_2)$ replaces the former J_1. Here

$$\sigma_1 = (\kappa^2 a^2 b^2/12c)[h(b) + 0.9074 + \ln(\kappa a/c^{1/2})] \quad (1)$$

$$\sigma^2 = \alpha\beta + (11\beta\kappa a/12c^{1/2}) - (\kappa ab\beta/8c^{1/2})[1.0170 + \ln(\kappa a/c^{1/2})] \quad (2)$$

$$h(b) = (2b^2 + 2b - 1)/b^3. \quad (3)$$

Define $\Lambda_\eta' \equiv (\Lambda_\eta + Sc^{1/2} - Ec \log c)$. For the Owen and Zeldes[4] data on potassium halides at 25°, a plot of Λ_η' against c is accurately linear. The intercept at $c = 0$ evaluates Λ_0 and the slope gives J. The $\mathring{a}$ — values found are: KCl, 3.07; KBr, 3.26; KI, 3.50. These values agree well with the sums of the corresponding crystallographic radii.

When association is not negligible, γ_0 is computed as before. Then Λ_η' becomes $(\Lambda_\eta + Sc^{1/2}\gamma_0^{-1/2} - Ec\,\gamma_0 \log c\,\gamma_0)$ where

$$\Lambda_\eta = \Lambda_0 - Sc^{1/2}\gamma_0^{1/2} + Ec\gamma_0 \log c\gamma_0 + Jc\gamma_0 - K_A c\gamma_0 f^2 \Lambda_\eta \quad (4)$$

The quantities $\Delta\Lambda$, y and x are *redefined* as follows: $\Delta\Lambda \equiv (\Lambda_\eta' - \Lambda_0) = (Jc\gamma_0 - K_A c\gamma_0 f^2 \Lambda_\eta)$; $y = \Delta\Lambda/c\gamma_0$ and $x = f^2\Lambda_\eta$. Again trial values of Λ_0 are used until the one is found which linearizes the y–x plot. The slope gives K_A; then from $y(0) = (J - K_A\Lambda_0)$, J and hence a are evaluated. If K_A is known (*e.g.*, by extrapolation of log K_A *vs.* $1/D$), define $\Lambda_J \equiv (\Lambda_\eta' + K_A c\gamma_0 f^2 \Lambda_\eta)$. A plot of Λ_J against $c\gamma_0$ is linear with slope and intercept equal to J and Λ_0, respectively. Alternatively, if a is known, define $\Lambda_K \equiv (\Lambda_\eta' - Jc\gamma_0)$. Then a plot of Λ_κ against $c\gamma_0 f^2 \Lambda_\eta$ determines Λ_0 and K_A. Equation 4 applied to the Mercier and Kraus[5] data for Bu_4NBr in dioxane–water lead to a-values $4.8 \leq a \leq 5.4$. The spread is much less than that reported before; we therefore believe the present equations represent a better approximation. Details will be presented later.

(1) R. M. Fuoss and L. Onsager, *J. Phys. Chem.*, **61**, 668 (1957).
(2) R. M. Fuoss, THIS JOURNAL, **79**, 3301 (1957).
(3) R. M. Fuoss and C. A. Kraus, *ibid.*, **79**, 3304 (1957).
(4) B. B. Owen and H. Zeldes, *J. Chem. Phys.*, **18**, 1083 (1950).
(5) P. Mercier and C. A. Kraus, *Proc. Nat. Acad. Sci.*, **41**, 1033 (1955).

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RETARDATION OF EXCHANGE PROCESSES BY MOLECULAR ASSOCIATION: METHYL ALCOHOL

Sir:

In high resolution nuclear magnetic resonance studies of liquids, exchange processes frequently preclude the observation of spin–spin multiplets.[1] In specific cases one can reduce the rate of exchange by carefully purifying the sample.[2-4] In some instances, however, an alternative procedure can be employed which provides information concerning exchange processes and molecular complexes in liquid systems. This is achieved by adding to the sample a complexing agent which preferentially forms a stable molecular complex with the sample under study and, consequently, decreases the rate of exchange.

As an example, consider the proton magnetic resonance spectrum of methyl alcohol. The spin–spin multiplets which should be observable in the spectrum of this molecule have long eluded detection, presumably because of exchange effects. On the other hand, in solutions containing sufficient quantities of acetone, hydrogen bonding increases the lifetime of –OH group protons in enough molecules to reveal the fine structure. Figure 1 shows the spectrum of methyl alcohol as observed at 40 Mc. in a solution of acetone containing 25% CH_3OH by volume. The theoretical spectrum for the special case of $J/\delta = 0.21$ is added for comparison. The experimental trace gives $J = 4.8$ sec.$^{-1}$ and $\delta = 22.8$ sec.$^{-1}$. It should be noted that two of the lines in the observed –OH multiplet are not predicted theoretically. One of these has been shown to be water; the other is attributed to an additional impurity.

Supplementary experiments with methanol and other molecules have shown that (a) by varying the acetone concentration the internal chemical shift can be changed and the concomitant alterations in fine structure observed and compared

(1) H. S. Gutowsky, D. W. McCall and C. P. Slichter, *J. Chem. Phys.*, **21**, 279 (1953).
(2) R. A. Ogg, *ibid.*, **22**, 560 (1954).
(3) I. Weinberg and J. R. Zimmerman, *ibid.*, **23**, 748 (1955).
(4) J. T. Arnold, *Phys. Rev.*, **102**, 136 (1956).

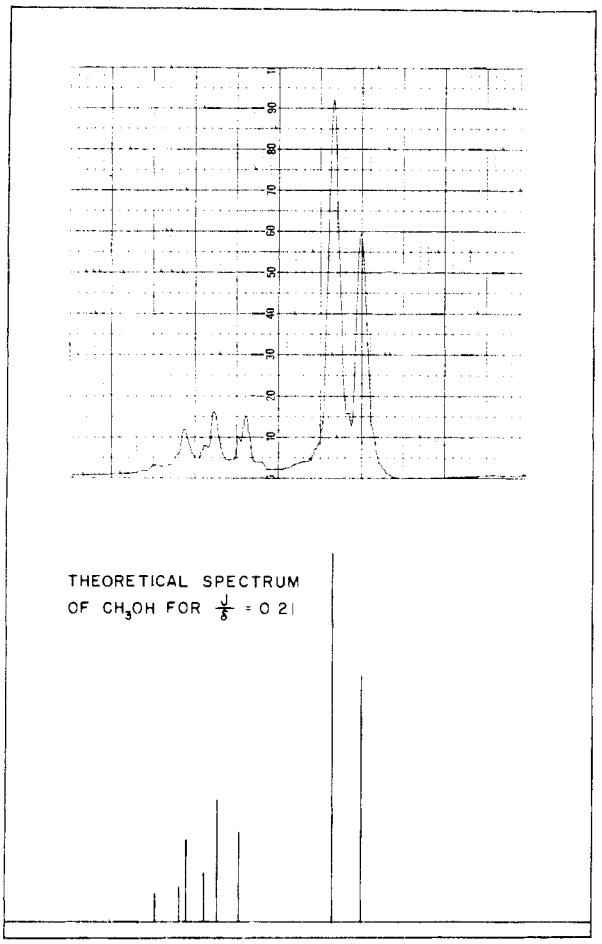

Fig. 1. - Proton magnetic resonance spectrum of methyl alcohol at 40×10^6 sec^{-1}.

with the theoretical spectra; (b) in some instances, molecules which are free to exchange and molecules which are complexed to acetone can be observed simultaneously and intensity measurements provide a measure of the number of molecules in each state; (c) the effect of acetone concentration on exchange rate can be studied.

A full report on these experiments is being prepared and will be published in the near future.

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EVIDENCE FOR AN INTERMEDIATE IN THE HYDROLYSIS OF ATP BY MUSCLE PROTEINS

Sir:

Muscular contraction has been shown to involve the interaction of an energy source, ATP, with the muscle proteins actin and myosin,[1] but the nature of the interaction has remained obscure. Isotopic studies reported here give evidence on the existence and properties of an intermediate formed during superprecipitation of actomyosin, the gel analogy of muscular contraction.

(1) (a) A. Szent-Gyorgyi, "Chemistry of Muscular Contraction," New York, 2nd edition, 1951; (b) S. V. Perry, *Symposia Soc. Experimental Biol.*, **9**, 203 (1955); (c) J. Hanson and H. E. Huxley, *ibid.*, **9**, 228 (1955); (d) H. H. Weber, *ibid.*, **9**, 271 (1955).

In Table I the results of experiments in which ATP was hydrolyzed by muscle proteins in H_2O^{18} are presented. Superprecipitation was observed during reaction with the actomyosins. It is seen that the phosphate produced in the reactions with intact lobster muscle, purified actomyosin, synthesized actomyosin and myosin had an O^{18}-content greatly in excess of the value (0.25) to be expected for simple cleavage of the terminal bond. That the exchange occurred at a stage intermediate

TABLE I

O^{18}-CONTENT OF H_3PO_4 PRODUCED IN THE HYDROLYSIS OF ATP (ADENOSINE TRIPHOSPHATE) BY MUSCLE PROTEINS

Conditions: 0.05*M* tris, 0.01*M* $MgCl_2$, 0.005*M* ATP, 0.1*M* KCl, *p*H 7.3, atom % excess of medium H_2O, *ca.* 1.0

Expt. no.	Protein	$\frac{O^{18}\text{ atom \% excess } H_3PO_4}{O^{18}\text{ atom \% excess } H_2O}$
1	Actin, rabbit muscle	0.004[a]
2	Myosin, rabbit muscle	0.77
3	Actomyosin, synthesized from actin and myosin preparations	0.52
4	Actomyosin, isolated from rabbit muscle	0.52
5	Lobster muscle strips[5]	0.71

[a] O^{18}-content of phosphate in unhydrolyzed ATP.

between the starting material and final product was shown by the (a) absence of exchange (less than 0.01 atom % excess) with inorganic phosphate added alone or during ATP hydrolysis and (b) absence of O^{18} in unhydrolyzed ATP (less than 0.02%) isolated after stopping the reaction at *ca.* 50% hydrolysis. Thus, the intermediate cannot be in rapid mobile equilibrium with either the starting material or the product. Furthermore, the high exchange in these experiments with Mg^{++} and the lack of exchange in previously reported[2] experiments with Ca^{++} show that a striking change in the properties of the intermediate is caused by the activating metal ion.

Despite the extensive purification of the individual proteins, the possibility of a contaminant required special attention in view of the known scavenging properties of myosin[3] and the O^{18}-exchanges in other systems.[4] The formation of actomyosin from myosin increased the rate of hydrolysis tenfold. If an impurity unrelated to actin were causing the O^{18}-exchange in myosin the amount of exchange in actomyosin prepared from it should be decreased by approximately this factor. Accordingly, actomyosin was synthesized[1a] from the purified actin and myosin preparations and the decrease in exchange was found to be too small to be accounted for by such an impurity (*cf.* experiments 1, 2 and 3, Table I). Further support for this conclusion was obtained by the agreement in exchange rates with intact lobster muscle,[5] isolated actomyosin and synthesized actomyosin.

(2) D. E. Koshland, Jr., Z. Budenstein and A. Kowalsky, *J. Biol. Chem.*, **211**, 279 (1954).

(3) H. M. Kalckar, *ibid.*, **153**, 358 (1944).

(4) M. Cohn and G. R. Drysdale, *ibid.*, **216**, 831 (1955); P. D. Boyer, A. B. Falcone and W. H. Harrison, *Nature*, **174**, 401 (1954); M. Cohn, *J. Biol. Chem.*, **230**, 369 (1958).

(5) D. E. Koshland, Jr., and E. Clarke, *ibid.*, **205**, 917 (1953).