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Studies on Molecular Interaction in Solution. III.[1] Salting Behavior of Cyclic Amides and Cyclic Conjugated Ketones in Tetraalkylammonium Salt Solutions

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Salting behavior of alkylxanthines, other cyclic amides, and cyclic conjugated ketones was investigated by the solubility, partition, and permeation methods. These compounds were found to be salted-out by tetramethyl- and tetraethylammonium salts against the common belief that nonelectrolytes are salted-in by these salts. The above compounds were, however, salted-in by large ammonium salts and the effect was attributed to complex formation between the large ammonium ions and the nonelectrolytes. Salts were also shown to affect equilibrium concentrations of nonelectrolytes across a lipoid membrane and the rate of permeation of nonelectrolytes through the membrane.

Much interest has recently been focused on the physicochemical properties of tetraalkylammonium salt solutions and their effects on the activity coefficients of nonelectrolytes.[3,4] The cause and nature of the salt effect has been subject of several investigations[4,5] and a review.[6] The concept underlying it has been developed along two lines. The first line of thought associates the salt effect with some change in the state of water.[5] This salt induced medium effect is believed to arise from the effect of the electrolyte upon the internal pressure of the solvent. According to this theory the degree of salting-out or salting-in of a nonpolar nonelectrolyte is determined by the extent to which the solvent medium is compressed or loosened when ions are present. Thus the salting-in effect of tetraalkylammonium salts on nonpolar nonelectrolyte as well as the salting-out effect of inorganic salts on nonelectrolytes are attributed to the change in the medium properties due to the electrolytes. The second is to assume the presence of direct solute-ion interaction in the case of large ions.[4] According to this theory the salting-in effect of large organic ions on nonelectrolytes are attributed to a specific short-range ion-nonelectrolyte interaction. In the absence of the specific interaction, salts are considered to decrease the solubility of nonelectrolytes.

Tetraalkylammonium salts have been known to cause a significant increase in the solubility (salt-in) of nonelectrolytes whereas the presence of most inorganic salts tends to decrease the solubility (salt-out) of nonelectrolytes.[6] As a part of investigations into medium effects on

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[1] Part I: K. Kakemi, H. Sezaki, T. Mitsunaga, and M. Nakano, *Chem. Pharm. Bull.* (Tokyo), **16**, 2018 (1968); Part II: K. Kakemi, H. Sezaki, E. Suzuki, and M. Nakano, *ibid.*, **17**, 242 (1969).

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[3] *a*) S. Lindenbaum and G.E. Boyd, *J. Phys. Chem.*, **68**, 911 (1964); *b*) D.R. Robinson and M.E. Grant, *J. Biol. Chem.*, **241**, 4030 (1966); *c*) W.Y. Wen and K. Nara, *J. Phys. Chem.*, **71**, 3907 (1967).

[4] *a*) A.G. Leiga and J.N. Sarmousakis, *J. Phys. Chem.*, **70**, 3544 (1966); *b*) J.E. Gordon and R.L. Thorne, *ibid.*, **71**, 4390 (1967); *c*) J.E. Desnoyers, G.E. Pelletier, and C. Jolicoeur, *Can. J. Chem.*, **43**, 3232 (1965).

[5] *a*) W.F. McDevit and F.A. Long, *J. Am. Chem. Soc.*, **74**, 1773 (1952); *b*) R.L. Bergen, Jr. and F.A. Long, *J. Phys. Chem.*, **60**, 1131 (1956); *c*) N.C. Deno and C.H. Spink, *ibid.*, **67**, 1347 (1963); *d*) E. Grunwald and A.F. Butler, *J. Am. Chem. Soc.*, **82**, 5647 (1960).

[6] F.A. Long and W.F. McDevit, *Chem. Rev.*, **51**, 119 (1952).

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molecular interaction,[7] the effects of tetraalkylammonium salts on the solubilities of xanthine derivatives and the related compounds have been examined. Xanthine derivatives were studied because their behavior in aqueous solutions seemed to be different from those of benzenoid compounds with respect to complex formation.[8] In the course of the present investigation it was revealed that not only xanthine derivatives but also some other cyclic amides and cyclic conjugated ketones are actually salted-out in the presence of some tetraalkylammonium salts. These novel observations are presented and some implications are discussed in this communication. It is hoped that these results will furnish us with new information on the nature of the salt effect and the mechanism of molecular interactions in aqueous solution.

Theoretical

Solubility

The theoretical relationship between the solubility of a nonelectrolyte in a salt solution and the concentration of the salt is given by Eq. 1.[6]

$$\log \frac{f}{f^0} = \log \frac{S^0}{S} = k_s C_s + k_i (S - S^0) \qquad (1)$$

where f and f^0 are the molar activity coefficients of the nonelectrolyte in the salt solution and in a salt-free solution, respectively;[9] S and S^0 are the solubilities in moles per liter of the nonelectrolyte in the salt solution and in pure water, respectively; C_s is the molar concentration of the electrolyte, k_s is the salting-out parameter, and k_i is the nonelectrolyte self-interaction parameter. If the solubilities S and S^0 are small, the last term of Eq. 1 can be neglected. For this case of low nonelectrolyte concentration, Eq. 2 is obtained:

$$\log \frac{S^0}{S} = k_s C_s \qquad (2)$$

The emipirical Setschenow equation given by Eq. 3 will often hold even when the last term of Eq. 1 is large:[10]

$$\log \frac{S^0}{S} = k_{\text{app}} C_s \qquad (3)$$

Thus the slope of the line obtained when log (S^0/S) is plotted against C_s corresponds to an emipirical salting-out parameter k_{app}. When S and S^0 are small k_{app} approximates k_s.

Partition

If the partition coefficient of a nonelectrolyte between an immiscible nonaqueous reference phase and pure water is defined by $K^0 = C^R/C^0$ and that between the reference phase and a salt solution by $K = C^R/C$, where C^0, C, C^R, and C^r are equilibrium concentrations of the nonelectrolyte in pure water, the salt solution, the reference phase in equilibrium with the salt solution, and the reference phase in equilibrium with the salt-free solution, and if the expected concentration of nonelectrolyte in the salt-free solution which would be in equilibrium with C^R is designated as C_{ex}^0, then the partition coefficient of the nonelectrolyte would be $K^0 = C^R/C_{ex}^0$. Since the following equations hold

$$f^0_{ex} C^0_{ex} = f^R C^R = fC \quad i.e. \quad \frac{f}{f^0} = \frac{C_{ex}^0}{C} = \frac{C^R/K^0}{C^R/K} = \frac{K}{K^0}$$

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[7] K. Kakemi, H. Sezaki, E. Suzuki, and M. Nakano, *Chem. Pharm. Bull.* (Tokyo), **17**, 242 (1969).
[8] K. Kakemi, H. Sezaki, T. Mitsunaga, and M. Nakano, *J. Pharm. Sci.*, in press.
[9] The relationship between activity a and solubility s in moles per liter can be expressed by $a = fs$.
[10] J. Setschenow, *Z. Physik. Chem.*, **4**, 117 (1889).

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where $f_{ex}{}^0$ and f^R are the molar activity coefficients of the nonelectrolyte in pure water which is equal to f^0 and in the reference nonaqueous medium, respectively, Eq. 4 can be otbained

$$\log\frac{f}{f^0}=\log\frac{K}{K^0}=k_sC_s \tag{4}$$

Thus the slope of the line obtained when log (K/K^0) is plotted against C_s corresponds to the salting-out parameter.

Permeation

If a nonelectrolyte in a salt solution is separated by a lipoid membrane from that of an identical concentration in salt-free water (Chart 1), the net movement of the nonelectrolyte occurs either from the salt solution to the salt-free aqueous solution or in the opposite direction depending upon whether the nonelectrolyte is salted-out or salted-in by the salt under investigation. Since the following relation holds at equilibrium (the terms should be self-evident from Chart 1),

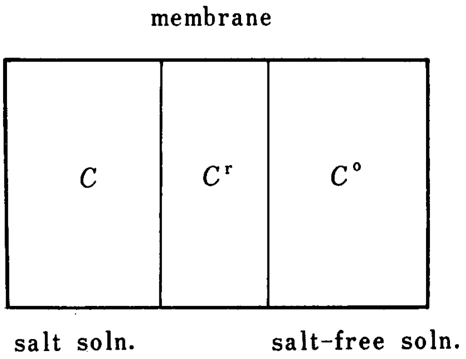

Chart 1

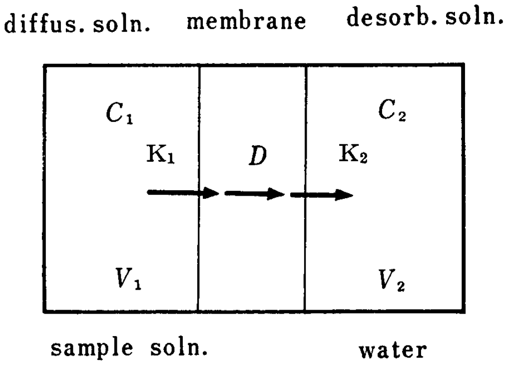

Chart 2

$$fC=f^rC^r=f^0C^0$$

or

$$\frac{f}{f^0}=\frac{C^0}{C}$$

the salting-out parameter can be obtained from Eq. 5.

$$\log\frac{C^0}{C}=k_sC_s \tag{5}$$

When a nonelectrolyte in a solution is, on the other hand, separated from pure water by a lipoid membrane (Chart 2), it will partition into the membrane, diffuse through the membrane, and partition out of the membrane into water. If a salt is added into the aqueous solution of the nonelectrolyte, the partition coefficient between the membrane and the salt solution will be different from that of between the membrane and the pure aqueous solution. Since permeation of a molecule from the solution to another solution through the membrane is dependent on both the partition coefficient of the molecule between the membrane and the solution as well as the diffusion coeffecient of the molecule through the membrane, the salt will affect the rate of permeation of the nonelectrolyte. The rate of permeation is given by Eq. 6[11]

$$\frac{dC_2}{dt}=\frac{DS(K_1C_1-K_2C_2)}{XV_2} \tag{6}$$

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[11] E.R. Garrett and P.B. Chemburkar, *J. Pharm. Sci.*, **57**, 949 (1968).

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where C_1 and C_2 are the concentrations of a nonelectrolyte in the diffusing solution and in the desorbing solution, respectively; K_1 and K_2 are the partition coefficient of a nonelectrolyte between the membrane and the diffusing solution and between the membrane and the desorbing solution, respectively; D is the diffusion coefficient of the nonelectrolyte in the membrane, S is the available surface area for diffusion, X is the thickness of the membrane, and V_2 is the volume of the desorbing solution. Since salts modify the pratition coefficient of the nonelectrolyte, the effect of salts would be reflected on the rate of permeation of the nonelectrolyte.

Experimental

Reagents——The ammonium salts were reagent grade chemicals from commercial sources. They were thoroughly washed with diethyl ether and kept away from moisture. Most of the nonelectrolytes used in this study were commercially available chemicals and purified whenever necessary.

Solubility Method——The salt solutions were saturated with nonelectrolytes at 25° by mechanically shaking these solutions in vials with an excess of nonelectrolytes in a thermostated water bath for about 40 hours. For every nonelectrolyte–salt pair, the experiments were done for at least three salt concentrations in the range of 0.125—0.500 M. After equilibration, the solutions were quickly filtered through sintered–glass funnels. An aliquot of the filtered solution was then appropriately diluted for spectrophotometric determination at the wavelength of the maximum absorbance of the nonelectrolyte. Experimental salting–out parameters were calculated according to Eq. 3.

Partition Method——When nonelectrolytes were too soluble in water, the partition behavior of nonelectrolytes between an aqueous salt solution (salt concn., 0—0.5M) and carbon tetrachloride (reagent grade) was examined. The concentrations of nonelectrolytes in the organic layer were spectrophotometrically determined and the corresponding concentrations in the aqueous solution were estimated from the original concentrations (4 mM) and the the concentrations in the organic layer at equilibrium. The experimental salting-out parameters were calculated according to Eq. 4.

Permeation Method——The diffusion cell designed by Patel and Foss[12] was used with dimethyl polysiloxane membranes (Silastic of Dow–Corning) in labeled thickness of 5 mil (5×10^{-3} inch$=12.7\times10^{-3}$ cm). (a) In the first set of experiments, a 10 ml portion of a nonelectrolyte solution (4mM) was placed in one compartment of the cell and the same volume of the nonelectrolyte solution with a salt (0.5M) in the other. The cell had been mounted on a shaker and both the cell and solutions had been preequilibrated at 25° in a low temperature incubator prior to the addition of the solutions into the compartments. One half milliliter sample was withdrawn from the aqueous and salt solutions at appropriate intervals until equilibrium was attained and the concentrations of nonelectrolytes were determined spectrophotometrically. Experimental salting–out parameters were calculated according to Eq. 5 from the equilibrium data. (b) In the kinetic experiments, one compartment of the diffusion cell was filled with 10 ml of water and the other with a 10 ml portion of one of the following nonelectrolyte diffusing solutions (4 mM); in pure water, in 1.2 M NaCl, and 1.2M $N(CH_3)_4Cl$. The cell was shaken in a constant temperature water bath kept at 30°. The same cell with the same membrane was used throughout for each nonelectrolyte with the three different types of diffusing solutions. One half milliliter portion of the desorbing solution was pipetted out at appropriate intervals and the increase in concentration of the nonelectrolyte in the desorbing solution was determined spectrophotometrically.

Results and Discussion

The first major finding of this study is that xanthine derivatives were salted–out even of tetramethylammonium salts solution. This is contrary to the common belief that nonelectrolytes are salted–in by tetraalkylammonium salts.[6] Figure 1 illustrates that the nonelectrolytes are either salted–in or salted–out by tetramethylammonium salt depending upon the nature of the nonelectrolytes. With caffeine a lagre positive salting–out parameter may be suspected to result from a large contribution of the self–interaction terms in Eq. 1 because of its large solubility in water.[13] Theobromine and 8-chlorocaffeine which are much less soluble in water than caffeine were, however, also found to be salted–out by the same salt. The results of further experiments carried out in order to elicit structural features for this unusual effect revealed that the phenomenon is not confined to the xanthine derivatives only, but glycine anhydride

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[12] N.K. Patel and N.E. Foss, *J. Pharm. Sci.*, **53**, 94 (1964).

[13] D.E. Guttman and T. Higuchi, *J. Am. Assoc., Sci. Ed.*, **46**, 4 (1957).

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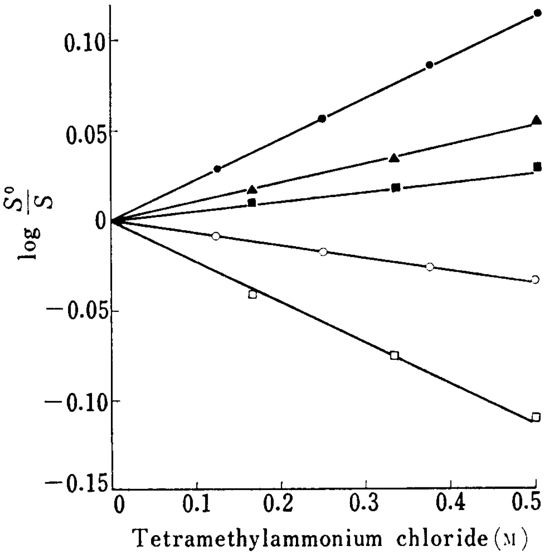

Fig. 1. Setschenow Plots for Several Nonelectrolytes in Tetramethylammonium Solution at 25°

key: ●: caffeine, ▲: glycine anhydride, ■: 2-hydroxyethylphthalamide, ○: N,N-dimethylcinnamamide, □: salicylamide

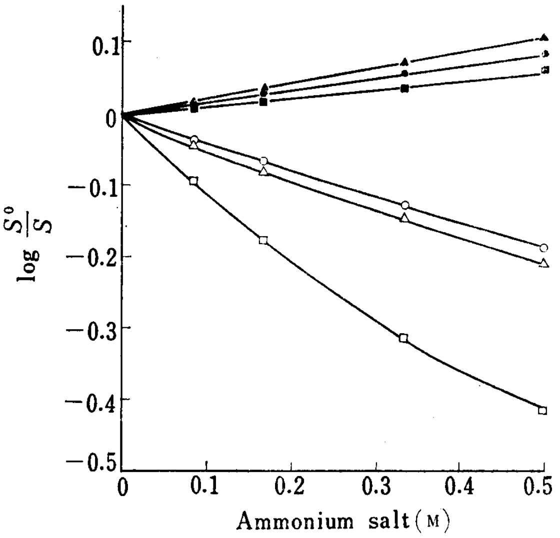

Fig. 2. Setschenow Plots for 8-Methoxycaffeine in Ammonium Salt Solutions at 25°

key: ▲: $N(CH_3)_4Cl$, ●: NH_4Cl, ■: $N(C_2H_5)_4Cl$, ○: $N(n\text{-}C_4H_9)_4Cl$, △: $N(CH_3)_3C_6H_5Cl$, □: $N(CH_3)_3CH_2C_6H_5Cl$

and uracil were also slated-out of tetramethylammonium chloride solutions. 2-Hydroxyethylphthalimide, antipyrine, and even quinones (benzoquinone and menadione) were also found to display the same phenomenon. Since cinnamamides, salicylamide, other amides, and a lactone (coumarin) were, however, found to be salted-in by the same salt, the phenomenon seems to be the characteristic related to cyclic amides and quinones. The salting-out parameters of the nonelectrolytes investigated are listed in Table I.

The second finding is that the salting-out parameters of alkylxanthines decrease with the increasing size of ammonium ions, the nagative value being observed in tetrabutylammonium

Table I. Salting-out Parameters at 25°

Nonelectrolyte	Salting-out parameter[a)] $N(CH_3)_4Cl$	$N(C_2H_5)_4Cl$
Caffeine	0.23	0.14
Theophylline	0.19	0.094
Theobromine	0.14	0.098
8-Methoxycaffeine	0.23, 0.22[b)]	0.11
8-Chlorocaffeine	0.14	0.051
Uracil	0.054	0.021
Glycine anhydride	0.11	—
Antipyrine	0.032[c)]	—
2-Hydroxyethylphthalimide	0.058	−0.092
Benzoquinone	0.098[c)]	—
Menadione	0.015	−0.18
2,6-Dimethyl-γ-pyrone	0.11[b)]	—
Coumarin	−0.068	—
Phenylacetamide	−0.086	—
Benzamide	−0.14	—
Cinnamamide	−0.22	−0.67
N,N-Dimethylcinnamamide	−0.068	—
Salicylamide	−0.22, −0.22[b)]	−0.52

a) by the solubility method unless otherwise specified
b) by the permeation method c) by the partition method

Table II. Salting-out Parameters Determined by the Solubility Method at 25°

Ammonium chloride	Salting-out parameter		
	8-Methoxycaffeine	8-Chlorocaffeine	Theobromine
Ammonium chloride	0.18	0.12	0.042
Tetramethyl-	0.23	0.14	0.14
Tetraethyl-	0.11	0.051	0.098
Tetra-*n*-butyl-[a]	−0.43 (1.0)	−0.34 (0.88)	−0.38 (0.76)
Trimethylphenyl-[a]	−0.53 (1.2)	−0.54 (1.3)	−0.67 (1.6)
Trimethylbenzyl-[a]	−1.1 (3.1)	−1.1 (3.1)	−1.3 (3.3)

a) Since the sating-out parameter changes with salt concentration, the limiting values is given. The number in parentheses corresponds to the apparent stability constant of a complexes when ion-nonelectrolyte complexation is assummed.

salt solution. Setschenow plots for 8-methoxycaffeine in the presence of various ammonium ions are shown in Fig. 2. 8-Methoxycaffeine was salted-out of $N(CH_3)_4Cl$ and $N(C_2H_5)_4Cl$ solutions as well as of NH_4Cl solution, whereas it was salted-in by large ammonium ions. Table II shows the salting-out parameters for 8-chlorocaffeine and theobromine as well as for 8-methoxycaffeine in various ammonium salt solutions. With the exception of ammonium salt (NH_4Cl), the salting-out parameters for these alkylxanthines decrease with increasing size of ammonium ions. The dependence of the salting effect on the size of the alkyl group may be rationalized by the relative importance of the salting-out contribution due to the charge bearing nitrogen atom and halide ion and the solubility-increasing contribution due to the association of the nonelectrolyte with the organic part of the ion. When the alkyl group is small (methyl or ethyl) the salting-out effect predominates because of little interaction of the polar nonelectrolyte with the small alkyl group, while with ammonium salts bearing large alkyl groups, the solubility-increasing effect outweighs the salting-out effect resulting in net salting-in.

Table III reviews the salting behaviors of nonelectrolytes in two kinds of salt solutions, *i.e.* in NaCl and $N(CH_3)_4Cl$ solutions. The fact that tetramethylammonium salt behaves differently depending upon the nature of nonelectrolytes may imply that the salt induced medium effect theory[5] cannot totally account for the behavior of the nonelectrolytes in tetraalkylammonium salt solutions. What is then the property of cyclic amides and cyclic conjugated ketones which distiguishes themselves from benzene and naphthalene derivatives with regard to the behavior of nonelectrolytes in tetramethylammonium chloride solutions. The salting-in behavior of benzene and naphthalene derivatives in tetraalkylammonium salt solutions is considered to be due to the interaction of these nonelectrolytes with the ammonium salts.[4] Alkylxanthines are, on the other hand, known to show little binding affinity toward organic cations.[14] They do not appear to bind with the small ammonium salts either. Alkylxanthines are, therefore, considered to be salted-out because they do not interact with small ammonium ions. Although the reason why alkylxanthines do not complex with the small tetraalkylam-

Table III. Salting Behavior of Nonelectrolytes in Sodium Chloride and Tetramethylammonium Chloride Solutions

Nonelectrolyte	Ion	Salting behavior
Benzene or naphthalene derivatives	NaCl	out
	$N(CH_3)_4Cl$	in
Cyclic amides and cyclic conjugated ketones	NaCl	out
	$N(CH_3)_4Cl$	out

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[14] K. Kakemi, H. Sezaki, T. Mitsunaga, and M. Nakano, *Chem. Pharm. Bull.* (Tokyo), **16**, 2018 (1968).

monium ions is not known, it is clear that the electronic distribution within the molecular domain of cyclic amides and cyclic conjugated ketones are much more irregular than that in benzenoid molecules. This irregularly distributed electrons over these molecules may be speculated to repel ions and other molecules similar to themselves. This molecular property of cyclic amides and cyclic conjugated ketones may be responsible for little tendency of these molecules to complex with small cations and with other cyclic amides.[8] They are, however, known to bind with organic cations when a bulky group is present in the cation.[14] Thus, the salting-out effect due to the ionic charge predominates in tetramethyl- and tetraethyl-ammonium salt solutions. With tetrabutyl- and benzene substituted ammonium salts, however, the solubility-increasing effect due to the specific interaction between alkylxanthines and the nonionic part of large ions may outweigh the salting-out effect resulting in net salting-in.

Specific ion-nonelectrolyte interactions are also indicated by the quantitative examination of the Setschenow plots (Fig. 2) and solubility plots (Fig. 3). The salting behavior of 8-methoxycaffeine in tetrabutyl-, trimethylphenyl-, and trimethyl-benzylammonium salt solutions does not follow straight line relationship (see Fig. 2) expected from the Setschenow equation (Eq. 3). The linear plots of solubility ratio S/S^0 against C_s (Fig. 3), however, gave straight lines expected for the formation of a 1:1 complex.[15] Apparent stability constants, K, obtained

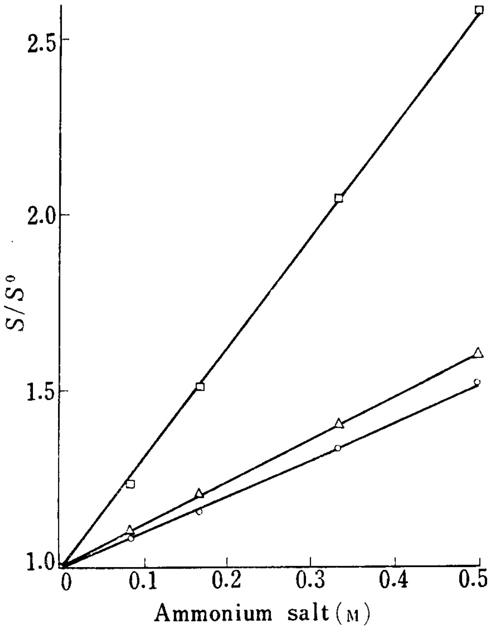

Fig. 3. Solubility Ratio for 8-Methoxycaffeine in Ammonium Salt Solutions at 25°

key: □: $N(CH_3)_3CH_2C_6H_5Cl$, △: $N(CH_3)_3C_6H_5Cl$, ○: $N(n\text{-}C_4H_9)_4Cl$

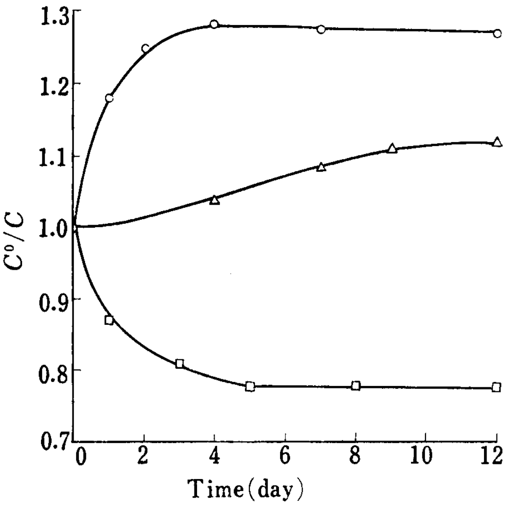

Fig. 4. Concentration Ratio (Concn. in Salt-Free Soln./Concn. in 0.5M $N(CH_3)_4Cl$ soln.) of Nonelectrolyte (Initial Concn., 4 mM) at 25° Plotted against Time

key: ○: 8-methoxycaffeine, △: 2,6-dimethyl-γ-pyrone, □: salicylamide

$$\frac{S}{S^0}=KC_s+1 \tag{7}$$

from Eq. 7 are shown in Table II.

Additional experiments were performed to obtain some information as to how salts affect the equilibrium state and the rate of permeation of nonelectrolytes across a membrane. In the equilibrium experiments the nonelectrolyte in water was separated by the membrane from a salt solution of the identical nonelectrolyte concentration. In Fig. 4 are presented typical data

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15) D.E. Guttman, *J. Pharm. Sci.*, **51**, 1162 (1962).

for such studies. 8-Methoxycaffeine was salted-out of tetramethylammonium solution, resulting in higher concentration of the xanthine in the salt-free solution than in the salt solution. 2,6-Dimethyl-γ-pyrone also behaved in the same way. Salicylamide, on the other hand, was found to be salted-in by the ammonium salt, resulting in an increase in concentration of the amide in the salt solution and a decrease in the salt-free solution. Salting-out parameters calculated according to Eq. 5 agreed with those obtained by the solubility method (see Table I). In the kinetic experiments, the rate of permeation of nonelectrolytes from solutions (with and without salts) to pure water was determined. The results recorded in Fig. 5 indicate that the rate of permeation of 8-methoxycaffeine was increased both by NaCl and by $N(CH_3)_4$-Cl, indicating salting-out of the xanthine by these salts. The rate of permeation of dimethylcinnamamide illustrated in Fig. 6, on the other hand, was found to be increased by NaCl while it was decreased by $N(CH_3)_4Cl$ indicating salting-out by NaCl and saling-in by $N(CH_3)_4Cl$. These results are in accord with the salting behavior of these compounds studied by the solubility method.

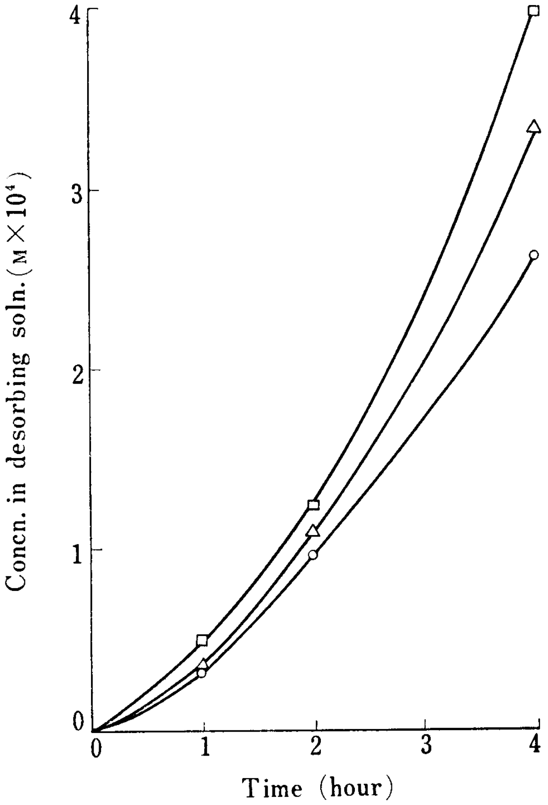

Fig. 5. Permeation of 8-Methoxycaffeine (Initial Concn., 4 mM) through the Membrane at 30°
key: □: 1.2M $N(CH_3)_4Cl$, △: 1.2M NaCl, ○: salt-free

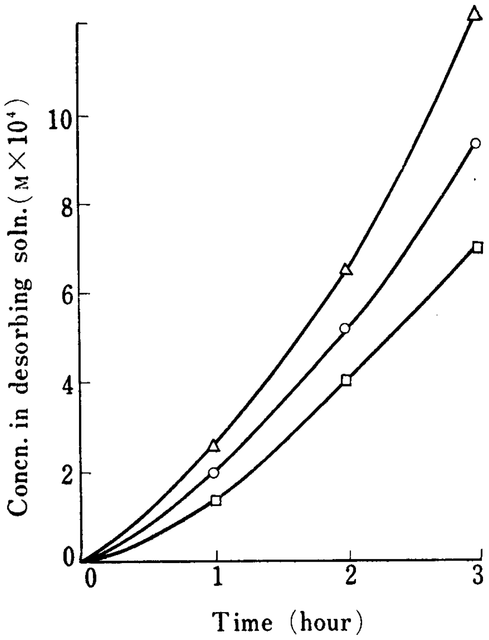

Fig. 6. Permeation of N,N-Dimethylcinnamamide (Initial Concn., 4 mM) through the Membrane at 30°
key: △: 1.2M NaCl, ○: salt-free, □: 1.2M $N(CH_3)_4Cl$