Effects of Ionic Protein Denaturants on Micelle Formation by Nonionic Detergents[1]

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Abstract: Critical micelle concentrations (cmc's) were determined for two *p-tert*-octylphenoxy(polyethoxy)-ethanols, OPE_{30} and OPE_{9-10}, containing an average of 30 and 9–10 ethoxy groups, respectively, in aqueous solution at 25° at various concentrations of several inorganic electrolytes and tetraalkylammonium halides commonly used in protein conformational studies. The salt effects are virtually independent of the polyethoxy chain length for these two detergents. The cmc's are lowered by all inorganic salts except LiI, which raises the cmc of OPE_{30}. The salting out effectiveness of the inorganic ions follows approximately the Hofmeister series, *viz.*, $0.5SO_4^{2-} > F^- > Cl^- > ClO_4^- > Br^- > NO_3^- > I^-$, SCN^- and $Na^+ > K^+ > Li^+ > 0.5Ca^{2+}$. Tetraalkylammonium bromides raise the cmc, in the order of effectiveness $(C_3H_7)_4N^+ > (C_2H_5)_4N^+ > (CH_3)_4N^+$, whereas $(CH_3)_4NCl$ lowers the cmc of OPE_{30} slightly. The logarithm of the cmc varies linearly with the salt concentration (<1 *M*). The salt effect constant, defined as the slope of such plots, appears to be additive with respect to contributions of the individual ions. From the data of Gratzer and Beaven, it is shown that this additivity holds also for the effect of guanidinium sulfate, chloride, and thiocyanate on the cmc of OPE_{9-10}. The salt effects can be accounted for mostly in terms of the mechanism proposed by Mukerjee, *i.e.*, the salting out or salting in of the hydrocarbon moiety of the monomeric detergent molecule. However, deviations from this theory are observed. Most likely causes of these deviations are (i) unequal extents of salting out the polyethoxy chain in the monomeric form and in the micellized form and (ii) specific interaction of the inorganic ions with the polyethoxy chain and hydrophobic adsorption of the tetraalkylammonium ions on the micellar surface. Other possible but less likely sources of these deviations also are discussed. The general pattern of the salt effects on a wide variety of systems seems to reflect differences in the interactions of these ions with water, although specific ion–solute interactions of the ion–dipole or hydrophobic type may also be involved.

Neutral salts can influence the solution conformations of proteins, polypeptides, and other macromolecules by affecting hydrophobic interactions, hydrogen bonding, and/or ionic interactions.[2] Solubility studies of a number of model solutes in aqueous salt solutions indicated that nonpolar groups are salted out[3–6] and peptide groups are salted in[7,8] by most electrolytes. However, salts of very large organic ions such as tetraalkylammonium halides[4,6,9–11] or complex ion electrolytes[12] such as *trans*-[Co(en)$_2$-NCSCl]X, where X = Cl, Br, or I, can cause salting in of some purely nonpolar solutes such as benzene and its homologs.

The reversible association of detergent monomers into micelles in aqueous solutions provides a very good example of hydrophobic interactions.[13] The monomer–micelle equilibria can, therefore, act as suitable model systems for studying the effects of salts and other additives on such interactions. The detergent ion or molecule is amphipathic in nature, consisting of a hydrocarbon tail and a hydrophilic head group that can be ionic, zwitterionic, or nonionic. Micelles are approximately spherical in structure at moderate detergent concentrations, with the hydrocarbon tails forming the core of the micelle and the polar head groups remaining on the exterior, free to interact with the surrounding medium.

The effects of inorganic salts on the cmc's of ionic detergents are rather drastic, the logarithm of the cmc decreasing linearly with the logarithm of the total counterion concentration. They have been studied in detail.[14–18] These effects can be accounted for largely in terms of electrostatic interactions,[19,20] although it has been pointed out recently[13,21] that salting out of the hydrocarbon chains of the detergents, neglected so far, may be appreciable, too.

The effects of salts on the cmc's of nonionic detergents are expected to be less complicated, due to the absence of charge interactions. However, such studies have been less systematic and the nature of the effects frequently was ill understood. The cmc's of nonionic detergents also are lowered by added salts, but to much lesser degrees than those of ionic detergents.[22–29]

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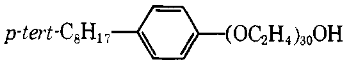
Various interpretations have been proposed for the observed effects. According to Hsiao, *et al.*,[22] the cmc is lowered due to the decreased hydration of the ethylene oxide chains, caused by added electrolytes, and probably due to the breaking of hydrogen bonds. Shinoda, *et al.*,[23] attributed the change in cmc to a decreased hydration of the detergents, resulting from an increase in their effective concentrations and due to the volume of added salts, including hydration water. Becher[24] first interpreted the lowering of the cmc as due to a decrease in the water activity, but later discounted this possibility.[25] Schick[26-29] attributed the effect to the salting out of the ethylene oxide chains. However, the most plausible interpretation so far seems to be that of Mukerjee[13,21] who showed that the existing data can be reasonably explained primarily in terms of the salting out of the hydrocarbon moiety of the detergents, and not of the hydrophilic head group as proposed by Schick.[27]

This paper reports the results of our systematic investigation of the effects of a large number of inorganic salts and four tetraalkylammonium halides, frequently used in protein conformational studies, on the cmc's of a nonionic detergent, *p-tert*-octylphenoxy(polyethoxy)ethanol, OPE_{30}, containing 30 ethoxy residues in the polyethoxy chain (I). Some of the measurements have been extended to OPE_{9-10}, which possesses the identical nonpolar tail as OPE_{30}, but only 9–10 ethoxy residues as the polar moiety. It will be shown that the results can be interpreted best qualitatively in terms of the mechanism proposed by Mukerjee.[13,21]



I

Quantitatively, however, some discrepancies exist between the effects observed and those estimated from a comparison with benzene[3,4,6] using the McDevit–Long theory,[3] based on the assumed proportionality of the salting out parameters to the partial molal volumes of the hydrocarbon moieties. Several possible causes for this discrepancy will be discussed later.

The effects of the cations and the anions seem to be additive for both the inorganic salts and the salts of large organic cations. Literature data[30] on the effects of guanidinium salts, another class of powerful protein denaturants, on the cmc of OPE_{9-10} are shown to obey the same additivity relationship.

The nonionic detergents used in our studies were commercially available samples, polydisperse with respect to the polyethoxy chain lengths. However, for reasons discussed elsewhere,[31] the general conclusions drawn from the results obtained with these samples can be considered to be applicable to monodisperse materials as well.

Experimental Section

Materials. OPE_{30} (Triton X-305) and OPE_{9-10} (Triton X-100) were supplied by Rohm and Haas Co. They were used as received. All salts used were reagent grade.

Method. The critical micelle concentrations were determined by a difference spectrophotometric technique developed in this laboratory.[32] The cmc's obtained were reproducible within ±1%.

All measurements were carried out at 25°, in a Cary 14 spectrophotometer equipped with a 0–0.1–0.2 slide wire. The cell compartments and the cell chambers were thermostated, and the temperatures of the solutions were maintained within ±0.05°.

Results

The cmc's of OPE_{30} and OPE_{9-10} are summarized in Table I. In most cases, the logarithm of the cmc varied linearly with the salt concentration (Figures 1–3) and the following empirical relationship[21] was obeyed

$$\log \text{cmc} = \text{constant} - k_s C_s \tag{1}$$

where C_s = the concentration of the salt in moles per liter and k_s = the salt effect constant. Such an empirical relationship was observed before in the case of alkylbetaines,[33] which are zwitterionic. Schick[27,28]

Table I. Critical Micelle Concentrations for *p-tert*-Octylphenoxy(polyethoxy)ethanols in Aqueous Salt Solutions at 25°

Salt	C_s, M	Cmc, $M \times 10^4$ OPE_{30}	OPE_{9-10}
	0.00	5.33	2.40
Na_2SO_4	0.10		1.60
	0.20	2.44	1.09
	0.40	1.13	
NaF	0.25		1.68
	0.50	2.26	1.12
	1.00	0.994	
NaCl	0.50	3.17	1.41
	1.00	1.86	0.834
	2.00	0.863	
$NaClO_4$	0.50	3.80	
	1.00	2.71	
NaBr	0.50	3.98	
	1.00	3.17	
$NaNO_3$	0.50	4.43	
	1.00	3.30	
NaI	1.00	4.20	
NaSCN	0.50	4.57	2.08
	1.00	4.16	
LiCl	0.50	3.93	
	1.00	2.94	
LiBr	0.50	4.73	2.08
	1.00	4.20	
	2.00	3.44	
LiI	1.00	6.55	
KCl	0.50	3.44	
	1.00	2.26	
KBr	1.00	3.62	
	2.00	2.39	
KSCN	1.00		1.70
$CaCl_2$	0.25	4.02	
	0.50	3.17	
TMACl	0.50	4.97	
	1.00	4.30	
TMABr	0.50	6.42	2.89
	1.00	7.66	
TEABr	0.50	10.0	4.49
	1.00	15.7	
TPABr	0.25	8.83	
	0.50	13.7	

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Table II. Salt Effect Constants and Salting Out Parameters at 25°

Salt	$k_{OPE_{30}}$[a]	$k_{OPE_{9-10}}$[a]	$k_{C_6H_6}$[b]	k_{NMA}[c]	k_{NMP}[c]	k_{ATGEE}[d]
Na_2SO_4	1.69	1.72	0.55	0.372	0.486	0.48
NaF	0.73	0.66	0.25			
NaCl	0.46	0.46	0.20	0.099	0.164	0.046
$NaClO_4$	0.29		0.106			
NaBr	0.24		0.155	0.065	0.118	0.00
$NaNO_3$	0.19		0.119			
NaI	0.103		0.095	0.018	0.056	−0.23
NaSCN	0.11_1	0.12		−0.023	0.025	−0.25
LiCl	0.26		0.14	0.033	0.077	0.021
LiBr	0.10	0.12	0.096[e]	−0.004	0.025	−0.17
LiI	−0.089		0.047[e]			
KCl	0.37		0.17			
KBr	0.17		0.119			
KSCN		0.14				
$CaCl_2$	0.46			0.076	0.166	−0.09
Gu_2SO_4		1.06[f]				
GuCl		−0.13[f]				
GuSCN		−0.41[f]				
TMABr	−0.16	−0.16	−0.15			
TEABr	−0.50	−0.52	−0.26			
TPABr	−0.85		−0.37			
TMACl	+0.08		−0.11[g]			

[a] This work. [b] References 4 and 6. [c] Reference 8. [d] Reference 7. [e] Estimated from ref 4, assuming additivity of individual ion effects. [f] Estimated from ref 30. [g] Estimated from ref 4 and 6, assuming additivity of individual ion effects.

reported a linear dependence of log cmc on C_s for nonylphenoxy(polyethoxy)ethanols and, at low temperatures, for $C_{12}H_{25}(OC_2H_4)_{30}OH$ ($C_{12}E_{30}$), but nonlinear dependence for the latter detergent at 25° or above. At high salt concentrations, we detected some deviations from linearity. For example, the cmc of OPE_{30} was lowered in 2 *M* NaCl by a factor of 6.2 instead of 8.4, expected from the initial linearity.

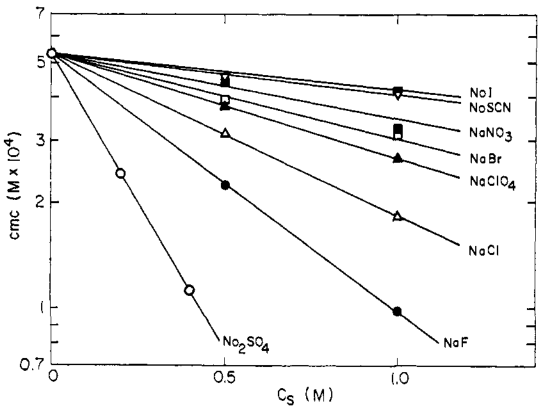

Figure 1. Plots of the logarithm of the cmc of OPE_{30} against salt concentration.

The salt effect constants $k_{OPE_{30}}$ and $k_{OPE_{9-10}}$, as obtained from the slopes of the log cmc *vs.* C_s plots, are summarized in Table II. The salting out parameters for benzene,[4,6] *N*-methylacetamide,[8] NMA, *N*-methylpropionamide,[8] NMP, and acetyltetraglycine ethyl ester,[7] ATGEE, also have been included in Table II for comparison. All the inorganic salts, except LiI, are found to lower the cmc, and hence the k_s values are positive. LiI is the only inorganic salt reported so far to cause an increase in the cmc of a nonionic detergent.

The only tetraalkylammonium halide whose effect on the cmc of a nonionic detergent was studied before[27,29] is tetramethylammonium chloride, TMACl. This salt was reported to lower the cmc's of $C_{12}E_{30}$ and of *p-tert*-nonylphenoxy(polyethoxy)ethanols very

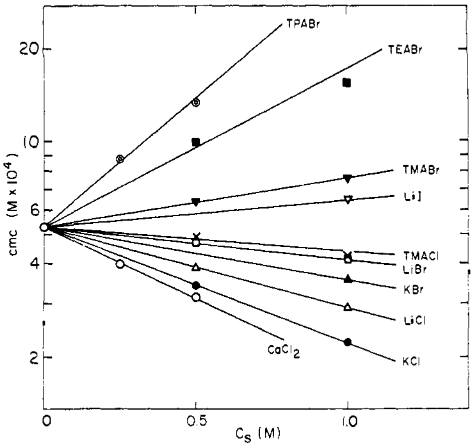

Figure 2. Plots of the logarithm of the cmc of OPE_{30} against salt concentration.

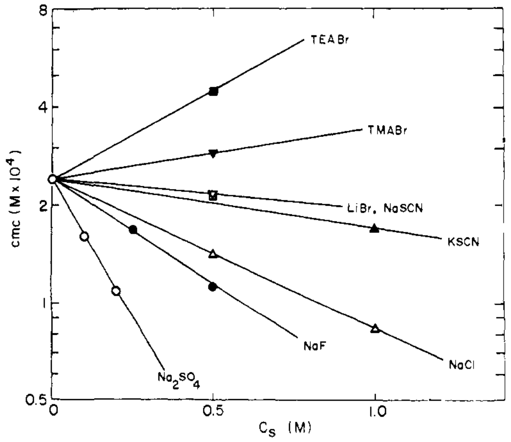

Figure 3. Plots of the logarithm of the cmc of OPE_{9-10} against salt concentration.

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strongly, corresponding to positive values for k_s. We have used TMACl, as well as tetramethyl-, tetraethyl-, and tetrapropylammonium bromides, TMABr, TEABr, and TPABr. TMACl lowers the cmc of OPE_{30} slightly. All three bromides raise the cmc of OPE_{30}, giving negative values for k_s. Similar results were obtained with OPE_{9-10} and TMABr and TEABr.

Discussion

Salt Order. The effectiveness of the salts in lowering the cmc of OPE_{30} follows approximately the lyotropic or Hofmeister series[2] of the anions for a given cation, *viz.*

$$0.5SO_4^{2-} > F^- > Cl^- > ClO_4^- > Br^- > NO_3^- > I^-, SCN^-$$

For a given anion, the cation order observed was as follows

$$Na^+ > K^+ > Li^+ > 0.5Ca^{2+}$$

Approximately similar salt orders are also observed in the salting out of model hydrophobic solutes,[4] amides and peptides,[7,8] and in the coil–helix transitions of fibrous and globular proteins.[2] It is worth mentioning that similar salt orders were observed for the ionic partial molal volumes, estimated by Mukerjee[34,35] from the differences between the intrinsic volumes of an ion and the electrostriction of the solvent. These values of the ionic partial molal volumes were used by Deno and Spink[6] to compute the salting out parameters for benzene, using the McDevit–Long equation. They obtained very good agreement between observed and calculated salt orders. This implies that the salt order, observed in connection with a variety of phenomena, basically reflects intrinsic differences in the interactions of various ions with the aqueous solvent.

The effectiveness of the tetraalkylammonium bromides for *increasing* the cmc of OPE_{30} increased with the chain length of the alkyl group, *viz.*

$$(C_3H_7)_4N^+ > (C_2H_5)_4N^+ > (CH_3)_4N^+$$

Such an order is also observed in the salting in of model hydrophobic solutes[6,9,10] and in the helix–coil transitions in proteins.[2]

Additivity of Individual Ion Effects. The effects of the ions of a salt on the solubilities of both polar and nonpolar solutes have long been known to be additive,[2,4,8] *i.e.*, the sum of the individual effects of the constituent ions of a given salt. Both inorganic salts and tetraalkylammonium halides, acting on OPE_{30} and OPE_{9-10}, obey fairly well this rule of additivity (Table II).

Effects of Guanidinium Salts. The effects of guanidinium sulfate, chloride, and thiocyanate on the cmc of OPE_{9-10} have been studied by Gratzer and Beaven.[30] We have estimated the $k_{OPE_{9-10}}$ for these salts from their data at low salt concentrations (1 *M*), as shown in Table II. These numbers can be compared with the $k_{OPE_{9-10}}$ values for Na_2SO_4, NaCl, and NaSCN (Table II)

$$0.5k_{Gu_2SO_4} - k_{GuCl} = 0.66 \qquad k_{GuCl} - k_{GuSCN} = 0.28$$

and

$$0.5k_{Na_2SO_4} - k_{NaCl} = 0.40 \qquad k_{NaCl} - k_{NaSCN} = 0.34$$

Thus the additivity relationship seems to hold approximately in the case of guanidinium salts too.

The parallel behavior observed between the relative effects of the three guanidinium salts on the cmc[30] of OPE_{9-10} and on the thermal transition of ribonuclease[36] suggests that the individual ion effects on the protein conformations are also additive. Tanford, *et al.*, proposed[37–39] that most proteins are completely denatured by 6 *M* GuCl. Several lines of evidence are, however, cited by Tanford[40,41] which suggest that unfolding is not complete in all cases even after denaturation by 6 *M* GuCl. This is likely to be caused by higher resistance of local nonpolar regions to unfolding. According to the data cited here, the existence of local folded regions is likely because, even though 6–8 *M* guanidinium ion is strongly destabilizing, 6–8 *M* chloride ion can cause considerable salting of the nonpolar residues of the protein. This is supported by the observation that 6–8 *M* GuCl is not able to completely disorganize the helical segments of polyleucine and polyphenylalanine, which have large nonpolar side chains, when incorporated into polypeptides that are water soluble.[42,43]

The salting in of nonpolar solutes by the guanidinium ion may be mediated through changes caused in the structure of water, as is believed to be the case with tetralkylammonium or other large organic ions.[4,6]

Effect of Polyethoxy Chain Length. A comparison of the salting out parameters for OPE_{30} with those of OPE_{9-10} (Table II) reveals that a difference of the polyethoxy chain length by 20 ethoxy residues causes negligible differences or none at all in the observed salting out parameters. This proves clearly that the salting out of the polyethoxy chains, as proposed by Schick,[27] cannot be the major mechanism for the observed salt effects on these systems. However, the salting out of a few ethoxy residues adjacent to the hydrocarbon moiety cannot be ruled out completely. This would be consistent with the higher than expected slopes observed in plots of k_{OPE_n} *vs.* $k_{C_6H_6}$ (to be discussed below), and with evidence for partial desolvation of some of these residues upon micelle formation.[31,44]

Application of the McDevit–Long Theory.[3] The salting out of nonpolar nonelectrolytes is correlated by the empirical equation

$$\log f = k_s C_s \tag{2}$$

where f = the activity coefficient of the nonelectrolyte and k_s = the salting out parameter known as the Setschenow constant. Various theories have been proposed to explain salting out phenomena, as discussed in several

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reviews.[3,4,45] The theory of McDevit and Long[3] appears to be more satisfactory than the older electrostatic theories because it predicts the correct salt order and the correct signs of k_s for purely nonpolar solutes.[45] According to McDevit and Long,[3] the energy required to create the volume in water which is needed to accommodate a nonpolar solute is increased in electrolyte solutions, due to ion–water interactions. This results in an increase of the activity of the nonelectrolyte and hence in its salting out. McDevit and Long derived the following equation for k_s

$$k_s = \overline{V}(V_s - \overline{V}_s)/2.303RT\beta_0 \qquad (3)$$

where $\overline{V}$ and $\overline{V}_s$ are the partial molal volumes of the nonelectrolyte and the salt in aqueous solutions, respectively, V_s is the liquid molal volume of the pure salt, β_0 is the compressibility of water, and T is the absolute temperature. However, for benzene, the observed magnitudes of k_s are smaller by a factor of 2–3 than the theoretical values[4,6] calculated on the basis of eq 3. This discrepancy was attributed by McDevit and Long to the finite size of the nonelectrolyte molecule, and a correction factor $a/(a + b)$ was introduced into eq 3, where a = the radius of the ion, and b = the radius of the nonelectrolyte.

The McDevit–Long theory has been used by Mukerjee[13,21] to interpret the effect of salts on monomer–micelle equilibria. Assuming (i) the validity of the mass-action model for micelle formation and (ii) additivity of the salting out coefficients, k_s, with respect to the polar and the nonpolar parts of the detergent molecule in solution and in the micellized form, the following equation was derived by Mukerjee[21]

$$\begin{aligned} \log \text{cmc} &= \Delta G/2.303RT - C_s(k_a + k_b - k_{bm}) \\ &= \text{constant} - k_sC_s \qquad (4) \end{aligned}$$

where ΔG = the standard free energy change per monomer, C_s = the salt concentration, and k_a, k_b, and k_{bm} are salting out parameters for the nonpolar and polar parts for the monomer in solution and for the polar part of the monomer in the micellized form, respectively. The corresponding k_{am} was assumed to be zero, since the nonpolar part in the core of the micelle is almost completely removed from any contact with the surrounding medium.

Since the polar group interacts with the salt solution both when the monomer is in solution and on the micellar surface, k_b and k_{bm} can be expected to cancel largely, but not entirely. For octyl glucosides in NaCl solution, cancellation was shown to be virtually exact.[13,21] This proved that the salting out of the hydrocarbon was primarily responsible for the observed salt effect in that case.

Gordon[46] recently criticized the use of the mass-action model by Mukerjee[21] and showed that the salt effects on alkyl glucosides and alkylbetaines can be explained very well if the McDevit–Long equation is applied to the two-phase model for micelle formation. According to the two-phase model, however, the salting out of the polyethoxy chains in the alkyl- or alkylaryl(polyethoxy)-ethanols would be more important than that of the hydrocarbon moieties, whereas the present data indicate that the observed salt effects depend very little on the polyethoxy chain length. Thus, our data are more consistent with the mass-action model.[47]

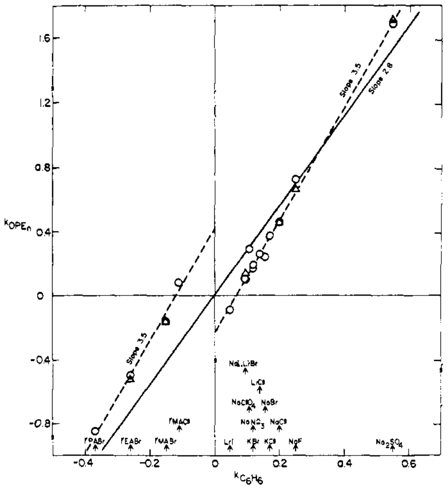

Figure 4. Plots of k_{OPE_n} *vs.* $k_{\mathrm{C_6H_6}}$ for various electrolytes, as indicated on the graph: ○, OPE_{30}; △, OPE_{9-10}.

If one assumes that the $k_b - k_{bm}$ term is negligible for the polyethoxy derivatives as well, so that k_s can be approximated[21] by k_a, it follows from eq 3 that for a given salt

$$k_{\mathrm{OPE}_n} = k_{\mathrm{C_6H_6}}\, \overline{V}_{\mathrm{OP}}/\overline{V}_{\mathrm{C_6H_6}} \qquad (5)$$

where k_{OPE_n} is the salt effect constant for OPE_{30} or OPE_{9-10}, $k_{\mathrm{C_6H_6}}$ is the salting out parameter for benzene, and $\overline{V}_{\mathrm{OP}}$ and $\overline{V}_{\mathrm{C_6H_6}}$ are the partial molal volumes of the *p-tert*-octylphenyl group only and of benzene, respectively.

Figure 4 shows a plot of $k_{\mathrm{OPE}_{30}}$ and $k_{\mathrm{OPE}_{9-10}}$ against $k_{\mathrm{C_6H_6}}$. The line drawn through the origin is the theoretical line of slope 2.8, calculated from $\overline{V}_{\mathrm{C_6H_6}}$ = 86 cm^3/mol (ref 6) and the estimated value $\overline{V}_{\mathrm{OP}}$ = 240 cm^3/mol. The latter was estimated assuming 86 cm^3/mol for $\overline{V}_{\mathrm{C_6H_4}}$, 25.5 cm^3/mol for the partial molal volume of each of the five CH_3 groups, 15.9 cm^3/mol for one CH_2 group,[8,48] and 5 cm^3/mol for each of the two quaternary C atoms. It is seen that (a) both inorganic salts and tetraalkylammonium halides give linear plots of equal slopes (3.5) when k_{OPE_n} is plotted against $k_{\mathrm{C_6H_6}}$, but (b) the observed slopes are higher than the theoretically expected slope of 2.8, and (c) the intercepts are nonzero for both series and have opposite signs, being negative for the inorganic and positive for the tetraalkylammonium salts.

Considering the wide variety of salts that are included in our studies, the extent of agreement between the experimental and theoretical k_s ratios is remarkable, indicating that the salting out of the hydrocarbon moiety is probably the most important factor. The very small differences observed between the $k_{\mathrm{OPE}_{30}}$ and $k_{\mathrm{OPE}_{9-10}}$ values indicate that the salting out of the last 20 ethoxy residues in OPE_{30} is certainly not involved. Also, assuming that the partial molal volume of a single ethoxy residue is 72 cm^3/mol, as estimated below, the salting out of the first ten ethoxy residues alone would give a slope greater than 8 on the k_{OPE_n} *vs.* $k_{\mathrm{C_6H_6}}$ plot. The

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[47] P. Mukerjee, *ibid.*, 74, 3824 (1970).

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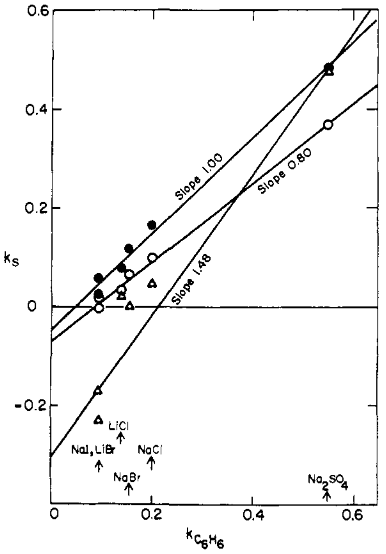

Figure 5. Plots of k_{NMA}, k_{NMP}, and k_{ATGEE} *vs.* $k_{C_6H_6}$: ○, NMA; ●, NMP; △, ATGEE. Based on data from ref 4, 7, and 8.

observed slopes (3.5) are much closer to the slope expected (2.8) on the basis of the salting out of the hydrocarbon moiety alone.

The deviations of slopes and intercepts may arise from several causes, to be discussed below.

(i) Incomplete Cancellation of Salting Out Effects of the Polyethoxy Chain. The salting out of a part of the polyethoxy chain of the monomer in solution but not in the micellar form would cause an increase of the slope. The salting out parameters, k_{NaCl}, for butane[49] and diethyl ether[47,50] are 0.22 and 0.31, respectively, at 25°. Based on additivity of the salting out parameters with respect to the constituent groups of the nonelectrolyte,[8,21] k_{NaCl} for the ether oxygen is obtained as 0.09. A comparison with benzene, for which $k_{NaCl} = 0.195$ and $\bar{V} = 86$ cm^3/mol, gives as the effective partial molal volume of the ether oxygen $\bar{V}_O = 40$ cm^3/mol. Assuming $\bar{V}_{O\text{-}CH_2\text{-}CH_2} = \bar{V}_O + 2\bar{V}_{CH_2}$, the partial molal volume of a single ethoxy residue should be 72 cm^3/mol. To account for the increase in slope from 2.8 to 3.5 (Figure 4), a volume contribution of about 60 cm^3/mol is needed above $\bar{V}_{OP}$. Thus, the salting out of a fraction of an ethoxy residue could account for the difference in slopes. However, the nonzero intercepts still are not explained by this effect.

(ii) Specific Interaction between Ions and the Nonelectrolyte. (a) Inorganic Salts. In Figure 5, k_{NMA}, k_{NMP}, and k_{ATGEE} are plotted against $k_{C_6H_6}$. It is interesting to note that for NMA and NMP the observed slopes are higher than the theoretical slopes (0.78 and 0.60, respectively) based on the salting out of only the nonpolar alkyl groups, but for ATGEE, the observed slope is very close to the expected slope of 1.52. All three are, however, characterized by negative intercepts, similar to those observed in the k_{OPE_n} *vs.* $k_{C_6H_6}$ plot (Figure 4). The effects of the inorganic salts on these amides have been interpreted in terms of the salting out of the alkyl groups and the salting in of the amide groups due to ion-dipole interactions.[7,8]

By analogy with the amides, it may be tempting to say that specific interactions of the inorganic ions with the ether oxygens of the polyethoxy chains are involved. Since the differences between $k_{OPE_{9-10}}$ and $k_{OPE_{30}}$ are negligible, any such interaction, causing the observed deviations from the theoretically expected values, would therefore have to involve only the initial few ethoxy residues. The possible interactions of the last 20 residues in OPE$_{30}$ probably are nearly the same in the monomer and on the micellar surface. However, as discussed earlier, the ether oxygen in diethyl ether is salted out by NaCl rather than salted in. This would argue against the existence of any significant specific interaction between the ether oxygens and the various inorganic ions. A more detailed study of the effects of various inorganic electrolytes on the solubility of diethyl ether would be needed, however, to reach a definite conclusion.

Inorganic electrolytes are known to lower the viscosities and the upper temperature limit of solubilities (T_p) of poly(ethylene oxides) in aqueous solutions.[51] The results were interpreted in terms of the salting out of the polyethoxy chains, and the conclusion was reached that no specific interactions exist between the inorganic ions and the ether oxygens in aqueous solutions, although such interactions occur in methanol.[52] However, the observed effects of the salts in the aqueous solutions possibly may be the sum of the salting out effects on the ethylene groups and salting in effects (if any at all) on the ether oxygens, and it may not be easy to separate the two. T_p values of the poly(ethylene oxides) are lowered by the inorganic salts, and the effects seem to be additive with respect to those of the individual ions (Figure 5 of ref 51). By extending this additivity relationship, it is estimated that LiI should increase T_p; this would be consistent with our observation that LiI raises the cmc of OPE$_{30}$, while most other inorganic electrolytes lower the cmc. It would be interesting to study the effects of LiI on the T_p and the viscosity behavior of poly(ethylene oxides).

On the other hand, butyl alcohol is salted in[53] by NaSCN. This raises the possibility that specific interaction of the inorganic ions with the terminal OH groups of the polyethoxy chain is involved. If such effects are incompletely canceled out between the monomer in solution and the molecule in the micellized form, deviations of the experimental k_s from the theoretical values may be explained. A comprehensive study of the effects of various inorganic electrolytes on the solubilities of some monohydric alcohol of low solubility in water may throw more light on the problem of specific interaction.

(b) Tetraalkylammonium Halides. The tetraalkylammonium halides are believed[4,6,54] to alter the structure of water so as to make $(\bar{V}_s - V_s) < 0$. Purely nonpolar solutes, such as benzene, are salted in by these salts.[6,9,10,55] The plot of k_{OPE_n} *vs.* $k_{C_6H_6}$ in Figure 4,

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[54] W.-Y. Wen and S. Saito, *ibid.*, **68**, 2639 (1964).

[55] At salt concentrations below about 1.4 *M*, the observed salting out parameters for benzene in these solutions agree very well with those calculated from the McDevit-Long equation, after the latter is multiplied by a factor of 0.3, as shown by Deno and Spink.[6] However, at higher salt concentrations, the solubility of benzene increases much more

however, indicates that OPE_n is much less salted in with respect to benzene than is expected on the basis of the salting in of its hydrocarbon moiety. The observed slope (3.5) can probably be accounted for in the same way as in the case of inorganic salts, *viz.*, by the salting out of a part of the first ethoxy residue. The positive intercept observed with this series of salts, as opposed to the negative intercept for the inorganic salts, suggests that the nonzero intercepts must have different origins for the two series of salts.

Tetraalkylammonium bromides and iodides raise the viscosities of poly(ethylene oxide) in aqueous solutions. This has been interpreted in terms of weak specific association between these salts and the polyethoxy chains.[52] The observed effect on the viscosity follows the order $I^- > Br^- > Cl^-$ and $(C_3H_7)_4N^+ > (C_2H_5)_4N^+ > (CH_3)_4N^+$. Even in the absence of any specific interaction between these salts and the polymers, the viscosity is expected to increase, since the tetraalkylammonium salts cause salting in of the ethoxy residues. However, should specific association also occur, such effects could cancel out largely between the ethoxy chains in the monomeric and micellized forms.

In anionic detergents, however, the tetraalkylammonium ions, when present as counterions, lower the cmc relative to Na^+, whereas an increase in cmc would be expected on the basis of the sizes of the tetraalkylammonium ions.[56] This was interpreted[56] in terms of stabilization of micelles by hydrophobic interactions between the micellar surface and the hydrophobic exterior of these ions.

Similar adsorption of the quaternary ions on the surface of the nonionic micelles is possible. It would stabilize the micelles, making the salt effect constant less negative than expected on the basis of a net salting in of the hydrocarbon moiety of the detergents only.[57] Thus, the positive deviations of the experimental points from the theoretical curve and hence the positive intercept in the k_{OPE_n} *vs.* $k_{C_6H_6}$ plot in Figure 4 could be explained. However, a less ambiguous interpretation has to await experimental studies of the effects of these salts on the solubilities of diethyl ether, *n*-alkyl alcohols, and the amides. It would be particularly interesting to know whether these solutes, especially the amides, are also relatively less salted in by these halides with respect to benzene.

(iii) Self-Interaction of Polyethoxy Chains. Self-interaction between polyethoxy chains in solution presumably is negligible, since monomer concentration is at most $1\text{–}20 \times 10^{-4}$ *M* at 25°. It may be more significant on the surface of the micelle, where the effective local concentration is high, $\gtrsim 1$ *M*. Self-interaction[4] usually lowers the salt parameter and therefore, if it occurs on the micellar surface alone, it could lower k_{bm} and hence increase $k_a + k_b - k_{bm}$. However, this would only cause an increased slope and could not account for the nonzero intercepts.

(iv) Limitations of the McDevit–Long Theory. It was pointed out clearly by Long and McDevit[3,4] that their theory is applicable only to very small nonpolar nonelectrolytes. Even in the case of benzene, the absolute values of k_s are larger by factors of 2–3 than the predicted values; this discrepancy was ascribed by them to the finite size of the benzene molecule. In view of the larger sizes and the partially polar nature of the alkylphenoxy(polyethoxy)ethanol molecules, it is possible that the observed deviations are caused by some inherent limitations of the McDevit–Long theory itself, which assumes that the partial molal volume is the only solute parameter important in salt effects. It has been shown recently[12] that dispersion forces play a significant role in the salting in of aromatic hydrocarbons by complex ion electrolytes.[58] Similar conclusions have been reached by Morrison and Johnstone.[5] They also suggested that structural changes of water may play a role.

In conclusion, the effect of ions studied here upon micelle formation by nonionic detergents can be explained largely in terms of the salting out of the hydrocarbon moiety of the detergent monomer in solution.[13,21,47] However, deviations from this theory are observed. Several possible sources for these deviations have been discussed: (i) imperfect cancellation of the salting out of the polyethoxy chain in monomeric and micellized forms, (iia) specific interaction of the inorganic salts with the polyethoxy chains, (iib) hydrophobic adsorption of the quaternary ammonium ions on the surface of the micelle, (iii) self-interaction of the polyethoxy chains on the surface of the micelle, and (iv) some inherent inadequacies of the McDevit–Long theory. As discussed above, factors i and ii are the more likely causes of the deviations.

The overall picture that emerges is that essentially similar salt orders are observed in studies of phenomena as diverse as ionic partial molal volumes, solubility of benzene, solubility of amides, stabilities of nonionic micelles, and conformational transitions in proteins, polypeptides, and other biological macromolecules. Thus the general pattern of salt effects seems to reflect a common cause, *viz.*, differences in the interactions of the various ions with water, as influenced by charge, size, polarizability, and other effects. In addition, specific effects may occur due to association of the ions with some moiety of the organic solutes. This may be caused by ion–dipole or hydrophobic interactions, in addition to possible charge–charge interactions.[59]

Acknowledgment. We thank Professor P. Mukerjee for helpful comments.

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rapidly.[10,11] According to Arnett, *et al.*,[11] the behavior of the solution approaches that of an organic nonelectrolytic solvent at very high salt concentrations.

(56) P. Mukerjee, K. J. Mysels, and P. Kapauan, *J. Phys. Chem.*, 71, 4166 (1967).

(57) It is conceivable that the adsorption of the tetraalkylammonium ions to the surface of protein molecules plays a similar role in influencing the conformational stability of proteins.

(58) J. O'M. Bockris, J. Bowler-Reed, and J. A. Kitchener, *Trans. Faraday Soc.*, 47, 184 (1951).

(59) NOTE ADDED IN PROOF: Nuclear magnetic resonance measurements (peak areas of proton signals) indicate that the OPE_{30} sample used contains 31% water by weight: F. Podo, personal communication. The cmc data for OPE_{30}, shown in Table I and in Figures 1 and 2, are corrected to the actual amount of detergent present. No water is contained in the OPE_{9-10}.