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POSSIBILITY OF ANALYTICAL APPLICATION OF THE PARTITION IN AQUEOUS BIPHASIC POLYMERIC SYSTEMS TECHNIQUE

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Summary

The partition behaviour of a number of ionic and nonionic surface-active substances in the dextran-polyethylene glycol system was examined. The strictly linear dependence of the logarithm of the partition coefficient on the alkyl chain length for a homologous series of nonionic surfactants provides a measure of the difference in the relative hydrophobicity between the two phases of the system, in terms of the free energy of transfer of a CH_2 group from the bottom phase to the top phase of the system. This difference is found to be altered in the presence of NaCl or KCl depending on the salt concentration. It is concluded that the influence of the salt composition of the system on the distributed solutes' behaviour may be due to the effect of the ions on the hydrophobicity difference between the phases.

The partition of ionic amphiphiles is found to be dependent on the relative hydrophobicity of the compounds as well as on their charge. It is shown that at salt concentrations up to about 0.1 M NaCl the charged solute partition is determined by its charge as well as its relative hydrophobicity, in the presence of 0.1—0.2 M NaCl the substance distribution is highly dependent on its charge and slightly on its lipophility. At the salt concentrations above 0.2 M the solute partition is determined just by its hydrophobic character and seems to be totally independent of its charge. It is concluded that the partition technique can be used for analytical purposes. The method seems to be unique in providing quantitative information on the amphiphilic surface properties of the solutes being partitioned.

Introduction

The partition of cells, cell organelles and biopolymers in aqueous two-phase polymeric systems [1] is widely used at present for separation and purification of different biological materials [2,3].

It was pointed out [1,4] that the partition coefficient of a given substance may be considered to be a characteristic constant of the substance particularly indicating its surface properties. It follows, therefore, that the partition technique can be used for analytical purposes. A major difficulty in the analytical application of the method lies in the lack of an approach to interpret the partition coefficient value characterizing the distribution behaviour of any given molecule. It seems that to overcome this difficulty the partition behaviour of some model low molecular weight compounds should be studied.

According to the theoretical considerations given in ref. 1, the phases of the system differ in their relative hydrophobicity and, being compared with a number of conventional solvents listed according to their hydrophobic-hydrophilic nature with increasing hydrophobicity the polymer solutions fall within a very narrow part of the solvent spectrum between an aqueous salt solution and a water/acetone mixture. It must be stressed, however, that the considerations of the differences in the relative hydrophobicity between the phases of an aqueous two-phase polymer system are only qualitative in character and no quantitative criteria for assessing these differences have been reported up to the present. Nonetheless, some arguments for the concept can be found in the literature. It has been shown by us [5] that the hydrophobicity of the two-phase system including dextran, *i*-propanol and water is high enough to distribute lipids as well as proteins and nucleic acid.

It is the purpose of the present communication to demonstrate that the partition technique can be used for analytical purposes. Experiments were undertaken to study the partition behaviour of a number of low molecular weight model compounds to obtain experimental evidence for the presence of some difference in the relative hydrophobicity between the phases of a two-phase polymer system and to estimate this difference in quantitative terms.

The experiments have been made with a system containing 7% (w/w) dextran-500 and 4.4% (w/w) poly(ethylene glycol)-6000. The system was chosen since it is most widely used for separation of biopolymers [1,4,6–10].

As the low molecular weight model compounds to be partitioned in the system surfactants have been chosen as substances of a dual character. This feature of surface-active compounds provides insight into the contributions of the hydrophilic and hydrophobic portions of the partitioned amphiphile to the distribution process.

Materials and Methods

Dextran 500 ($M_r = 5 \cdot 10^5$) was supplied by Serva (Heidelberg, G.F.R.). Poly(ethylene glycol)-6000 was obtained from Merck (Schuchardt, G.F.R.).

Alkyltrimethylammonium bromides $[R_nN(CH_3)_3]Br$ ($R_n = C_nH_{2n+1}$; $n =$ 8,10,12,14,16) were prepared by the method described in ref. 11, alkylbenzyldimethylammonium chlorides $[R_nN(CH_3)_2CH_2C_6H_5]Cl$ ($n = 8,10,12,14$) were synthesized as in ref. 12 and sodium alkyl sulfates R_nOSO_3Na ($n = 6,8,10,11,$ $12,13,14,15$) were obtained by the method of Dreger et al. [13]. All salts and chemicals used in the study were of analytical grade.

To find the critical micelle concentrations of the surfactants in water, the solubilization technique with eosin for cationics [14] and pinacyanol for anionics [15] was used. All experimental critical micelle concentrations are found to be in good agreement with literature data.

The technique of the system preparation was described elsewhere [6—10]. The system included 7% (w/w) dextran, 4.4% (w/w) polyethylene glycol and 88.6% (w/w) water or salt solution.

The partition coefficient, K, is defined as the ratio of sample concentration in the top phase to sample concentration in the bottom phase [1]. Top phase and bottom phase aliquots (0.1 ml) were carefully pipetted from the system and the concentrations of the partitioned compound were determined.

To measure the concentrations of the cationic compounds the method described in ref. 16 was used and in the case of sodium alkyl sulfates the technique of ref. 17 was applied.

The partitioned compounds were distributed in a series of experiments at different concentrations not exceeding their critical micelle concentration/2 values. The series of experiments were performed 2—3 times, the value for the partition coefficient, K, was obtained by the graphical method according to ref. 5 and the measured deviation from the average K value did not exceed 3% for all the compounds studied.

Results and Discussion

The first point to emerge from the obtained results is that all surfactants under study have more affinity for the top polyethyleneglycol-rich phase. It strongly suggests that the top phase of the system is more hydrophobic than the bottom dextran-rich phase.

The relationship found between the logarithm of the partition coefficient of the cationic compounds ($\ln K$) and their alkyl chain length (n) for two homologous series involving different polar groups at $\mathrm{pH} > 8.0$ are linear and parallel to each other supporting the obvious conclusion that the slope of the curve represents the free energy of transfer of a CH_2 group from the bottom phase to the top phase of the system. It should be noted that at $\mathrm{pH} > 8.0$ these compounds appear to be in the uncharged form and can be considered as non-ionics [18].

To evaluate the contributions of the hydrophilic and hydrophobic portions of the distributed amphiphile to the free energy of transfer of the compound molecule from the bottom phase to the top phase, ΔG_{tr}, it is possible to use the known relation $\Delta G_{\mathrm{tr}} = -RT\ln K$ (Eqn. 1), where K is the partition coefficient and T is the absolute temperature. The commonly used phase separation approach to the thermodynamics of surfactant micelle formation utilizes the concept that the contributions of the hydrophilic and hydrophobic parts of the surfactant to the free energy of micellization should be nearly independent [19] and can be expressed by the relation: $\Delta G_{\mathrm{mic}} = \Delta G_{\mathrm{head}} + \Delta G_{\mathrm{tail}} + m \cdot \Delta G_{\mathrm{CH_2}}$ (Eqn. 2), where ΔG_{mic} is the free energy of micellization, ΔG_{head}, $\Delta G_{\mathrm{CH_2}}$ and ΔG_{tail} are the contributions of the polar hydrophilic head group, that of a CH_2 group and that of the hydrophobic part of the molecule which cannot be expressed as a sum of CH_2 groups respectively, and m is the total number of CH_2 groups in the molecule structure. It seems possible to use the analog of Eqn. 2 for an approximate estimate of the contributions of the above portions of the distributed amphiphile to the free energy of transfer between the system phases, ΔG_{tr}.

The estimates of these separate contributions give the following values characterizing the interphasic transfer of a CH_2 group: $\Delta G_{CH_2} = -18.4$ cal/mol and that of the hydrophilic $-\overset{|}{\underset{|}{N}}{}^{+}OH^{-}$ group $\Delta G_{head} = +55.2$ cal/mol. These values appear to provide a measure of the difference in the relative hydrophobicity between the two phases of the system.

The effect of the ionic composition on the biopolymers partition is generally attributed to the unequal distribution of the ions present in the system which creates a potential difference between the phases [1,20]. This potential difference depending on the ionic composition, even if it is small, is believed to have a strong influence on the partition of polyelectrolytes. This concept seems to us to be correct but not the only probable one. Since various inorganic salts are known to affect the water properties differently [21] it appears to be reasonable to assume that the salts can influence the difference in the relative hydrophobicity between the system phases to a large extent.

To verify the assumption, the partition of the homologous series of alkylbenzyldimethylammonium chlorides in the system in the presence of 0.1 M NaCl and in the presence of 0.1 M KCl was examined. The obtained relationships between the logarithm of the partition coefficient (ln*K*) and the total number of CH_2 and CH_3 groups in the molecule structure (*m*) are given in Fig. 1.

The above approach permits one to estimate the contributions of a CH_2 group in the presence of different salts as follows: the ΔG_{CH_2} is -18.4 cal/mol in the absence of added salts, -21.3 cal/mol in the presence of 0.1 M NaCl and -22.8 cal/mol in the presence of 0.1 M KCl. The results indicate that the effect of the ionic composition of the system on the difference in the relative hydro-

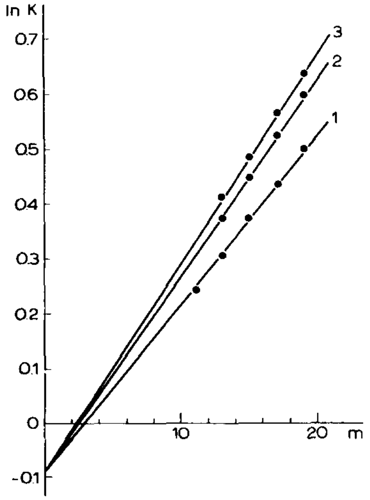


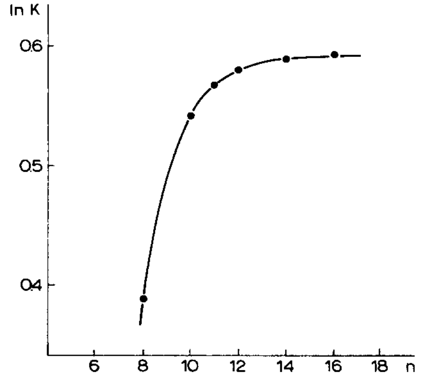
Fig. 1. Logarithm of the partition coefficient, ln*K*, for alkylbenzyldimethylammonium chlorides as a function of the total number of CH_2 and CH_3 groups, *m*, in the distributed molecule. The phase system composition: 7% (w/w) dextran 500; 4.4% (w/w) polyethyleneglycol-6000. 1, in the absence of added salt; 2, in the presence of 0.1 M NaCl; 3, in the presence of 0.1 M KCl.

phobicity between the system phases may be at least as important as its influence on the electrical potential difference between the phases. It follows also from the data in Fig. 1 that the addition of potassium chloride to the system affects the difference in the relative hydrophobicity between the phases more than that of sodium chloride at the same concentration. The replacement of KCl by NaCl in this system was reported [4] to lower the *K* values of several proteins partitioned in the system at pH values corresponding to their isoelectic points. The ionic composition of the system appears not to affect the contribution of the hydrophilic $-\overset{|}{\underset{|}{N}}{}^{+}OH^{-}$ group and to influence the contribution of a phenyl residue as follows: $\Delta G_{C_6H_5}$ is —35.6 cal/mol in the absence of added salt, —41.5 cal/mol in the presence of 0.1 M NaCl and —44.8 cal/mol in the presence of 0.1 M KCl.

To study the rules governing the partition of ionic compounds, the partition behaviour of the above cationic surfactants in the system was examined at $pH < 6.0$, i.e., pH values at which they are in the cationic form. It was found that at pH 6.0 these compounds have the same value of the partition coefficient, *K*, namely 1.28, independent of the hydrocarbon chain length. It was concluded that *K* for these compounds is determined by their charges and, to eliminate the charge effect, sodium chloride was added to the system. It was found that the surface-active cations' behaviour in the system in the presence of 1.0 M NaCl follows a linear relationship between the ln*K* and *m* similar to that observed in the case of the non-ionic compounds. It appears, furthermore, that in the presence of 1.0 M NaCl the compounds with the same value of *m* are characterized by the same partition coefficient, *K*, at both $pH < 6.0$ and > 8.0. The above treatment of the obtained data leads to the following estimates of the CH_2, C_6H_5 and $-\overset{|}{\underset{|}{N}}{}^{+}X^{-}$ ($X = OH, Cl, Br$) groups' contributions: in the presence of 1.0 M NaCl the contribution of a CH_2 group is —24.9 cal/mol, that of a phenyl residue is —48.6 cal/mol and that of the hydrophilic $-\overset{|}{\underset{|}{N}}{}^{+}X^{-}$ ($X = OH, Cl, Br$) group in the dissociated, as well as in the associated, form amounts to +55.2 cal/mol.

The relationship between the logarithm of the partition coefficient of sodium alkyl sulfates and their alkyl chain length (n) found in the system in the absence of added salt is plotted in Fig. 2. As it can be seen from Fig. 2, the relationship is not linear as is the case of the cationic compounds. The obtained results indicate that in contrast to the above surface-active cations at $pH < 6.0$, the anions' partition behaviour does depend on their relative hydrophobity. It suggests that the sodium alkyl sulfate distribution in the system depends on the mutual influence of the charge and the hydrocarbon chain size of the solute.

The mutual effect of the hydrophobic and hydrophilic regions of the amphiphilic molecule structure on the compound's properties is usually displayed to the highest degree when the surfactant's micelle formation is studied [22]. The critical micelle concentration is known [22] to be depend on the hydrophobic-hydrophilic nature, the structural features and the other specific properties of a given compound being considered as one of the primary characteristics of ionic surfactants. It must be stressed that all experiments on the surfactants' partitioning in the system were performed at concentrations much lower than those equal to the compounds' critical micelle concentrations. The alkyl sulfates behaviour in the system in the absence of added salt was found to be inter-



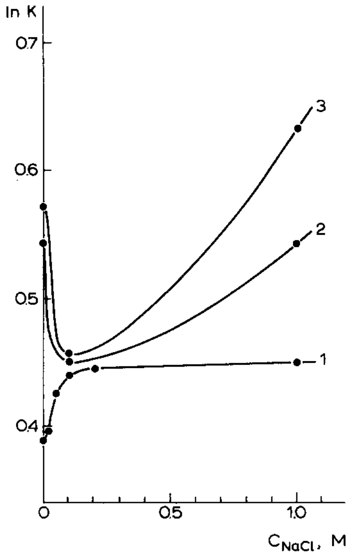


Fig. 2. Logarithm of the partition coefficient, ln*K*, for sodium alkyl sulfates as a function of the alkyl chain length, *n*. The phase system composition is given in Fig. 1.

Fig. 3. Logarithm of the partition coefficient, ln*K*, for several sodium alkyl sulfates as a function of the concentration of NaCl in the system. The phase system composition is given in Fig. 1. 1, sodium octyl sulfate; 2, sodium decyl sulfate; 3, sodium dodecyl sulfate.

locked with the alkyl chain length (*n*) corresponding to the relation $\ln K = a - b \cdot e^{-B \cdot n}$ (Eqn. 3), where *a*, *b*, *B* are constants, the physical significance of which is unknown at present.

The partitioning of a number of sodium alkyl sulfates with increasing NaCl concentration is shown in Fig. 3. The results indicate that the anions' behaviour in the system containing 0.1 M NaCl is similar to that of the surface-active cations in the absence of added salt, i.e., their partition coefficient appears to be independent of the alkyl chain length and amounts to 1.57. This implies that although in the presence of 0.1 M NaCl the sulfate group's influence on the relative lipophility of the anion appears to be restricted to a large extent, the effect of the distributed compound charge on the compound partitioning greatly exceeds that of the hydrophobic-hydrophilic nature of the compound. In order to eliminate this effect of the anion charge, by analogy with the cationic surfactants the sodium alkyl sulfates partitioning in the presence of 1.0 M NaCl was studied.

The relationship between the logarithm of the partition coefficient and the alkyl chain length in the presence of 1.0 M NaCl is found to be linear indicating that the partition coefficient of the anions is primarily determined by their relative hydrophobicity, and the contribution of a CH_2 group to the free energy of transfer from the bottom phase to the top phase amounts to —24.9 cal/mol, i.e., is equal to the value found above for the quaternary ammonium compounds in the presence of 1.0 M NaCl. This finding strongly supports the view that the alkyl chain length dependence of the partition coefficient for a homologous series of surfactants provides a measure of the difference in the relative hydrophobicity between the two phases of the system. It should be noted also that the contribution of the sulfate group to the free energy of transfer between the phases of the system containing 1.0 M NaCl appears to be —76.4 cal/mol.

Thus, it appears that the effect of the distributed compound's charge on the compound partition may be eliminated in the presence of an abundance of salt.

The data shown in Fig. 3 indicate that the partition coefficient tends to depend on the ionic strength to a greater degree as the alkyl chain length of the partitioned surfactant increases. The results obtained here on model amphiphilic compounds' behaviour are similar to those reported for proteins [1]. Thus, it seems likely that there is one general mechanism which governs the partitioning of biopolymers such as proteins and nucleic acids as well as of low molecular weight compounds.

The results obtained here with model compounds appear to be consistent with the conclusions made on the basis of the theoretical consideration of the distribution of charged macromolecules in the polymer two-phase systems in the presence of added salts [1,20]. The following important conlusions can be drawn from our results. The partitioning of a given charged amphiphilic compound at an ionic strength below 0.1 M appears to be influenced by its charge as well as its relative hydrophobicity. In the presence of 0.1—0.2 M NaCl the amphiphile's partition coefficient is highly dependent on its charge and only slightly dependent on its hydrophobic character. At salt concentrations above 0.2 M, the solute partition coefficient appears to be determined by the solute's hydrophobic character which partially depends on the presence of hydrophilic charged groups in the solute. The results obtained here, as compared with previously reported data on the influence of the ionic strength on proteins' partitioning behaviour [1,3,4,6,7,23—26], appear to support the conclusion that the above rules are general for the partitioning of any soluble substance independent of its molecular weight.

The above conclusion that the influence of the surface amphiphility of the distributed protein on its partition behaviour increases at high salt concentrations appears to be supported by many data reported on the experimentally found optimal conditions for separation of different natural materials [27—30]. It seems that the best separation of natural mixtures of proteins, nucleic acids and other biopolymers is usually achieved at the ionic strength up to about 4.0—5.0 M [27—30].

From the results of this work and the analysis of the data reported in literature it follows that partitioning in aqueous two-phase polymer systems can be used not just as a convenient preparative method for separation and purification of biological materials but as a unique analytical technique as well. The method is unique in that one can obtain quantitative information on the hydrophobic-hydrophilic properties (amphiphility) of the partitioned molecule's surface and to study the relation of these to media conditions. The technique may provide a means of studying conformational changes of biopolymers which are inaccessible by other methods since the change of the partition coefficient value is sensitive to the change of the composition of the partitioned macromolecule's surface. It appears that there is no other method able to detect this kind of change in macromolecular structure at present.

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