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CHEMICAL AND SOLVENT EFFECTS ON THE INTERACTION OF TETRAPHENYLBORATE WITH LIPID BILAYER MEMBRANES

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Alkali metal salts of tetraphenylboron dissociate in aqueous solution to yield the hydrophobic anion, BPh_4^-, which is strongly adsorbed at the surfaces of lipid bilayer membranes. Upon application of a transmembrane voltage pulse these anions cross the membrane without appreciable desorption, thereby exhibiting a transient electrical conductance. The relaxation time of this transient is governed by the height of the central potential barrier which the anions must surmount in crossing the membrane. Because of discrete charge effects, the barrier height and hence the observed relaxation time increase markedly with increasing surface density of adsorbed BPh_4^-. Since adsorbed BPh_4^- are in partition equilibrium with the same species dissolved in the aqueous phase, measurement of the relaxation time for BPh_4^- membrane conductance can be used to assay the aqueous-phase concentration of the hydrophobic anion. In this way we have observed the precipitation of $KBPh_4$ in water, obtaining a solubility concentration product of $1.0 \cdot 10^{-7}$ mol$^2 \cdot$dm^{-6} for the precipitation reaction at 25°C. This result is larger by a factor of two than the most directly comparable published values from other sources. In additional experiments we have reduced the polarity of the aqueous phases bathing the membrane by adding varying amount of ethylene glycol to the water. Using the same conductance relaxation assay, we have determined that partitioning of BPh_4^- into the membrane/solution interfaces is lessened as the polarity of the bathing solutions is reduced. This result is attributed to a lowering of the chemical potential of the BPh_4^- in the less polar medium.

Introduction

Hydrophobic anions such as are produced in aqueous solutions of dipicrylamine and salts of tetraphenylboron (BPh_4^-) have been shown to adsorb strongly at the surfaces of lipid bilayer membranes [1]. This and subsequent studies [2,3] have established the utility of hydrophobic ions as probes of the electrostatic energy barrier which they must surmount in crossing the membrane. Application of a transmembrane voltage step results in a transient current flow having a characteristic relaxation time which increases with increasing height of the energy barrier. Steady-state hydrophobic ion current is negligible because of slow desorption of hydrophobic ions from the membrane/solution interfaces into the surrounding aqueous phases. Still more recent studies [4,5] have shown that, because of discrete charge effects, the height of the central barrier, and hence the characteristic time for relaxation of hydrophobic ion conductance, increases with increasing density of these ions adsorbed onto the membrane/solution interfaces. Since these adsorbed ions are also in partition equilibrium with the same species dissolved in the aqueous solutions bathing the membrane, it is possible to use measurement of conductance relaxation time to monitor their aqueous-phase concentration in situations where this quantity is not known a priori.

In the present paper we describe the use of this approach to observe the precipitation of $KBPh_4$. The limited solubility of this salt, relative to Li and Na salts of tetraphenylboron, has been of considerable importance to the analytical chemistry of the alkali metals [6]. Our measured value for the solubility concentration product of $KBPH_4$ at 25°C, $1.0 \cdot 10^{-7}$ $mol^2 \cdot dm^{-6}$, is larger by a factor of two than the most nearly comparable literature values available. Possible reasons for this discrepancy are discussed below.

Additional studies utilizing this approach have examined the effect of bathing-solution polarity on the partitioning of BPh_4^- into the membrane/solution interfaces. To do this we have formed membranes in water/ethylene glycol binary solutions ranging to 60 wt.% ethylene glycol. It is observed that lowered polarity of the bathing medium reduces the partitioning of hydrophobic ions into the membrane/solution interfaces. Thermodynamic considerations lead to the conclusion that reduced partitioning is due primarily to a lowering of the chemical potential of BPh_4^- in the bathing medium as its polarity is reduced.

Materials and Methods

Bilayer membranes were formed by the brush technique using diphytanoyl phosphatidylcholine (Avanti Biochemicals, Inc., Birmingham, AL) as the lipid. It was dissolved in decane (Aldrich Chemical Co., Milwaukee, WI) to a concentration of 20 $mg \cdot cm^{-3}$. Salts used in the preparation of electrolyte solutions were of reagent grade. Distilled water was redistilled in glass prior to use. Reagent grade ethylene glycol (Mallinckrodt, Inc., Paris, KY) was used as supplied to prepare the binary solvent solutions employed. Sodium tetraphenylborate (Aldrich Chemical Co., Milwaukee, WI) was dissolved in reagent-grade dimethyl sulfoxide (Mallinckrodt) at concentrations up to 10^{-2} $mol \cdot dm^{-3}$ to provide stock solutions which were then added to the membrane bathing solutions to produce the desired final concentration of tetraphenylborate. In no case did the aqueous concentration of dimethyl sulfoxide exceed 0.5 vol%, an amount which does not significantly affect membrane properties. Both the $NaBPh_4$ as supplied and the stock solutions prepared from it were stored at 0°C when not in use to minimize decomposition.

Membranes were formed on a 1.5-mm diameter aperture in a septum separating two compartments of a Teflon (E. I. du Pont de Nemours, Inc., Wilmington, DE) cell. One end of the cell was fitted with a quartz window to permit visual observation of the membrane. The cell was water-jacketed, with its temperature controlled at 25 ± 0.04°C by use of a Lauda Model K-2R circulating constant temperature bath (Brinkmann Instruments, Inc., Westbury, NY).

Electrical contact to the membranes was provided through Ag/AgCl electrodes immersed in the bathing solutions. The output of a pulse generator (Model DM-4, Continental Specialities Corp., New Haven, CT) was applied through a voltage divider and series resitor to the electrodes. Transient hydrophobic ion currents were measured by monitoring the voltage drop across the series resitor using a Textronix (Beaverton, OR) Model 7313 storage oscilloscope equipped with Model 7A22 differential amplifier plug-ins. Current transients were photographed from the oscilloscope screen for subsequent measurements of relaxation times. Graphical analysis of the photographed current transients permitted the determination of relaxation times with an estimated maximum random error of $\pm 5\%$. Values of current-sensing resistors were always chosen to be low enough so that the membrane charging time was short when compared to the relaxation time of the hydrophobic ion current.

Results

(a) Precipitation of $KBPh_4$ in water

A compendium of solubilities of tetraphenylborates has recently appeared [7]. For $NaBPh_4$ in water at 25°C the cited solubility is 0.947 $mol \cdot dm^{-3}$. The corresponding value for $KBPh_4$ is $1.76 \cdot 10^{-4}$ $mol \cdot dm^{-3}$. For the determination of these values, data from a number of sources using conventional analytical methods have been evaluated. These methods include evaporation and weighing of residue, conductometric measurement and spectrophotometric techniques. Since these values pertain to pure water as solvent, the solubility concentration products are given by squaring the values cited.

Our determination of the solubility product of

$KBPh_4$ is illustrated in Fig. 1. Here we have plotted measured values of τ_0, the relaxation time for the decay of BPh_4^- membrane conductance, versus the aqueous phase concentration of added sodium tetraphenylborate. The four plots shown illustrate the results obtained with varying proportions of NaCl in aqueous solution. In all cases the total ionic strength has been held constant at 1.0 $mol \cdot dm^{-3}$ to minimize surface potential effects [8]. The plot for 1.0 $mol \cdot dm^{-3}$ NaCl shows a monotonic and rapid increase of τ_0 with added $NaBPh_4$. This behavior has been interpreted theoretically in terms of the discrete charge effect [4,5] and has been previously observed experimentaly for both dipicrylamine (2,9,10] and tetraphenylborate [11]. Nowhere on our plot for 1.0 $mol \cdot dm^{-3}$ NaCl does the concentration product $[Na^+] \cdot [BPh_4^-]$ approach the solubility product for this ion pair, 0.897 $mol^2 \cdot dm^{-6}$, deducible from the solubility data given above. We therefore expect added $NaBPh_4$ to be fully ionized in this case. Activity corrections, which do not affect this conclusion, will be considered below.

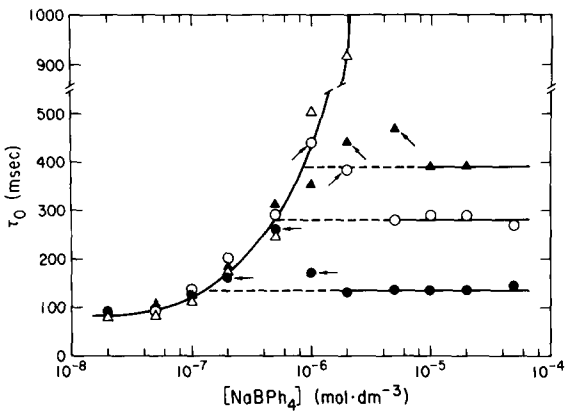

Fig. 1. The relaxation time, τ_0, characteristic of the decay of transient BPh_4^- conductance, is plotted versus the concentration of added $NaBPh_4$. In the presence of K^+ in the aqueous phases, the concentration of BPh_4^- is limited by the formation of a $KBPh_4$ precipitate. This limitation imposes a corresponding limit on the increase of τ_0 with $[NaBPh_4]$, at levels which decrease with increasing K^+ concentration. Arrows identify those data points for which the system appeared to be supersaturated, the dashed lines indicate the shape of the curves to be expected had thermodynamic equilibrium with respect to precipitation of $KBPh_4$ been maintained throughout. Aqueous-phase ionic strength is held fixed at 1.0 $mol \cdot dm^{-3}$. ●, 1.0 M KCl; ○, 0.8 M NaCl + 0.2 M KCl; ▲, 0.9 M NaCl + 0.1 M KCl; △, 1.0 M NaCl.

The plot of τ_0 versus added tetraphenylborate obtained with 1.0 $mol \cdot dm^{-3}$ of KCl in the aqueous phases, referring again to Fig. 1, shows a markedly different form. The relaxation time, τ_0, reaches a limiting value of 135 ms, which is maintained at all concentrations of added tetraphenylborate in excess of 10^{-7} $mol \cdot dm^{-3}$. This implies that the concentration of BPh_4^- cannot exceed 10^{-7} $mol \cdot dm^{-3}$ because of the formation of $KBPh_4$ precipitate. Three data points belonging to the 1.0 M KCl plot do, however, show τ_0 values substantially in excess of 135 ms. They are indicated by arrows and are believed to result from supersaturation. The dashed line portion of the plot indicates the range of tetraphenylborate concentration over which thermodynamic equilibrium conditions were not achieved, and indicates the shape of the curve to be expected had such conditions prevailed.

Two additional plots shown in Fig. 1 illustrate results for binary salt solutions containing 0.1 M and 0.2 M KCl, respectively. As the KCl concentration is reduced the 'break' in the plots of τ_0 versus added tetraphenylborate rises to progressively higher concentrations of the latter. This indicates that progressively higher aqueous phase concentrations of BPh_4^- are reached before precipitation of $KBPh_4$ commences. All three of the plots in Fig. 1 for which KCl is present in the aqueous phases are consistent with a single solubility product (of concentrations) for $[K^+] \cdot [BPh_4^-]$ of $1.0 \cdot 10^{-7}$ $mol^2 \cdot dm^{-6}$. The corresponding product inferred form the solubility limit data for $KBPh_4$, cited above, is $3.1 \cdot 10^{-8}$ $mol^2 \cdot dm^{-6}$. Activity corrections and other factors which may contribute to the discrepancy are considered in the discussion.

In Fig. 2 we show plots of τ_0 versus added tetraphenylborate for aqueous solutions containing 0.1 M NaCl and 0.1 M KCl. The data for 1.0 M NaCl, plotted in Fig. 1, are also repeated here to facilitate comparison. When the latter curve is compared with that for 0.1 M NaCl, we see that the 0.1 M curve is displaced toward higher concentrations of added $NaBPh_4$, and rises less steeply than does the curve for 1.0 M NaCl. This difference is most readily explained in terms of an enhanced surface potential effect at lower ionic strength [5,8,12]. At lower ionic strength a given surface density of adsorbed BPh_4^- will produce a

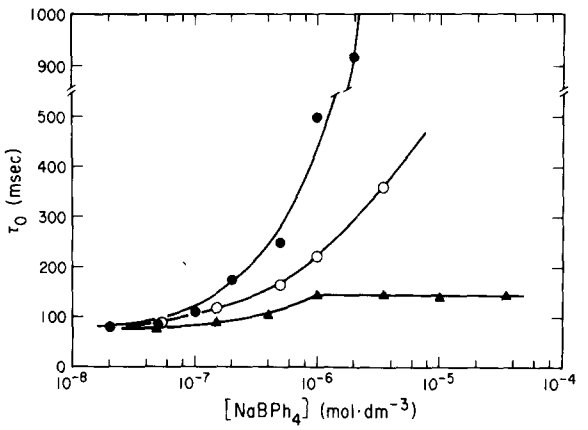

Fig. 2. Plots of τ_0 versus [$NaBPh_4$] in the presence of 1.0 M and 0.1 M NaCl permit a comparison of the effects of changing ionic strength (see text). Saturation of τ_0 in the presence of 0.1 M KCl again indicates the formation of a $KBPh_4$ precipitate in the aqueous phases. ●, 1.0 M NaCl; ○, 0.1 M NaCl; ▲, 0.1 M KCl.

negative surface potential of greater magnitude, which in turn will more effectively limit the adsorption of additional BPh_4^-.

The third plot in Fig. 2 shows that, with 0.1 M KCl in the bathing solutions, the relaxation time, τ_0, again reaches a limiting value as $NaBPh_4$ is added at nominal concentrations in excess of 10^{-6} mol · dm^{-3}. Hence the solubility product for [K^-] · [BPh_4^-], again given as a product of concentrations, is $1.0 \cdot 10^{-7}$ mol^2 · dm^{-6}, in good agreement with the results obtained at an ionic strength of 1.0 M. A complication is evident, however, in that the presaturation segment of the plot for 0.1 M KCl is shifted to the right relative to the plot for 0.1 M NaCl. No such shift is evident when comparing plots for 1.0 M NaCl and 1.0 M KCl presented in Fig. 1. If the curve for 0.1 M NaCl (Fig. 2) is shifted to the right by an amount corresponding to an increase of $NaBPh_4$ concentration by a factor of three on the logarithmic scale, it superposes well on the presaturation segment of the curve for 0.1 M KCl. One possibility is that, in the presence of 0.1 M KCl, not all of the added $NaBPh_4$ yields BPh_4^- in solution, even prior to the formation of $KBPh_4$ precipitate. The effective concentration of BPh_4^- could be lowered by the formation of a soluble $KBPh_4$ complex, for example. Arguments against this possibility are: (1) no comparable displacements are observed at 1.0 M salt, (b) the solubility products for [K^+] · [BPh_4^-] in 1.0 M and in 0.1 M electrolyte agree well, and (c) electrolytic conductivity measurements of the solubility of $KBPh_4$ in pure water, assuming full dissociation of the soluble fraction [13], agree, within 5%, with the solubility measured spectrophotometrically [7]. The relative horizontal displacement of the plots for 0.1 M salts, seen in Fig. 2, is most likely due to differences of interface structure which give rise to different surface potentials in the presence of Na^+ and K^+.

(b) Solvent effects on BPh_4^- transport

In Fig. 3 we present plots of the relaxation time, τ_0, for the decay of BPh_4^- conductance, versus concentration of $NaBPh_4$ added to bathing solutions consisting of water, and of water/ethylene glycol binary solutions containing 20, 40 and 60 wt.% ethylene glycol ($C_2H_6O_2$). In each case the solvent medium contains 0.1 M NaCl as well. At 80 wt.% of ethylene glycol membrane stability was insufficient to permit measurements. Mem-

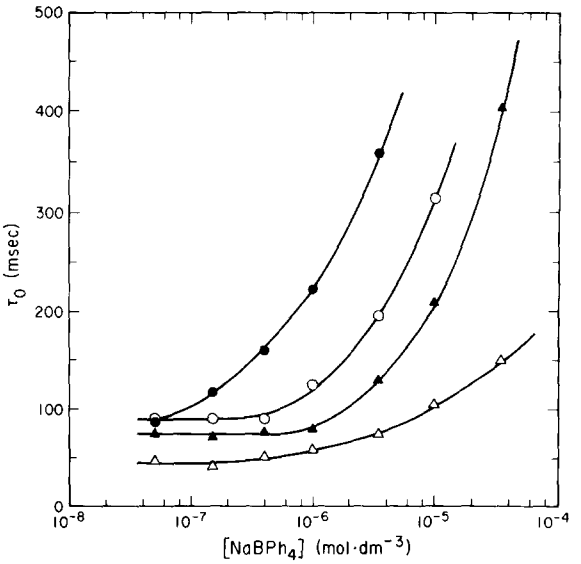

Fig. 3. Plots of τ_0 versus [$NaBPh_4$] in the presence of increasing percentages of ethylene glycol in the bathing media are presented. A shift of the curves to the right indicates a lowered partitioning of BPh_4^- into the membrane/solution interfaces as the solution polarity is reduced by ethylene glycol addition. The electrolyte is 0.1 M NaCl in all cases. ●, 0 wt.%; ○, 20 wt.%; ▲, 40 wt.%; △, 60 wt.% ethylene glycol.

branes could not be formed at all at 100% ethylene glycol. Two effects of ethylene glycol addition are clearly evident: (a) the plots are shifted to the right by an amount roughly proportional to the amount of added ethylene glycol, and (b) the relaxation time observed at low BPh_4^- concentration is lowered by added ethylene glycol, particularly at 60 wt.% of the latter. The partition coefficient for adsorption of BPh_4^-, defined as the ratio of their surface density in the membrane/solution interfaces to their volume density in the bathing media, is reduced by addition of ethylene glycol to the latter, as evidenced by point (a), above. This is so because the relaxation time, τ_0, on the steeply rising parts of the curves is uniquely determined by the interfacial density of adsorbed BPh_4^- [5], while the volume density of the latter is plotted on the abscissa. Thus the fractional change in partition coefficient accompanying addition of ethylene glycol to the bathing media may be evaluated by noting the shift on the logarithmic abscissa scale required to superpose the steeply rising segments of the plots. In this way we establish by reference to Fig. 3 that the partition coefficient for the case of water only is decreased by factors of 6, 16 and 50 as 20, 40 and 60 wt.%, respectively, of ethylene glycol is added to the bathing media. Thus we see that the affinity of the bathing media for hydrophobic BPh_4^- is enhanced by addition of ethylene glycol. It is expected that the hydrophobic ion entering the glycol-containing media would distort fewer hydrogen bonds on average than would be the case upon entering pure water, i.e., the glycol-containing solutions are more hydrophobic. Furthermore, this hydrophobicity increases with increasing glycol content. We observe that purely electrostatic considerations, on the other hand, would suggest that partitioning of BPh_4^- into the interface should increase with addition of ethylene glucol to the bathing solutions. This is so because the solution dielectric constant decreases with added ethylene glycol [14], making entry of ions into the less polar medium more difficult. We conclude that the hydrophobic effect [15] is dominant in this case.

As noted in point (b) above, Fig. 3 indicates that the concentration-independent values of τ_0 observed at low BPh_4^- concentration decrease with increasing ethylene glycol content of the bathing

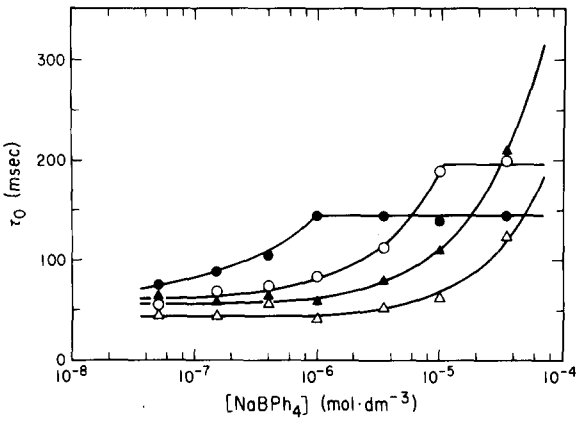

Fig. 4. With 0.1 M KCl in the bathing media, plots of τ_0 versus $[NaBPh_4]$ in the presence of increasing percentages of ethylene glycol illustrate the increase of the solubility product for the precipitation of $KBPh_4$ as the solution polarity is decreased. ●, 0 wt.%; ○, 20 wt.%; ▲, 40 wt.%; △, 60 wt.% ethylene glycol.

solutions. Since BPh_4^- adsorption is ideal in this range, and since τ_0 is sensitive to the height of the central barrier which the ions must surmount in crossing the membrane, we attribute the observed decrease in τ_0 to structural changes of the membrane caused by the presence of ethylene glycol. One possibility is that ethylene glycol dissolves in the membrane to an extent sufficient to raise its dielectric constant and thereby lower the height of the central barrier. Such a structural change could also account for the loss of membrane stability at high external ethylene glycol concentrations.

In Fig. 4 we present results analogous to those of Fig. 3, except that here the bathing solutions contain 0.1 M KCl. Precipitation of $KBPh_4$ is again evident, but the solubility product increases rapidly with ethylene glycol content in the solutions. Reference to Fig. 4 indicates that addition of 20 wt.% ethylene glycol raises the solubility product, $[K^+]\cdot[BPh_4^-]$, to $1.0\cdot10^{-6}$ mol$^2\cdot$dm^{-6}, an order of magnitude increase over the value for a purely aqueous bathing medium. Precipitation was not observable at higher concentrations of ethylene glycol. Enhanced solubility of $KBPh_4$ in the less polar binary solutions parallels increased partitioning of BPh_4^- into these solutions.

Discussion

We begin by presenting well-known chemical potential relationships [16] appropriate to the sys-

tem being considered. Strictly speaking, we should consider electrochemical potentials, but we will ignore surface potentials in this discussion. In the solvent media adjacent to the membrane the chemical potentials of K^+ and BPh_4^- are

$$\mu_s(K^+) = \mu_s^0(K^+) + RT \ln \lambda(K^+) \quad (1)$$

and

$$\mu_s(BPh_4^-) = \mu_s^0(BPh_4^-) + RT \ln \lambda(BPh_4^-) \quad (2)$$

respectively, where λ represents the absolute activity of each ion species. The standard-state chemical potentials, μ_s^0, are the chemical potentials of each ion species at unit molarity under hypothetical conditions where the activity coefficient in each case is equal to unity. As such it is important to bear in mind that the standard-state chemical potentials are sensitive only to ion-solvent interactions. Ion-ion interactions are accounted for by the absolute activity terms. When K^+ and BPh_4^- are in thermodynamic equilibrium with $KBPh_4$ precipitated throughout the solvent medium, we know that

$$\mu_s(KBPh_4 \downarrow) = \mu_s(K^+) + \mu_s(BPh_4^-) \quad (3)$$

Combining Eqns. 1–3 gives

$$RT \ln K_s = \mu_s(KBPh_4 \downarrow) - \mu_s^0(K^+) - \mu_s^0(BPh_4^-) \quad (4)$$

where

$$K_s = \lambda(K^+) \cdot \lambda(BPh_4^-) \quad (5)$$

is the solubility product, expressed as a product of absolute activities.

Since BPh_4^- in the solvent is in equilibrium with the same species adsorbed at the membrane/solution interfaces, we have in addition,

$$\mu_s(BPh_4^-) = \mu_m(BPh_4^-) = \mu_m^0(BPh_4^-) + RT \ln \rho(BPh_4^-) \quad (6)$$

where again μ_m^0 is a reference state sensitive only to the interaction of the adsorbed ion species with the surrounding membrane/solution interfacial structure, and ρ is the surface density of adsorbed ions. Here we are assuming ideal adsorption, known to be valid only at low enough surface density so that the molar interaction energy between adsorbed ions is small in comparison to RT [5]. Combining Eqns. 2 and 6 yields

$$RT \ln K_p = \mu_s^0(BPh_4^-) - \mu_m^0(BPh_4^-) \quad (7)$$

where

$$K_p = \frac{\rho(BPh_4^-)}{\lambda(BPh_4^-)} \quad (8)$$

is a partition coefficient describing the distribution

TABLE 1

CALCULATED IONIC ACTIVITY COEFFICIENTS AND ACTIVITY PRODUCTS AT 25°C

Ionic strength	1.0 mol·dm^{-3}	0.1 mol·dm^{-3}			
Weight percent of ethylene glycol	0	0	20	40	60
BPh_4^-	0.76	0.83	0.83	0.83	0.82
K^+	0.55	0.75	0.75	–	–
	0.88 [a]	0.78 [a]	0.78 [a]	–	–
$[K^+]\cdot[BPh_4^-]$ (mol^2·dm^{-6})	$5.0 \cdot 10^{-8}$	$6.2 \cdot 10^{-8}$	$6.2 \cdot 10^{-7}$	–	–
	$8.0 \cdot 10^{-8}$ [a]	$6.5 \cdot 10^{-8}$ [a]	$7.1 \cdot 10^{-7}$ [a]	–	–

[a] Including correction term linear in ionic strength.

of BPh_e^- between the solvent medium and the membrane surfaces.

In further discussion we will make two assumptions, namely (a), the chemical potential of the precipitate, $\mu_s(KBPh_4 \downarrow)$, is at most a function of temperature and pressure only, and hence is a constant in our experiments, and (b), the absolute activity of the large hydrophobic tetraphenylborate ion may be calculated from the Debye-Hückel theory assuming an ion radius of 10 Å. The first assumption ignores surface effects which could be significant when the precipitate is highly dispersed. In making the second assumption we follow Popovych and Friedman [17]. In Table I we list calculated activity coefficients for BPh_4^- in water at 25°C [18]. Similar calculations of activity coefficients for K^+, assuming an effective ionic radius of 3Å [18], are also presented in Table I. In this case we have applied both the unmodified Debye-Hückel theory, and a modification in which the logarithm of the absolute activity is reduced by a term linear in the ionic strength. This semi-empirical term is intended to account for solvation and short-range non-Coulomb interactions between ions [19]. Finally, using the data on concentration products from Figs. 1, 2 and 4, we calculate the ion activity products also listed in Table I.

Comparison of the activity products given in the first two columns of Table I with the value $3.0 \cdot 10^{-8}$ mol$^2 \cdot$ dm^{-6}, cited for $KBPh_4$ in water at 25°C by Popovych [7], indicates that the best agreement is obtained by omission of the linear correction term. The cited value is obtained by solubility measurements in water containing no other added salt. The relatively poor agreement is most likely attributable to lack of precision in our determination of activity coefficients. Other data more directly comparable to our own are that of McClure and Rechnitz [20], whose molar solubility value for $KBPh_4$ in 0.1 M Tris buffer at 24.8°C leads to a concentration product of $5.3 \cdot 10^{-8}$ mol$^2 \cdot$ dm^{-6}. Siska [21] gives molar solubility data for $KBPh_4$ at 20°C in aqueous solutions containing up to 2 mol $\cdot$ dm^{-3} of dissolved Na_2SO_4. Solubility increases with added electrolyte to 0.1 mol $\cdot$ dm^{-3}, then decreases as the concentration of Na_2SO_4 is increased further. Siska's values are low overall, however, and it is likely that equilibrium was not achieved in his experiments [7].

Turning to a consideration of solvent effects we note first that the calculated activity coefficients do not change significantly upon addition of ethylene glycol. This is so in spite of reduction of the solvent dielectric constant from 78.5 to 59.4 upon proceeding from pure water to a binary solution containing 60 wt.% ethylene glycol [14]. This lack of change results from the fact that, at an ionic strength of 0.1 mol $\cdot$ dm^{-3}, the Debye-Hückel equation for the activity coefficient is insensitive to variation of the dielectric constant. The ionic activity product, however, increases by an order of magnitude when pure water as solvent is replaced by one containing 20 wt.% ethylene glycol. It is therefore evident from Eqn. 4 that this increase of activity product must be attributed to a decreased reference-state chemical potential of either K^+ or BPh_4^-, since the chemical potential of the precipitate remains constant. A decrease for the relatively small and hydrophilic K^+ seems unlikely in view of the decreasing dielectric constant. The large hydrophobic BPh_4^-, on the other hand, could very well be expected to experience a reduction in chemical potential upon transfer to a more hydrophobic solvent medium containing ethylene glycol. This would imply that fewer hydrogen bonds would have to be distorted, on average, to accomodate the tetraphenylborate ion in the more hydrophobic medium.

Further support for a postulated reduction in standard chemical potential of BPh_4^- in the solvent medium is provided by the observed 50-fold reduction in its partition coefficient for adsorption at the membrane/solution interface, upon passing from pure water to a binary solution containing 60 wt.% ethylene glycol. In this view, the observed reduction of the partition coefficient, K_p, is attributed to a reduction of $\mu_s^0(BPh_4^-)$, appearing on the right of Eqn. 7. Eqn. 7 suggests that a change of $\mu_m^0(BPh_4^-)$ may also be involved, implying that structural changes of the membrane/solution interface accompany the addition of ethylene glycol to the bathing medium. Experimental evidence in support of this possibility includes, (a) the fact that membranes become unstable at high concentrations of ethylene glycol, and (b) the concentration-independent relaxation time for the membrane translocation of BPh_4^- decreases by

about 40% upon addition of 60 wt.% ethylene glycol (See Figs. 3 and 4). The much larger decrease of partition coefficient suggests, however, that a decrease of $\mu_s^0(BPh_4^-)$ upon addition of ethylene glycol is the dominant effect observed in our experiments. The free energy decrease at room temperature corresponding to a 50-fold reduction in partition coefficient is about 2–3 kcal/mol.

Acknowledgements

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References

1 Ketterer, B., Neumcke, B. and Läuger, P. (1971) J. Membrane Biol. 5, 225–245
2 Bruner, L.J. (1975) J. Membrane Biol. 22, 125–141
3 Andersen, O.S. and Fuchs, M. (1975) Biophys. J. 15, 795–830
4 Wang, C.-C. and Bruner, L.J. (1978) Biophys. J. 24, 749–764
5 Kleijn, W.B. and Bruner, L.J. (1983) J. Theor. Biol. 100, 139–152
6 Flaschka, H. and Barnard, A.J., Jr. (1960) in Advances in Analytical Chemistry and Instrumentation, Vol. 1 (Reilley, C.N. ed.), pp. 1–117, Interscience Publishers, New York
7 Popovych, O. (1981) in Solubility Data Series, Vol. 18, Tetraphenylborates (Popovych, O., ed.), pp. 3–4, 7–14, Pergamon Press, Oxford
8 McLaughlin, S. (1977) in Current Topics in Membrane Transport (Bronner,F. and Kleinzeller, A., eds.), pp. 71–144, Academic Press. New York
9 Wang, C.-C. (1978) Ph.D. Thesis, University of California, Riverside
10 Kleijn, W.B., Bruner, L.J., Midland, M.M. and Wisniewski, J. (1983) Biochim. Biophys. Acta 727, 357–366
11 Andersen, O.S., Feldberg, S., Nakadomari, H., Levy, S. and McLaughlin, S. (1978) Biophys. J. 21, 35–70
12 Wang, C.-C. and Bruner, L.J. (1978) J. Membrane Biol. 38, 311–331
13 Rüdorf, W. and Zannier, H. (1953) Z. Naturforsch. 8B, 611–612
14 Harned, H.S. and Owen, B.B. (1958) The Physical Chemistry of Electrolytic Solutions, 3rd. Eds., p. 151, Reinhold Publishing Corporation, New York
15 Tanford, C. (1963) The Hydrophobic Effect: Formation of Micelles and Biological Membranes, pp. 1–3, John Wiley and Sons, New York
16 Moore, W.J. (1962) Physical Chemistry, pp. 344–347, Prentice-Hall, Inc., Englewood Cliffs, NJ
17 Popovych, O. and Friedman, R.M. (1966) J. Phys. Chem. 70, 1671–1673
18 Dean, J.A. (1979) Lange's Handbook of Chemistry, 12th Edn., Sect. 5, pp. 3–6, McGraw-Hill, New York
19 Guggenheim, E.A. (1935) Philos. Mag. 19, 588–643
20 McClure, J.E. and Rechnitz, G.A. (1966) Anal. Chem. 38, 136–139
21 Siska, E. (1976) Magy. Kem. Foly. 82, 275–278