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Journal of Chromatography B, 711 (1998) 45–52

JOURNAL OF
CHROMATOGRAPHY B

Consistent view of electrolytes in aqueous two-phase systems

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Abstract

The effects of electrolytes in aqueous two-phase systems are investigated. It is shown that macroscopic and molecular models give a consistent view of electrolytes at interfaces. The electrostatic potential difference $\Delta\varphi$ between coexisting phases is a common property at interfaces even though the phases are strictly electroneutral and $\Delta\varphi$ can not be measured. It is shown how $\Delta\varphi$ can be quantified under controlled conditions. Additionally, a molecular picture is presented based on computer simulations. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Partitioning; Aqueous two-phase systems; Electrolytes

1. Introduction

The dramatic effect of electrolytes on the partition behaviour of charged species in aqueous two-phase systems has been demonstrated in many publications (e.g., [1–4]). This influence has been attributed to the existence of electrostatic potential differences between the two liquid phases in equilibrium [1]. Thus several authors set out to determine the potential difference experimentally (e.g., [5–8]) leading to a discussion on whether it exists at all and whether it can be measured. The goal of this work was to clarify some of the aspects of the electrostatic potential difference between coexisting liquid phases. It should be noted that other models than those presented here can be derived to describe the effects of electrolytes. Nevertheless, the results of these models should be consistent with the ideas developed in this work even if they circumvent the

explicit definition of an electrostatic potential difference.

2. Graphic model

The first questions raised are where the electrostatic potential differences originate from and if the phases are electroneutral. To answer these questions let us imagine an aqueous two-phase system as depicted in Fig. 1. If now a salt is added to the system as the only salt present, which we may assume to be NaCl for simplicity, it will dissociate into the appropriate ions: Na^+ and Cl^- . These ions form individual species which will have different affinities for the two phases in equilibrium as would, in general, have any other components in the system. This affinity is to be understood in terms of partition coefficients and not a complete partitioning into one of the phases. For example, Na^+ might tend to partition into the top phase and Cl^- to the bottom

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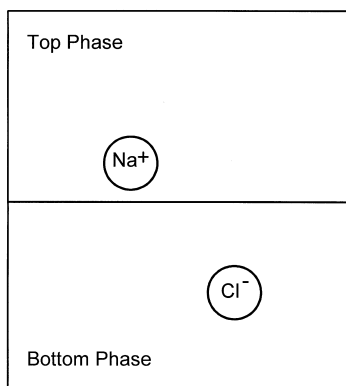


Fig. 1. Schematic two-phase system with NaCl added.

phase. If the ions would behave according to these affinities they would separate as shown in Fig. 1 resulting in a system with bulk phases carrying excess charges.

To investigate the properties of such charged phases, the Poisson equation may be considered for one spatial dimension:

$$-\frac{\partial^2 \varphi}{\partial x^2} = \frac{1}{\varepsilon} \rho \quad (1)$$

where ρ is the excess (or net) charge density, ε the dielectric permittivity of the phase, φ the electrostatic potential at a given location in the phase and x the distance from the interface. In a charged phase ρ is nonzero. If for a nonzero ρ Eq. (1) is integrated twice with respect to x , keeping in mind that thermodynamicists think of bulk phases as being infinitely extended, infinite values for the electrostatic potential and its derivative with respect to x will result. Since the force acting on an ion is given by the product of this derivative and the charge of the ion, infinite forces will occur. Thus a phase with nonzero excess charge density could never be kept stable in a beaker but would rather be driven apart by the infinite forces. Since this behaviour is generally not observed when performing experiments with aqueous two-phase systems containing salts, the only conclusion can be that the excess charge density of any bulk phase has to be exactly zero. The bulk phases are strictly electroneutral.

Thus the picture shown in Fig. 1 has to be modified: anion and cation of the single salt present

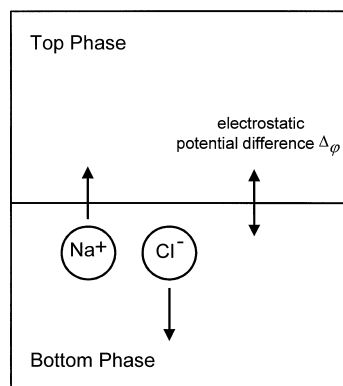


Fig. 2. Schematic two-phase system with NaCl added and electroneutral phases.

in the system have to find an agreement how to partition between the phases. The partition coefficients of Na^+ and Cl^- have to be identical to guarantee electroneutrality of the phases. This is shown in Fig. 2. Here the ions agree to partition together slightly into the bottom phase. Still they are drawn toward the phase they have the greater affinity for. This will set the system under a tension which manifests itself as electrostatic potential difference $\Delta\varphi$ between the phases. Thus the driving force for $\Delta\varphi$ is the individual behaviour of the ions on one side and the electroneutrality forcing the ions to partition together on the other. This very graphic explanation has of course its limitations but nevertheless may prove to be useful for the basic understanding of $\Delta\varphi$. Interestingly enough these ideas can directly be moulded into equations which have been put forward for aqueous two-phase systems by Albertsson [1].

3. Albertsson model

The derivation given by Albertsson starts with the equilibrium condition for charged species in a two-phase system [1,2,9]:

$$\tilde{\mu}_i' = \tilde{\mu}_i'' \quad (2)$$

where $\tilde{\mu}_i$ is the electrochemical potential of species i , ' and '' indicating the two phases in equilibrium. $\tilde{\mu}_i$ can be written as

$$\tilde{\mu}_i = \mu_i + Fz_i\varphi \quad (3)$$

where μ_i is the chemical potential of component i , F the Faraday constant, z_i the number of elementary charges of species i and φ the electrostatic potential of a phase. Now the activity a_i of species i can be introduced, which leads to

$$\mu_i = RT \ln a_i \quad (4)$$

where R is the universal gas constant and T the absolute temperature. a_i can be split further into concentration c_i and activity coefficient γ_i :

$$a_i = \gamma_i c_i \quad (5)$$

By substituting Eqs. (3)–(5) into Eq. (2) and defining the partition coefficient K_i of species i ,

$$K_i = \frac{c_i'}{c_i''} \quad (6)$$

one obtains

$$\ln K_i = \ln K_i^0 - \frac{z_i F}{RT} \Delta\varphi \quad (7)$$

where $\Delta\varphi$ is the electrostatic potential difference between the phases,

$$\Delta\varphi = \varphi' - \varphi'' \quad (8)$$

and K_i^0 summarizes the influence of the activity coefficients:

$$K_i^0 = \frac{\gamma_i''}{\gamma_i'} \quad (9)$$

Eq. (7) shows that the partition of charged species depends on the electrostatic potential difference between the coexisting phases weighed with the charge number of the species as well as other interactions with the two-phase system summarized in K_i^0 . K_i^0 can be explained in two different ways. Either K_i^0 is the partition coefficient of the species of interest when there is no $\Delta\varphi$ present. This interpretation is somewhat problematic since $\Delta\varphi$ is not a well defined property as will be shown. Or K_i^0 can be understood as the partition coefficient of a hypothetically uncharged species i . This is not a very clear definition either. To clarify matters it appears appropriate to state which interactions are described by K_i^0 and which are not. From Eq. (9) it can clearly be seen that K_i^0 includes all “ordinary” interactions

with the phases like van der Waals forces, polar interactions or hydrogen bonding. But K_i^0 also includes ionic interactions e.g., between charged species and dipolar molecules in the hydration shell of the ions. The only interaction not included in K_i^0 is that between the charge of the ion and $\Delta\varphi$. Eq. (9) makes also clear that in principle K_i^0 is a function of all variables influencing γ_i , e.g., the temperature and the concentrations of all species in the system.

Based on these simple equilibrium considerations of charged species, the origin of the electrostatic potential difference can be deduced along the same lines as in the graphic model. If a single salt is added to an otherwise non-ionic system then the equilibrium conditions apply for cation and anion. For simplicity, it is again assumed that the salt is of the form A^+B^- and that it is fully dissociated. Then the equilibrium condition can be written as

$$\ln K_{A^+} = \ln K_{A^+}^0 - \frac{z_{A^+} F}{RT} \Delta\varphi \quad (10)$$

and

$$\ln K_{B^-} = \ln K_{B^-}^0 - \frac{z_{B^-} F}{RT} \Delta\varphi \quad (11)$$

To obtain electroneutral phases the partition coefficients of both ions have to be identical,

$$K_{A^+} = K_{B^-} \quad (12)$$

Substituting Eqs. (10) and (11) into Eq. (12) and solving for $\Delta\varphi$ one obtains

$$\Delta\varphi = \frac{RT}{(z_{B^-} - z_{A^+})F} \ln \frac{K_{B^-}^0}{K_{A^+}^0} \quad (13)$$

Here again it can be seen that the difference between the K_i^0 of the individual ions which is a quantitative measure of their affinities for the phases together with the electroneutrality condition is responsible for the occurrence of $\Delta\varphi$. Two limiting cases of Eq. (13) shall be mentioned briefly. If the concentration of the salt added tends to zero there will still be a $\Delta\varphi$ present. This holds at least in principle, where one has to bear in mind that all contributions to $\Delta\varphi$ resulting from non-ionic interactions are not included in the Albertsson model. Secondly Eq. (13) shows that $\Delta\varphi$ will also be present if the K_i^0 tend to zero or to infinity as long as

they are different. Thus also in aqueous–organic two-phase systems the effects of $\Delta\varphi$ are to be expected.

4. Measurement of $\Delta\varphi$

Having derived Eq. (13) it would be desirable to measure $\Delta\varphi$. Unfortunately, Gibbs realized that $\Delta\varphi$ can not be measured with any currently used experimental techniques [10]. This was more clearly stated by Guggenheim [11]: “the electric potential difference between two points in different media can never be measured and has not yet been defined in terms of physical realities; it is therefore a conception which has no physical significance”. Since the two phases of two-phase systems are different media this statement directly applies to the measurement of $\Delta\varphi$. In the restless minds of researchers this statement apparently provoked contradiction. We set out to discuss what can be done about Guggenheim’s statement concerning the measurement of $\Delta\varphi$ and why we nevertheless believe that $\Delta\varphi$ is of physical significance.

Why can $\Delta\varphi$ not be measured? Several equivalent arguments can be given. From Eq. (11) or Eq. (13) together with Eq. (9) it becomes clear that determination of $\Delta\varphi$ can only be achieved if the activity coefficients of the individual ions in the phases and thus K_i^0 could be measured. Currently, only the activity coefficient for a salt can be determined which is averaged over anion and cation. Thus the information necessary to determine $\Delta\varphi$ along this route is not available. Also direct measurement of $\Delta\varphi$ with electrodes immersed into the two phases will not give unequivocal results. At each electrode new interfaces with the phases are created which themselves are associated with an electrode potential. Since the driving forces for these potentials are, among other, also the different activities of the ions, the difference between the electrode potentials in the different phases will not cancel and result in a contribution of the detected signal of the same order of magnitude as the $\Delta\varphi$ to be measured. Thus a possible error of 100% is introduced.

A third approach to determine $\Delta\varphi$ is to add test ions (index T) to the system and to modify the charge of the test ion partitioned in the two-phase

system. This can be achieved either by changing pH and choosing an amphoteric test ion, or by modifying the chemical structure of the test ion slightly. If it is then assumed that K_T^0 remains unchanged during this operation K_T^0 as well as $\Delta\varphi$ can be backed out from Eq. (7) written for the test ion:

$$\ln K_T = \ln K_T^0 - \frac{z_T F}{RT} \Delta\varphi \quad (14)$$

Here the constancy of K_T^0 is questionable since the hydration shell around the ion changes if the charge is varied and thus also K_T^0 is modified. Unfortunately this effect can hardly be neglected because it appears that partitioning especially in aqueous two-phase systems is very sensitive to slightest changes in the structure of the molecules to be partitioned. For example, changing the sequence of amino acids in small peptides will result in different partitioning. While this high resolving power is the advantage of aqueous two-phase systems it impairs the determination of $\Delta\varphi$.

The question arises if anything quantitative can be stated about $\Delta\varphi$ or if it is indeed a conception without any physical significance. It turns out that it is possible to gain some further insight, if two two-phase systems are compared with a different salt added to each of them as depicted in Fig. 3 [2,3]. If a test ion is added to both systems, its partition coefficient will, in general, be different for both systems. By solving Eq. (14), an expression for the $\Delta\varphi$ in these systems is obtained:

$$\Delta\varphi = -\frac{RT}{z_T F} (\ln K_T - \ln K_T^0) \quad (15)$$

If now the difference between the $\Delta\varphi$ in the two systems is taken, the following equation results:

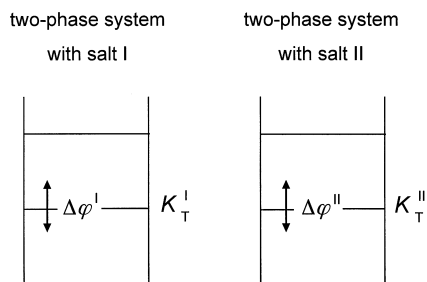


Fig. 3. Determination of $\Delta(\Delta\varphi)$ between two two-phase systems.

$$\begin{aligned}\Delta(\Delta\varphi) &= \Delta\varphi' - \Delta\varphi'' \\ &= -\frac{RT}{z_T F} [(\ln K_T - \ln K_T^0)' - (\ln K_T - \ln K_T^0)''] \end{aligned} \quad (16)$$

By choosing very low salt concentrations the difference in K_T^0 between the two systems vanishes since under these conditions the thermodynamic properties of the systems approach each other [2]. The only remaining difference between the systems is that in $\Delta\varphi$, which according to the Albertsson model will be present up to infinite dilution of the salt. Fortunately, exactly the interaction between the charge of the test ion with $\Delta\varphi$ is not included in K_T^0 as explained above. Thus under these conditions Eq. (16) can be simplified:

$$\Delta(\Delta\varphi) = -\frac{RT}{z_T F} (\ln K_T' - \ln K_T'') \quad (17)$$

This equation can be regarded as a recipe for the determination of $\Delta(\Delta\varphi)$: prepare two identical two-phase systems, add different salts and a test ion with known z_i , determine the partition coefficients of the test ion in the two systems and calculate $\Delta(\Delta\varphi)$ from Eq. (17). The concentration of the salts should be varied in order to check if it is small enough and the K_T^0 cancel and the concentration of the test ion should be lower than that of the salt added by at least a factor of 10 [2].

For our measurements, we have chosen charged dyes as test ions since their concentration can easily be determined by spectroscopy [2]. The results with alizarine yellow for four salts are shown in Fig. 4. The partition coefficients of alizarine yellow for different salts added to the systems are shown as function of salt concentration. The points are the experimental partition coefficients. The behaviour of the curves is unexpected at first sight. According to the simple Albertsson model for a single salt in the system, four horizontal lines at different levels would be expected below a certain salt concentration. Instead, the partition coefficients approach each other at low salt concentrations. This result can be explained by ionic impurities present in the system which dominate the behavior at low concentrations of the added salt. The salt has been determined to be mainly sodium lactate which stems from the pro-

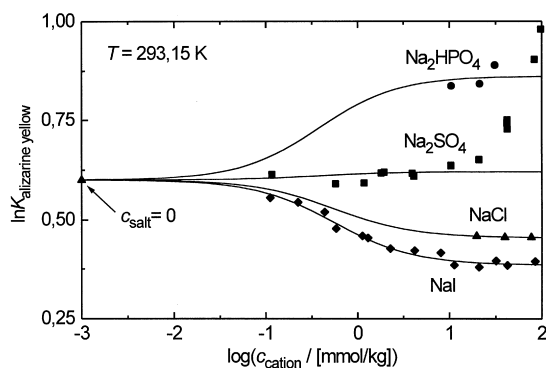


Fig. 4. Partition of a charged dye in a system water+PEG 3000+dextran 500.000 with different salts added. The experimental procedure is described in Ref. [2].

duction of poly(ethylene glycol) (PEG). According to ion chromatography, it is present in the two-phase systems at concentrations of approximately 0.5 mmol/kg, which agrees nicely with the inflection point of the curves for the different salts. This impurity effect could not be explained by taking into account other ionic species present in the system, e.g., the ionic dye, H^+ or OH^- from the autodissociation of water, or HCO_3^- from the CO_2 in the air, because their concentrations are significantly lower. The curves were calculated with an extended Albertsson model which takes into account the ionic impurity [2]. Above 5 mmol/kg, the expected plateau values with the different salts can be seen. At roughly 50 mmol/kg, the curves start to deviate indicating that the K_T^0 do not cancel any more. This upper limit appears to vary strongly between the salts. Thus in a range between approximately 5 mmol/kg and 50 mmol/kg the added salt determines the potential difference and Eq. (17) can be evaluated. This has been done for a variety of salts as shown in Fig. 5. Only the results calculated from the extended Albertsson model are shown. Fig. 5 shows that the differences in $\Delta\varphi$ to be expected in aqueous two-phase systems are of the order of 10 mV.

To verify that the $\Delta(\Delta\varphi)$ are a property of the systems and do not depend on the properties of the test ion, different test ions were used. The results for $\Delta(\Delta\varphi)$ between systems with Na_2SO_4 and $NaCl$ are compared in Table 1. The results show that by this method $\Delta(\Delta\varphi)$ can be determined independent of the test ion with an accuracy better than 0.5 mV.

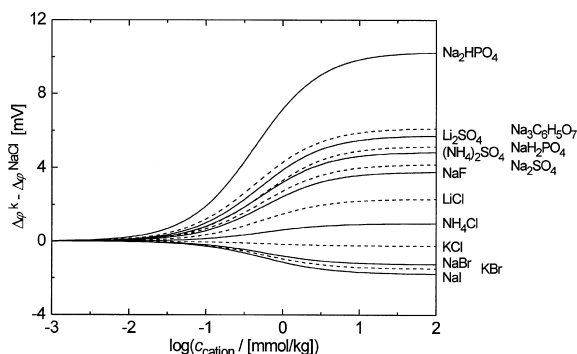


Fig. 5. $\Delta(\Delta\varphi)$ for various salts in the same system as in Fig. 4 where the reference system is chosen to be that with NaCl added. The curves are calculated with the extended Albertsson model [2].

Table 1
Comparison of $\Delta(\Delta\varphi)$ determined with different test ions

Test substance	z_T	$\Delta\varphi^{\text{Na}_2\text{SO}_4} - \Delta\varphi^{\text{NaCl}}$ (mV)
Alizarine yellow	-1	4.2
Diamine light blue	-3.34	4.6
Na[B(C ₆ H ₄) ₄]	-1	4.1
Dipeptides	-1 to +1	4.8

The results for Na[B(C₆H₄)₄] and the dipeptides are taken from Ref. [3].

Since the $\Delta(\Delta\varphi)$ are also expected to occur at aqueous–organic two-phase systems as mentioned above, we used the same experimental technique for a system water + 1-butanol with the test ion aniline blue. In Fig. 6 the results of these measurements are compared with those presented above. The linear relationship may be fortuitous but nevertheless it can be seen that the $\Delta(\Delta\varphi)$ in the aqueous–organic two-

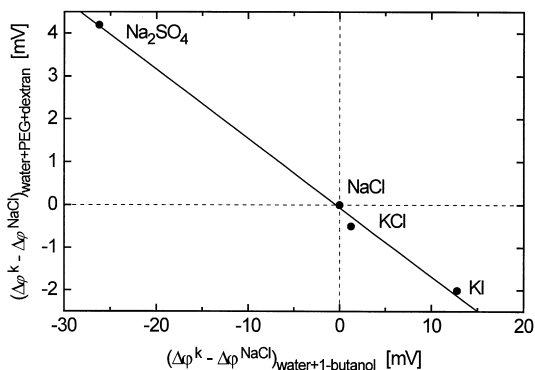


Fig. 6. Comparison of $\Delta(\Delta\varphi)$ in different two-phase systems.

phase system are almost by a factor of 10 larger than those in the aqueous two-phase systems. This can be understood since the difference between the phases, in e.g., the hydrophobicity, is much higher in aqueous–organic two-phase systems. Since these differences are the driving force for the partitioning of the species, all properties related to partitioning and thus also $\Delta(\Delta\varphi)$ will be more extreme in these systems.

5. Computer simulations of ions at liquid–liquid interfaces

The question remains how two-phase systems composed of electroneutral phases can display electrostatic potential differences. To investigate this, Monte-Carlo computer simulations have been performed for the behaviour of ions near an interface. The basic system investigated consists of two liquid phases where periodic boundary conditions are applied. The only interactions accounted for are the partition coefficient for the ions if no $\Delta\varphi$ is present, that is K_i^0 , and the interaction between the ions and the electrostatic potential. The effect of the solvents and other uncharged species in the phases are thus lumped into the value of K_i^0 . The electrostatic potential is obtained by evaluating the net charge density ρ as function of the distance x from the interface and performing the integration of the Poisson equation (Eq. (1)). φ results as a function of x where φ is set to zero in the center of one of the phases.

The results of one of these simulations for a low salt concentration are shown in Figs. 7–9. From Fig. 7 it becomes clear that indeed the bulk phases are electroneutral. The electrostatic effects originate from slight shifts of the ion concentrations in the close vicinity of the interface. These diffusive double layers at the interface lead to a nonzero net charge density near the interface as seen in Fig. 8. The resulting φ as function of the distance from the interface is compared with the results of the Albertsson model in Fig. 9. The excellent agreement between macroscopic model and molecular interpretation is obvious. The slight deviation is well within the numeric uncertainty of approximately 1 mV of the simulation. Fig. 9 shows that in the bulk phases φ is independent of the position inside the phase. Since

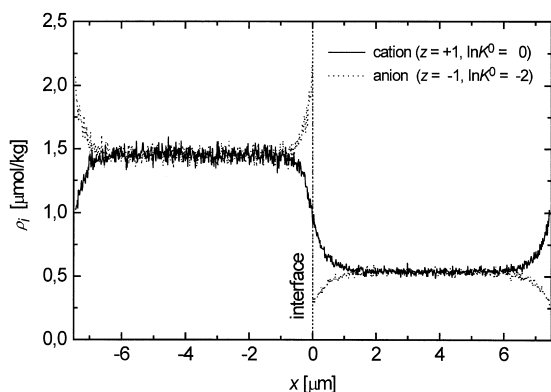


Fig. 7. Ion distribution between coexisting phases from Monte-Carlo simulation.

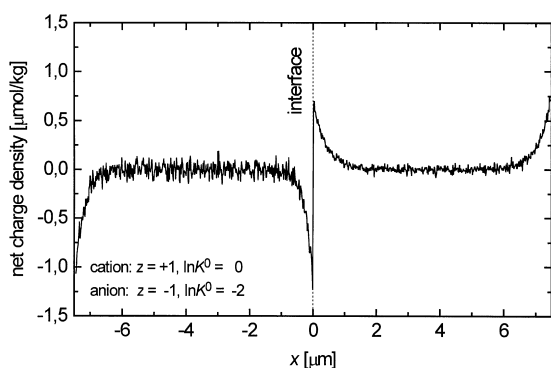


Fig. 8. Net charge density across the interface.

a bulk phase should be homogeneous with respect to all its properties this result is expected. Across the interface the electrostatic potential difference be-

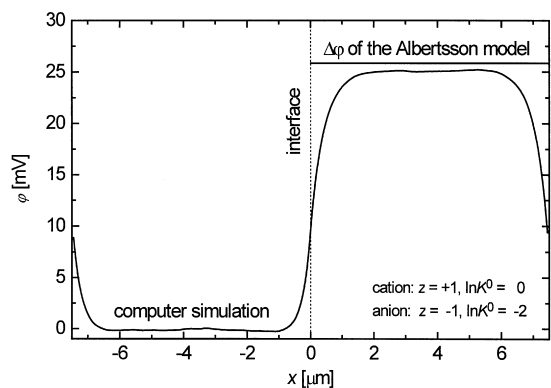


Fig. 9. Electrostatic potential from computer simulation compared with the Albertsson model.

tween the phases is built up. Thus the $\Delta\varphi$ of about 25 mV is a difference of Galvani potentials between the bulk phases. The simulations together with the Albertsson model show that the properties of the phases (K_i^0) influence the electrostatic potential difference between the phases which is built up in the close vicinity of the interface. Since all particles in the system decide at the interface with which probability they partition between the phases, this, in turn, determines their partition coefficient.

Because the ion concentration used for the simulation is extremely low, the resulting thickness of the electrostatic interface is of the order of 1 μm . As the salt concentration is increased, the extension of the inhomogeneous region in the vicinity of the mathematical interface will shrink to a few nm and less at salt concentrations of some 100 mmol/kg usually encountered in partition experiments.

Fig. 9 shows also that, contrary to the statement by Guggenheim cited above, $\Delta\varphi$ appears to be of physical significance. It should be stated though that in the computer simulations $\Delta\varphi$ can be calculated unambiguously because the K_i^0 if the ions are set to specific values whereas they can not be determined individually by experiment.

6. Conclusions

So far it has been shown that macroscopic models and molecular interpretation result in a consistent view of the behaviour of electrolytes at liquid–liquid interfaces. The results may be summarized as follows:

1. Bulk phases are strictly electroneutral; nevertheless a potential difference $\Delta\varphi$ exists between the coexisting phases.
2. Currently this $\Delta\varphi$ can not be determined experimentally.
3. $\Delta(\Delta\varphi)$ can be determined from measurements in systems with different salts added and with test ions.
4. K_i^0 of the Albertsson model is independent of concentration up to salt concentrations of about 50 mmol/kg to 100 mmol/kg, where this upper limit strongly depends on the salt chosen.

5. Electrostatic effects are present at every interface.

Which conclusions can be drawn from this for the investigation of partitioning of charged species in aqueous two-phase systems and the interpretation of the results of such experiments?

To define the electrostatic potential difference between the phases a salt should be present in the system at ion concentrations higher than those of any other ionic species by at least a factor of 10. Here all ionic species are to be taken into account including pH-buffer ions and proteins, as well as their counterions. The salt defining $\Delta\varphi$ should be chosen such that the state of dissociation is well defined in the system.

If the salt effect is to be investigated, a system with a specified salt can be taken as reference system. For the reference system $\Delta\varphi$ can be set to zero. Systems with other salts added instead can then be modelled relative to the reference system. Thus quantitative statements concerning the effect of salts on the partitioning of charged species are possible even though $\Delta\varphi$ can not be determined for any individual system. If the results are to be interpreted with the Albertsson model, the concentrations of all ionic species should be kept below approximately 50 mmol/kg to 100 mmol/kg depending on the salt.

Acknowledgements

We thank the Deutsche Forschungsgemeinschaft for financial support and Dr. D. Woermann for helpful discussions.

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