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ESTABLISHMENT AND DETERMINATION OF INTERFACIAL POTENTIALS AND STATIONARY PHASE DIELECTRIC CONSTANT IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

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SUMMARY

Salts added to the mobile phase in reversed-phase chromatography are used to establish an electrostatic potential difference between mobile phase and stationary phase. This electrostatic potential may be easily measured using k' values for three solutes that are assumed to be identical except for charge, namely tetraphenylsilane, tetraphenylarsonium cation and tetraphenylborate anion. The same results can be used to measure the stationary phase dielectric constant.

Results show a stationary phase dielectric constant of 22. The data strongly suggest a minor influence of ion specific effects, *e.g.* ion exchange and ion pairing. The major effect of the ionic hetaerons employed is the electrostatic potential difference between the mobile phase and the stationary phase. These potentials are more negative than the very approximate theory suggests, reflecting the influence of stationary phase anionic sites.

INTRODUCTION

All chromatographic separations are based on the equilibria that control solute distribution between phases. For charged solutes a major contributing factor to such equilibria is the electrostatic potential difference between two phases. It is well understood that electrostatic phenomena play a large role in the practice of modern reversed-phase liquid chromatography (RPLC). Horváth *et al.*[1] put forth a theory that accounted for the effects of a charge on a solute, based on earlier work[2] in the hydrophobic theory for RPLC. Later work, especially with so-called ion-pairing systems, brought forth a number of theoretical treatments based on equilibria. Much of this work was reviewed by Tomlinson *et al.*[3] and some key insights on the ion-pair problem were provided by Bidlingmeyer *et al.*[4] and by Knox and Hartwick[5]. More recently Stranahan and Deming[6] have developed a four-parameter equation to describe the retention of solutes in the presence of hetaerons in RPLC, one of which is an electrostatic term. The consideration of the electrical double layer formed at an interface on which charged species are adsorbed has led Cantwell and co-workers[7,8] to describe electrostatic effects on unmodified and modified polystyrene–divi-

nylbenzene gel particles. In this work the effective surface electrostatic potential in the system was measured *ex-situ* by using microelectrophoresis and also by measurement of adsorption isotherms of charged species followed by treating the data using the double-layer concept.

We have recently been exploring various electrostatic effects in RPLC[9,10]. An important property, the interphase potential, would be useful in understanding these effects if it were known. The consideration of potential differences between phases is by no means new. Indeed work in the fields of ion exchange[11], membranes[12] and solvent extraction[13] is relevant. The work of Alexandrowicz and Katchalsky[14] on rod-like ionic polymers has shown that ion exchange sites can be treated electrostatically as surface charges. This theoretical work has been successfully used to understand the experiments of Marinsky[11]. Many workers have used the concepts of interphase potentials in solvent extraction[13] and membrane electrochemistry[12]. There may be specific adsorption at the interface leading to an inner Helmholtz plane[1,7,8,12] and there may also be dipole orientation at the interface creating a surface potential. The ionic distribution given by the Poisson-Boltzmann equation is valid for low concentrations of ions and this has been used to derive potential distance relationships[12].

We are certain that such possibilities exist in the superficially similar RPLC system. It is therefore of some importance to be able to measure the interphase, or interfacial, potential under a variety of circumstances to more fully understand electrostatic effects in chromatography. Accordingly we have developed a method to do this, and introduce it here. Furthermore, using this method, we have measured the electrostatic potential difference between mobile phase and stationary phase with various salts in the mobile phase. This allows conclusions to be drawn regarding the establishment of the observed potentials.

THEORY

The method suggested is based on work in the field of solvation. Consider that the measurement of the distribution of a neutral molecule between two phases may be carried out, and the results may be interpreted on thermodynamic grounds. However the measurement of the same quantity for a charged species is not such a simple matter since the transfer of a cation from one phase to another must be accompanied by the transfer of an anion, and the total free energy for the salt is what one measures. One must have at hand at least one measurement of the transfer free energy of a single ion in order to calculate the single ion free energies of transfer for other single ions from data acquired on salts. This is not possible within the framework of thermodynamics, so an extra-thermodynamic assumption is required. There have been many assumptions put forward, and their merits have been hotly debated. The currently favored extra-thermodynamic assumption is called the tetraphenylarsonium-tetraphenylborate assumption. Here it is assumed that these two molecules are different only in their charge, that there are no chemical (*i.e.* solvation) or size differences between them[15,16]. This assumption allows one to measure the free energy of transfer of the salt Ph_4AsPh_4B (Ph = phenyl) and then simply divide the energy by two to yield the (equal) energies of each. Then measurement of the distribution of the salt Ph_4AsCl yields the value for Cl^-, and so on. We accept the assumption of

the essential equality of the two ions on which the assumption is based.

One can use this reasoning chromatographically. One prepares a RPLC system for which the interphase potential is desired, for example, by allowing the stationary phase to come to equilibrium with a salt-containing mobile phase. One then measures the k' for three solutes, *viz.*, Ph_4Si, Ph_4B^-, Ph_4As^+. The charged solutes are injected as salts into a mobile phase containing an excess of another salt. The latter salt, called the buffer salt, can create an electrostatic potential between the mobile and stationary phases. By breaking down the energetic contributions to the distribution process one can see how the assumption of the similarity of the tetraphenyl compounds under consideration allows the determination of some fundamental electrostatic properties of the buffer-salt-containing chromatographic system.

The free energy of transfer of each of the solute ions is made up of three terms:
(1) A solvation term, ΔG_s
(2) A sign independent, charge dependent (Born) term, ΔG_b
(3) A sign dependent, charge dependent (potential) term, ΔG_p

The solvation energy is the free energy of transfer of the hypothetical molecules Ph_4B° and Ph_4As°. We assume that this energy is adequately given by the free energy of transfer of Ph_4Si. The Born term is the result of the charge moving between media of different dielectric constants. While it is not necessary to invoke the approximate theory put forward by Born, we do assume that a molecule's charge can influence its solvation energy in a manner that is independent of the sign of the charge[1,15,16]. The last term is due to electrostatic potential differences between phases, so that

$$\Delta G_p = -zF\psi \tag{1}$$

where the differences are taken as right minus left for the free energy and left minus right for the potential, ψ.

For the three solutes one has, where φ is the phase ratio, R is the gas constant and T is the temperature

$$Ph_4Si: \quad \ln k' = \ln \varphi - \Delta G_s/RT \tag{2}$$

$$Ph_4B^-: \quad \ln k' = \ln \varphi - \Delta G_s/RT - \Delta G_b/RT + F\psi \tag{3}$$

$$Ph_4As^+: \quad \ln k' = \ln \varphi - \Delta G_s/RT - \Delta G_b/RT - F\psi \tag{4}$$

Therefore using α values referred to the k' of Ph_4Si one has

$$Ph_4B^-: \quad \ln \alpha_B = -\Delta G_b/RT + F\psi \tag{5}$$

$$Ph_4As^+: \quad \ln \alpha_{As} = -\Delta G_b/RT - F\psi \tag{6}$$

And finally by suitable combination one has

$$1/2 \ln \alpha_{As}\alpha_B = -\Delta G_b/RT \tag{7}$$

$$1/2 \ln \alpha_B/\ln \alpha_{As} = F\psi \tag{8}$$

Here we report preliminary results based on this method of measuring electrostatic effects.

EXPERIMENTAL

NaPh$_4$B (Gold Label, Aldrich), Ph$_4$AsCl (Aldrich), and Ph$_4$Si (Petrarch), KCl, Bu$_4$NCl (Bu = butyl) were used without further purification. KClO$_4$ and Bu$_4$NClO$_4$ were prepared by adding HClO$_4$ to KOH and Bu$_4$NCl respectively, and washing the resulting precipitate with about 70 ml of cold glass-distilled and deionized water ten times. Test solutes were initially prepared with the predominant counter ion in the mobile phase. Bu$_4$NPh$_4$B, KPh$_4$B and Ph$_4$AsClO$_4$ were prepared by mixing equal volumes of equimolar aqueous solutions of Bu$_4$NCl and NaPh$_4$B for the first; KCl and NaPh$_4$B for the second and Ph$_4$AsCl and HClO$_4$ for the third, and washing as above. All salts were dried at room temperature *in vacuo* until no water was trapped in an isopropanol–dry ice cooled trap.

Mobile phases were prepared as follows. Acetonitrile (MCB "omnisolv") and glass distilled and deionized water were separately filtered (0.45 μm). Exactly 2 l of each were mixed. The appropriate weight of dried salt (KCl, KClO$_4$, Bu$_4$NCl or Bu$_4$NClO$_4$) to make the solution 0.010 M was dissolved in a sufficiently large volume of acetonitrile–water (50:50, v/v) so that all injections could be made with the same solvent. The mobile phase was deaerated continuously with helium that had been saturated with acetonitrile–water (50:50).

Injections were made with the probe solute below 5% of the buffer salt concentration for late eluting compounds and below 1% of the buffer salt concentration for early eluting solutes. At these concentrations, increasing the solute concentration by a factor of two had no significant effect on k'. Initially care was taken to inject the test solute ion as a salt with the counterion in the mobile phase. Later comparison experiments showed this to be an unnecessary precaution, undoubtedly because in all the systems studied the anion and cation of the injected salt were quickly separated. In systems in which the retentions of anion and cation of the injected salt are similar, an effect due to the counterion may be expected.

The dead volume of the system was determined using fructose as a solute.

Experiments in Trial 1 were done at ambient temperature (22 ± 2°C) and those of Trial 2 were done at 25°C. A possible source of error is that the temperature inside the column may at times have been higher than the temperature outside the column. This is because high (*e.g.* 6 ml/min) flow-rates were sometimes used for some solutes because of large k' values. For example the k' for Ph$_4$Si is about 55 in our experiments.

In Trial 1 one hetaeron was rinsed out of the system with several hundred ml of acetonitrile–water (50:50) before the next hetaeron was introduced. In Trial 2 300 ml of 0.1% HClO$_4$ in acetonitrile–water (50:50) were pumped through the column overnight, and this was rinsed out with 150 ml of acetonitrile–water (50:50). Then 150 ml of hetaeron-containing mobile phase was pumped through the system before injections were made. This was sufficient mobile phase as determined by measurements of breakthrough curves and by seeing reproducible k' values for ionic solutes. Each trial used a different column, but both were 10-μm ODS Spherisorb packed by HPLC Technology.

Conductance measurements were carried out in acetonitrile–water (50:50) using Job's method. The cell was thermostatted at 25°C, and the bridge used was a Beckman PC18A.

RESULTS AND DISCUSSION

There are two aspects to this work, namely the establishment of an electrostatic potential and the measurement of the Born term and the established potential. First the establishment of the potential will be discussed. Following this the measurement of the electrostatic effects will be discussed.

Establishment of conditions

The development of an electrostatic potential between two phases occurs when ions in the system have different free energies of transfer between the phases[13]. For bulk systems eqn. 9 holds

$$\psi = -\Delta G_t/2F \tag{9}$$

where ΔG_t is the difference (anion minus cation) of free energies of transfer (phase two to phase one) of monovalent ions and ψ is the potential difference (phase two minus phase one) between phases. The values for single ion free energies of transfer from water to acetonitrile for the buffer ions used (Table I) are recommended values taken from a recent critical review[17]. Also shown are these energies divided by 2.5. This division is a crude attempt at allowing for the fact that in our chromatographic system the ions are going from a medium of 50% acetonitrile to one of perhaps 90% acetonitrile in the stationary phase[18] which is a change in acetonitrile concentration of 40%, and the data in ref. 17 are for a change of 100%. The ions in Table I were chosen because of the large range of ΔG_t shown by them. The salts of these ions should, therefore, show a range of interphase potentials when used in the mobile phase. Of course the potentials calculated for a given salt in a two phase system consisting of acetonitrile–water (50:50) and acetonitrile–water (90:10) only approximate the potential expected for the chromatographic system described for at least two reasons. One is the fact that the chromatographic system consists of regions of the two phases that are of the same dimensions as the double layer and the calculated potentials are for bulk systems. The magnitude of the bulk potentials will be larger[13]. The second reason is the stationary phase is certainly not simply a mixture of acetonitrile–water (90:10).

Measurements

Data for all the buffer salts using two different Spherisorb columns were obtained under similar, but not identical conditions, and are shown in Table II and

TABLE I

FREE ENERGIES OF TRANSFER

Ion	ΔG_t^*	$\Delta G_t/2.5$
Cl^-	42.1	16.8
ClO_4^-	2	0.8
K^+	8.1	3.2
Bu_4N^+	−31	−12.4

* Free energy of transfer from water to acetonitrile, kJ/mol at 25°C.

TABLE II

ALPHA VALUES AND THE BORN TERM

Salt	Trial 1			Trial 2		
	a_B	a_{As}	$-0.5ln\ \alpha_B\alpha_{As}$	a_B	a_{As}	$-0.5ln\ \alpha_B\alpha_{As}$
KCl	0.629	0.056	1.67	0.044	0.744	1.71
KClO$_4$	0.038	0.630	1.88	0.024	1.13	1.81
Bu$_4$NCl	0.162	0.136	1.91	0.975	0.037	1.66
Bu$_4$NClO$_4$	0.705	0.038	1.81	0.649	0.061	1.62
Mean			1.82			1.70

* Referred to k' of Ph$_4$Si.

Table III. The set of data called Trial 1 in the Tables is primarily shown as an indication of the importance of environmental influences on certain of the data acquired. Several points should be mentioned in this regard. According to Verzele and Dewaele[19] the Spherisorb material is not particularly well deactivated, and is prone to adsorbing metal ions. Both of the columns had been used before these studies began, and we have observed dramatic chromatographic consequences on these columns following an injection of Fe^{3+}, thus trace metals and the history of the column will play a role in determining the retention of sensitive solutes. The Trial 1 data in Tables II and III are taken from a set that showed changes in retention parameters with time. Because of this irreproducibility in Trial 1, in Trial 2 the column was washed with 0.1% perchloric acid before introducing a new hetaeron to the mobile phase in order to remove trace metals, and also to remove tightly bound hetaeron from the previous experiment. Thus, although both sets of data are valid, it is likely that the second set is more truly representative of the chromatographic system in the absence of large amounts of impurities.

According to eqn. 7 the natural log of the product of the α values for Ph$_4$As$^+$ and Ph$_4$B$^-$ yields the energy required to move a charged species of the radius of the solute [5.0 Å (ref. 20)] from the bulk, with an assumed dielectric constant of 60, to the stationary phase. Since the type of salt in the mobile phase should have a minor influence on this, this energy should not vary greatly with the salt. Each of the two sets of data can be seen to yield values in agreement with one another. The free energy is 4.3−4.4 kJ/mol for the transfer of a positive or negative species to the

TABLE III

MEASUREMENT OF POTENTIAL

Salt	Trial 1			Trial 2		
	$0.5ln\ \alpha_B/\alpha_{As}$	$\psi_{exp.}\ (V)$	$\psi_{calc.}\ (V)$	$0.5ln\ \alpha_B/\alpha_{As}$	$\psi_{exp.}\ (V)$	$\psi_{calc.}\ (V)$
KCl	1.21	0.031	0.069	−1.42	−0.036	0.069
KClO$_4$	−1.40	−0.036	−0.012	−1.94	−0.050	−0.012
Bu$_4$NCl	0.09	0.002	0.149	1.64	0.042	0.149
Bu$_4$NClO$_4$	1.46	0.037	0.067	1.19	0.030	0.067

stationary phase from the mobile phase. The insensitivity of this property of the column to the buffer ions and impurities is noteworthy. One can calculate a stationary phase dielectric constant of 22 using eqn. 15e from Horváth *et al.*[1] and the following data: bulk dielectric constant = 60; lambda (ratio of solute–octadecyl complex molar volume to solute molar volume) = 2. This is consistent with Horváth *et al.* who found a value of 35 using a variety of ionizable substituted benzenes in a 100% aqueous mobile phase.

A possible drawback of the current treatment is the simple idea that all the effects of ionic hetaerons are embodied in the potential created at the interface by those ions. Certainly there is the possibility of an ion-exchange contribution and of an ion-pairing contribution[7–10]. Indeed, conductance measurements show significant ion-pair formation constants for two of the ion combinations resulting from the buffer salts and the solutes chosen. The formation constant for Ph_4AsClO_4 in acetonitrile–water (50:50) is 16 M^{-1} and that for Bu_4NPh_4B is $3 \cdot 10^3$ M^{-1}. Such contributions to the overall chromatographic process do not fit into the preconceptions of the method espoused here. This treatment assumes that the effect of a change in the chromatographic system on Ph_4B^- retention is exactly the opposite of the effect on Ph_4As^+ retention. In a process like ion pairing and partitioning of the ion pair this symmetry of the effect is not necessarily the rule since an ion of one charge may be affected while an ion of opposite charge remains unaffected in such a process. Due to the "unsymmetrical" (with respect to charge) nature of these processes, their influence would be seen as an increase in ln $\alpha_B\alpha_{As}$ (a smaller value of $-\ln \alpha_B\alpha_{As}$ in Table II) over that expected for Born effects alone, since an increase in the retention of one ion is not compensated by a decrease in the retention of an ion of opposite charge. That such specific effects are not seen here can be appreciated by noting the remarkable homogeneity of the values of ln $\alpha_B\alpha_{As}$ over the various experiments. One may argue that the values of $-\ln \alpha_B\alpha_{As}$ for the Bu_4N^+ salts in Trial 2 (Table II) are smaller than those for the K^+ salts as one would expect for ion pairing of Bu_4N^+ with Ph_4B^-, however the difference may not be statistically significant. At least one can say that any such effect is causing less than 0.3 RT of a shift in the adsorption energy of the solute. In part this is in agreement with the work of Afrashtehfar and Cantwell[8] who found that the predominant influence of a charged hydrophobic hetaeron on polystyrene–divinylbenzene was not its ability to ion pair, but rather was the electrostatic potential developed at the interface. Although somewhat surprising, it seems to be the case that the data may be interpreted in the simple fashion outlined above.

Knowing that the influence of ion specific processes is minor allows the straightforward interpretation of the value of ln a_B/a_{As} in terms of the interphase potential (eqn. 8). The values of ln a_B/a_{As} and the experimentally determined potential values are shown in Table III along with calculated bulk potentials (eqn. 9). The calculated potentials contain a number of assumptions that obviate the discernment of small differences in the numbers, but nonetheless some information is obtained by their comparison to the experimental data, particularly Trial 2. Here it is seen that the rank order of the potentials created by the salts in bulk solution ($\psi_{calc.}$) and in RPLC ($\psi_{exp.}$) is similar in both cases. From this one may infer that the solvation by acetonitrile plays a role in the distribution of ions. This indicates that it may be possible to fine tune buffer solutions by adding or replacing ions in the buffers using

tables of single ion free energies of transfer as a guide.

The most noticeable feature of the comparison is the negative shift in the experimental data as compared to the calculated data. This shift is most pronounced for the less hydrophobic K^+ salts than for the Bu_4N^+ salts. This shift may be estimated quantitatively. The calculated potentials for the system KCl and Bu_4NClO_4, when added, equal the sum of the potentials for the salts $KClO_4$ and Bu_4NCl. The same is roughly true for the experimental values, except the sum is more negative by about 0.14 V (0.137 calculated *vs.* -0.007 experimental). This shift must represent the influence of the anionic sites on the column or differences in the solvation energy of ions in the stationary phase from the assumed 90% acetonitrile, or both. Since we have proof of anionic sites on these columns[9] we can attribute part of the negative shift to these.

These observations are consistent with the expectations one has for reversed-phase chromatography with ions in the mobile phase[4–6]. The most in depth study of similar phenomena is that of Afreshtehfar and Cantwell[8], in which an XAD resin and an anion exchanging QXAD were studied. Our data show a negative stationary phase potential for K^+ containing salts and a positive stationary phase potential for Bu_4N^+ salts. Analogously, Afrashtehfar and Cantwell found that the retention of *p*-nitrobenzenesulfonate was greatly decreased when the buffer salt NaCl was replaced by $NaClO_4$. This was attributed to specific adsorption of ClO_4^-, whereas we attribute the effects of K^+ and Bu_4N^+ on transfer free energy differences. Nonetheless the effects are similar in that the dominant electrostatic property of the stationary phase, the ion-exchange sites, can be modulated by the presence of ions that have an affinity for the hydrophobic portion of the stationary phase.

Another remarkable feature in the data is the similarity of the calculated potentials for the salts KCl and Bu_4NClO_4, and the dissimilarity of the experimental potentials. The calculated potentials are similar because in the salt KCl, K^+ has only a small ΔG_t while Cl^- has a large and positive one, while in Bu_4NClO_4 it is ClO_4^- that has a negligible ΔG_t and Bu_4N^+ has a large negative one. In the chromatographic case, where the anionic sites create a negative potential, it is apparently not possible to effectively counteract these sites with a negative surface excess of anions (the KCl case), rather a positive surface excess of cations is required.

It should be emphasized that these results are preliminary, and the approach is a simple one, but not without merits. Work underway includes the measurement of these electrostatic effects as a function of the concentration of the hetaeron, improvements in the reproducibility of the measurements and calculation of the electrostatic potential distribution in a chromatographic particle due to the differential solvation of the ions in the mobile phase so that our experimental results can be compared to a sound theoretical Poisson-Boltzmann treatment.

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