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NON-AQUEOUS LIQUID CHROMATOGRAPHY OF SOME METALLOPOR-PHYRINS ON A REVERSED-PHASE COLUMN

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SUMMARY

The effect of the hetaerons tetraphenylarsonium chloride (Ph_4AsCl) and tetra-phenylarsonium perchlorate (Ph_4AsClO_4) on the retention of neutral and cationic metalloporphyrins on a reversed-phase column with an acetonitrile–tetrahydrofuran (85:15, v/v) mobile phase was investigated. The adsorption properties of the two hetaerons on the stationary phase were dramatically different. While Ph_4AsCl adsorbed, reaching saturation at a concentration of 4 mM, Ph_4AsClO_4 did not adsorb. The retention of neutral metalloporphyrins was unaffected, while cationic metallo-porphyrins demonstrated decreasing retention with an increase in the concentration of either hetaeron. The possible equilibria involved are discussed.

INTRODUCTION

Porphyrins and metalloporphyrins are important biological molecules. Heme, chlorophyll and vitamin B_{12} are examples of metal-containing porphyrins vital to life processes. Thin layers of silica gel or alumina[1,2], paper[3] and column chromatography have been useful in separations in the study of porphyrin chemistry. Different packings have been used in column chromatography including silica gel[4], alumina[5], calcium carbonate[6] and magnesium silicate–cellulose mixture[7]. Thin-layer and high--performance thin-layer chromatography (TLC and HPTLC) of metal tetraphenyl-porphyrin chelates have been investigated[8,9].

Much research has centered on the biologically important porphyrins such as heme and chlorophyll but little work has been done in applying high-performance liquid chromatography (HPLC) to the separation of metalloporphyrins containing metals other than magnesium or iron. It would be an advantage to apply HPLC to the separation of metalloporphyrins owing to the speed and good resolution obtainable with this technique. Many metalloporphyrins are relatively insoluble in polar solvents such as methanol or water so that conventional reversed-phase liquid chromatography (RPLC) would not be a good choice. Non-aqueous liquid chromatography with a reversed-phase column has been applied to tetraphenylporphyrin chelates by Saitoh *et al.*[10]. Their separation of tetraphenylporphyrin chelates was accomplished using a mobile phase of acetonitrile–acetone (60:40). Good peak shapes

were obtained for all the neutral [*i.e.* metal(II)] porphyrins. The Fe(III)tetraphenyl-porphyrin chloride had a very low retention, and the Mn(III)tetraphenylporphyrin chloride was unique in having a very broad peak.

The non-ideal behavior of solutes in reversed-phase systems is of interest to us[11]. The results of Saitoh *et al.*[10] provide an unambiguous example of the effects of charge on a molecule. It is particularly useful that the metalloporphyrin solutes are not strong hydrogen bond acceptors or donors, since hydrogen bonding with silica can be a cause of non-ideality in reversed-phase systems. It is with this background in mind that we explored the chromatography of several metalloporphyrins in the presence of ionic hetaerons.

Previous work[11] has shown that the columns that we employed contain a significant number (10–20 μmoles) of anionic sites that act as cation-exchange sites. We anticipated that the influence of the positive charge on a metalloporphyrin would be chiefly felt through an ion-exchange process. Thus we chose hetaerons with a cationic counterion that would be hydrophobic enough to distribute to the stationary phase from a nonaqueous mobile phase. In particular we used tetraphenylarsonium chloride (Ph$_4$AsCl) and tetraphenylarsonium perchlorate (Ph$_4$AsClO$_4$). The Ph$_4$As$^+$ ion has the added advantage that it is detectable with good sensitivity, thus its distribution in the chromatographic system is easily determined.

EXPERIMENTAL

Apparatus

The chromatographic system consisted of an IBM Instruments, LC/9533 ternary gradient liquid chromatograph, a Gilson (Middleton, WI, U.S.A.) Model HM variable-wavelength UV detector with an 8-μl detector cell, and a thin-layer amperometric detector with a glassy carbon electrode following the UV detector. The Spherisorb 10 μm ODS column (25 cm × 5.0 mm) was obtained from HPLC Technology (Lomita, CA, U.S.A.).

Reagents

Acetonitrile was supplied by MCB Manufacturing Chemists, (Cincinnati, OH, U.S.A.), tetrahydrofuran was from Waters Assoc. (Milford, MA, U.S.A.), Ph$_4$AsCl was from Aldrich (Milwaukee, WI, U.S.A.), *meso*-tetraphenylporphine was from Alfa (Danvers, MA, U.S.A.). The metalloporphyrins, Co(II) octaethylporphyrin, Ni(II) octaethylporphyrin, Bi(III) octaethylporphyrin and Mn(III) etioporphyrin were obtained from Dr. S. A. Asher, University of Pittsburgh. Pb(II) tetraphenyl-porphyrin was prepared by the reaction of *meso*-tetraphenylporphine and lead(II) acetate in refluxing N,N-dimethylformamide and purified by extracting from a Soxhlet thimble with benzene. Ph$_4$AsClO$_4$ was prepared by dissolving Ph$_4$AsCl in water forming a saturated solution and then adding an excess of perchloric acid. The resulting precipitate was recrystallized from acetonitrile.

Chromatographic conditions

Chromatography of the metalloporphyrins was done in a mobile phase of acetonitrile–tetrahydrofuran (85:15, v/v) to which Ph$_4$AsCl, Ph$_4$AsClO$_4$ or no salt was added. The concentration range of study for both Ph$_4$AsCl and Ph$_4$AsClO$_4$ was

0.2–4.0 mM. The flow-rate was 2.0 ml/min. Injections of 20 μl of the metallopor-
phyrins dissolved in the mobile phase (*ca.* 1 mg/ml) were made. The solutes were
detected by absorbance at 420 nm.

Break-through curves
    The break-through of both the Ph_4As^+ and Cl^- ions were done simultaneous-
ly. Ph_4As^+ was detected by absorbance at 254 nm. Cl^- was detected electrochemi-
cally at a potential of +1.86 V *vs.* silver–silver chloride, 3 M sodium chloride ref-
erence. Cl^- break-through was confirmed by visual observation of the formation of
a silver chloride precipitate. This is necessary since the electrochemical detector may
respond to conductance changes as well as to changes in chloride concentration. In
between each experiment the column was flushed with 0.01 M nitric acid.

RESULTS AND DISCUSSION

    Since the distribution of the ionic hetaerons is important in understanding the
consequences of their presence, we have measured break-through curves for the two
hetaerons. Following this, the effect of hetaeron concentration on the chromato-
graphic separation of some metalloporphyrins was determined.

Break-through curves
    In order to determine whether Ph_4As^+ was acting as an ion-exchange coun-
terion, break-through curves of Ph_4AsCl and Ph_4AsClO_4 were measured. By meas-
uring the break-through of both anion and cation one can determine the extent of
adsorption of Ph_4As^+ due to ion exchange. The ion-exchange sites were in the H^+
form prior to each experiment. When a solution of Ph_4AsCl is pumped through the
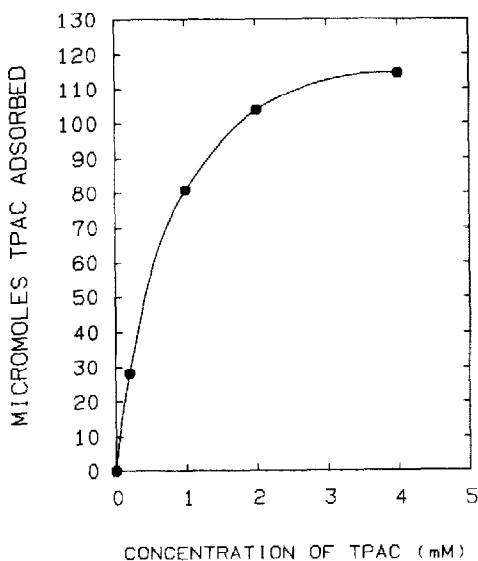

Fig. 1. Amount of Ph_4AsCl (TPAC) adsorbed (μmol) *vs.* concentration (mM) of Ph_4AsCl.

TABLE I

GIBBS FREE ENERGIES OF TRANSFER OF SINGLE IONS FROM WATER TO NON-AQUEOUS
SOLVENTS

Ion	ΔG_T (kJ/mol at 25°C), transfer from water to	
	Acetonitrile	Tetrahydrofuran
Cl^-	42.2	30
ClO_4^-	2.5	

column, Ph_4As^+ can exchange for H^+ on the ion-exchange sites as well as distribute
to the stationary phase as Ph_4AsCl. Thus the difference between the break-through
volumes for Ph_4As^+ and Cl^- is a measure of the extent to which Ph_4As^+ is acting
as an ion-exchange counterion. The break-through curve of Ph_4AsCl showed co-
elution of both Ph_4As^+ and Cl^-. Thus Ph_4As^+ does adsorb to the stationary phase
but not as a counterion in ion exchange for if it were, the elution of Ph_4As^+ would
occur after that of Cl^-. Fig. 1 shows that the quantity of Ph_4AsCl adsorbed increases
as the concentration of Ph_4AsCl is increased. The same data were replotted to con-
firm a Langmuir adsorption isotherm. For the Langmuir plot it was assumed that
at a concentration of 4 mM Ph_4AsCl, total coverage of the stationary phase occurs.

The break-through curve of Ph_4AsClO_4 was different from that of Ph_4AsCl,
for Ph_4AsClO_4 did not absorb. Thus the break-through curves of both Ph_4AsCl and
Ph_4AsClO_4 showed that Ph_4As^+ does not act as a counterion to stationary phase
ion-exchange sites. Since Ph_4AsClO_4 does not even adsorb on the stationary phase
the effect of the salt is not a stationary phase effect. The contrasting adsorption
behavior of Ph_4AsCl and Ph_4AsClO_4 is intriguing and one may explain this differ-
ence using the Gibbs free energies of transfer (ΔG_T) of Cl^- and ClO_4^-. The ΔG_T
values listed in Table I are recommended values taken from a recent critical review[12].
The ΔG_T values in Table I suggest that Cl^- strongly prefers an aqueous over a
non-aqueous environment while ClO_4^- exhibits virtually no preference. It is known
that even with maximized surface coverage and "capping" techniques approximately
50% of the surface hydroxyls remain unreacted[13,14]. Thus one may argue that traces
of water present in the mobile phase will adsorb to the silaceous support and therefore
drive Cl^- to the stationary phase.

One must conclude that the ion-exchange sites on the column remain in their
H^+ form during these experiments. If the striking difference in the adsorption be-
haviour of the chloride salt and the perchlorate salt is reflected in the chromatography
of the porphyrins, then it is likely that the hetaeron exerts its effect by stationary
phase modification (although apparently not through acting as an ion-exchange
counterion). On the other hand, if the hetaerons both have similar effects on the
chromatography, then one must conclude that any alteration of the stationary phase
by Ph_4AsCl plays a minor role.

Effect of the hetaerons on the metalloporphyrin chromatography

The free base porphyrin ligand has an overall charge of $2-$. Thus metal com-
plexes formed with bivalent metals are neutral and those formed with trivalent metals
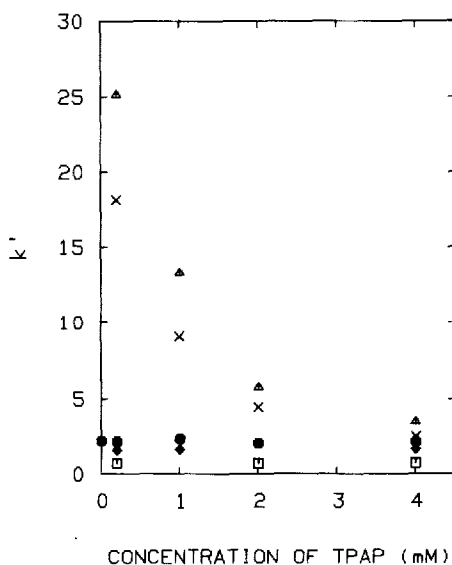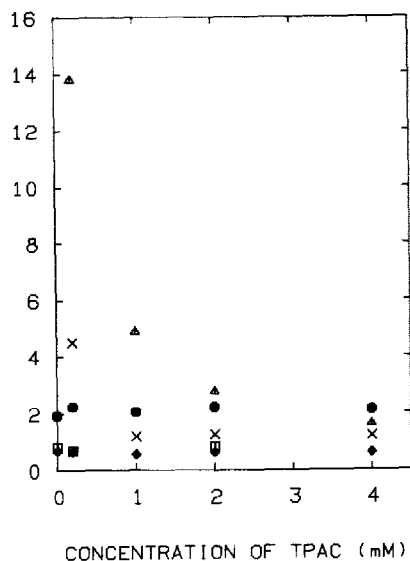are positively charged.

Fig. 2. Plot of *k'* *vs.* concentration (m*M*) of Ph$_4$AsCl (TPAC). $\triangle$ = Bi(III) octaethylporphyrin; $\times$ = Mn(III) etioporphyrin; $\bullet$ = Ni(II) octaethylporphyrin; $\blacklozenge$ = Pb(II) tetraphenylporphyrin; $\square$ = Co(II) octaethylporphyrin.

Fig. 3. Plot of *k'* *vs.* concentration (m*M*) of Ph$_4$AsClO$_4$ (TPAP). Symbols the same as Fig. 2.

In a mobile phase of acetonitrile–tetrahydrofuran (85:15) neutral metalloporphyrin complexes eluted whereas the positively charged metal porphyrin complexes did not elute. The addition of either Ph$_4$AsCl or Ph$_4$AsClO$_4$ to the mobile phase brought about elution of all the metalloporphyrins studied. Figs. 2 and 3 are plots of *k'* *vs.* concentration of Ph$_4$AsCl and Ph$_4$AsClO$_4$ respectively. As seen from the plots the retention of the neutral metal complexes is unaffected by the presence of either Ph$_4$AsCl or Ph$_4$AsClO$_4$ in the mobile phase. The *k'* values of Bi(III) octaethylporphyrin and Mn(III) etioporphyrin decrease as the concentration of either Ph$_4$AsCl or Ph$_4$AsClO$_4$ is increased. The *k'* values for both Mn(III) etioporphyrin and Bi(III) octaethylporphyrinn are greater with Ph$_4$AsClO$_4$ in the mobile phase than for Ph$_4$AsCl.
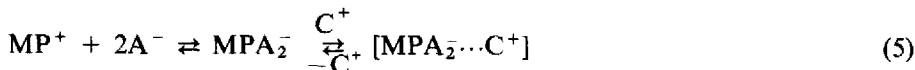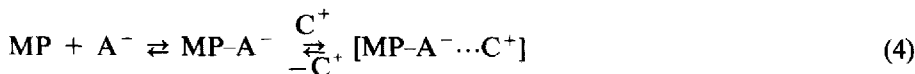
The results indicate that the effect of the hetaeron is not primarily a stationary phase effect. Thus, although our initial thinking was in terms of Ph$_4$As$^+$ as a counterion to anionic sites on the stationary phase, this reasoning is incorrect. Other equilibria must be considered. In particular, the most obvious effect of the hetaeron (Figs. 2 and 3) is that it makes charged porphyrins appear neutral. Such equilibria should be considered.

There are two classes of equilibria that may be involved. The first involves only the anionic component of the hetaeron. The anions Cl$^-$ or ClO$_4^-$ (A$^-$) may form axial ligands (MP–A) or ion pair ([MP$^+$···A$^-$]) with cationic metalloporphyrins (MP$^+$) and may form axial ligands with neutral metalloporphyrins (MP)

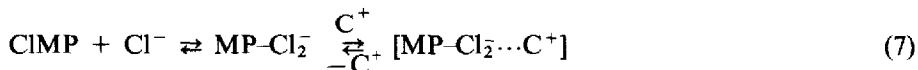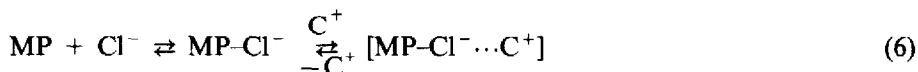$$MP^+ + A^- \rightleftharpoons MP\text{–}A \tag{1}$$

$$MP^+ + A^- \rightleftarrows [MP^+\cdots A^-] \qquad (2)$$

$$MP + A^- \rightleftarrows MP\text{-}A^- \qquad (3)$$

The second set of equilibria involves the cation Ph_4As^+ (C^+)

$$MP + A^- \rightleftarrows MP\text{-}A^- \underset{-C^+}{\overset{C^+}{\rightleftarrows}} [MP\text{-}A^-\cdots C^+] \qquad (4)$$

$$MP^+ + 2A^- \rightleftarrows MPA_2^- \underset{-C^+}{\overset{C^+}{\rightleftarrows}} [MPA_2^-\cdots C^+] \qquad (5)$$

Let us consider the latter equilibria first.

The Ph_4As^+ ion has been used for many years as an ion pairing reagent. Willard and Smith[15] were among the first to recognize the value of Ph_4AsCl as an analytical reagent when they found that mercury could be precipitated as $[Ph_4As]_2HgCl_4$ in the presence of Ph_4AsCl. The same authors also found that the perrhenate anion reacts with the Ph_4As^+ to form a white crystalline precipitate of Ph_4AsReO_4[16]. Dwyer et al.[17] studied a series of quaternary arsonium salts as precipitates for cobalt. Bane and Grimes[18] and Potratz and Rosen[19] also used Ph_4As^+ as an inorganic reagent in the determination of cobalt and bismuth. Uranium(VI) can be extracted by Ph_4AsCl in chloroform from benzoate solutions as the complex $[Ph_4As^+][UO_2(C_6H_5COO)_3]$[20]. The utility of Ph_4As^+ lies in its ability to form organic solvent soluble ion pairs with ionic oxides[16], halides[15], thiocyanates[17] or other compounds of many metals. Although the Ph_4As^+ ion has been used extensively as an ion pairing reagent in the extraction of various elements it is not commonly used as an ion pairing reagent in HPLC.

The unique feature of equilibria 4 and 5 is that the solutes must be made anionic and then the anion pairs with the Ph_4As^+ ion. Since ClO_4^- is a weak donor it probably does not form anionic complexes with neutral metalloporphyrins (reaction 4) nor would it form the bis complex with a cationic porphyrin (reaction 5). For perchlorate the equilibria represented by eqns. 4 and 5 are not primarily responsible for the observed chromatographic effect. The case for Cl^- is not so clear cut. The one piece of evidence that is the most convincing is that the neutrals are not affected by the hetaeron Ph_4AsCl. Reactions 6 and 7 are very similar.

$$MP + Cl^- \rightleftarrows MP\text{-}Cl^- \underset{-C^+}{\overset{C^+}{\rightleftarrows}} [MP\text{-}Cl^-\cdots C^+] \qquad (6)$$

$$ClMP + Cl^- \rightleftarrows MP\text{-}Cl_2^- \underset{-C^+}{\overset{C^+}{\rightleftarrows}} [MP\text{-}Cl_2^-\cdots C^+] \qquad (7)$$

Thus if a cationic metalloporphyrin had its retention altered by reaction 7 one would expect that a neutral metalloporphyrin would have its retention altered by reaction 6. Experimentally the neutral metalloporphyrins show no alteration in retention as a function of hetaeron concentration.
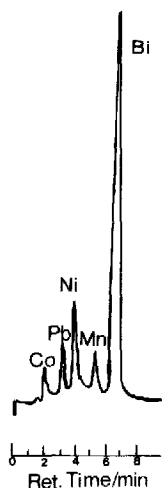
Fig. 4. Chromatogram of a mixture of metalloporphyrins with 4 mM Ph$_4$AsClO$_4$ in the mobile phase. Conditions as described in the experimental section.

The process most likely responsible for decreasing k' for the porphyrin complexes of Bi(III) and Mn(III) involves the interaction of the anion (Cl$^-$ or perchlorate) with the charged metal complex (reactions 1–3). The Cl$^-$ can form an axial ligand and bind to the metal in the porphyrin complex forming an uncharged complex or Cl$^-$ or ClO$_4^-$ could ion pair with the metal complex. For iron(III) porphyrin complexes the iron(III) porphyrin–counterion binding strengths increase in the order ClO$_4^-$ < Br$^-$ < Cl$^-$ < N$_3^-$ < F$^-$ (ref. 21). Also, Boucher and Garber[22] carried out the reduction of Mn(III) chloride porphyrin complexes in acetonitrile with a perchlorate salt as the supporting electrolyte. Thus Cl$^-$ is much better at forming an axial ligand bond with the metal than ClO$_4^-$. It is possible that the process for reducing k' for the charged metalloporphyrins is not the same for Cl$^-$ and ClO$_4^-$. The Cl$^-$ ion could reduce k' by forming an axial ligand while ClO$_4^-$ could produce the same effect by ion pairing with the metal complex. Alternatively both Cl$^-$ and ClO$_4^-$ could form ion pairs with the charged metal complex in the mobile phase. Since the process of decreasing k' for the charged metal complexes involves mainly the anion and not Ph$_4$As$^+$ other salts such as the tetraalkylammonium salts should also produce the same effect.

In a practical sense, the conditions explored herein allow rapid separation of a number of metalloporphyrins independent of the electrostatic charge on the complex. Figs. 2 and 3 indicate that many mobile phases will be effective. The perchlorate salt is preferred over the chloride because it is less corrosive. Fig. 4 is a chromatogram of a mixture of Co(II) octaethylporphyrin, Pb(II) tetraphenylporphyrin, Ni(II) octaethylporphyrin, Mn(III) etioporphyrin and Bi(III) octaethylporphyrin with 4 mM Ph$_4$AsClO$_4$ in the mobile phase. This rapid separation demonstrates the utility of this procedure in following syntheses or in analysis of mixtures containing metalloporphyrins, e.g. geological material. Work is continuing with a larger set of metalloporphyrins to allow us to understand the selectivity of the chromatographic system towards the metal and the porphyrin ring.

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