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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF BIS[1,2-BIS-(DIPHENYLPHOSPHINO)ETHANE]GOLD(I) CHLORIDE, A POTENTIAL ANTINEOPLASTIC AGENT

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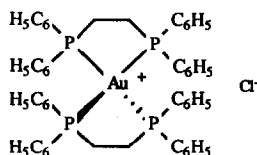
SUMMARY

The chromatography of $[\text{Au}(\text{dppe})_2]^+$ (I), a potential antineoplastic drug, was studied on a variety of stationary phases (ODS Hypersil, PLRP-S, Partisil SAX and Partisil SCX) using aqueous mobile phases containing 60% acetonitrile, 15% tetrahydrofuran and various electrolytes. The effects of both the concentration (0–20 mM) and the nature of the electrolytes, added to the mobile phase, on the chromatography of I were investigated. A wide variety of electrolytes were investigated in which the hydrophobicity of both the anion and the cation were varied. The analyte of interest was found to be unretained by the like-charged Partisil SAX column. On the other hand, I was retained on the Partisil SCX by an ion-exchange mechanism and retention could be controlled by manipulating the electrolyte composition of the mobile phase. I was retained on the two reversed-phase materials by a mixture of solvophobic and electrostatic interactions but, under the conditions studied, the latter mechanism was the dominant one. The retention of I on the two reversed-phase materials was influenced much more by the nature and concentration of the cation added to the mobile phase than it was by the nature and concentration of the anion. Therefore, manipulation of the nature and concentration of the cationic species in the mobile phase appears to afford the most useful means of manipulating the retention of I, and presumably its analogues, on reversed-phase columns.

INTRODUCTION

Bis[1,2-bis(diphenylphosphino)ethane]gold(I) ($[\text{Au}(\text{dppe})_2]^+$) is a cationic, organometallic, complex of gold(I). This complex is an experimental anticancer drug, having been synthesized as an analogue of auranofin which itself has only weak antineoplastic activity *in vitro* and *in vivo*¹. The complex has been formulated as an aqueous solution of the chloride salt ($[\text{Au}(\text{dppe})_2]\text{Cl}$, I, Fig. 1). The preformulation and prepharmacokinetic studies on I required the development of high-performance liquid chromatographic (HPLC) methods for the determination of the complex in aqueous formulations and biological fluids, respectively.

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[Au(dppe)₂]⁺Cl⁻, I

Fig. 1. The structure of bis[1,2-bis(diphenylphosphino)ethane]gold(I) ([Au(dppe)₂]⁺Cl⁻, I).

The reversed-phase HPLC of organometallic complexes, inorganic metal complexes and metal ions have been studied by a number of workers²⁻⁴ and the whole subject has been reviewed by Willeford and Veening². In general, ion-pair chromatography is the method of choice for the separation of metal ions or ionic metal complexes²⁻⁴. For example, Valenty and Behnken³ separated several derivatives of tris(2,2'-bipyridyl)ruthenium(II) on a C₁₈ bonded phase by the addition of alkylsulfonates to the mobile phase. Similarly, Buckingham *et al.*⁴ found that adding *p*-toluenesulfonate or hexanesulfonate to the mobile phase facilitated the separation of Co³⁺ complexed with various amino acids and ethylenediamine.

Despite being a positively charged species, initial attempts to elute I from silica-based or polymeric reversed-phase columns were unsuccessful. Consequently, a thorough investigation of the chromatographic properties of I was initiated so that the retention mechanism could be characterized and the factors which allow manipulation of the retention could be identified. In addition, the results obtained should be applicable to the assay development of pharmaceutically relevant analogues of I.

EXPERIMENTAL

Chemicals and reagents

Crystalline I was kindly provided by Smith Kline and French, Philadelphia, PA, U.S.A. HPLC-grade acetonitrile and Spectranalyzed tetrahydrofuran (THF) were obtained from Fisher Scientific. All the tetraalkylammonium salts were obtained from Fluka, Ronkonkoma, NY, U.S.A., with the exception of tetrabutylammonium hydrogensulfate (TBA · HS) which was obtained from Aldrich, Milwaukee, WI, U.S.A. The following tetraalkylammonium salts were used: tetramethylammonium hydrogensulfate (TMA · HS) and bromide (TMA · Br), tetraethylammonium hydrogensulfate (TEA · HS) and bromide (TEA · Br), tetrabutylammonium hydrogensulfate (TBA · HS), bromide (TBA · Br), nitrate (TBA · N.), methanesulfonate (TBA · Ms), hydrogenphosphate (TBA · Ph), perchlorate (TBA · Pe), *p*-toluenesulfonate (TBA · Ts) and tetraphenylborate (TBA · TPB), tetrapentylammonium bromide (TPA · Br), tetrahexylammonium bromide (THxA · Br), tetraheptylammonium bromide (THpA · Br) and tetraoctylammonium bromide (TOA · Br). The sodium ethanesulfonate (SES) was obtained from American Tokyo Kasei, Harborgate, OR, U.S.A. The sodium pentanesulfonate (SPS) and the sodium octanesulfonate (SOS) were obtained from Eastman Kodak, Rochester, NY, U.S.A. The other chemicals were at least reagent grade and were obtained from various sources.

Equipment

The chromatography was performed on a modular system consisting of an Altex 110A pump (Beckman Instruments, Chicago, IL, U.S.A.), an Altex 210 injector fitted with a 20- μ l loop and either a Beckman 153 (254 nm) or a Waters 450 (280 nm) detector (Waters Assoc., Milford, MA, U.S.A.). The chromatograms were recorded on a Spectra-Physics 4290 integrator (Spectra-Physics, San Francisco, CA, U.S.A.). The following HPLC columns were obtained from their respective manufacturers: PLRP-S (5 μ m, 15 cm \times 4.6 mm I.D., Polymer Labs., Amherst, MA, U.S.A.), Partisil SAX (10 μ m, 25 cm \times 4.6 mm I.D. Whatman, Nutley, NJ, U.S.A.), Partisil SCX (10 μ m, 25 cm \times 4.6 mm I.D.). ODS Hypersil (5 μ m, 15 cm \times 4.6 mm I.D., Keystone Scientific, State College, PA, U.S.A.) was packed in the upward direction at 48 MPa (7000 p.s.i.) using chloroform as the slurry solvent and methanol as the packing solvent.

Procedures

Measurements of the chromatographic parameters were made at least in duplicate by injecting (20 μ l) solutions of I dissolved in acetonitrile. The effects of adding various salts to a mobile phase of acetonitrile-THF-water (60:15:25) on the chromatography of I were investigated. After each experiment the column was flushed with 10–15 column volumes of water. The columns were equilibrated until constant retention times were achieved with fresh mobile phase prior to recording of the results. This was generally after 10–15 column volumes.

RESULTS AND DISCUSSION

In the absence of added electrolytes, I (Fig. 1) was completely retained by both a silica-based stationary phase (ODS Hypersil) and a macroporous copolymer of poly(styrene-divinylbenzene) (PLRP-S), using hydro-organic mobile phases containing either acetonitrile (0–100%) or THF (0–100%). The strong retention of I may be attributed, at least in part, to solvophobic interactions^{5,6} between the phenyl groups of the analyte and the hydrophobic regions of the stationary phases. However, the presence of a positive charge on I suggested that interactions with the negatively charged silanol groups on the silica backbone might also be involved in the retention process^{7–9}. This was supported by the results of preliminary experiments which showed that I could be displaced from both types of reversed-phase column by the addition of electrolytes, such as KBr or TBA \cdot Br, to the mobile phase.

Following these initial observations, a series of experiments were conducted to characterize the retention behavior of I on reversed-phase columns. For comparison and to characterize the chromatography further, the retention behavior of I on a strong cation (Partisil SCX) and a strong anion (Partisil SAX) exchanger was also studied. Throughout these experiments the organic modifier composition of the mobile phase was maintained constant (acetonitrile-THF-water; 60:15:25) and the effects of various electrolytes were investigated.

Initially, the retention behavior of I on ODS Hypersil was studied by investigating the effects of adding KBr, TBA \cdot Br and TBA \cdot HS (0–20 mM) to the mobile phase. It can be seen from Table I and Fig. 2, that in all cases the retention of I decreased with increasing electrolyte concentration. There was little difference in the effects of TBA \cdot Br and TBA \cdot HS on the retention of I; KBr however was much less

TABLE I

THE EFFECTS OF ADDING TBA · Br, TBA · HS AND KBr TO THE MOBILE PHASE ON THE RETENTION OF I ON ODS HYPERSIL

Temperature: ambient. Mobile phase: acetonitrile-THF-water (60:15:25) containing TBA · Br, TBA · HS or KBr.

[TBA · Br] (mM)	k'		[TBA · HS] (mM)	k'		[KBr] (mM)	k'	
	Obs.	Calc.*		Obs.	Calc.*		Obs.	Calc.*
5.0	10.7	10.6	1.0	35.0	37.0	5.0	38.0	33.0
7.5	7.44	7.88	5.0	8.50	8.77	10.0	22.0	21.8
10.0	6.47	6.38	10.0	5.10	5.14	20.0	13.0	13.2
12.5	5.47	5.45	20.0	3.26	3.14			
20.0	4.00	4.01						

* Calculated from eqn. 3 and the constants in Table II.

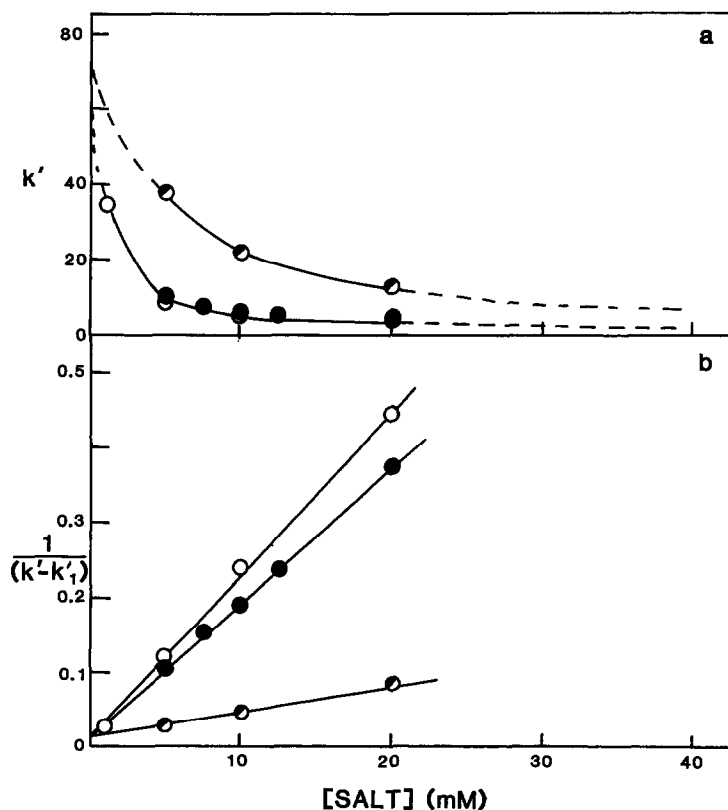


Fig. 2. The relationships between the capacity ratio (k') of I on ODS Hypersil and the concentrations of KBr (○), TBA · Br (●) and TBA · HS (○) added to the mobile phase. The data in the upper section (a) have been replotted in the lower section (b) according to eqn. 3. The points are experimental and the lines have been drawn using eqn. 3 and the constants given in Table II. Temperature: ambient. Mobile phase: acetonitrile-THF-water (60:15:25).

effective in displacing I from ODS Hypersil. This indicates that the nature of the cation plays a more important role than the nature of the anion in determining the retention of I on this type of column. The results presented so far are consistent with a mixed mechanism of solvophobic and silanophilic interactions as described by Horváth and coworkers⁷⁻⁹. The latter is presumed to arise from an electrostatic attraction between the residual silanols on the surface of the stationary phase and the cationic solute. Thus the effects of adding electrolytes may be explained in terms of competition between the cation added to the mobile phase and the analyte for the oppositely charged stationary phase. This mixed mechanism of retention has been described, phenomenologically⁷⁻⁹, by the following equation:

$$k' = k'_1 + k'_2/(1 + K_A/[A^+]) \quad (1)$$

where k'_1 and k'_2 are the incremental solvophobic and silanophilic retention factors⁷, respectively, and $[A^+]$ is the concentration of the competing cation added to the mobile phase. The reversible interaction of the competing cation, A^+ , with the stationary phase is given by⁷:

$$K_A = [\text{SiO} \cdot \text{A}]/([\text{SiO}^-] \cdot [\text{A}^+]) \quad (2)$$

To test the appropriateness of this model to the present study, the data (Table I, Fig. 2a) relating retention to the concentrations of competing ions were fit by least squares linear regression to eqn. 3, which is a linearized form of eqn. 1, to obtain the values of k'_1 , k'_2 and K_A .

$$1/(k' - k'_1) = 1/k'_2 + (K_A/k'_2) \cdot [A^+] \quad (3)$$

The results of this analysis are shown in Table II. In addition, the values of the constants (k'_1 , k'_2 and K_A) were used to recalculate the values of k' and it can be seen that agreement between the observed and the predicted values is excellent (Fig. 2 and Table I). By definition (eqn. 2), K_A is dependent on the natures of the stationary phase and the competing ion and is independent of the natures of the analyte and the

TABLE II

VALUES OF THE FORMATION CONSTANTS, K_A , FOR THE ASSOCIATION OF TBA IONS (AS THE BROMIDE AND HYDROGENSULFATE SALTS) AND POTASSIUM IONS WITH ODS HYPERSIL

The values of k'_1 , k'_2 and the recommended concentrations of the salts, C_A , to be added to the mobile phase, are also provided. Temperature: ambient. Stationary phase: ODS Hypersil. Mobile phase: see Table I. The parameters were taken from the data in Table I and Fig. 2, using eqn. 3.

Salt	$K_A \cdot 10^{-3}$ (M^{-1})	C_A^* (mM)	k'_1	k'_2
TBA · Br	1.08	9.3	1.37	59.2
TBA · HS	1.43	7.0	1.00	63.3
KBr	0.25	40.0	1.37	71.4

* C_A is defined as $10/K_A$.

counterion to the competing ion. This is confirmed by the values of K_A which were found to be $1.1 \cdot 10^3 M^{-1}$ and $1.4 \cdot 10^3 M^{-1}$ for TBA · Br and TBA · HS and only $2.5 \cdot 10^2 M^{-1}$ for KBr. Bij *et al.*⁷ have suggested that 90% of the silanophilic interactions are abolished if the concentration of competing ions in the mobile phase, C_A , is equal to $10/K_A$. Accordingly, Table II gives the recommended values for the concentrations (C_A) of KBr, TBA · Br and TBA · HS which would satisfy this condition. It is clear from Table II that the concentration of KBr ($\approx 40 mM$) required to abolish 90% of the silanophilic interactions is much greater than that for the two TBA salts ($\approx 10 mM$), reflecting the difference in the affinities of K^+ and TBA^+ for the stationary phase (Table II).

The results obtained with K^+ and TBA^+ salts (Table II) suggest that while the driving force for the interaction of competing ion and the silanol groups may be electrostatic, there is clearly a hydrophobic (or solvophobic) component to this interaction as described previously by Bij *et al.*⁷. To probe this phenomenon further, the effect of the hydrophobicity (total carbon number) of the tetraalkylammonium ions, added to the mobile phase, on the retention of I was investigated and the results are shown in Fig. 3. It was found that the retention of I decreased with increasing hydrophobicity of the competing ion and this could be described by the empirical relationship

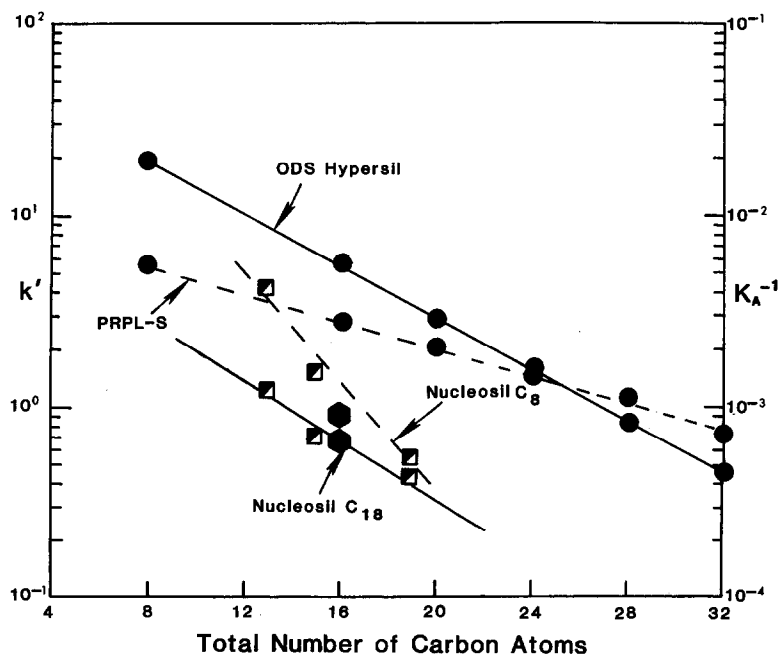


Fig. 3. The relationships between the capacity ratio (k') of I and the total number of carbon atoms in the tetraalkylammonium bromides, added to the mobile phase (circles), and the relationship between the reciprocal of the association constant (K_A^{-1}) of and the total number of carbon atoms in the trimethylalkylammonium ions, added to the mobile phase (squares). The retention data were obtained in this study using the chromatographic conditions given in Fig. 2. The values of K_A were taken from the work of Bij *et al.*⁷. For comparison the values of K_A^{-1} obtained in this study using TBA · Br and TBA · HS are also shown (●).

$$\log k' = -0.069n + 1.85 \quad (4)$$

$$r = 0.9994$$

These results indicate that although the effect of adsorption of competing cations onto the surface of silica-based stationary phases is to mask the residual silanol groups, there is a significant solvophobic contribution to this interaction. These observations are consistent with those of Bij *et al.*⁷ who found that the value of the association constant for the interaction of alkyltrimethylammonium ions with Nucleosil C₈ and Nucleosil C₁₈ increased with increasing length of the alkyl chain (Fig. 3). The data obtained in this study are compared with those obtained previously⁷ in Fig. 3. It is interesting to note that the values of K_A obtained by Bij *et al.*⁷ exhibit a stronger dependency on hydrophobicity in the interaction of the alkyltrimethylammonium ions with Nucleosil C₈ compared with the interaction with Nucleosil C₁₈. What is of more interest, however, is that the values of K_A obtained here with tetraalkylammonium ions are consistent with those obtained previously⁷ with the alkyltrimethylammonium ions. This may suggest that the ability of an ammonium ion to mask residual silanols is more dependent on its hydrophobicity than the distance separating the charge centers.

The phenomenological model described by eqn. 1 assumes that the cations added to the mobile phase compete with the analyte for one of the two sites of retention, presumed here to be the silanol groups. Adsorption of a hydrophobic cation onto the surface of the ODS Hypersil could also have the effect of masking the solvophobic sites of interaction and these two models would be mathematically equivalent since k'_1 and k'_2 (eqn. 1) would simply be reversed. However, if this alternative were true then it is to be expected that k'_1 (eqn. 1) would be significantly effected by the nature of the electrolytes, which appears not to be the case (Table I and Fig. 2).

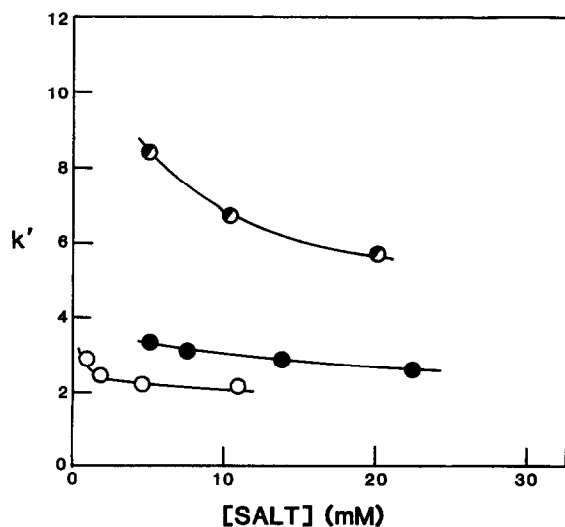


Fig. 4. The relationships between the capacity ratio (k') of I on PLRP-S and the concentrations of KBr (●), TBA · Br (●) and TBA · HS (○) added to the mobile phase. Other conditions as Fig. 2.

It has been well established^{10,11} that ions are strongly adsorbed onto the surface of hydrophobic polymers such as PLRP-S. Fig. 4 shows that the retention of I decreased with increasing concentration of KBr, TBA · Br and TBA · HS, consistent with the previous observations of Cantwell and Puon¹⁰. These workers have shown that the retention of such analytes may be described by the Stern–Gouy–Chapman theory of double layer adsorption and they are displaced from the column by the addition of like-charged molecules to the mobile phase. This phenomenon is similar to that seen for the retention behavior of I on the silica-based reversed-phase material, however it was experimentally impossible to quantify these observations in the same fashion. Very low concentrations (1 mM) of electrolytes, particularly the tetraalkylammonium ions, were extremely effective at displacing I from the column (Fig. 4) and it was not possible to obtain an adequate range of k' values to fit the data to a mathematical model.

It was expected that the retention of I on the PLRP-S column would be dependent on the nature (hydrophobicity) of the competing cation added to the mobile phase. This was supported by the data in Fig. 4 in which the effects of KBr and TBA · Br are compared and confirmed more substantially by the results in Fig. 3. Fig. 3 shows that the retention of I decreased with increasing hydrophobicity of the tetraalkylammonium ions added to the mobile phase at a concentration of 10 mM. Again this could be described empirically by a logarithmic relationship between k' and the total number of carbon atoms in the competing ions, n :

$$\begin{aligned} \log k' &= -0.0355n + 1.01 \\ r &= 0.9974 \end{aligned} \quad (5)$$

It is interesting to note that the hydrophobic contribution to the interaction is less (slope = -0.0355) in the case of the PLRP-S column compared with the silica based ODS Hypersil column (slope = -0.069).

TABLE III

THE EFFECTS OF THE NATURE OF ANION ON THE RETENTION OF I ON ODS HYPERSIL AND PLRP-S COLUMNS ELUTED WITH MOBILE PHASES CONTAINING VARIOUS TETRA-BUTYLAMMONIUM SALTS (TBA · X)

Temperature: ambient. Mobile phase: acetonitrile–THF–water (60:15:25) containing TBA · X (10 mM).

Anion (X^-)	k'	
	ODS Hypersil	PLRP-S
(C ₆ H ₅) ₄ B ⁻	11.3	26.8
ClO ₄ ⁻	6.65	3.46
CH ₃ SO ₃ ⁻	6.37	2.40
Br ⁻	6.25	2.43
<i>p</i> -TsO ⁻	6.12	2.64
NO ₃ ⁻	5.85	2.52
H ₂ PO ₄ ⁻	5.63	2.06
HSO ₄ ⁻	5.14	2.21

TABLE IV

THE EFFECTS OF ADDING SODIUM ALKYL SULFONATES (SES, SPS AND SOS) TO THE MOBILE PHASE ON THE RETENTION OF I ON THE PLRP-S COLUMN

Temperature: ambient. Mobile phase: acetonitrile-THF-water (60:15:25) containing SES, SPS or SOS.

Alkylsulfonate	k'
SES (5 mM)	4.76
SPS (5 mM)	4.86
SOS (1 mM)	6.71
SOS (5 mM)	5.70
SOS (10 mM)	5.83
SOS (15 mM)	6.08

The ratio k'_2/k'_1 reflects the relative contributions of the two interactions in a dual retention process in the absence of a masking agent⁷⁻⁹. It can be seen (Table II and Fig. 2) that, under the conditions studied here, the solvophobic contribution to retention on the ODS Hypersil column is between 1 and 2% of the total. This can be increased to about 20% if the contribution of the silanophilic interactions is reduced substantially by the addition of 10 mM TBA to the mobile phase (Table III). Under these conditions it is reasonable to assume that the retention of I might be somewhat influenced by the nature of the counter ion to TBA, due to the effects of ion-pairing^{12,13}. Table III shows the effect of various salt forms of TBA on the retention of I on ODS Hypersil. For comparison, the retention of I on the PLRP-S column with various salt forms of TBA added to the mobile phase is also shown. In addition, Table IV shows the effect of the chain length of sodium alkylsulfonates on the retention of I on the PLRP-S column. In general, it may be concluded from the results of these experiments (Table III and IV) that the nature of the anion added to the mobile phase is relatively unimportant compared with the nature of the cation. The exception to this is the tetraphenylborate ion which is expected to form strong solvent reinforced ion pairs¹³ with I and hence resulted in a significant enhancement of retention.

To complete the chromatographic characterization of I, its retention was investigated on Partisil SAX and Partisil SCX using aqueous mobile phases containing 60% acetonitrile and 15% THF. Not unexpectedly, I was completely unretained by the like-charged Partisil SAX and completely retained by the oppositely-charged Partisil SCX, in the absence of added electrolyte. No further experiments on Partisil SAX were conducted. It was found that I could be displaced from the Partisil SCX column by the addition of TBA · Br to the mobile phase and that the capacity ratio of I was linearly related to the reciprocal of the concentration of the added electrolyte with a negligible intercept, according to the relationship:

$$\begin{aligned} k' &= 0.034/[TBA] + (0.113) \\ r &= 0.999 \end{aligned} \quad (6)$$

This result is consistent with a single mechanism of ion exchange for I on Partisil SCX. The effect of the size of competing ion on the retention of I on Partisil SCX was also studied, by adding tetraalkylammonium (TMA, TEA, TBA, TPA, THxA, THp and TOA) bromides to the mobile phase at a concentration of 10 mM. It was found that, in

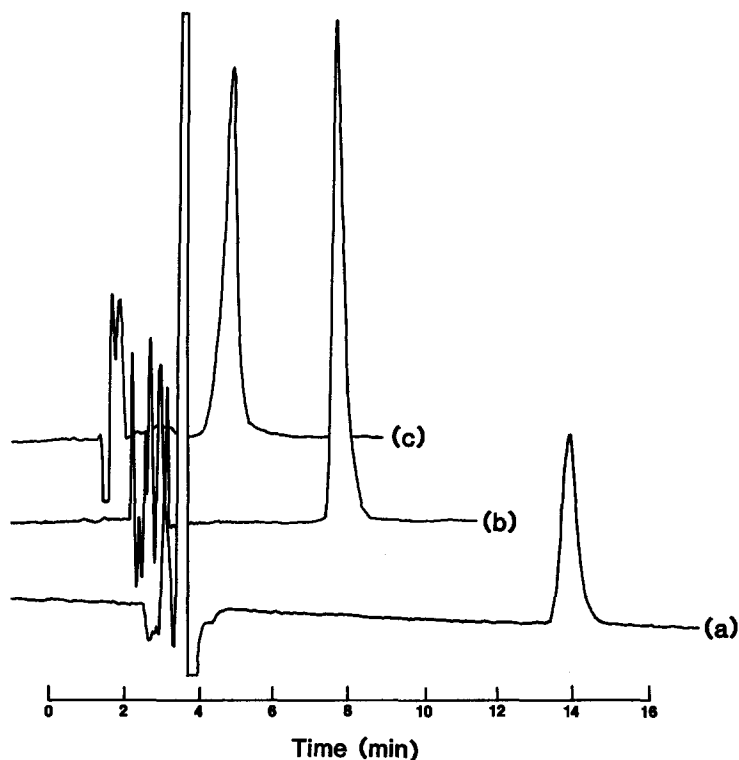


Fig. 5. Chromatograms of I on (a) ODS Hypersil, (b) Partisil SCX and (c) PLRP-S. Mobile phase: acetonitrile-THF-water (60:15:25) containing (a and c) 16 *mM* TBA · HS, (b) 15 *mM* TBA · Br. Flow-rate: 1.0 ml/min. Detection: 254 nm. Injection volume: 20 μ l. Solute concentrations: (a and b) 12 μ g/ml, (c) 36 μ g/ml.

contrast with the reversed-phase materials (ODS Hypersil and PLRP-S), the retention of I increased with increasing size of the competing ion, reflecting the importance of electrostatic interactions and charge separation on the retention of cations by Partisil SCX. Although the retention of I on Partisil SCX increased with increasing size of the tetraalkylammonium bromides added to the mobile phase, no mathematical relationship describing this effect could be developed.

In conclusion, the cationic gold complex, I, was strongly adsorbed onto both silica-based and polymeric reversed-phase materials, apparently by mixed mechanisms of retention. However, the affinity of the analyte for these columns and also for the strong cation exchanger (Partisil SCX) could be substantially reduced by the addition of electrolytes to a mobile phase containing acetonitrile-THF-water (60:15:25). By appropriate choice of the electrolyte and its concentration in the mobile phase, good chromatography with almost symmetrical peaks and efficiencies between 7000 and 20 000 plates/m could be obtained on each of the columns (Fig. 5).

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