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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF INORGANIC PLATINUM(II) COMPLEXES USING SOLVENT-GENERATED ANION EXCHANGERS

II. THE EFFECT OF ELECTROLYTES ON SOLUTE RETENTION

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SUMMARY

The chromatographic properties of bivalent neutral platinum complexes on solvent-generated anion exchange columns were investigated using cisplatin (*cis*-dichlorodiammineplatinum; a clinically useful anti-neoplastic agent) as a model compound. Solute retention was controlled by the addition of electrolytes to totally aqueous mobile phases. The effect of salts on retention was rationalized in terms of the Stern–Gouy–Chapman theory of electrical double layers and the application of solvophobic theory. The presence of bromide or nitrate in the mobile phase decreased retention, apparently by decreasing the thermodynamic activity of the cationic sites in the stationary phase due to the creation of an inner Helmholtz plane in the electrical double layer. At low concentration, citrate and acetate (at pHs where the carboxylate is appreciably ionized) caused increased retention of cisplatin owing to the effect of the added salt on the surface tension of the mobile phase as rationalized by solvophobic theory. At higher concentrations of citrate, retention of the solute decreased owing to the secondary contribution of electrostatic effects. Retention was explained in terms of ion–dipole interactions reinforced by hydrophobic contributions.

INTRODUCTION

Interest in the clinical analysis of neutral bivalent platinum species has been stimulated by laboratory and clinical evidence demonstrating the dramatic anti-neoplastic activity of cisplatin, [*cis*-dichlorodiammineplatinum(II)], $[Pt(NH_3)_2Cl_2]^0$ (CDDP), toward many solid malignancies which are refractory to other drug therapy[1]. Such analysis requires a chromatographic component since these compounds need to be separated from biological matrices and are highly reactive toward nucleophiles both in aqueous solution and in biological fluid[1].

Most clinically applicable methods for cisplatin are non-selective and respond to total platinum in the sample without regard to the ligands co-ordinated to the metal. Basolo *et al.*[2] first described separation of cisplatin from other platinum com-

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plexes on a cellulose support and observed that retention was increased with increasing concentration of organic modifier in the mobile phase. More recently, we have reported that cisplatin is retained on chemically bonded and solvent-generated anion exchange columns[3–5]. In both instances, retention is primarily due to ion–dipole interactions. Whereas the addition of organic modifier to the chemically bonded system increased the capacity ratio (k') of cisplatin, organic modifiers facilitated elution of the solute from the solvent-generated exchanger. This difference in behavior was attributed to the effect of organic modifiers on the activity coefficients of the solute and cationic surfactant in the stationary phase. However, on solvent-generated exchangers, the maximum value of k' obtainable only approached unity.

The addition of electrolytes to the mobile phase produced profound effects on the chromatographic behavior of cisplatin on the solvent-generated and the chemically bonded anion exchange system, at similar salt concentrations. The potential for controlling the chromatographic properties of bivalent platinum compounds on solvent-generated anion exchange columns by the manipulation of electrolyte composition and concentration in purely aqueous mobile phases is the subject of the present study. The opportunity for carrying out such separations in the absence of organic modifiers is particularly attractive because these systems are being interfaced with detectors whose performance is compromised by the presence of organic solvents in the mobile phase.

EXPERIMENTAL

High-performance liquid chromatography (HPLC)

The liquid chromatograph consisted of an Altex Model 110A pump (Altex, Berkeley, CA, U.S.A.) and an Altex Model 210 injector fitted with a 20-μl loop. The eluent composition was monitored with an Altex Model 156 differential refractometer and an Altex Model 153 UV detector (280 nm) connected in series. The detector outputs were recorded on a dual pen potentiometric chart recorder. A flow-rate of 1.0 ml min^{-1} was used throughout.

A μBondapak C_{18} column (10 μm; 300 × 3.9 mm I.D.) was obtained from Waters Assoc. (Milford, MA, U.S.A.) and an ODS Hypersil column (Shandon & Southern, Sewickley, PA, U.S.A.; 5 μm, 100 × 4.6 mm I.D.) was slurry packed according to the method described by Bristow *et al.*[6]. The column temperature was maintained at (30 ± 0.1)°C as described previously[5]. Solute capacity ratios, k', were calculated[5] using deuterium oxide for the determination of t_0.

Solvent-generated anion exchangers were prepared by the adsorption of hexadecyltrimethylammonium bromide (HTAB) onto the hydrophobic stationary phases[5]. After the column had been pre-loaded, stability was maintained by the addition of 10^{-4} mol dm^{-3} HTAB to the mobile phase.

Materials

HTAB was obtained from Aldrich (Milwaukee, WI, U.S.A.) and used without further treatment. Distilled water was used throughout. All other chemicals were of at least reagent grade and used as received from various sources.

Crystalline samples of cisplatin and *trans*-dichlorodiammineplatinum(II) were obtained from the National Cancer Institute (Bethesda, MD, U.S.A.). Stable solu-

tions of these solutes were prepared in 0.1 mol dm^{-3} sodium chloride. Aqueous solutions containing a mixture of *cis*-$[Pt(NH_3)_2BrCl]^0$ and *cis*-$[Pt(NH_3)_2Br_2]^0$ were prepared *in situ* by incubating cisplatin in 1 mol dm^{-3} sodium bromide for *ca.* 2 h at 30°C. Similarly, an aqueous solution containing a mixture of *cis*-$[Pt(NH_3)_2Cl(H_2O)]^+$ and *cis*-$[Pt(NH_3)_2(H_2O)]^{2+}$, was prepared by incubating cisplatin in pure water.

Kinetic studies

The degradation of cisplatin in aqueous solutions containing various anions (as their sodium salts) was investigated at 30°C. The initial concentration of cisplatin was either 0.1 or 0.2 mg ml^{-1}. The effect of nucleophile type and concentration was studied over the following ranges: bromide, 0.01–1.00 mol dm^{-3}; nitrate, 0.01–1.00 mol dm^{-3}; acetate 0.05–0.30 mol dm^{-3}; citrate 0.01–0.80 mol dm^{-3}. In the cases of acetate and citrate, the effects of pH were also studied over the range 2.8–6.0. Cisplatin disappearance was monitored by HPLC with UV detection comparing the peak heights of the drug with standard solutions prepared in 0.1 mol dm^{-3} sodium chloride.

RESULTS

Effect of monovalent ions

The effect of the addition of the monovalent anions, nitrate, bromide and acetate (as their sodium salts) on the retention of cisplatin was investigated over the concentration range 0–0.1 mol dm^{-3}. In the case of acetate, the effect of pH was also determined.

Fig. 1 shows the relationship between the reciprocal of the capacity ratio of cisplatin, the concentration of added salt, and, in the case of acetate, the effect of pH. The addition of sodium nitrate or sodium bromide to the mobile phase resulted in a decrease in the retention of cisplatin. This effect was more pronounced for nitrate than for bromide. For both these salts, retention decreased more rapidly between 0 and 0.02 mol dm^{-3} added salt than at higher concentrations. In the case of added acetate buffer (pK_a 4.76), retention was dependent upon both pH and concentration. At pH 7.0, cisplatin retention increased with increasing concentration of acetate buffer. At pH 5.00, the retention of cisplatin increased slightly between 0 and 0.02 mol dm^{-3} acetate and then remained constant at higher concentrations; whereas at pH 3.5, cisplatin retention decreased with increasing buffer concentration.

Effect of multivalent ions

The effect of citrate (pK_a 3.13, 4.76 and 5.40) on the retention of cisplatin was investigated as a function of buffer concentration and pH, and the results are shown in Fig. 2. The addition of low concentrations of citrate (0–$5 \cdot 10^{-3}$ mol dm^{-3}) produced a dramatic enhancement of the retention of cisplatin, and this effect increased with increasing pH. The retention of the drug decreased gradually with increasing concentration of citrate over the range $5 \cdot 10^{-3}$ to $1 \cdot 10^{-1}$ mol dm^{-3}.

The addition of the dibasic anion, sulphate, produced a similar enhancement of the retention of cisplatin. With addition of 10^{-1} mol dm^{-3} sodium sulphate, cisplatin eluted with a capacity ratio of 3.22. However, inclusion of sodium sulphate in the

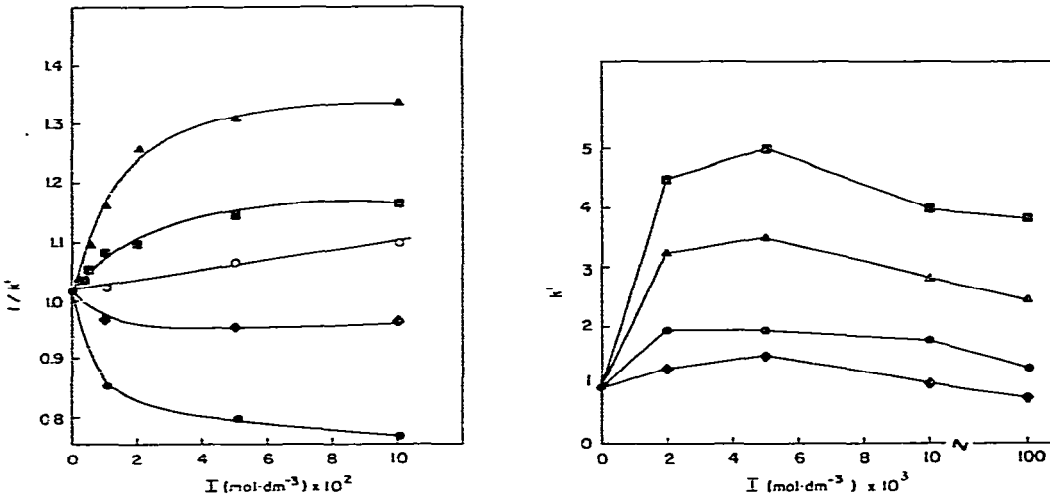

Fig. 1. The effect of salt concentration (I) on the capacity ratio (k') of cisplatin. Stationary phases, μBondapak C_{18} loaded with 1.31 μmol m^{-2} HTAB; mobile phase, 10^{-4} mmol dm^{-3} HTAB in water plus sodium bromide (■), sodium nitrate (▲) or sodium acetate–acetic acid buffers at pH 7.0 (●), 5.0 (◆) and 3.5 (○); temperature, 30 ± 0.1°C; solute concentration, 1.0 mg ml^{-1}.

Fig. 2. The effect of citrate buffer concentration (I) on the capacity ratio (k') of cisplatin. Stationary phases, μBondapak C_{18} loaded with 1.31 μmol m^{-2} HTAB; mobile phases, 10^{-4} μmol dm^{-3} HTAB in water plus citrate buffer at pH 7.0 (■), 5.5 (▲), 4.0 (●) and 3.00 (◆); temperature, 30 ± 0.1°C; solute concentration, 1 mg ml^{-1}.

mobile phase was associated with gradually increasing column back-pressure on repeated injection of cisplatin. This column blockage precluded further investigations into the effects of sulphate.

DISCUSSION

By measurement of breakthrough times a monolayer of 1.31 μmol m^{-2} HTAB was found on the stationary phase (μBondapak C_{18}), in equilibrium with a mobile phase of 10^{-4} mol dm^{-3} HTAB. Such systems are capable of retaining anionic solutes as a result of electrostatic interactions[8–10] and are often termed "solvent-generated" anion exchangers. Such systems are also useful in the resolution of neutral inorganic solutes such as cisplatin[5] with a high degree of functional group selectivity (Table I). Evidence has been presented[5] suggesting that retention of neutral platinum-(II) species arises from ion–dipole interactions between solute and the cationic stationary phase. Further evidence for ion–dipole mediated retention is provided here since the apolar transplatinum is less well retained than the more polar *cis*-isomer in these systems (Table I). However, the elution order of the three halogenated complexes ($[Pt(NH_3)_2Cl_2]^0$, $[Pt(NH_3)_2BrCl]^0$ and $[Pt(NH_3)_2Br_2]^0$ suggests that these ion–dipole interactions are reinforced by a hydrophobic effect[11]. Clearly, electrostatic repulsive forces are responsible for the poor retention and the lack of separation of the two aquated species $[Pt(NH_3)_2Cl(H_2O)]^+$ and $[Pt(NH_3)_2(H_2O)_2]^{2+}$. The increased HTAB uptake observed on the column used in this study is consistent with

TABLE I

THE CAPACITY RATIOS, k', OF SOME PLATINUM(II) COMPLEXES

Stationary phase, μBondapak C_{18} + 1.31 μmol m^{-2} HTAB; mobile phase, 10^{-4} mol dm^{-3} HTAB in water; temperature, 30 ± 0.1°C.

Solute	k'*
cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$	0.10
cis-$[Pt(NH_3)_2Cl(H_2O)]^+$	0.10
trans-$[Pt(NH_3)_2Cl_2]^0$	0.30
cis-$[Pt(NH_3)_2Cl_2]^0$ (CDDP)	0.98
cis-$[Pt(NH_3)_2BrCl]^0$	1.35
cis-$[Pt(NH_3)_2Br_2]^0$	2.50

* $k' = (t_{CDDP} - t_0)\, t_0^{-1}$; t_0 determined with ^2H_2O.

batch-to-batch variation between columns and accounts for the higher retention of cisplatin compared with that seen previously[5].

Retention model for cisplatin

The effects of adding salt to the mobile phase on the retention of ionic solutes in ion exchange systems is well documented (*e.g.*, ref. 12). These effects may be described in terms of competition between the solute ions and mobile phase ions for the oppositely charged stationary phase ligands. Thus, increasing the ionic strength of the mobile phase decreases the retention of ionic solutes such that their capacity ratios are reciprocally related to the activity of the competing ions in the mobile phase[12].

It is clear from Figs. 1 and 2 that the modifying influence of added anions on the retention of cisplatin arises from at least two effects. One effect apparently arises from the influence of the anions on the electronic character of the stationary phase, which can be rationalized in terms of the Stern–Gouy–Chapman (SGC) theory of electrical double layers[13]. A second "salting out" effect arises from the influence of added salt on the mobile phase surface tension, which may be explained by the application of solvophobic theory[14–16].

According to the SGC theory, the positively charged stationary phase would have an associated electrical double layer of anions which preserves electrical neutrality. Within this double layer, the electrical potential decays linearly between the surface and the outer Helmholtz plane (OHP) and nearly exponentially between the OHP and the bulk mobile phase. Increasing the electrolyte concentration may result in a concomitant increase in the anion concentration in the double layer, followed by increased adsorption of quaternary ammonium ions to maintain electrical neutrality. These effects, however, appear to play a minor role in the present system, since the rapid column equilibration times observed on changing the salt concentration were inconsistent with further adsorption of the cationic surfactant.

A second consequence of an increase in the ionic concentration of the mobile phase is specific adsorption of dehydrated anions producing a inner Helmholtz plane (IHP). Adsorption of anions at the IHP results in ion-pair formation[17] and a reduction in the thermodynamic activity of the sorbed cationic surfactant. This postulation

is consistent with the findings of Cantwell and Puon[17] and provides the most likely explanation for the decrease in retention of cisplatin with increasing salt concentration.

Basolo *et al.*[2] have shown previously that increased ionic strength reduces the interaction of cisplatin with polar adsorbents such as cellulose as a result of effects of the ionic cloud of the solute. Thus, it may be concluded that increases in the ion concentration in the stationary phase may reduce retention in the present system owing to a reduction in the activity coefficients of both the solute and quaternary ammonium groups in the stationary phase.

A priori, increases in the size, charge and polarizability of the anionic species present in the two chromatographic phases of the solvent-generated anion exchanger would be expected to decrease the retention of cisplatin. This was not observed in the cases of acetate and citrate, which produced complex changes in retention. Increased citrate concentration produced an initial increase in retention to $5 \cdot 10^{-3}$ mol dm^{-3} citrate with a decrease in retention at higher concentrations. The form of the relationship between k' and salt concentration did not change with pH, but decreased in magnitude with decreasing pH, presumably owing to a reduction in the charge on the citrate ion. At pH 3.0, the retention of cisplatin was relatively unaffected by citrate concentration, indicating that un-ionized citric acid has little effect on cisplatin retention.

At higher pH, acetate caused a slight enhancement of the retention of cisplatin, as opposed to the decrease observed with bromide and nitrate. However, at low pH, the presence of undissociated acetic acid produced a decrease in retention. This latter effect probably arises from the ability of acetic acid to behave as an organic modifier, in a fashion analogous to methanol, which has been shown to reduce the retention of cisplatin in solvent-generated anion exchange systems[5]. At pH 5.0, the retention of cisplatin was relatively independent of acetate concentration since the protonated and unprotonated species are present in approximately equal proportions and the two effects tended to cancel.

It is likely that the salting-out effects responsible for increased retention observed with citrate, sulphate and acetate arise from a hydrophobic effect due to the influence of salt on mobile phase surface tension. Further evidence of a hydrophobic effect influencing the retention of platinum(II) complexes is shown by the elution order of the brominated solutes (Table I). Replacement of one or both of the chloro groups in cisplatin by the more hydrophobic bromo group results in increased retention. Furthermore, the reduced retention of transplatinum, which presents a smaller hydrophobic surface than cisplatin, may be due to solvophobic effects as well as differences in dipole moments.

Horváth and co-workers[15,16] have applied solvophobic theory[14] to reversed-phase HPLC systems employing secondary equilibria. They have shown that the retention of solutes in these systems may be described by an equation of the form:

$$\ln k' = k_s + k_p + k_h(\Delta A) \qquad (1)$$

which is a summation of all possible solute–solvent–stationary phase interactions that may contribute to the retention of the solute. The term k_s depends only on the properties of the mobile and stationary phases (*i.e.*, it is solute independent); k_p in this

system describes the ion–dipole interactions between cisplatin and the cationic stationary phase. The third term, $k_h(\Delta A)$, is a measure of the hydrophobic interactions, since k_h is given by $\gamma(RT)^{-1}$ where γ is the surface tension of the mobile phase, and ΔA is the decrease in hydrophobic surface area on binding of the solute to the stationary phase[15,16].

The addition of salt to the mobile phase results in an increase in surface tension[15-18] according to

$$\gamma = \gamma_0 + \tau m \tag{2}$$

where γ_0 is the surface tension of the mobile phase in the absence of salt, m is the molal salt concentration, and τ is a constant related to the nature of the added salt. Combining eqns. 1 and 2 gives eqn. 3

$$\ln k = k_s + k_p + (\gamma_0 + m\tau)(\Delta A)(RT)^{-1} \tag{3}$$

which predicts a linear relationship between ln k' and τ at a fixed salt concentration. Table II shows the relationship between the capacity ratio of cisplatin at a fixed salt concentration and the values[18] of τ, which is given by

$$\ln k' = 0.85\tau - 1.23 \qquad r = 0.998 \qquad n = 4 \tag{3a}$$

TABLE II

THE RELATIONSHIP BETWEEN THE CAPACITY RATIO, k', OF CISPLATIN AT A FIXED ELECTROLYTE CONCENTRATION IN THE MOBILE PHASE AND THE τ VALUES OF THE ADDED SALTS

Electrolyte	k'*	τ**
Sodium nitrate	0.74	1.06
Sodium bromide	0.86	1.32
Acetate buffer (pH 7.0)	1.31	***
Sodium sulphate	3.22	2.73
Citrate buffer	3.97	3.12

* Stationary phase, μBondapak + 1.31 μmol m^{-2} HTAB; mobile phase, 10^{-4} mol dm^{-3} HTAB + 0.1 mol dm^{-3} electrolyte; temperature, 30°C.

** See eqn. 3 in ref. 18.

*** Not reported in the literature.

These results indicate that the complex effects of salts on the retention of cisplatin in solvent-generated anion exchangers arise from two effects, as described by eqn. 3. Increasing in the ionic concentration of the mobile phase results in a reduction in the thermodynamic activity of the cationic binding sites leading to a decrease in the contributions of k_p and k_s (eqn. 3) and decreased retention of cisplatin. However, addition of salt to the mobile phase may also lead to an enhancement of retention owing to its influence on the hydrophobic term, $k_h(\Delta A)$.

For salts with a large value of τ (*e.g.*, trisodium citrate), the retention of cis-

platin is governed by the hydrophobic term, $k_h(\Delta A)$. Conversely, the terms k_p and k_s dominate the effect of salts with small values of τ (*e.g.*, sodium nitrate). Although no values of τ have been reported for sodium acetate and the mono- and bivalent sodium citrate, the data presented by Melander and Horvath[18] suggest that τ values decrease with decreasing charge and ionic size. This would account for the decrease in retention of cisplatin with decreasing pH of the citrate buffer in the mobile phase and the lower retention observed for acetate compared with citrate.

Stability of cisplatin

The rate of degradation of cisplatin from aqueous solution (Fig. 3) was limited by its rate of aquation, and thus was independent of pH and the presence of citrate, acetate and nitrate[4,19]. Conversely, in the presence of bromide, degradation shows a first-order dependency on bromide concentration (second-order rate constant, k_2 = 0.56 h^{-1} mol^{-1} dm^3 at 30°C)[20,21] as well as proceeding via aquation, *i.e.*, k_{obs} = $k_2[Br^-] + k_1$ (where k_1 is the intrinsic rate constant due to aquation). Thus, within the elution times of cisplatin under all mobile phase conditions, less than 5% cisplatin was degraded on the column.

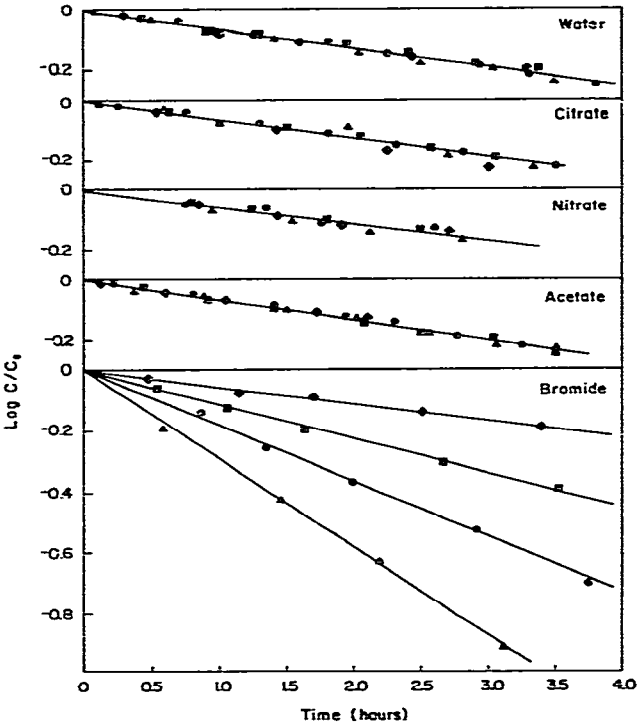

Fig. 3. Degradation of cisplatin with time from aqueous solutions containing various anionic species (at 30°C). Citrate: ■, 0.01 mol dm^{-3}, pH 2.57; ●, 0.10 mol dm^{-3}, pH 5.74; ◆, 0.01 mol dm^{-3}, pH 4.16; ▲, 0.80 mol dm^{-3}, pH 2.78. Nitrate: ■, 0.01 mol dr ; ●, 0.10 mol dm^{-3}; ◆, 0.50 mol dm^{-3}; ▲, 1.00 mol dm^{-3}. Acetate: ●, 0.05 mol dm^{-3}, pH 4.76; ▲, 0.10 mol dm^{-3}, pH 3.62; ◆, 0.20 mol dm^{-3}, pH 2.84; ■, 0.30 mol dm^{-3}, pH 5.75. Bromide: ◆, 0.01 mol dm^{-3}; ■, 0.30 mol dm^{-3}; ●, 0.60 mol dm^{-3}; ▲, 1.00 mol dm^{-3}. For reaction studied in pure water, symbols represent each of four separate determinations. The concentration of cisplatin is expressed in terms of the fraction of the initial concentration, C/C_0.

The HPLC systems developed for the assays of cisplatin were capable of separating the degradation products of cisplatin formed during the stability studies. In most cases it was possible to monitor the rate of production of these degradation products as well as the rate of loss of cisplatin. Some of the assays were performed on a short (100 mm) column packed with ODS Hypersil, which produced identical separations to those obtained on μBondapak C_{18} but offered significant advantages in terms of improved peak shapes and shortened analysis times (Fig. 4).

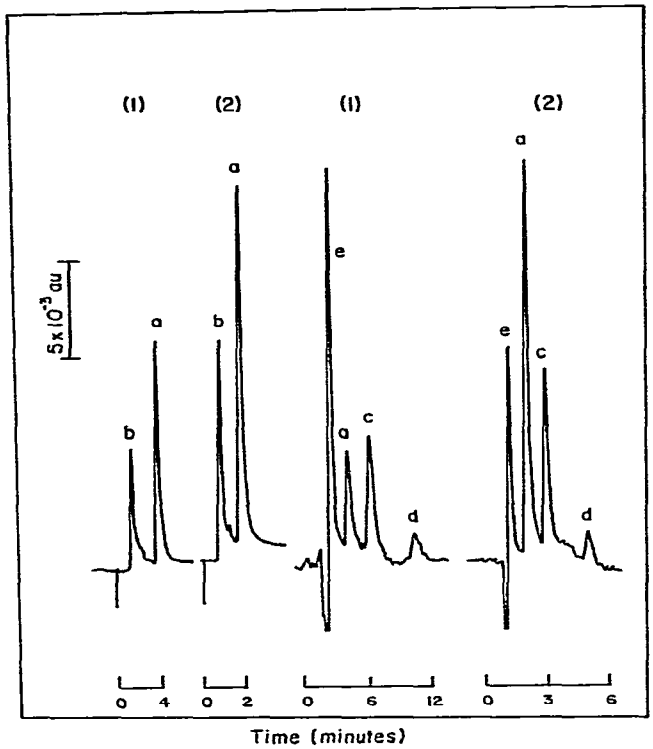

Fig. 4. Separation of platinum(II) complexes on solvent-generated anion exchangers, supported by μBondapak C_{18} (1) and ODS Hypersil (2). Mobile phase, 10^{-4} mol dm^{-3} HTAB in water. Stationary phases: 1, μBondapak C_{18} + HTAB (10 μm; 300 × 3.9 mm I.D.); 2, ODS Hypersil + HTAB (5 μm; 100 × 4.6 mm I.D.). Temperature, 30°C. Flow-rate, 1.0 ml min^{-1}. Peaks: a = $[Pt(NH_3)_2Cl_2]^0$; b = $[Pt(NH_3)_2Cl(H_2O)]^+$ and $[Pt(NH_3)_2(H_2O_2]^{2+}$; c = $[Pt(NH_3)_2ClBr]^0$; d = $[Pt(NH_3)_2Br_2]^0$; and, e = solvent.

In conclusion, bivalent platinum complexes are well separated on solvent-generated anion exchangers, and the mechanism may be described in terms of ion–dipole interactions in the stationary phase reinforced by a hydrophobic effect, and retention may be manipulated by controlling the composition and concentration of electrolyte in the mobile phase.

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