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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF *cis*-DICHLORODIAMMINEPLATINUM(II) USING CHEMICALLY-BONDED AND SOLVENT-GENERATED ION EXCHANGERS

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SUMMARY

cis-Dichlorodiammineplatinum(II) (cisplatin), a neutral square planar platinum(II) complex useful in the clinical management of a variety of neoplasms, was found to be retained on chemically-bonded and solvent-generated anion exchangers. The solvent-generated anion exchanger was prepared by the adsorption of hexadecyltrimethylammonium bromide onto the surface of a hydrophobic stationary phase. Investigations into the effects of ionic strength, organic modifiers and temperature revealed certain fundamental differences between the two systems. However, the retention mechanism of cisplatin on both types of cationic stationary phases was most readily explained in terms of ion–dipole interaction.

INTRODUCTION

cis-Dichlorodiammineplatinum(II) (cisplatin, DDP) is a neutral square planar complex of platinum(II) and a potent antineoplastic agent[1]. Cisplatin has been determined in aqueous solutions and biological fluids by X-ray fluorescence[2], flameless atomic absorption[3] and high-performance liquid chromatography (HPLC)[4]. However, these methods are non-selective and only permit the determination of total platinum concentrations.

Recently, we have reported a selective HPLC method for the separation of cisplatin from a number of potential degradation and biotransformation products[5,6]. The drug was found to be retained on a strong anion-exchange column (Partisil 10 SAX) and its retention increased with increased methanol concentration in the aqueous mobile phase[5,6]. Previously Basolo *et al.*[7] have shown that the retention of cisplatin on cellulose increases with increasing concentration of hydroxylic modifiers in aqueous eluents. They rationalized this behavior in terms of the effect of the solvent on the ionic cloud of the analyte.

Very hydrophobic ionic surfactants can be adsorbed onto the surface of alkylsilicas from aqueous mobile phases[8–11] (following a Langmuir isotherm[8,11]) to produce stationary phases which may then function as ion exchangers[11]. To maintain the stability of the system, surfactant must also be present in the mobile phase and retention of solutes may therefore also be influenced by ion–ion interactions in the

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mobile phase[11–13]. Gilbert[10] has shown that such solvent generated ion exchangers are prepared most conveniently by preloading the stationary phase from a high concentration (*e.g.*, 1%) of ionic surfactant in water. Stability is then maintained by the use of lower mobile phase concentrations of the previously adsorbed surfactant.

The present study is concerned with the retention mechanisms of cisplatin on cationic stationary phases. The effects of organic modifiers, ionic strength and temperature on cisplatin retention are described and a comparison is made between its retention mechanism on chemically-bonded and solvent-generated[8–10] ion exchangers. The solvent-generated ion exchanger was prepared by the adsorption of a cationic surfactant onto the surface of μBondapak C_{18} which is a hydrophobic reversed-phase material.

EXPERIMENTAL

Chemicals and reagents

HPLC grade methanol, tetrahydrofuran (THF), 2-propanol and *n*-heptane were supplied by Manufacturing Chemists (Cincinnati, OH, U.S.A.) and Fisher Scientific (Fair Lawn, NJ, U.S.A.). Hexadecyltrimethylammonium bromide (HTAB) was obtained from Aldrich (Milwaukee, WI, U.S.A.) and was used without further treatment. All other chemicals were of analytical grade and were used as received from various sources. Deionized water was used after further distillation from an all glass still.

Crystalline cisplatin was obtained from the National Cancer Institute and was used without further treatment. Stock solutions (1.0 mg ml^{-1}) of cisplatin were prepared in 0.9% w/v sodium chloride, stored at 4°C and discarded after 7 days.

High-performance liquid chromatography

The liquid chromatograph comprised an Altex constant-flow-rate pump (Model 110A, Altex, Berkeley, CA, U.S.A.), a fixed wavelength (280 nm) ultraviolet detector (Altex, Model 153), and an Altex injector (Model 210) fitted with a 20-μl loop. The detector output was monitored with a 10-mV input potentiometric recorder. A flow-rate of 1.0 ml min^{-1} was used throughout.

Pre-packed μBondapak C_{18} (10 μm, 300 × 3.9 mm) and Partisil PXS 10/25 SAX (10 μm, 250 × 4.6 mm) columns were obtained from Waters Assoc. (Milford, MA, U.S.A.) and Whatman (Clifton, NJ, U.S.A.), respectively. A Partisil 5 column (250 × 4.6 mm) was slurry packed according to the method described by Bristow *et al.*[14]. The column temperature was controlled (±0.1°C) by its enclosure in a water jacket (Alltech, Deerfield, IL, U.S.A.) and the use of a recycling water heater (Haake, Saddle Brook, NJ, U.S.A.). For column thermostating below 25°C, a Haake cooling unit was used in conjunction with the water heater. Stainless-steel columns were used since this material was previously shown[5] not to interact with cisplatin. In fact, it is the material of choice if cisplatin must be exposed to metal surfaces.

Mobile phases containing organic modifiers were prepared by mixing the appropriate volumes of water and organic solvent. Concentrations of organic modifiers in water are expressed on a volume fraction basis, where the volume fraction of an organic modifier, φ_x is given by

$$\varphi_x = V_x (V_x + V_{H_2O})^{-1} \quad (1)$$

where V_x and V_{H_2O} are the volumes of organic modifier and water, respectively.

Cisplatin capacity ratios, k'_{DDP}, were calculated at least in duplicate according to eqn. 2:

$$k'_{DDP} = (t_{DDP} - t_0)\, t_0^{-1} \quad (2)$$

where t_{DDP} and t_0 are the elution times of cisplatin and an unretained solute, respectively. Depending on the mobile phase being used, independent injections of methanol or water were made to determine t_0. These values were consistent with column porosities of about 0.7 (ref. 15).

Solvent-generated anion exchangers[8-10] were prepared according to the method described by Gilbert[10]. A 75-ml volume of $2.7 \cdot 10^{-2}$ mol dm^{-3} HTAB in water was passed through the μBondapak C_{18} column at 1.0 ml min^{-1} and 30°C. By setting the detector attenuation to 0.010, a rise in baseline was observed after the passage of about 30 ml. This rise in baseline has been attributed to "breakthrough" of HTAB and equilibration of the system[16]. After pre-loading the stationary phase, lower concentrations of HTAB (10^{-3}–10^{-5} mol dm^{-3}) were used in the mobile phase to maintain the stability of the modified stationary phase.

At the end of each working day, the modified reversed-phase column was washed with 100 ml of water to remove any inorganic material, followed by 100 ml of acetone to remove the adsorbed surfactant. The acetone was removed from the column by flushing with 100 ml of methanol followed by 200 ml of water. The Partisil 10 SAX column was regenerated periodically according to the manufacturer's specifications. Prior to studies on the retention behavior of cisplatin, the acetate form of the bonded ion exchanger was generated by the passage of 200 ml of 0.5 mol dm^{-3} sodium acetate followed by 100 ml of water. To expedite these cleaning procedures higher flow-rates were used.

The presence of residual silanol groups on the surface of the Partisil 10 SAX and μBondapak C_{18} columns was evaluated by determining the capacity ratio of nitrobenzene, $k'_{\varphi NO_2}$, using a mobile phase of *n*-heptane[17].

RESULTS AND DISCUSSION

According to the manufacturer's specifications, Partisil 10 SAX is a strong anion exchanger consisting of tetraalkylammonium groups chemically bonded via Si–O–Si linkages to silica gel. With a mobile phase of *n*-heptane, a capacity ratio of 2.20 was obtained for nitrobenzene indicating the presence of significant silanol concentration on the surface of Partisil 10 SAX[15]. The absence of significant numbers of silanol groups is generally associated with $k'_{\varphi NO_2} \leqslant 0.5$ (ref. 17). It follows then that three potential sites for the interaction of cisplatin with Partisil 10 SAX can be envisaged; (a) the quaternary ammonium functionalities; (b) the silanol moieties; and (c) the hydrocarbon groups.

To identify the site(s) of interaction of cisplatin with the stationary phase, the effect of methanol on its retention was investigated using Partisil 10 SAX, a hydrophobic stationary phase (μBondapak C_{18}) and silica gel (Partisil 5). Fig. 1 shows that the retention of cisplatin on a bonded anion-exchange column increases with increasing methanol concentration over the range $\varphi = 0.00$ to $\varphi = 0.80$. With the other two

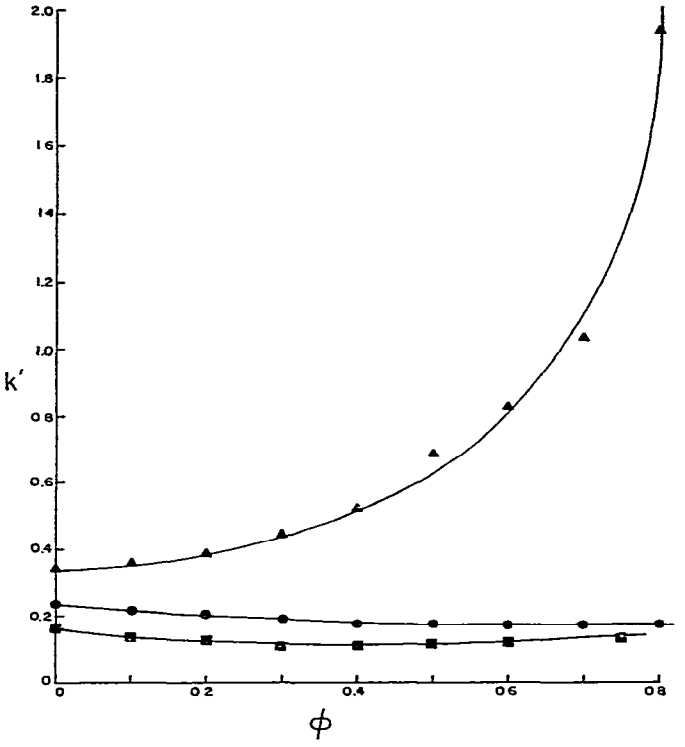

Fig. 1. The effect of methanol volume ratio (φ) on cisplatin capacity ratio (k') on strong anion-exchange (▲), reversed-phase (●) and silica gel (■) columns.

stationary phases, cisplatin was poorly retained and its retention decreased slightly with increasing methanol concentration.

These results suggest that the drug is retained on Partisil 10 SAX primarily as a result of interactions with the bonded quaternary ammonium groups and that these interactions are strengthened by the presence of methanol in the mobile phase. These interactions are probably ion–dipole in nature since cisplatin has a significant dipole moment (5.3 D, ref. 18). The fact that the corresponding *trans*-isomer of cisplatin which has no dipole[18] is unretained in these systems[19] adds support to this hypothesis.

The poor retention of cisplatin on silica indicates that dispersion forces arising from dipole–dipole interactions play a minor role in the retention of cisplatin on Partisil 10 SAX. Similarly, the results obtained on the hydrophobic stationary phase indicate that solvophobic[20,21] interactions of cisplatin with the hydrocarbon moieties of Partisil 10 SAX are relatively unimportant. The results obtained here may be compared with those of Basolo *et al.*[7] who found that the retention of cisplatin on cellulose increased with increasing concentration of ethanol in the aqueous eluent. The retention of cisplatin on cellulose presumably arises from dipole–dipole (dispersion) interactions, whereas, the retention of cisplatin on Partisil 10 SAX arises primarily from ion–dipole interactions. However, both these interactions are apparently strengthened by the presence of hydroxylic modifiers in the mobile phase.

The results obtained with the chemically-bonded ion exchanger suggested that a more flexible approach might be to use a solvent-generated ion exchanger as described by Kraak *et al.*[9] and others[8,10].

In the present study, a reversed-phase column (μBondapak C_{18}) was preloaded by the passage of 0.027 mol dm^{-3} HTAB in water at 30°C. By the observation of "breakthrough" times[16] and assuming a surface area of 300 m^2 g^{-1} (manufacturer's specifications) and packing weight of 2.37 g it was found that 0.99 mol m^{-2} of HTAB was adsorbed onto the surface of the stationary phase. This value is in good agreement with that obtained by Knox and Laird[8] for the adsorption of HTAB onto SAS Hypersil. After preloading the column, lower concentrations of HTAB were used in the mobile phase. In some cases, changing to higher organic modifier concentrations produced a displacement peak attributed to desorption of HTAB. With methanolic mobile phases, no displacement of HTAB was observed when $\varphi < 0.15$ and the HTAB mobile phase concentration was 10^{-4} mol dm^{-3}. With 10^{-5} mol dm^{-3} HTAB in the mobile phase, no displacement was observed when $\varphi_{MeOH} < 0.10$. Successive increases in methanol above these concentrations resulted in significant detectable displacement of some of the adsorbed surfactant from the column. With THF and 2-propanol, HTAB was partially displaced at all concentrations above $\varphi = 0.05$ with 10^{-4} mol dm^{-3} HTAB in the mobile phase.

Cisplatin was retained on the solvent-generated ion exchanger ($k'_{DDP} = 0.84$) with a purely aqueous mobile phase containing 10^{-4} mol dm^{-3} HTAB. This represents a four-fold increase in retention compared with that obtained on the same

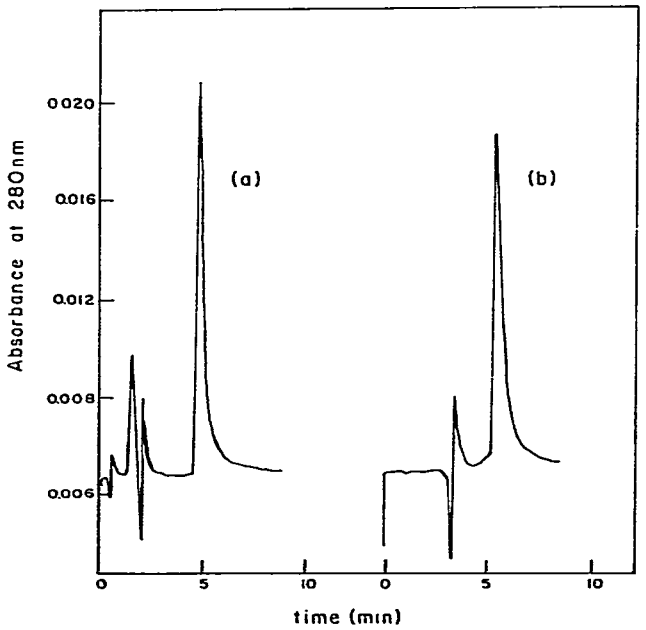

Fig. 2. Chromatogram of cisplatin. (a) Solvent-generated anion exchanger; stationary phase: C_{18} column loaded with 0.99 μmol m^{-2} HTAB; mobile phase: 10^{-4} mol dm^{-3} HTAB in water. (b) Partisil 10 SAX column; mobile phase: methanol–water ($\varphi = 0.60$). For both systems $T = 30$°C; flow-rate = 1 ml min^{-1}; solute concentrations 1 mg ml^{-1}.

stationary phase in the absence of adsorbed HTAB ($k'_{DDP} = 0.21$, Fig. 1). Fig. 2 shows a chromatogram of cisplatin using the solvent-generated ion-exchange stationary phase and an aqueous mobile phase. For comparable retention of cisplatin on the chemically-bonded ion-exchange column a high concentration ($\varphi = 0.60$) of methanol was required (Fig. 2).

The differences in the retention behavior of cisplatin on the two ion exchangers were explored further by investigating the effects of ionic strength, organic modifiers and temperature.

Ionic strength

If cisplatin is retained in these systems by ion–dipole interactions, then it is to be expected that its retention will be influenced by the electronic environment of the stationary phase quaternary ammonium groups and the nature of their counter ions. To confirm this hypothesis, the effect of increasing sodium nitrate concentration on the retention of cisplatin was investigated and the results are shown in Table I. In the case of the solvent-generated ion exchanger, retention of cisplatin remained constant at 0 and 10^{-2} mol dm^{-3} sodium nitrate and decreased when sodium nitrate concentration was increased to 0.1 mol dm^{-3}. With Partisil 10 SAX, retention decreased consistently with increasing ionic strength.

TABLE I

EFFECT OF SODIUM NITRATE CONCENTRATION ON THE RETENTION OF CISPLATIN USING CHEMICALLY-BONDED AND SOLVENT-GENERATED ANION EXCHANGERS

Sodium nitrate concentration (mol/dm^3)	k'_{DDP}	
	Chemically-bonded ion exchanger*	*Solvent-generated** ion exchanger*
0	0.84	0.84
10^{-3}	0.74	0.89
10^{-2}	0.69	0.83
10^{-1}	0.65	0.67

* Mobile phase: methanol–water (6:4) + $NaNO_3$. Temperature: 30°C ± 0.1°C. t_0 determined by independent injection of methanol.

** Mobile phase: 10^{-4} mol dm^{-3} HTAB + $NaNO_3$. Temperature: 30°C ± 0.1°C. t_0 determined by independent injection of water.

The decrease in retention with increasing ionic strength may be rationalized in terms of increased charge shielding of the quaternary ammonium groups with increasing concentration of the oppositely charged nitrate ions. The difference in response to increased ionic strength observed for the two systems probably arises from the fact that the bonded ion exchanger was initially in the acetate form whereas the solvent-generated ion exchanger was in the bromide form and a bromide concentration of 10^{-4} mol dm^{-3} was also present in the mobile phase. The softness of the bromide ion relative to acetate makes it more difficult to be displaced by nitrate[17].

From a practical point of view, added salt is not required for good chromatography in either system. Additionally, high sodium nitrate concentrations were as-

sociated with a high background absorbance at 280 nm and broad "solvent peaks" which interferred with cisplatin resolution.

Organic modifiers

The effects of methanol, 2-propanol and THF concentration on the retention of cisplatin in the two ion exchange systems were investigated and the results are shown in Fig. 3. With increasing methanol and 2-propanol concentration, retention increased on the bonded ion exchanger and decreased on the solvent-generated ion exchanger. Increased THF concentration resulted in decreased retention in both systems. Fig. 3 also shows the effect of methanol concentration in the solvent-generated ion-exchange system at two concentrations of HTAB in the mobile phase. At low methanol concentrations, the retention of cisplatin was independent of HTAB concentration, indicating a negligible contribution of cisplatin–HTAB interactions in the mobile phase. Furthermore, the initial decrease in the retention of cisplatin with increasing methanol concentration cannot be attributed to desorption of HTAB. At

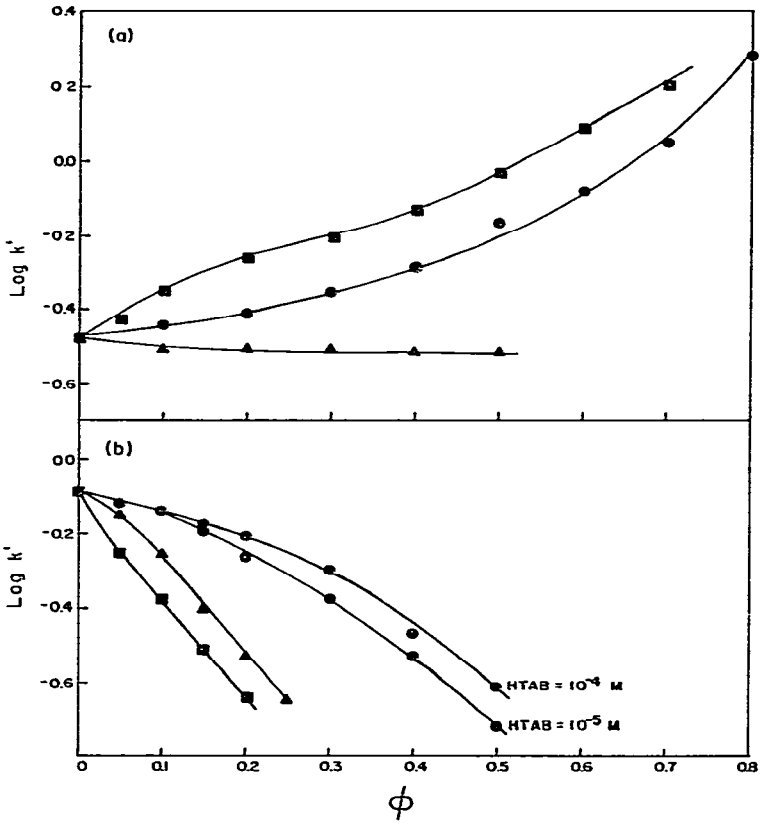

Fig. 3. The effect of organic modifier and organic modifier concentration on the retention of cisplatin on (a) a Partisil 10 SAX column and (b) solvent-generated anion-exchange column (C_{18} column loaded with 0.99 μmol m^{-2} HTAB). Mobile phase consisted of water containing methanol (●), tetrahydrofuran (▲) or 2-propanol (■) as organic modifier. Systems were thermostated at 30°C; flow-rate: 1 ml min^{-1}; solute concentration: 1 mg ml^{-1}.

higher methanol concentrations a sharper decrease in retention occurs, consistent with and corresponding to displacement of HTAB from the column (as indicated earlier in this section). Compared with methanol, retention decreased more rapidly on the solvent-generated ion exchanger with increasing concentrations of THF and 2-propanol. This effect presumably reflects the greater strength of these two modifiers to desorb HTAB compared with methanol.

The results and the differences between the two ion-exchange systems may be explained in terms of the different environments of the two stationary phases in which cisplatin–HTAB interactions occur. With a purely aqueous mobile phase, interactions in the stationary phase of the chemically-bonded ion exchanger occur in a polar environment in close proximity to solvated silanol groups. In contrast, the interactions in the stationary phase of the solvent-generated ion exchanger occur in an apolar hydrophobic environment. When organic modifiers are added to the mobile phase, they will be adsorbed onto the stationary phase[22–24]. These organic molecules will be adsorbed onto the hydrated silanols of the Partisil 10 SAX material as a secondary layer[22,23]. At low organic modifier concentrations, in the solvent-generated ion-exchange system, the organic molecules will be adsorbed onto the alkyl surface[24] and cause a re-orientation of the adsorbed surfactant molecules. At higher concentrations of organic modifiers the adsorbed surfactant will be displaced.

The adsorption of organic modifiers from the mobile phase can be expected to cause a change in the dielectric constant of the stationary phase resulting in a concomitant change in the activity coefficients of both cisplatin and the quaternary ammonium ions. In the case of the chemically-bonded stationary phase, adsorption of organic molecules from the mobile phase can be expected to decrease the polarity of the environment of the two interacting species, increasing their activity coefficients and promoting ion–dipole interactions. This is confirmed by the fact that 2-propanol

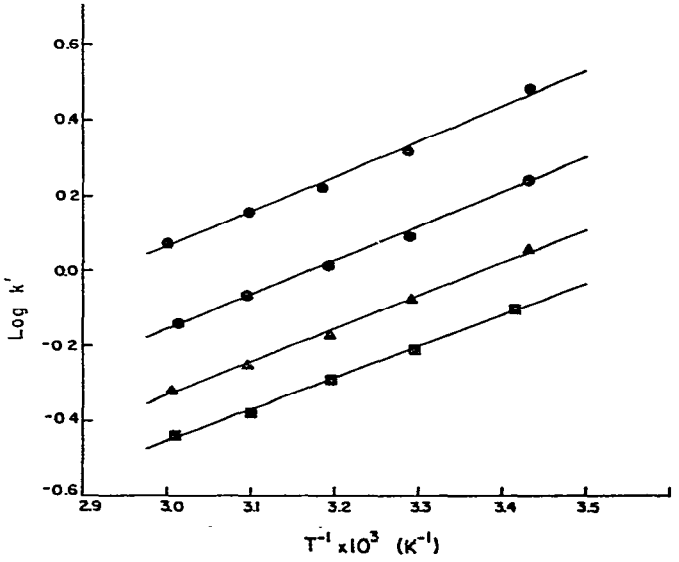

Fig. 4. Modified Van 't Hoff plots of k'_{DDP} on a Partisil 10 SAX column with mobile phase of water–methanol (φ = 0.50 ■; 0.60 ▲; 0.70 ◆; 0.80 ●).

has a greater effect on the retention of cisplatin on the chemically-bonded ion exchanger than does methanol, which is more polar. Conversely, adsorption of organic molecules onto the surface of the solvent-generated ion exchanger will increase the polarity of the stationary phase decreasing the activity coefficients of cisplatin and HTAB in this media resulting in a reduction in the strength of their interactions.

It is not clear from the present study whether the effects of the weakly electron-donating solvent, THF, in the two ion-exchange systems are due to cisplatin–THF interaction in the mobile phase or the influence of THF on cisplatin–HTAB interactions in the stationary phase. Investigations into the role of THF and other more strongly electron donating solvents on the retention of cisplatin on anion exchangers are presently being carried out in our laboratories.

Temperature

The combined effect of temperature and methanol concentration was investigated to obtain information on the thermodynamics of retention of cisplatin by the two ion-exchange systems. For the study of the chemically-bonded system, mobile phases containing volume fractions of 0.5 to 0.8 methanol were used. To prevent desorption of HTAB from the solvent-generated ion-exchange column and maintain

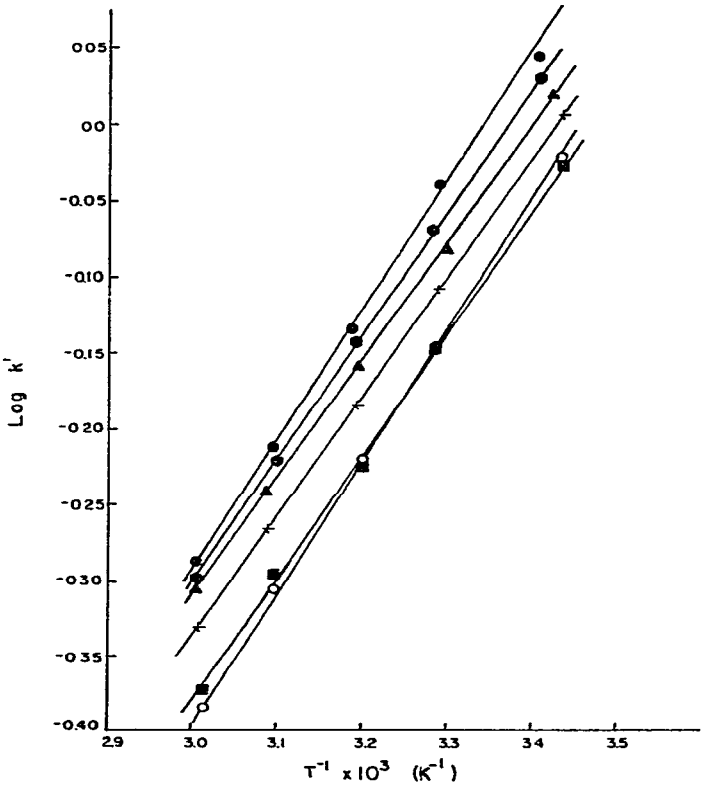

Fig. 5. Modified Van 't Hoff plots of k'_{DDP} on a solvent-generated anion-exchange column (C_{18} column loaded with 0.99 μmol m^{-2} HTAB) with mobile phase of water–methanol containing 10^{-3} mol dm^{-3} HTAB (φ = 0.0 ●; 0.02 ◆; 0.04 ▲; 0.06 +; 0.08 ■; 0.10 ○). Flow-rate: 1 ml min^{-1}; solute concentrations: 1 mg ml^{-1}.

a standard state, the maximum methanol volume fraction in this system was restricted to 0.1. Additionally, a higher HTAB concentration (10^{-3} mol dm^{-3}) was used in the mobile phase to prevent desorption of HTAB at elevated temperatures and higher mobile phase concentrations of methanol.

Under all conditions, retention decreased with increased temperature and the capacity ratios of cisplatin were related to the reciprocal absolute temperature, T, according to a modified Van 't Hoff equation (eqn. 3, Figs. 4 and 5).

$$\log k'_{DDP} = \frac{a}{T} + b \tag{3}$$

The slope and the intercept terms a and b are given by eqns. 4 and 5, respectively.

$$a = -\Delta H\,(2.3\,R)^{-1} \tag{4}$$

$$b = \Delta S\,(2.3\,R)^{-1} + \log V_s\,V_m^{-1} \tag{5}$$

where ΔH, ΔS, R and $V_s\,V_m^{-1}$ are the enthalpy of transfer, the entropy of transfer, the gas constant and the phase volume ratio, respectively. The data were analyzed in terms of eqns. 3 and 4 to obtain the values of b and ΔH in the two ion-exchange systems using different methanol concentrations in the mobile phase (Table II). Due to the difficulties involved in calculating $V_s\,V_m^{-1}$ in these systems[13] the b terms were not analyzed to obtain values of ΔS (eqn. 5).

With the chemically-bonded ion-exchange system, Fig. 4 and Table II show that the enthalpies of transfer become more negative with increasing retention (*i.e.*, increasing methanol concentration). Such systems are said to exhibit linear enthalpy–entropy compensation[25] on ΔG–ΔH coordinates[26,27] according to eqn. 6 (ref. 28):

$$\log k'_{DDP,T} = c\,\Delta H + d \tag{6}$$

where $k'_{DDP,T}$ is the capacity ratio of cisplatin at the harmonic mean temperature of the experiments (in this case $T = 313$°K). The slope and intercept terms for eqn. 6 are given by eqns. 7 and 8, respectively:

$$c = -(2.3\,R)^{-1}\cdot(T^{-1} - \beta^{-1}) \tag{7}$$

$$d = -\Delta G_\beta\,(2.3\,R\cdot\beta)^{-1} + \log V_s\,V_m^{-1} \tag{8}$$

where β is the compensation temperature[22] and ΔG_β is the free energy of transfer when $T = \beta$. Log $k'_{DDP,T}$ was found to be linearly related to ΔH (Fig. 6) over the methanol concentration range, $\varphi = 0.5$–0.8 for the chemically-bonded ion exchanger ($r = 0.998$, $c = -0.265$, $d = -4.45$). This is indicative of a constant retention mechanism for cisplatin on the chemically-bonded ion exchanger over this methanol concentration range. A value of -0.265 for c corresponds to a compensation temperature, β, of 536°K and compares with values of 550 to 770°K obtained by others for reversed-phase[28] and reversed-phase ion-pair[13] HPLC systems.

TABLE II

THERMODYNAMIC PARAMETERS DESCRIBING CISPLATIN RETENTION ON CHEMICALLY-BONDED AND SOLVENT-GENERATED ION EXCHANGERS AS A FUNCTION OF METHANOL VOLUME FRACTIONS

Experimental conditions described in text and Figs. 4 and 5.

Chemically-bonded ion exchanger				*Solvent-generated ion exchanger*			
φ	ΔH^* (kJ mol^{-1})	b^{**}	r^{***}	φ	ΔH^* (kJ mol^{-1})	b^{**}	r^{***}
0.50	−15.7	−2.93	0.997	0.00	−15.6	−2.74	0.998
0.60	−16.2	−2.89	0.998	0.02	−15.2	−2.69	0.999
0.70	−17.0	−2.83	0.999	0.04	−14.4	−2.66	0.999
0.80	−17.7	−2.72	0.999	0.06	−15.0	−2.70	0.999
				0.08	−15.2	−2.77	0.999
				0.10	−16.2	−2.93	0.999

* $a = -\Delta H\,(2.3\,R)^{-1}$ (eqn. 4).

** $\log k' = \frac{a}{T} + b$ (eqn. 3).

*** Correlation coefficient for eqn. 3.

In contrast to the chemically-bonded ion exchanger, cisplatin did not exhibit enthalpy–entropy compensation on the solvent-generated ion exchanger (eqn. 6: r = 0.197). The non-linearity of the relationship embodied in eqn. 6 for the solvent-generated ion exchanger may be due to the contribution of entropy effects arising

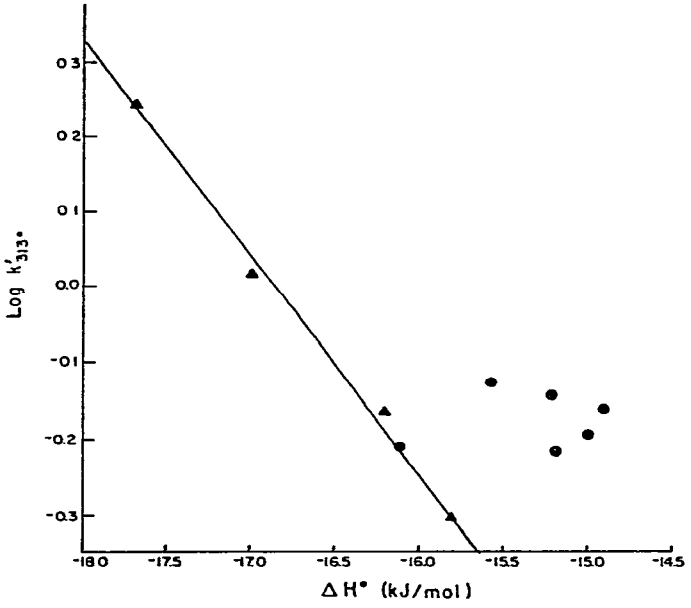

Fig. 6. Enthalpy–entropy compensation plot (log $k'_{313\,K}$–ΔH coordinates) for cisplatin on Partisil 10 SAX (▲) and solvent-generated anion-exchange columns (●). Regression line is for Partisil 10 SAX (eqn. 6). Chromatographic conditions as for Figs. 4 and 5.

from the re-orientation of the adsorbed surfactants with increasing methanol concentration or simply due to the narrow range of enthalpies measured.

In general, peak heights increased and peak shape improved with increasing temperature up to 50°C and this effect was more pronounced with the chemically-bonded ion exchanger (Figs. 7 and 8) than with the solvent-generated system. Very poor peak shape was observed at 70°C in both systems, hence, the upper temperature limit for this study was set at 60°C. The poor peak shape and the reduction in peak height at the higher temperatures was attributed to "on-column" degradation[19] of cisplatin.

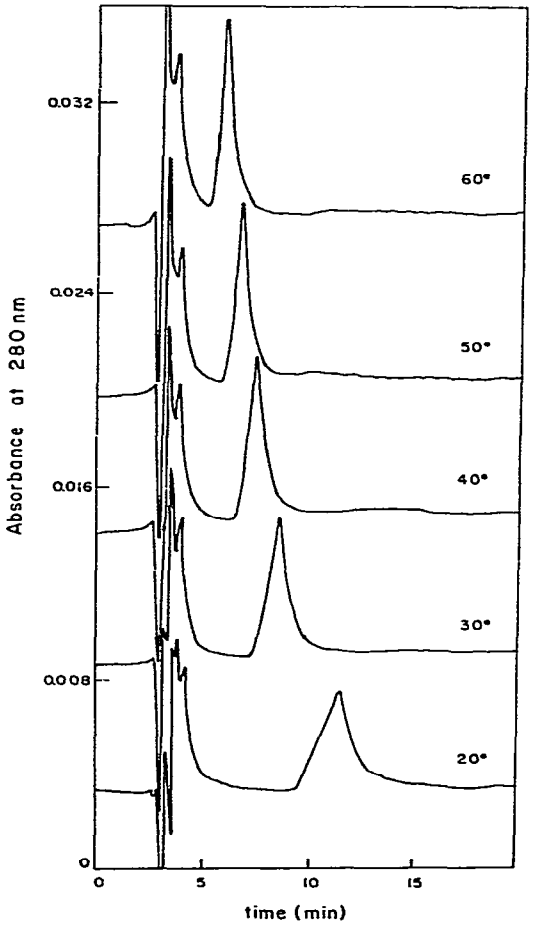

Fig. 7. Chromatograms of cisplatin (1 ml min^{-1}) on Partisil 10 SAX column as a function of temperature. A mobile phase of methanol–water (80:20) was used throughout. Flow-rate: 1 ml min^{-1}.

Cisplatin undergoes replacement of the chloride groups in aqueous solution by nucleophiles (*e.g.*, Br^-)[7,29]. Therefore, a stability study was performed to determine if cisplatin can be eluted intact from the solvent-generated ion-exchange system. Solutions ($6.6 \cdot 10^{-4}$ mol dm^{-3}) of cisplatin were prepared in (a) 0.1 mol dm^{-3} NaCl, (b) 0.1 mol dm^{-3} NaBr and (c) 10^{-4} mol dm^{-3} HTAB and incubated at 30°C ± 0.2°C.

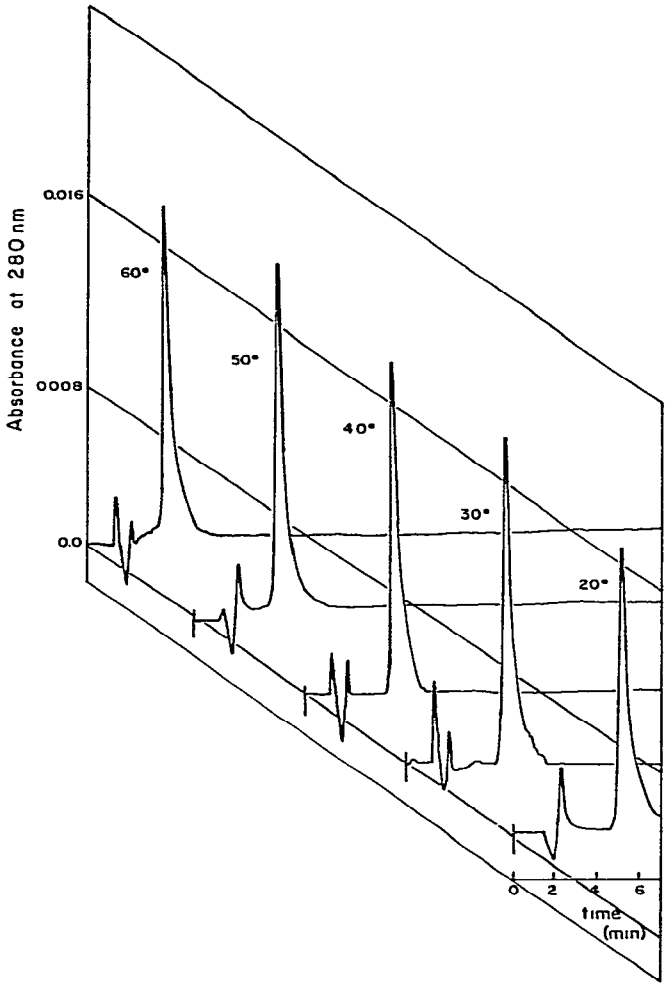

Fig. 8. Chromatograms of cisplatin (1 ml min^{-1}) on a solvent-generated anion-exchange column (C_{18} column loaded with 0.99 mol m^{-2} HTAB). A mobile phase of water containing 10^{-3} mol dm^{-3} HTAB was used throughout.

Aliquots of these incubates were taken over a period of 2 h and injected onto the solvent-generated ion-exchange HPLC system (mobile phase 10^{-4} mol dm^{-3} HTAB; 30°C). The cisplatin concentration was determined by peak height comparison with the solution of cisplatin prepared in 0.1 mol dm^{-3} NaCl. Fig. 9 shows that cisplatin undergoes pseudo-first order degradation; the rate of loss being dependent on the bromide ion concentration. In the case of cisplatin incubated in 0.1 mol dm^{-3} NaBr, a peak ($k' = 1.35$) presumably corresponding to $[Pt(NH_3)_2Br \cdot Cl]^\circ$ or $[Pt(NH_3)_2 \cdot Br_2]^\circ$ was observed and its height increased with incubation time. This peak was not observed in the case of cisplatin stored in 10^{-4} mol dm^{-3} HTAB. In this instance, the major degradation product eluted with a capacity ratio of 0.10 and corresponded to the positively charged aquation product $[Pt(NH_3)_2Cl, H_2O]^+$. These results suggest that the primary degradation product generated "on-column" in the

presence of a low concentration of bromide is the aquated derivative. The residency time of cisplatin on-column in these studies (3–6 min at 30°C) is sufficiently short that $<5\%$ degradation of parent drug would occur before elution. This conclusion is confirmed by the results obtained previously[6,19] which showed that the aquation reaction is the rate limiting step in the displacement of chloride from cisplatin in dilute aqueous solution of weak to moderate nucleophiles (*e.g.*, Br^-). These results further suggest that the poor peak shapes observed at 70°C arise in both ion-exchange systems from aquation reactions.

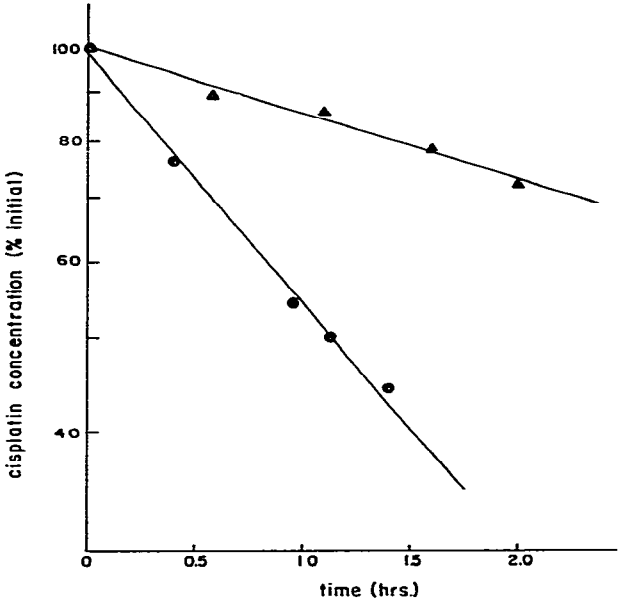

Fig. 9. Loss of cisplatin ($6.6 \cdot 10^{-4}$ mol dm^{-3}) from aqueous solutions which contained 0.1 mol dm^{-3} NaBr (●) and 10^{-4} mol dm^{-3} HTAB (▲) *vs.* time at 30°C. Other conditions as in the text.

CONCLUSIONS

Cisplatin is retained on both chemically-bonded and solvent-generated ion exchangers. The mechanism of retention in both systems is most readily explained in terms of ion–dipole interactions between cisplatin and the stationary phase quaternary ammonium groups. Interactions in the mobile phase or with other regions of the stationary phase appear to play minor roles in the retention mechanisms.

Hydroxylic modifiers appear to increase the strength of these ion–dipole interactions with quaternary ammonium ions bonded to silica and appear to decrease the strength of these interactions with quaternary ammonium ions physically adsorbed onto a hydrophobic surface. These effects may be explained in terms of the environment in which these ion–dipole interactions take place and the influence exerted by the alcohols which are extracted into the stationary phase. The role of electron donating solvents in these systems is less clear and is the subject of future study. In the case of the solvent-generated ion exchanger, organic modifiers can also influence the retention of the analyte due to their ability to displace the adsorbed surfactants.

Cisplatin exhibited linear enthalpy–entropy compensation over the range 0.5 to 0.8 (by volume) methanol in water on the chemically-bonded ion exchanger. Linear enthalpy–entropy compensation was not observed in the solvent-generated system, however, it should be noted that the study was performed at much lower methanol concentrations.

It is tempting to suggest that solvent-generated ion exchangers would behave similarly to chemically-bonded systems at higher methanol concentrations. However, concentrations of surfactant well in excess of their solubility in aqueous methanol would be required in the mobile phase to maintain a sufficient concentration of surfactant in the stationary phase to test this hypothesis.

Finally, the retention behavior of cisplatin on solvent-generated ion-exchange systems can be manipulated without the need for large amounts of organic modifiers, offering significant advantages in the interfacing of such systems with extraction-based reaction detectors. Such detectors are presently being developed in our laboratories to monitor trace levels of platinum in biological fluids.

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