Journal of Chromatography, 641 (1993) 101–113
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 031

Chromatographic behaviour of some platinum(II) complexes on octadecylsilica dynamically modified with a mixture of a cationic and an anionic amphiphilic modifier

M. Macka* and J. Borák

Lachema, Research Institute of Fine Chemicals, Karásek 28, 62133 Brno (Czech Republic)

(First received October 15th, 1992; revised manuscript received January 18th, 1993)

ABSTRACT

A method for the separation of anionic, uncharged and cationic platinum(II) complexes in one run was developed. The separation is based on an octadecylsilica sorbent dynamically modified with a mixture of an anionic (octanesulphonate, OS) and a cationic (tetrabutylammonium, TBA) amphiphilic modifier dissolved in aqueous mobile phase. Unless a tenfold excess of TBA is used, a sorbent with prevailing cation-exchange properties is generated. The retentions of the anionic complexes are, however, greater than retentions that would result from a pure ion-exclusion mechanism on a cation-exchange column and enable the anionic complexes to be separated also. Effects of the mobile phase composition parameters (concentration of the amphiphilic modifiers, phosphate and pH) on the separation were studied. Applications to the purity determination of cisplatin and to the reactions of the platinum(II) complexes in solutions are presented.

INTRODUCTION

Cisplatin [*cis*-diamminedichloroplatinum(II), CDDP] and carboplatin [*cis*-diammine-1,1-cyclobutanedicarboxylateplatinum(II), Pt-CBDCA] (Fig. 1) are widely used cytostatic agents [1]. Regarding the synthesis and stability of cisplatin and carboplatin, several other platinum(II) complexes (starting compounds, intermediates, side-products and products of ligand-exchange reactions in solutions) should be considered (Fig. 1).

Owing to the kinetic stability of platinum complexes [2], separation methods such as liquid chromatography can be applied. Most HPLC methods utilize reversed-phase sorbents dynamically modified with a cationic [3–9] or an anionic [6,10–17] amphiphilic modifier dissolved in the mobile phase. However, solutes having the same charge as the amphiphilic modifier generally elute with negligible retentions that do not allow the separation and quantification of these complexes. The charges of the platinum(II) complexes **I–XI** (Fig. 1) vary from −2 to +2 and therefore their simultaneous determination has not been possible using a reversed-phase sorbent modified with either a cationic or an anionic amphiphilic modifier.

Regarding the simultaneous separation of anionic and cationic platinum(II) complexes, tetrachloroplatinate and the positively charged aquation products of cisplatin (**X** and **XI**) have been previously separated by TLC on silica, however, the complexes **X** and **XI** were retained at the start [18].

In principle, a cation-exchange column plus an

* Corresponding author. Present address: Analytical Department WAQ-1, LONZA AG, CH-3930 Visp, Switzerland.

0021-9673/93/$06.00 © 1993 Elsevier Science Publishers B.V. All rights reserved

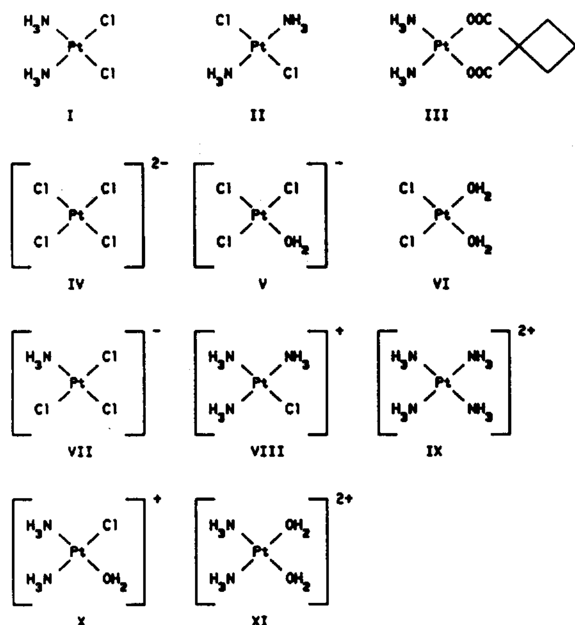

Fig. 1. Structures of platinum (II) complexes, their charges and symbols used in graphs if not specified: **I** = *cis*-diamminedichloroplatinum(II) CDDP), 0 (○); **II** = *trans*-diamminedichloroplatinum(II) (transplatin, TDDP) 0 (●); **III** = *cis* - diammine - 1,1 - cyclobutanedicarboxylateplatinum (II) (carboplatin, Pt-CBDCA), 0 (□); **IV** = tetrachloroplatinum(II), −2 (□); **V** = aquatrichloroplatinum(II), −1 (△); **VI** = *cis*-diaquadichloroplatinum(II), 0 (+); **VII** = amminetrichloroplatinum(II), −1 (▲); **VIII** = triamminechloroplatinum(II), +1 (◇); **IX** = tetraammineplatinum(II), +2 (◆); **X** = *cis*-diammineaquachloroplatinum(II), +1 (▽); **XI** = *cis*-diamminediaquaplatinum(II), +2 (▼).

anion-exchange column connected in series could be used to achieve sufficient retentions of both positively and negatively charged complexes. This arrangement was previously used in the separation of cisplatin and its aquation products **X** and **XI** [19]. In this case, however, the anion-exchange column was obviously needed to increase the retention of cisplatin ascribed to ion–dipole interactions of the quaternary ammonium groups and the uncharged but polar molecule (dipole moment 5.3 D) of cisplatin [3].

A mixture of both cationic (ammonium type) and anionic amphiphilic modifiers dissolved in a mobile phase was used to dynamically modify a silica sorbent [20–22]. This was based on the primary sorption of the long-chain alkylammonium amphiphilic modifier on the silica caused by the affinity to the silanol groups and secondary sorption of the anionic amphiphilic modifier due to hydrophobic interactions with the sorbed cationic modifier. On changing the ratio of the concentrations of the oppositely charged amphiphilic modifiers, the character of the sorbent changed from an anion exchanger to a cation exchanger and *vice versa*, making possible the generation of sorbents with a variety of selectivities.

Applications of mobile phases containing both anionic and cationic amphiphilic modifiers to chromatography on reversed-phase sorbents are limited to additions of triethylamine or tetramethylammonium to a mobile phase containing an anionic amphiphilic modifier, obviously to prevent peak tailing when chromatographing bases [23,24]. Another interesting application of a mobile phase containing both an anionic and a cationic amphiphilic modifier is to speed up the transition between two rotamers of the dipeptide enalapril, thus making possible the chromatography of the analyte at ambient temperature [25]. In this case the mobile phase, however, contained a high percentage (60–70%) of methanol, which usually shifts the sorption equilibria of surfactants on reversed-phase sorbents in favour of the mobile phase.

The aim of this work was to study a chromatographic system using an octadecylsilica sorbent dynamically modified with a mixture of a cationic and an anionic amphiphilic modifier dissolved in the mobile phase that could be used to achieve sufficient retentions of both the positively and negatively charged platinum(II) complexes. Such a separation system will presumably differ substantially from a mixed-bed ion exchanger or a pair of columns filled with cation exchanger and anion exchanger as the charges of the oppositely charged amphiphilic modifiers in the stationary phase will probably be considerably compensated for owing to the formation of ion pairs. However, the idea was that the retentions of the ions that under normal conditions would be excluded could be increased owing to mixed interactions with the dynamically generated polar stationary phase.

EXPERIMENTAL

Materials

Sodium octanesulphonate (NaOS), tetrabutylammonium hydrogensulphate ($TBAHSO_4$), sodium dihydrogenphosphate, potassium chloride and fructose were of analytical-reagent grade (Merck). Dextran T 2000 (standard for size-exclusion chromatography, $M_r = 2 \times 10^6$) was obtained from Pharmacia, (Uppsala, Sweden).

Potassium tetrachloroplatinate(II) (**IV**, Fig. 1) was purchased from Aldrich, (Milwaukee, WI, USA), assay >99.99%. It was used as a 1 mg/ml solution in 0.15 *M* potassium chloride. The assay in the solution was 87% (HPLC, internal normalization 210 nm); the remainder was the aquation product **V**.

Aquatrichloroplatinate(II) (**V**, Fig. 1) complex was prepared by aquation of a 1 mg/ml potassium tetrachloroplatinate(II) solution in water at 25°C. The solution was used after 3 days. The assay was 75%, the remainder was **IV** (15%) and **VI** (8%) (HPLC, internal normalization 210 nm).

cis-Diaquadichloroplatinum(II) (**VI**, Fig. 1) was prepared according to ref. 26 in alkaline medium. A 1 mg/ml solution of potassium tetrachloroplatinate(II) in 0.01 *M* sodium hydroxide was used after ageing for 1 day. The assay was 70% (HPLC, internal normalization 210 nm); the remainder was **V** (17%), an unknown compound (*ca.* 10%) and **IV** (*ca.* 3%). The solution was unstable and after several days solid Pt^0 was formed. The solution could not be acidified in order to improve the stability of the solution as the equilibrium was then shifted back to **V**.

Cisplatin (**I**, Fig. 1) and carboplatin (**III**, Fig. 1) were obtained from Lachema (Brno, Czech Republic). The assay was >99.5% (HPLC).

trans-Diamminedichloroplatinum(II) (**II**, Fig. 1) complex was prepared according to ref. 27. It contained <0.1% of CDDP and the assay was >99% (HPLC, internal normalization 210 nm).

cis-Diamminediaquaplatinum(II) (**XI**, Fig. 1) complex was prepared by a modified procedure according to Dhara [28]. To a CDDP solution (concentration 1 mg/ml), solid silver sulphate was added at a CDDP:Ag molar ratio of 1:2.2. The mixture was shaken and allowed to stand overnight. After centrifugation the supernatant was acidified with sulphuric acid to pH 2 and stored in a dark bottle. The chromatogram showed one major peak and less than 1% CDDP and *cis*-diammineaquachloroplatinum(II). The advantage of using silver sulphate over nitrate (which had been used previously [17] and partly also in this work) is that the reference sample does not contain a highly absorbing anion such as nitrate.

cis-Diammineaquachloroplatinum(II) (**X**, Fig. 1) complex was prepared by modifying the above procedure so that silver sulphate was added to CDDP in molar ratio of only 1:1.1. The chromatogram showed one major peak, 21% of *cis*-diamminediaquaplatinum(II) and 16% of CDDP.

Amminetrichloroplatinum(II) (**VII**, Fig. 1) was prepared as the potassium salt according to ref. 27. The assay was 99% (HPLC, internal normalization 210 nm).

Triamminechloroplatinum(II) (**VIII**, Fig. 1) was prepared by partial ammination of CDDP (concentration 1 mg/ml) in 3.3 m*M* ammonium chloride at 90°C. Small portions of 0.2 *M* ammonia solution were used, so that the pH did not exceed 7.5. The reaction was stopped when the amount of side-product, tetraammineplatinum(II), formed was about the same as that of unreacted CDDP. The product was purified three times by fractional precipitation from a water–2-propanol mixture. The assay of triamminechloroplatinum(II) was 55% (HPLC, internal normalization 210 nm); the remainder was CDDP (13%, HPLC, external standard), TDDP (9%, HPLC, external standard) and tetraammineplatinum(II) (26%, HPLC, external standard).

Tetraammineplatinum(II) (**IX**, Fig. 1) was prepared according to ref. 27. The assay was >99% (HPLC, internal normalization 210 nm).

As potentially all platinum(II) complexes are light sensitive [29] and some are even extremely sensitive [30], all solutions were prepared in vials of dark-brown glass. Also, the use of ultrasound to facilitate the dissolution of the platinum(II)

complexes was avoided, as at least in some instances decomposition by ultrasound is evident [30]. Unless specified, all solutions of platinum(II) complexes were prepared and stored at ambient temperature.

The HPLC method described here was used to characterize the reference samples of the platinum complexes **I**, **II** and **IV–VII**. The HPLC method reported recently [17] was used to characterize the reference samples of the platinum(II) complexes **III** and **VIII–XI**.

Apparatus and chromatographic conditions

The system used was a Hewlett-Packard Model 1090 chromatograph consisting of an HP 79880A diode-array detector, a DR5 binary pumping system, an HP 79846A variable-volume injector equipped with a 25-μl syringe and an HP 79847A autosampler; if not specified otherwise, volumes of 10 μl were injected. For system control and data evaluation an HP 79994A Workstation based on an HP 310 computer was used.

A stainless-steel column (250 × 4 mm I.D.) packed with Silasorb SPH C_{18} (surface area *ca.* 300 m^2/g, particle diameter 7.5 μm) (Lachema) was used. The column temperature was maintained at 30°C.

The mobile phases contained 0–4 m*M* octanesulphonate, 0–6 m*M* tetrabutylammonium and 5–200 m*M* dihydrogenphosphate. The pH of the mobile phase was adjusted with sodium hydroxide to the desired value. The composition of the final mobile phase for the analysis of impurities in cisplatin was as follows: (A) 4 m*M* sodium octanesulphonate, 6 m*M* tetrabutylammonium (pH 5.9) and (B) the same as A plus 0.20 *M* dihydrogenphosphate (pH 5.9).

Analyses were run under isocratic conditions (90% A–10% B) or with an ionic strength gradient as follows [17]:

gradient programme I:

time (min)	0	3	7	8	8.1
B (%)	5	5	100	100	5

gradient programme II:

time (min)	0	4	8	9	9.1
B (%)	10	10	100	100	10

The stationary phase was generated by pumping the mobile phase through the column until the retention times were constant (usually 1–2 h). If not specified otherwise, the flow-rate was 1.0 ml/min, resulting in a column inlet pressure of *ca.* 90 bar.

Determination of the limit of detection

Limits of detection (LODs) of the complexes were calculated for a signal equal to ten times the standard deviation of the baseline noise. The baseline noise was evaluated from the raw data by a program using statistics as described previously [22].

Determination of the void volume of the column

Fructose (10 μl of a 1% solution in the mobile phase) was injected and detected at 210 nm; the elution volume was 2.00 ± 0.02 ml. Further, dextran T 2000 (10 μl of a 1% solution in the mobile phase) was injected and detected at 190 nm or with an SP 6040 refractometric detector (Spectra-Physics, Darmstadt, Germany) connected to the workstation via an HP 35900 dual-channel interface. As detection both at 190 nm and with the refractometer gave the same values, only photometric detection was subsequently used. With all mobile phases the elution volume of dextran T 2000 was 1.50 ± 0.02 ml.

The thermodynamic definition of the capacity factor leads to negative capacity factors for excluded ions. However, if the elution volume of fructose were to be used as the value of the void volume, the capacity factors of the excluded anions would be $k' < 0$ and could not be used for log k' graphs. For this reason the elution volume of dextran T 2000 was used to calculate the capacity factor k' of all the solutes and is used throughout this paper to express retentions. Knowing the elution volumes of fructose and dextran T 2000 the capacity factors can be recalculated if needed.

Determination of the amounts of sorbed amphiphilic modifiers

The column was flushed with 20 ml of water and then the sorbed surfactants were eluted with 50 ml of methanol and the eluate was evaporated to dryness. The residue was analysed for nitro-

gen (Dumas) and sulphur (Schöninger). From the percentage of N and S and the mass of the eluted amphiphilic modifiers, the corresponding amounts of the sorbed OS and TBA were calculated. The completeness of the elution of TBA was checked by further elution (50 ml) with 0.1 g/l lithium perchlorate solution in methanol and evaporation to dryness. The residue was analysed for nitrogen (Kjeldahl). Its content was lower than *ca.* 5% relative to the amount of TBA determined in the first step.

After all the experiments had been completed the column was flushed with water and methanol and the sorbent was pressed out of the column and dried to constant mass at 110°C. The column contained 2.04 g of the sorbent.

RESULTS AND DISCUSSION

Choice of the modifiers

Preliminary studies on chromatographic systems using octadecylsilica and buffered mobile phases containing one anionic amphiphilic modifier (octanesulphonate or dodecyl sulphate) and one cationic amphiphilic modifier (tetrabutylammonium, dodecyltrimethylammonium or hexadecyltrimethylammonium) showed two major sources of problems:

(i) the precipitation of the oppositely charged long-chain ($n_C \geq 12$) amphiphilic modifiers (surfactants) in aqueous media limits their application either to extremely low concentrations or to micellar liquid chromatography with mobile phases containing both anionic and cationic surfactants but one (*e.g.*, sodium dodecyl sulphate) being in excess over the other (*e.g.*, HTMABr) and thus solubilizing the formally uncharged very lipophile ion pairs [22];

(ii) the lipophilicity of the two oppositely charged amphiphilic modifiers must be balanced, so that both amphiphilic modifiers can be sorbed on the reversed phase and influence the resulting sorbent ion-exchange properties. We preferred a column that would behave generally as a cation exchanger, because of the complexes to be separated the majority are cationic. Octanesulphonate and tetrabutylammonium were a suitable combination adopted in further work.

Effect of molar fraction of TBA on the amounts of sorbed TBA and OS

The amounts of the sorbed amphiphilic modifiers are plotted as a function of x_{TBA} are plotted in Fig. 2a. A pronounced synergistic effect on the sorption of the two oppositely charged amphiphilic modifiers is observed, which can be ascribed to the formation of formally uncharged ion pairs in the stationary phase. In the range $0.25 < x_{TBA} < 0.75$ the concentration of both amphiphilic modifiers in the stationary phase decreases with increasing x_{TBA}, suggesting that the sorption of OS is probably primary and TBA acts more as a counter ion. However, with increasing $x_{TBA} > 0.9$, TBA is likely also to be sorbed directly on the octadecylsilica.

The sorbed OS and TBA as calculated from the elemental analysis and further their difference as the approximate capacity of the column (charge compensation of the oppositely charged modifiers in the stationary phase) are shown in Fig. 2b. A sorbent with a significantly lower capacity is generated when a mixture of OS and TBA is used instead of only one of them.

Effect of molar fraction of TBA on the retention and selectivity

The influence of x_{TBA} as the molar fraction of the TBA in the total (TBA + OS) amphiphilic modifier concentration of 4 m*M* is illustrated for the cationic complexes and carboplatin in Fig. 3a and for the anionic and the remainder of the neutral complexes in Fig. 3b. As was expected, as a result of the presumable charge compensation of the oppositely charged amphiphilic modifiers in the stationary phase, it is possible to achieve significant retentions either of cationic ($x_{TBA} \rightarrow 0$) or anionic solutes ($x_{TBA} \rightarrow 1$). At $x_{TBA} < 0.8$ the column behaves generally as a cation exchanger, then it reverses its ion-exchange properties and at $x_{TBA} > 0.9$ the column behaves as an anion exchanger.

The curve for cisplatin in Fig. 3b shows slight "anion-like" behaviour. Such a behaviour of the formally uncharged molecule **VI** (Fig. 3b) is caused by the dissociation of the H_2O ligand, resulting in a negative charge of the molecule. The pK_a is reported to be 6.5 at 35°C [31]. With **I**, however, ion–dipole interactions are consid-

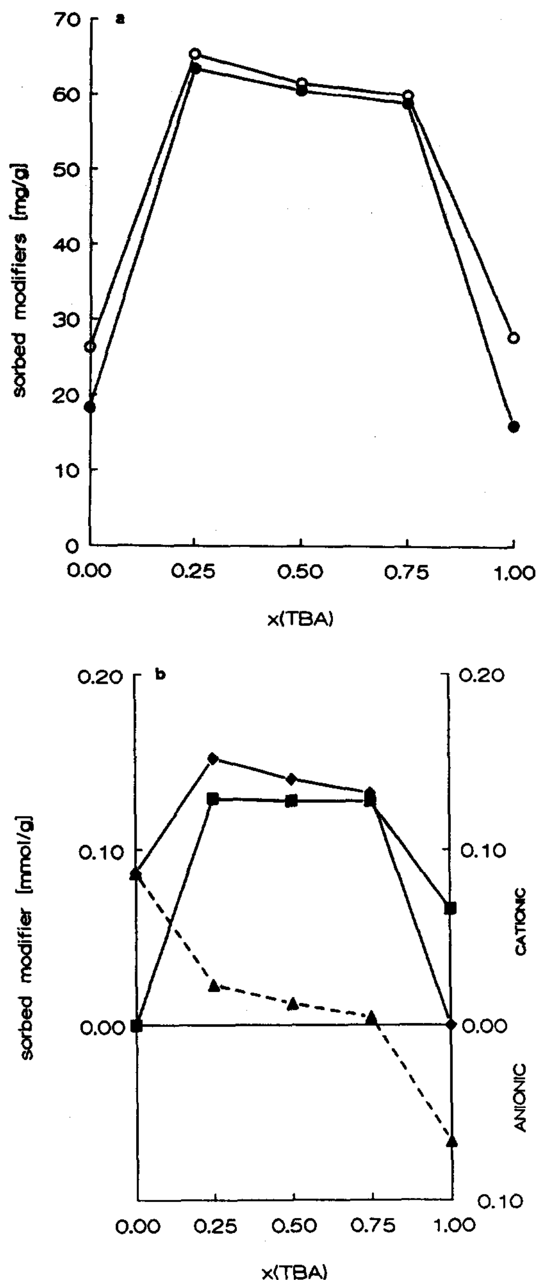

Fig. 2. (a) Amounts of sorbed modifiers *versus* x_{TBA}: (○) = mass of residue including potassium and phosphate as counter ions; ● = summed amounts of OS + TBA as calculated from the elemental analysis. Chromatographic conditions: eluent, OS and TBA in 0.02 *M* phosphate, pH adjusted to 5.9. (b) Amount of sorbed OS and TBA and the column capacity *versus* x_{TBA}: the amount of sorbed surfactant as calculated from the elemental analysis for (◆) OS and (■) TBA; ▲ = ion-exchange capacity of the sorbent. Chromatographic conditions as in (a).

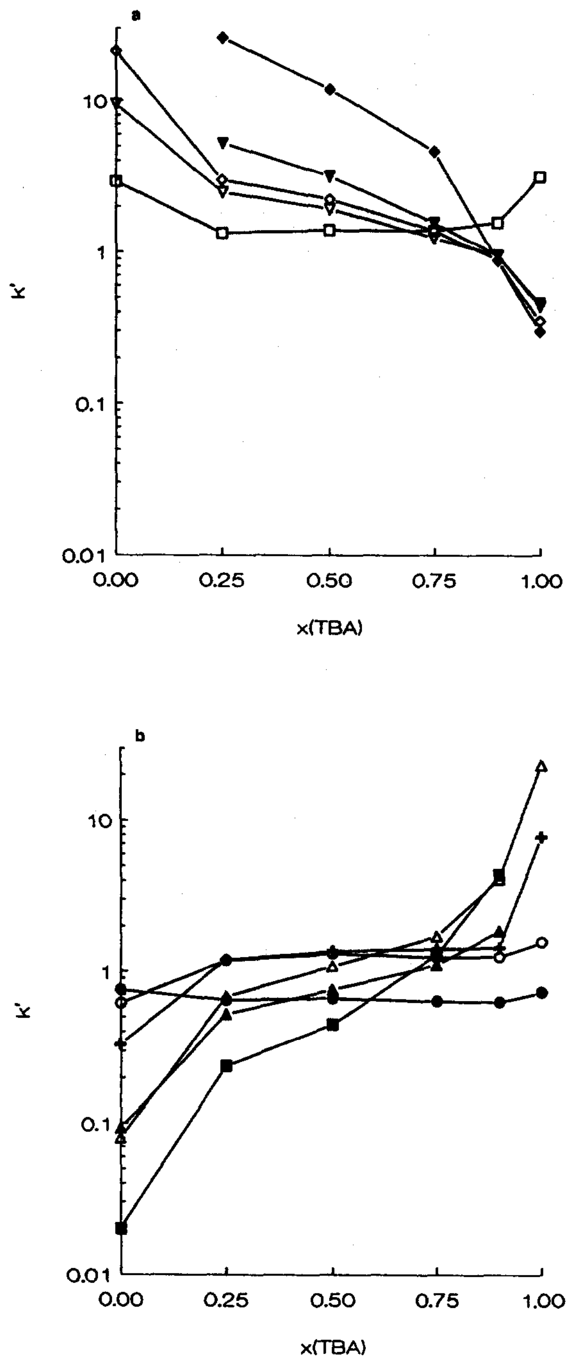

Fig. 3. (a) Retentions (k') of the cationic platinum(II) complexes (**VIII–XI**) and the uncharged **III** *versus* x_{TBA} as the fraction of TBA in the total (TBA + OS) amphiphilic modifier concentration (4 m*M*). (b) Retentions (k' values) of the anionic platinum(II) complexes (**IV**, **V** and **VII**) and the uncharged **I**, **II** and **VI** *versus* x_{TBA}. Symbols as in Fig. 1. Chromatographic conditions as in Fig. 2.

ered to be the cause of the behaviour [3]. In agreement with this, columns with anion-exchange properties (mostly reversed-phase sorbents dynamically modified with a cationic amphiphilic modifier) allowing sufficient retention of **I** to be achieved are generally used.

For a mixture of both cationic and anionic platinum(II) complexes a clearly better separation is achieved when using a mixture of both OS and TBA in the mobile phase instead of only OS or TBA. The separation of anions, expressed as the resolution of several pairs of anionic analytes (Fig. 4) has an optimum and then deteriorates again as the region $0.8 < x_{TBA} < 0.9$ is approached where the elution order changes. On the other hand, for the cationic complexes illustrated with the examples of **VIII** and **IX** it is not surprising that the highest resolution is achieved at $x_{TBA} \rightarrow 0$ as their retentions are maximum there. The resolution of the pair **VIII–IX** in the whole range $x_{TBA} < 0.8$ is, however, sufficient and hence the optimum range for the separation of both anionic and cationic solutes is $x_{TBA} \approx$ 0.25–0.65.

Effect of phosphate and TBA concentration at constant OS concentration

From the log k'–log $c(PO_4)$ dependence (Fig. 5) for the cationic analytes it can be concluded that their predominant retention mechanism is ion exchange. As expected, the slopes of the curves for the +1 (**VIII** and **X**) and the +2 (**IX** and **XI**) charged complexes clearly differ.

Concerning the retentions of anions (**IV**, **V** and **VII**) under these conditions, their capacity factors rapidly increase with addition of TBA (Fig. 6). From the character of the curves (IV, V and VII) at low TBA concentration ($c_{TBA} \leq 1$) and from the negative rather than positive slopes of their log k'–log $c(PO_4)$ dependence (Fig. 5), ion exclusion can be concluded to be the predominant retention mechanism. This is also supported by the fact that their capacity factors at low TBA concentration are less than 0.3, which is the capacity factor of the unretained low-molecular-mass solute fructose (high-molecular-mass dextran T 2000 is used as a void volume marker). However, for $c_{TBA} > 1$ the anions are sorbed on the stationary phase, also. It can be concluded

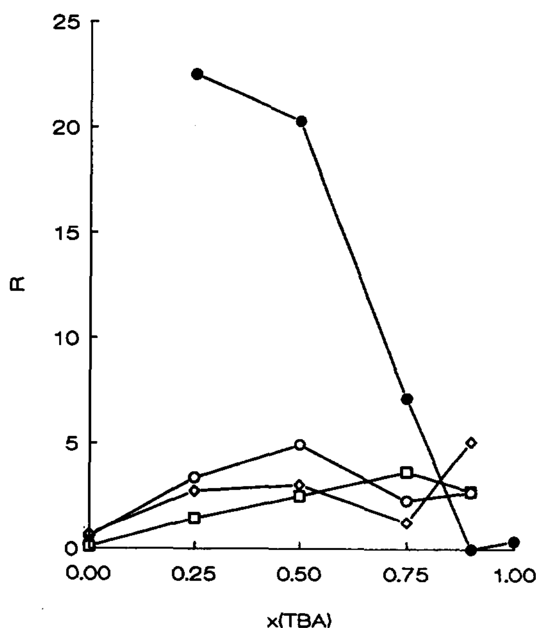

Fig. 4. Resolution (R value) of pairs of complexes *versus* x_{TBA}: ○ = R(IV–V); ◇ = R(IV–VII); □ = R(V–VII); ● = R(VIII–IX). Chromatographic conditions as in Fig. 2.

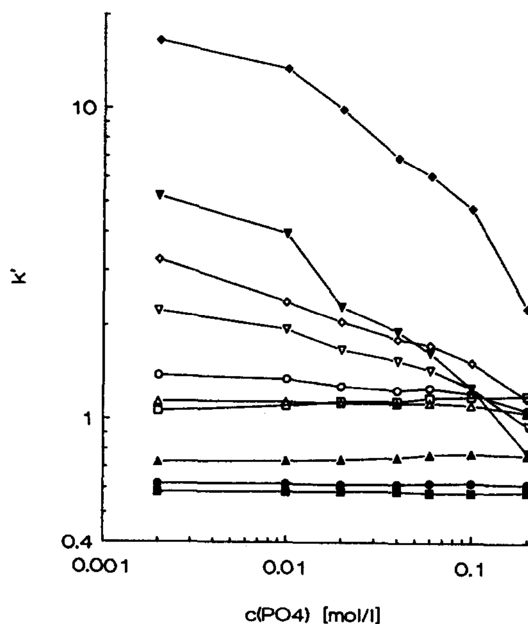

Fig. 5. Retentions (k') of the platinum(II) complexes *versus* phosphate concentration. Symbols as in Fig. 1. Chromatographic conditions: eluent, 0.002–0.2 M phosphate in 4 mM OS and 6 mM TBA, pH adjusted to 5.9.

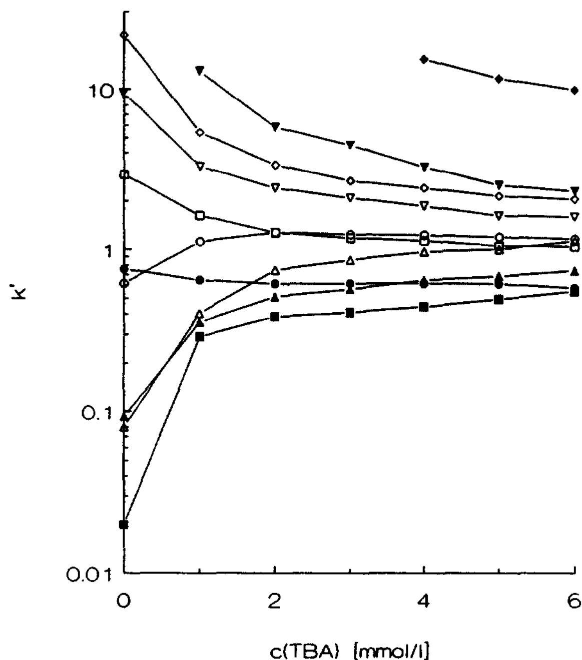

Fig. 6. Retentions (k') of the platinum(II) complexes *versus* concentration of TBA. Symbols as in Fig. 1. Chromatographic conditions: eluent, 0 and 6 m*M* TBA in 4 m*M* OS–0.02 *M* phosphate, pH adjusted to 5.9.

that the retention mechanism of anions under the conditions of prevailing cation-exchange character of the stationary phase is complex, involving ion exclusion, ion exchange and partitioning, probably based on ion–dipole interactions with the polar layer of amphiphilic modifiers covering the octadecylsilica.

The retention of the uncharged molecule of **II** possessing no dipole moment is low and almost uninfluenced by any parameter of the mobile phase composition.

The slightly negative slope of the $\log k'$–$\log c(PO_4)$ graph of the complex **III** (Fig. 5) can be explained by the salting-out effect due to the hydrophobic interactions of the cyclobutanedicarboxylate ligand. The molecule also shows a "cation-like" behaviour (Figs. 2a and 6). As a result of the decrease in retention after addition of TBA to the mobile phase the separation of carboplatin from cisplatin begins to be a problem, and therefore for the separation of cisplatin and carboplatin we preferred mobile phases with low TBA content or only with OS [17].

Effect of pH

The retentions of the complexes containing at least one water molecule as ligand (**V**, **VI**, **X** and **XI**) are pH dependent (Fig. 7), which is not the case with the remaining solutes. The reason is the deprotonation reaction on the water ligand that causes a change in the effective charge of the molecule. As the charge of the molecule becomes more negative with increasing pH of the mobile phase, their retentions decrease. For **XI**, pK_{a1} is reported to be 5.56 and pK_{a2} 7.32 at 20°C [32]. The effect of pH was advantageously applied to achieve the separation of **VIII** from **X** and of **IX** from **XI**, which originally failed at pH 3.2. A pH of 5.9 was found to be optimum for the separation of **I**, **X** and **VIII** (see Fig. 9).

The unknown peak **U1** in the chromatogram of a 16-month-old solution of **IV** in 1 m*M* hydrochloric acid (Fig. 8) obviously also corresponds to an aqua complex, because its retention is pH dependent (Fig. 7). It may be the *trans*-diaquadichloroplatinum(II) which is formed, as opposed to the *cis*-complex, much more slowly [25]. Note

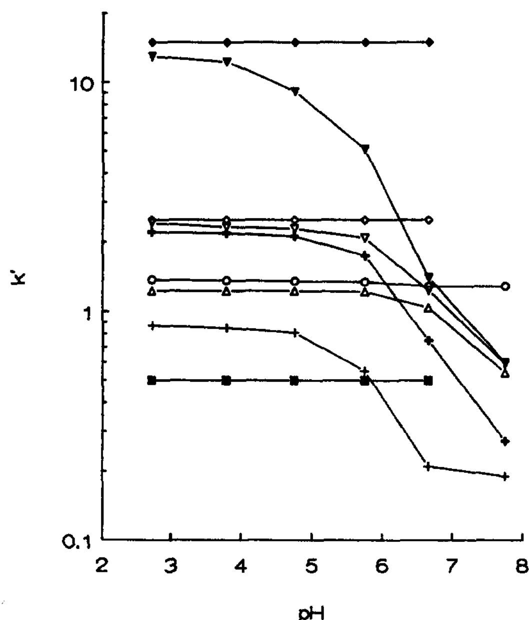

Fig. 7. Retentions (k') of the platinum(II) complexes *versus* pH of the mobile phase. + = Presumably *trans*-diaquadichloroplatinum(II); other symbols as in Fig. 1. Chromatographic conditions: eluent, 4 m*M* OS and 6 m*M* TBA in 0.02 *M* sodium dihydrogenphosphate, pH adjusted to desired value.

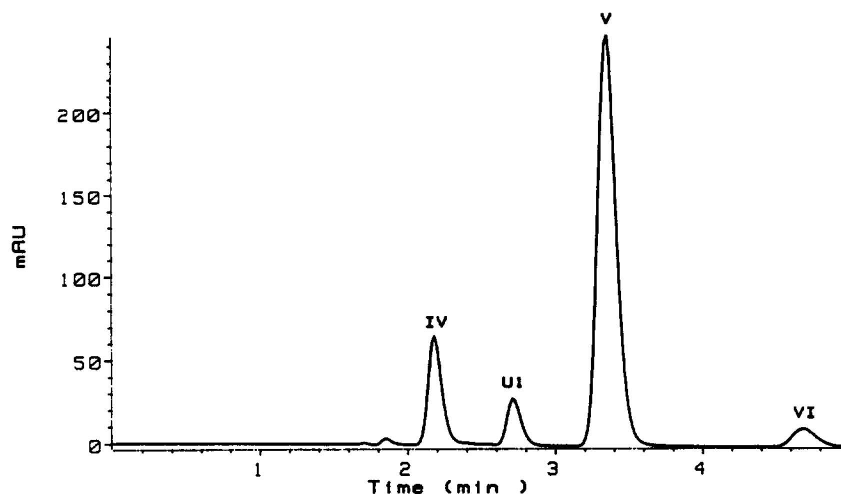

Fig. 8. Chromatogram of tetrachloroplatinate (1 mg/ml solution in 1 mM HCl, ambient temperature, 16 months). For peak identification, see Fig. 1. Chromatographic conditions as in Fig. 10, except pH = 4.8.

that here at pH 4.8 the complex **VI** is eluted later than at pH 5.9 (see Fig. 11).

Applications

The separation of a model mixture of the complexes **I**, **II**, **IV**, **V** and **VII–XI** shown in Fig. 9 illustrates the separation potential of the method.

Purity of cisplatin. According to the USP XXII [33], the impurities **II** and **VII** are determined in **I** using two different chromatographic systems. This method makes it possible to determine both **II** and **VII** in **I** in one run (Fig. 10). The limit of detection expressed relative to **I** is 0.005% for both **II** and **VII**. The method that has been used in our laboratory for more than 1 year offered reproducible selectivities and retentions from column to column. The application of the method to the study of the decomposition of cisplatin in aqueous solutions containing chlorides by ultrasonic energy and light will be reported separately [30].

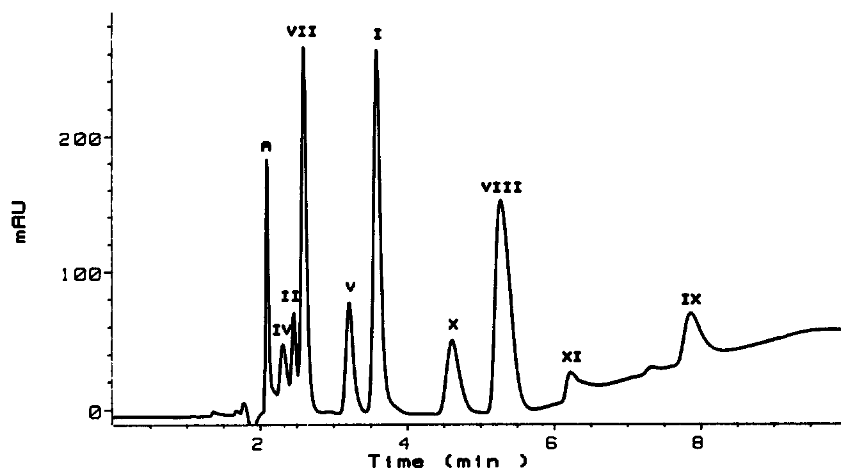

Fig. 9. Chromatogram of a model mixture of complexes **I**, **II**, **IV**, **V** and **VII–XI**. For peak identification, see Fig. 1; A = nitrate as impurity. Chromatographic conditions: injection, 20 μl; eluent, 4 mM OS and 6 mM TBA, pH adjusted to 5.9; ionic strength gradient I. For other conditions, see Experimental.

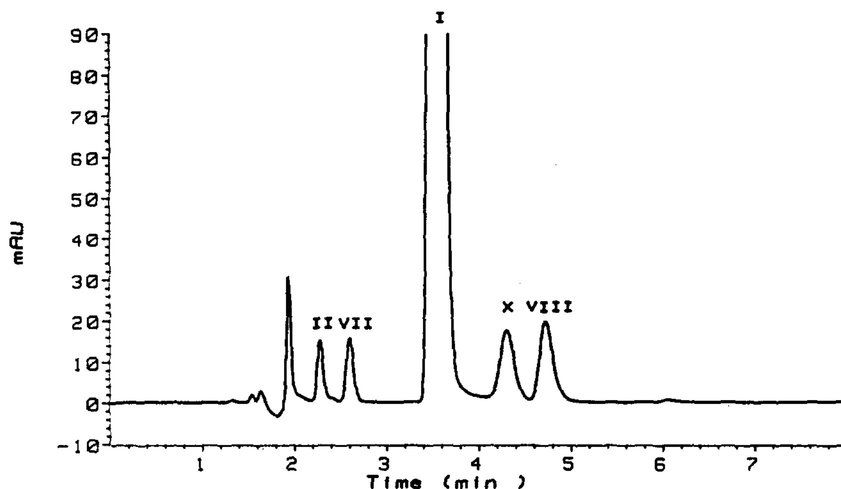

Fig. 10. Chromatogram of cisplatin drug substance, 1 mg/ml solution in 0.15 *M* KCl, with 0.5% of each impurity **II**, **VII** and **VIII** added. **X** is present as a result of aquation of **I**. For peak identification, see Fig. 1. Chromatographic conditions: injection, 20 μl; eluent, 4 m*M* OS and 6 m*M* TBA in 0.02 *M* phosphate, pH adjusted to 5.9. For other conditions, see Experimental.

Aquation of tetrachloroplatinate. The conversion of **IV** into **V** and **VI** in aqueous media [26] may be followed by the separation system described here. In Fig. 11a, b and c the chromatograms of tetrachloroplatinate **IV** in 0.15 *M* KCl, water and 0.01 *M* NaOH, respectively, are shown.

Ammination of tetrachloroplatinate (Fig. 12) and cisplatin (Fig. 13). Here **VII**, **I**, **VIII** and **IX** are formed consecutively from **IV**; **II** is the product of isomerization of **I** and/or the product of deammination of **VIII** [2]. The method has been used to study ammination reactions of **IV** in aqueous–organic solutions under various conditions [34].

Kinetic stability of the complexes

To obtain a true view of the analysed solution, it is important that during the chromatographic separation the extent of the ligand-exchange

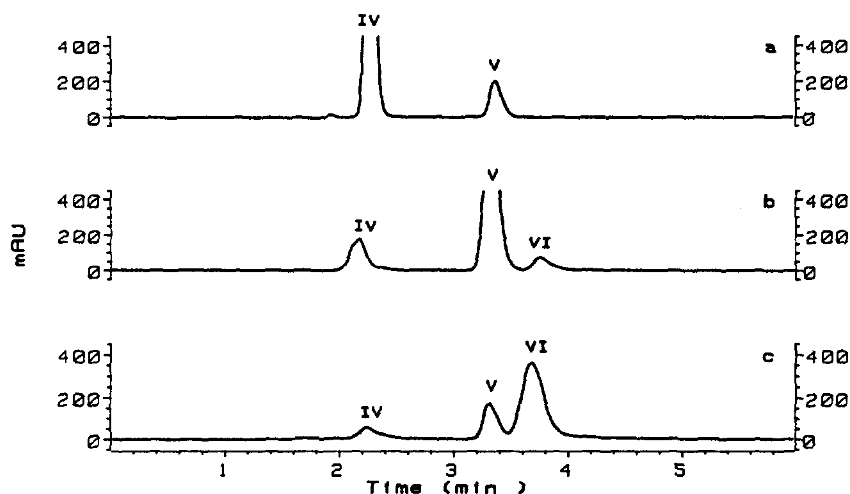

Fig. 11. Chromatograms of tetrachloroplatinate undergoing aquation in various solutions. For peak identification, see Fig. 1. Chromatographic conditions as in Fig. 10. For other conditions, see Experimental. (a) 1 mg/ml solution of **IV** in 0.15 *M* KCl, ambient temperature, 21 h; (b) 1 mg/ml solution of **IV** in water, ambient temperature, 14 days; (c) 1 mg/ml solution of **IV** in 0.01 *M* NaOH, ambient temperature, 21 h.

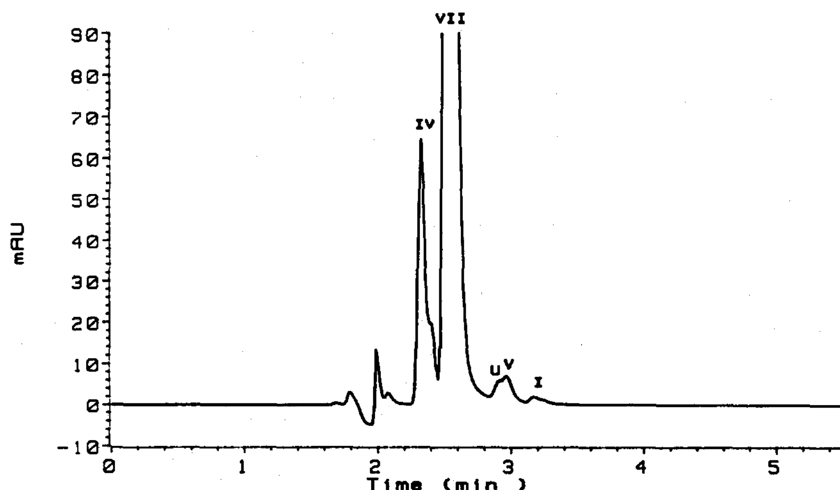

Fig. 12. Chromatogram of a crude preparation of **VII** prepared by ammination of tetrachloroplatinate (1 mg/ml solution of the sample in 0.15 *M* KCl). For peak identification, see Fig. 1. Chromatographic conditions as in Fig. 10, except 5.0 m*M* TBA and pH = 6.5.

reactions is negligible. Therefore, the aqua complexes with the least thermodynamically stable aquo ligands compared with the other ligands [29] should be checked for stability under the given conditions.

For practical purposes, the stability of the complexes in a mobile phase in the time range of *ca.* 10 min would be sufficient. Using the final mobile phase (pH 5.9) we examined the stability of **X** and **XI** in this medium by mixing 10 μl of the reference sample in the loop of the autosampler with the mobile phase. After waiting for 10 min the samples were injected. The peak area decreased to 99.7% and 95.4% of the original concentration for **X** and **XI**, respectively. Therefore, it can be concluded that the stability of **X** and **XI** in the final mobile phase during the analysis is acceptable.

If the reference solutions of the aqua complexes **X** and **XI** were mixed 1:1 (v/v) with the

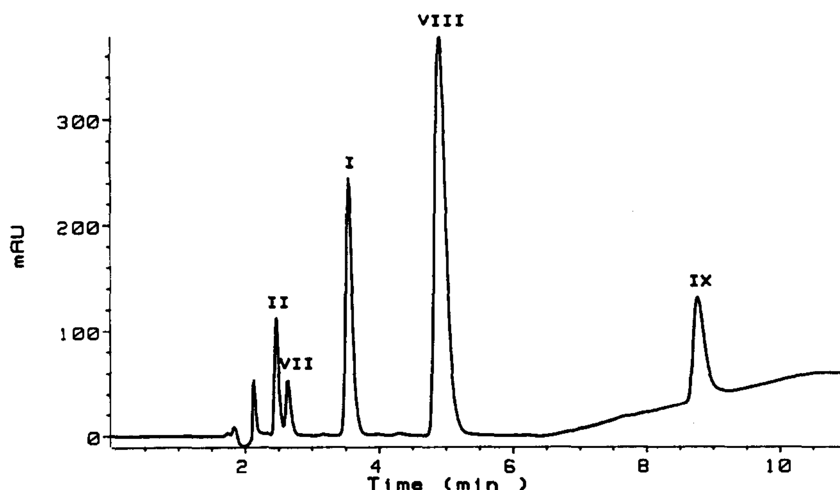

Fig. 13. Chromatogram of raw **VIII** prepared by ammination of cisplatin (1 mg/ml solution of the sample in 0.15 M KCl). For peak identification, see Fig. 1. Chromatographic conditions as in Fig. 9, except ionic strength gradient II. For other conditions, see Experimental.

mobile phase (0.020 *M* phosphate, pH 3.2) and stored for 1 h at ambient temperature, two unknown peaks appeared (one major at $k' = 1.7$ and one minor at $k' = 2.4$) and the amounts of **X** and **XI** in the solutions decreased to 62% and 50% of the original concentrations, respectively. As the spectrum of the major unknown peak is very similar to that of **XI** itself, the major unknown may be a complex with an oxo ligand, *e.g.*, phosphate, in place of the aquo ligand.

Electron absorption spectra

The absorption spectra of the platinum(II) complexes in the ultraviolet and visible region consist of an intense ($\epsilon \approx 10^4$) charge-transfer band near 200 nm and several d–d bands of low intensity shifted to the long-wavelength region [29]. The first band is used to achieve sensitive detection and the later bands are very useful for identification. Characteristic absorption maxima of the spectra for the complexes **I–XI** measured from the eluted peaks are given in Table I.

The measured maxima agree with those given in the literature [2,26,35–37]. The maxima follow the rule of shifting towards higher energy on replacing a Cl ligand with H_2O and then with NH_3 [29]. In the case of the aqua complexes (**V**, **VI**, **X** and **XI**) the weak d–d maxima shifted with increasing pH slightly to the short-wavelength region owing to the deprotonization of the water ligands, the largest shift being in the case of **XI**, the maxima of which at pH 3.2 were 252 and *ca.* 330 nm. Consequently, for identification of aqua complexes it is important to use reference spectra registered at the same pH.

CONCLUSIONS

The use of a reversed-phase sorbent and two oppositely charged ionic amphiphilic modifiers in the mobile phase makes it possible for sufficient retentions for both anionic and cationic solutes to be achieved, although in such a system cation- or anion-exchange properties may prevail.

Using this approach, dynamically generated sorbents with controlled cation- or anion-exchange character and controlled capacity may be prepared. Owing to the charge compensation of the oppositely charged modifiers such systems have a considerably lower capacity than sorbents modified with a cationic or an anionic amphiphilic modifier only. Such sorbents, especially the low-capacity ones, may find applications in ion chromatography. Also, the mobile phase concentration of a certain modifier needed to generate a sorbent of a certain capacity is considerably higher when using it in a mixture with an oppositely charged modifier. This may result in a shorter time being needed to generate the sorbent until the retentions are constant.

TABLE I

WAVELENGTHS OF ABSORPTION MAXIMA OF PLATINUM(II) COMPLEXES

pH = 5.9; for other conditions, see Experimental.

Complex	Formula	Maxima (nm)
I	*cis*-$[Pt(NH_3)_2Cl_2]^0$	*ca.* 275, 301.0, 365.5
II	*trans*-$[Pt(NH_3)_2Cl_2]^0$	270.5, 312.5
III	$[Pt(NH_3)_2(C_6H_6O_4)]^0$	*ca.* 230, 316
IV	$[PtCl]_4^{2-}$	330.5, 391.0, *ca.* 480
V	$[Pt(H_2O)Cl_3]^-$	318, *ca.* 380
VI	*cis*-$[Pt(H_2O)_2Cl_2]^0$	306.5
VII	$[Pt(NH_3)Cl_3]^-$	300.0, 344.5
VIII	$[Pt(NH_3)_3Cl]^+$	254.5, *ca.* 320
IX	$[Pt(NH_3)_4]^{2+}$	286.5
X	*cis*-$[Pt(NH_3)_2(H_2O)Cl]^+$	266.0, *ca.* 346
XI	*cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$	247.5, *ca.* 322
U1	(*trans*-$[Pt(H_2O)_2Cl_2]^0$)[a]	305.5, *ca.* 355

[a] Presumed.

ACKNOWLEDGMENTS

We thank Dr. K. Šlais for the valuable discussions on the manuscript, Dr. I. Závodná and Dr. F. Kiss for the preparation of the complexes **II** and **VI–IX** and Mrs. A. Doubravová and Mrs. M. Weisslampelová for their assistance.

REFERENCES

1 M. Nicolini (Editor), *Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy, Proceedings of XV ISPCC '87, Padua*, Martinus Nijhoff, Boston, 1988.
2 F.R. Hartley, *The Chemistry of Platinum and Palladium*, Applied Science, Barking, 1973.
3 C.M. Riley, L.A. Sternson and A.J. Repta, *J. Chromatogr.*, 217 (1981) 405–420.
4 C.M. Riley, L.A. Sternson, A.J. Repta and R.W. Siegler, *J. Chromatogr.*, 229 (1982) 373–386.
5 S.J. Bannister, L.A. Sternson and A.J. Repta, *J. Chromatogr.*, 273 (1983) 301–318.
6 C.M. Riley, L.A. Sternson, A.J. Repta and S.A. Slyter, *Anal. Biochem.*, 130 (1983) 203–214.
7 C.M. Riley, L.A. Sternson and A.J. Repta, *J. Pharm. Sci.*, 72 (1983) 351–355.
8 C. Gooijer, A.C. Veltkamp, R.A. Bauman, N.H. Velthorst, R.W. Frei and W.J.F. van der Vijgh, *J. Chromatogr.*, 312 (1984) 337–344.
9 K.C. Marsh, L.A. Sternson and A.J. Repta, *Anal. Chem.*, 56 (1984) 491–497.
10 P.T. Daley-Yates and D.C.H. McBrien, *Biochem. Pharmacol.*, 32 (1983) 181–184.
11 P.T. Daley-Yates and D.C.H. McBrien, *Biochem. Pharmacol.*, 33 (1984) 3063–3070.
12 P.J. Parsons and A.F. LeRoy, *J. Chromatogr.*, 378 (1986) 395–408.
13 P.J. Parsons, P.F. Morrison and A.F. LeRoy, *J. Chromatogr.*, 385 (1987) 323–335.
14 W.A.J. de Waal, F.J.M.J. Maessen and J.C. Kraak, *J. Chromatogr.*, 407 (1987) 253–272.
15 G.S. Baldew, K.J. Volkers, J.J.M. de Goeij and N.P.E. Vermeulen, *J. Chromatogr.*, 491 (1989) 163–174.
16 R. Kizu, M. Kaneda and M. Miyazaki, *Anal. Sci.*, 8 (1992) 145–150.
17 M. Macka, J. Borák and F. Kiss, *J. Chromatogr.*, 586 (1991) 291–295.
18 B. de Spiegeleer, G. Slegers, W. van den Bossche and P. de Moerloose, *J. Chromatogr.*, 315 (1984) 481–487.
19 A.A. Hincal, D.F. Long and A.J. Repta, *J. Parent. Drug Assoc.*, 33 (1979) 107–116.
20 B.A. Persson, S.O. Jansson, M.L. Johansson and P.O. Lagerstrom, *J. Chromatogr.*, 316 (1984) 291–300.
21 P. Helboe, S.H. Hansen and M. Thomsen, *Adv. Chromatogr.*, 28 (1989) 195–265.
22 M. Macka, J. Borák, L. Seménková, M. Popl and V. Mikeš, *J. Liq. Chromatogr.*, in press.
23 W.R. Sisco, C.T. Rittenhouse, L.A. Everhart and A.M. McLaughlin, *J. Chromatogr.*, 354 (1986) 355–366.
24 S.L. Richheimer and T.M. Amer, *J. Pharm. Sci.*, 72 (1983) 1349–1351.
25 J. Šalamoun and K. Šlais, *J. Chromatogr.*, 537 (1991) 247–257.
26 L.I. Elding, *Dissertation*, Carl Blooms Botryckerei, Lund, 1970.
27 I.I. Chernyaev, A.V. Babaeva, V.A. Golovnya, O.E. Zvyagintsev, L.A. Nazarova and I.A. Fedorov (Editors), Sintez Kompleksnykh Soedinenii Metallov Platinovoi Gruppy (*Spravochnik*), Nauka, Moscow, 1964.
28 S.C. Dhara, *Indian. J. Chem.*, 8 (1970) 193.
29 A.J. Thomson, R.J.P. Williams and S. Reslova, *Struct. Bonding (Berlin)*, 11 (1972) 28–29, and references cited therein.
30 M. Macka, J. Borák and L. Seménková, in preparation.
31 N.M. Nikolaeva, B.V. Ptitsyn and I.I. Gorbacheva, *Zhur. Neorg. Khim.*, 10 (1965) 1051.
32 K.A. Jensen, *Z. Anorg. Chem.*, 242 (1939) 87.
33 *The United States Pharmacopeia, XXII Revision*, Mack, Easton, PA, 1989.
34 F. Kiss, *Dissertation*, Research Institute of Fine Chemicals, Lachema Brno, 1990.
35 A.V. Babaeva and R.I. Rudyi, *Zhur. Neorg. Khim.*, 1 (1956) 921.
36 J. Chatt, G.A. Gamlen and L.E. Orgel, *J. Chem. Soc.*, (1958) 486.
37 H. Ito, J. Fujita and K. Saito, *Bull. Chem. Soc. Jpn.*, 42 (1969) 2863.