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Separation of some platinum(II) complexes by ionic strength gradient on a solvent-generated ion-exchange sorbent

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ABSTRACT

The compatibility of ionic strength gradient with solvent-generated ion-exchange chromatography on an octadecylsilica sorbent was proven for a mobile phase containing octanesulphonate. Only a slight baseline shift was observed during the gradient of the phosphate buffer, even at 210 nm. An equilibration time of 3 min between the runs was sufficient to obtain retention times with a reproducibility better than 1%. The compounds separated were cisplatin, carboplatin and related neutral and cationic platinum(II) complexes, including transplatin and the aquation products of cisplatin.

INTRODUCTION

Several platinum(II) complexes have been used extensively in cancer treatment [1]. Mainly neutral or positively charged complexes exist in aqueous solutions of cisplatin [*cis*-diamminedichloroplatinum(II), CDDP] and carboplatin [*cis*-diammine-1,1-cyclobutanedicarboxylateplatinum(II), Pt-CBDCA] (Fig. 1), the aquation products of cisplatin [2–5], *trans*-diamminedichloroplatinum(II) [6] and triamminechloroplatinum(II) and tetraammineplatinum(II) complexes [6].

Cisplatin and the related platinum complexes have been separated chromatographically on reversed-phase sorbents dynamically modified with cationic surfactants [7]. However, positively charged molecules have generally negligible retentions under these conditions. The current methods for the separation of CDDP and its cationic aquation products or other cationic platinum(II) complexes use an octadecylsilica sorbent dynamically modified with an anionic surfactant [7–10]. Isocratic elution is most often used. Nevertheless, for the analysis of complicated mixtures gradient techniques should give a better separation and/or shorter analysis time [11].

The use of a gradient of organic modifier concentration has been reported [9,12,13]. As the distribution coefficients of surfactants depend strongly on the organic modifier concentration, a change in surfactant concentration in the stationary phase caused by the gradient results. Consequently poor reproducibility [13] and long equilibration times after each run [9] can occur. For this reason, the application of an ionic strength gradient should be more convenient, especially in separation systems where the ion-exchange mechanism dominates.

In ion-exchange chromatography (IEC) on classical ion exchangers, the use of an ionic strength gradient is essential [11]. The application of an ionic strength gradient (at constant concentrations of organic modifier and surfactant) to solvent-generated IEC is not so common. De Waal *et al.* [9] used a mixed gradient of increasing organic modifier concentration and increasing ionic strength. When using the related separation principle of ion-exclusion chromatography on octadecylsilica dynamically modified with sodium dodecyl sulphate, a

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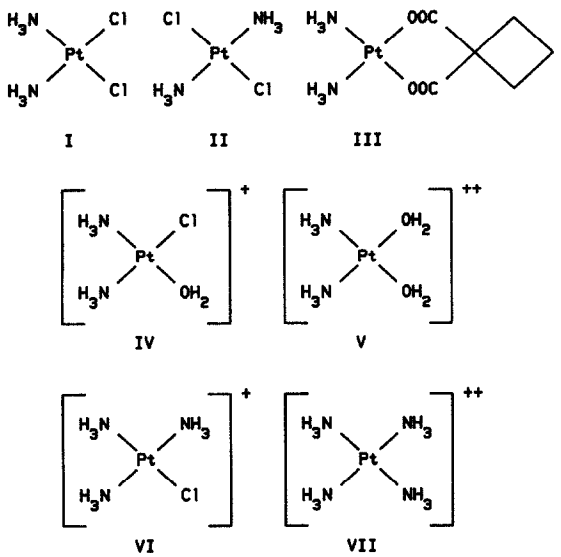

Fig. 1. Structures of platinum(II) complexes. I = *cis*-diamminedichloroplatinum(II) (cisplatin, CDDP); II = *trans*-diamminedichloroplatinum(II) (transplatin, TDDP); III = *cis*-diammine-1,1-cyclobutanedicarboxylateplatinum(II) (carboplatin, Pt-CBDCA); IV = *cis*-diammineaquachloroplatinum(II); V = *cis*-diamminediaquaplatinum(II); VI = triamminechloroplatinum(II); and VII = tetraammineplatinum(II).

sample-induced internal gradient of decreasing ionic strength has been applied [14].

This paper reports the separation of platinum(II) complexes applying an ionic strength gradient on an octadecyl column dynamically coated with octanesulphonate dissolved in the mobile phase. The convenience of the gradient technique is shown.

EXPERIMENTAL

Materials

Sodium octanesulphonate and sodium dihydrogenphosphate were of analytical-reagent grade (E. Merck).

Cisplatin and carboplatin were synthesized in the Research Institute of Pure Chemicals (Lachema). They were characterized according to USP XXII and the assay show them to be more than 99.5% pure by high-performance liquid chromatography (HPLC).

The *trans*-diamminedichloroplatinum(II) complex was prepared according to Chernyaev *et al.* [6]. It contained less than 0.1% CDDP and the assay was more than 99% pure (HPLC, internal normalization 210 nm).

The *cis*-diamminediaquaplatinum(II) complex was prepared according to Dhara [4]. To a CDDP solution (concentration 1 mg/ml), silver nitrate was added at a molar ratio of CDDP to Ag of 1:2.2. The mixture was shaken and allowed to stand overnight. After centrifugation the supernatant was acidified with nitric acid to pH 2 and stored in a dark bottle. The chromatogram showed one major peak (in addition to nitrate), an unknown peak (9%), and less than 1% CDDP and *cis*-diammineaquachloroplatinum(II).

The *cis*-diammineaquachloroplatinum(II) complex was prepared by modifying the above procedure so that silver nitrate was added to CDDP in molar ratio of only 1:1.1. The chromatogram showed (in addition nitrate) one major peak of 11% *cis*-diamminediaquaplatinum(II) and 16% CDDP.

Triamminechloroplatinum(II) was prepared by partial ammonolysis of CDDP (concentration 1 mg/ml) at 90°C. Small portions of 0.2 *M* aqueous ammonia were used so that pH did not exceed 7.5. The reaction was stopped when the amount of side-product formed, tetraammineplatinum(II), was about the same as that of the unreacted CDDP. The assay of triamminechloroplatinum(II) was approximately 40% pure (HPLC, internal normalization 210 nm); the remainder was CDDP and tetraammineplatinum(II).

Tetraammineplatinum(II) was prepared according to Chernyaev *et al.* [6]. The assay was more than 99% pure (HPLC, internal normalization 210 nm).

The HPLC method described here was used to characterize the reference samples of platinum complexes II and IV–VII.

Apparatus and chromatographic conditions

The system used was a Hewlett-Packard 1090 chromatograph consisting of an HP1040 diode-array detector and a DR5 binary pumping system. The samples were injected by a Rheodyne 7125 manual injection valve equipped with a 10-μl sample loop. For system control and data evaluation an HP-79994A workstation based on an HP-310 computer was used.

A stainless-steel column (4 × 250 mm) packed with Silasorb SPH C_{18}, $d(p)$ = 7.5 μm (Lachema) was used. The column temperature was 30°C.

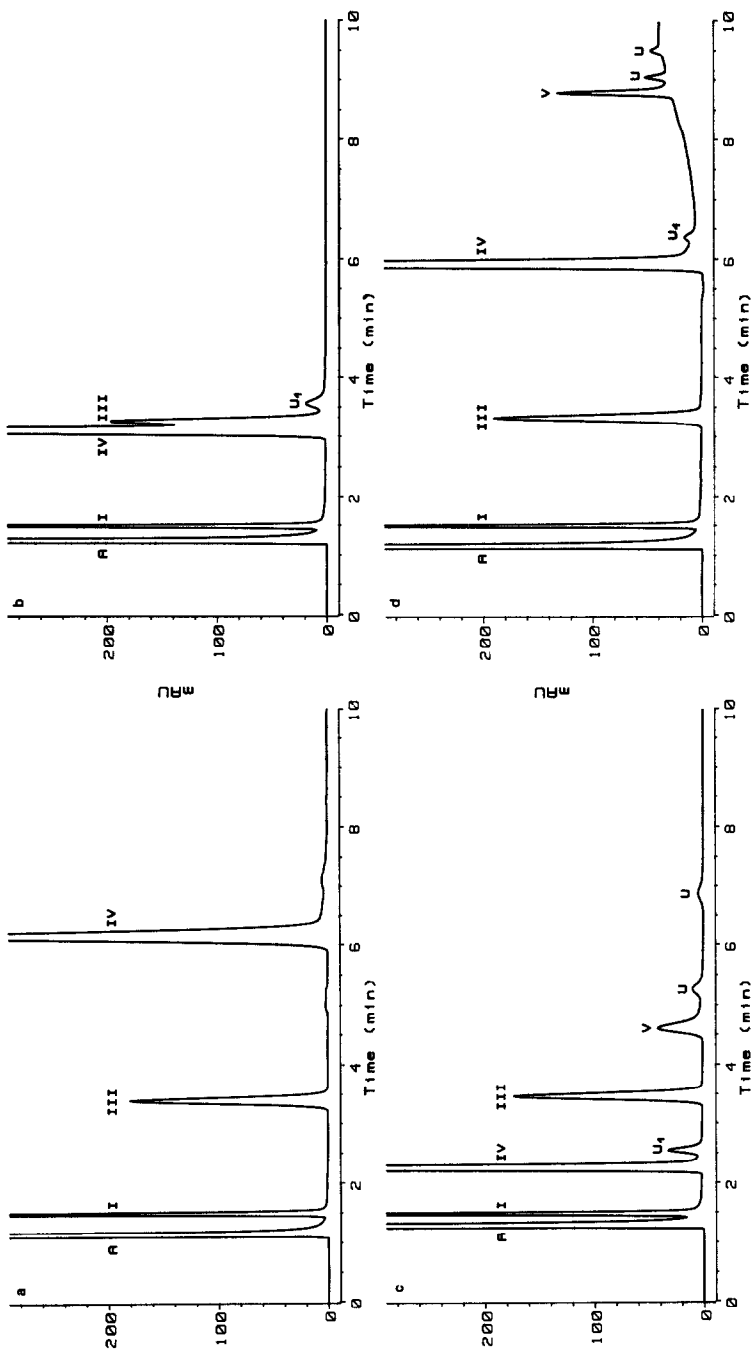

Fig. 2. Separation of Pt-CBDCA (III), CDDP (I) and its aquation products (IV, V). Isocratic analysis with a mobile phase containing (a) 10% B, (b) 30% B, (c) 50% B, (d) gradient analysis of standard mixture and (e) a real sample. Peak identification: A = anions; U, U_1 = unknown; for others, see Fig. 1. For other experimental conditions, see text.

The following mobile phases were used. Eluent A was sodium octanesulphonate (2 mM) and eluent B sodium octanesulphonate (2 mM), dihydrogen phosphate (0.5 M). The analyses were run under isocratic conditions (90% A and 10% B, pH 4.53) or with the following gradient: 10% B for $t = 0$ to $t =$ 4 min, 10–100% B from $t = 4$ to $t = 8$ min, 100% B from $t = 8$ to $t = 9$ min, 100–10% B from $t = 9$ to $t = 9.1$ min.

The stationary phase was generated by pumping the mobile phase until the retention times were constant (about 4 h). The flow-rate was 1.5 ml/min, resulting in a column inlet pressure of about $9 \cdot 10^6$ Pa.

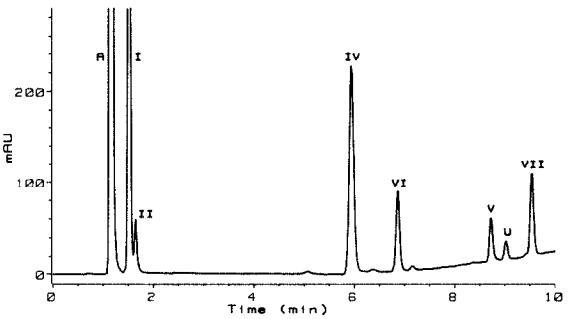

Fig. 3. Separation of a mixture of various amminechloroplatinum(II) complexes (I, II, VI and VII) and aquation products of CDDP (IV and V). Peak identification: A = anions; U = unknown; for others, see Fig. 1. For other experimental conditions, see text.

RESULTS AND DISCUSSION

The chromatograms for the isocratic separations of model mixtures of I, III, IV and V (Fig. 2) document the strong influence of ionic strength on the retention times of the charged complexes IV and V; complex V is not eluted within 10 min using a mobile phase containing less than 30% B. The retention of the uncharged complexes I and III remain almost unchanged. The standard mixture is baseline-resolved under isocratic conditions with a mobile phase containing 50% B (Fig. 2c). However, some real samples, e.g., the reaction mixture obtained by the reaction of CDDP and cyclobutanedicarboxylic acid, gave rise to unknown peaks interfering with complex IV. Gradient analysis allowed these interfering peaks from real samples to be separated (Fig. 2e). Another example in Fig. 3 shows the gradient separation of various amminechloroplatinum(II) complexes and the aquation products of CDDP.

It is important to determine whether there are changes in the surfactant sorption–desorption process caused by the ionic strength gradient which would shift the retention times in the next run or produce baseline disturbances when using photometric detection. Even at 210 nm only a slight baseline shift caused by the gradient was observed during the run. An equilibration time of 5 min between runs is sufficient to condition the system. The reproducibility of retention times for all the peaks detected was better than 1% during a single day.

An equilibration time of less than 3 min gave shorter and irreproducible retention times for the positively charged complexes. The required equilibration time can be related to washing out the higher concentration of the phosphate from the column rather than to changing the octanesulphonate concentration in the stationary phase, as the latter would require a much longer time. To estimate the extent of the change of the octanesulphonate stationary phase concentration as a result of the higher phosphate content in the mobile phase, a further experiment was carried out. The mobile phase, consisting of 100% B, was pumped for 2 h through the column, then the composition was switched to 10% B and after 5 min standard solutions of Pt-CBDCA, CDDP and its aquation products were injected. Only small changes in the retention times [maximum +5% for *cis*-diammineaquachloroplatinum(II) complex] resulted. Therefore it can be concluded that the change in the octanesulphonate stationary phase concentration with increasing phosphate concentration is not significant for the application of the ionic strength gradient.

This method was successfully applied to the analyses of samples related to technology development and to the analyses of drugs, including stability studies.

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