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DETERMINATION OF PLATINUM-CONTAINING DRUGS IN HUMAN PLASMA BY LIQUID CHROMATOGRAPHY WITH REDUCTIVE ELECTROCHEMICAL DETECTION

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SUMMARY

The platinum complex *cis*-diamminedichloroplatinum(II) (cisplatin or CDDP), which is used successfully to treat various kinds of tumour, can be determined in human plasma ultrafiltrate using liquid chromatography with reductive electrochemical detection (LC–ED). Polarographic analyses of other platinum-containing drugs have been carried out, and the results indicate that some of them might be good candidates for detection using the ED method. *cis*-Dichloro-*trans*-dihydroxo-*cis*-bis-(isopropylamine)platinum(IV) (CHIP or JM-9), diammine(1,1-cyclobutanedicarboxylato)platinum(II) (CBDCA or JM-8), and tetrachloro(*trans*-1,2-diaminocyclohexane)platinum(IV) (TCDCP), which are under evaluation for antitumour properties, have been investigated by this method. The results suggest that LC–ED may be a suitable technique for the determination of CHIP and TCDCP. The separation of cationic hydrolysis products from the neutral parent complex (CDDP) was carried out on reversed-phase columns, modified with alkylsulphonic acid ion-pair reagents. The rate of disappearance of CDDP in various media at 37.0°C was studied using this method. These results are in good agreement with those determined by other investigators using different methods. In addition, the monoaqua hydrolysis product was detected after incubation of CDDP with plasma ultrafiltrate and 100 m*M* sodium chloride. Atomic absorption spectrophotometry with electrothermal atomisation was used to determine the total platinum content in eluate fractions. This aided the identification of platinum-containing peaks in the LC–ED profile, and was also used to measure the percentage platinum recovered from the column.

INTRODUCTION

The growing interest in trace metals, particularly in the fields of environmental health and medicine, has led to development of sensitive analytical methods for trace metal determination in biological matrices. One of the most commonly employed

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techniques is atomic absorption spectroscopy with electrothermal atomisation (ETA-AAS), which is carried out after acid digestion of the sample matrix. Although sensitive, ETA-AAS only permits the determination of total metal content; no information is gained about the metal oxidation state, nor the kind of metal species present in the sample. Inorganic chromatography can provide information about metal species by combining the separation technology of high-performance liquid chromatography (HPLC) with sensitive metal determination. In aqueous solution, most metal species are cationic. However, the transition metals form complexes which, depending on the ligands coordinated to the metal, may be cationic, anionic, or neutral. In HPLC, charged species can be separated by ion exchange using either "chemically bonded" or "solvent-generated" ion-exchange columns. The latter, more commonly known as ion-pair chromatography, involves modifying a reversed-phase column with an ion-pair reagent, which is dissolved in the mobile phase. Metal species of different charge are eluted from the column and passed through a suitable detector. The theory and applications of inorganic chromatographic analysis have been the subject of a recent review[1].

Several metal-specific detectors are now available for HPLC. The development of conductivity detectors has led to the establishment of ion chromatography (IC). The adaptation of other techniques has given rise to several combined methods: liquid chromatography coupled with inductively coupled plasma-atomic emission spectrometry (LC–ICP-AES), flame atomic absorption spectrometry (LC–AAS), mass spectrometry (LC–MS) and electrochemical detection (LC–ED). Here, we describe the development and application of LC–ED methods to the determination of platinum species in human plasma. These species are derived from platinum-containing drugs which are now used in cancer chemotherapy.

Although antitumour activity has been identified for a number of platinum compounds, the most widely investigated has been the original complex discovered by Rosenberg[2], *cis*-diamminedichloroplatinum(II) (CDDP), which is also known as cisplatin in the formulated form. The determination of platinum-containing drugs by liquid chromatography has been the subject of several papers recently[3–8]; platinum detection was reportedly carried out by: direct absorbance at 214 nm[3], pre-column derivatisation with absorbance at 254 nm[4,5] or 346 nm[6], post-column derivatisation followed by absorbance at 290 nm[7], and quenched phosphorescence[8]. LC–ED has also been used for monitoring platinum[9–13]. Differential pulse amperometry (DPA) has been used with a hanging mercury drop electrode (HMDE)[9,10], current-sampled amperometry with a HMDE[11,13], and a thin-layer Au/Hg electrode[12]. In an earlier paper[13], we described the application of a similar LC–ED method to the determination of CDDP in human plasma, after protein removal by centrifugal ultrafiltration. Ion-pair chromatography was used with ED (HMDE) to analyse plasma ultrafiltrate for platinum species having a small relative molecular mass of $< 50\,000$. In this paper, we report an improved LC–ED method for the determination of *cis*-diammineaquachloroplatinum(II) ("monoaqua"), a hydrolysis product of CDDP, formed via nucleophilic substitution of one of the chloride ligands with water (Fig. 1).

Several second-generation platinum complexes are now under evaluation as antitumour agents. They include: *cis*-dichloro-*trans*-dihydroxo-*cis*-bis(isopropylamine)platinum(IV) (CHIP or JM-9), diammine(1,1-cyclobutanedicarboxylato)plat-

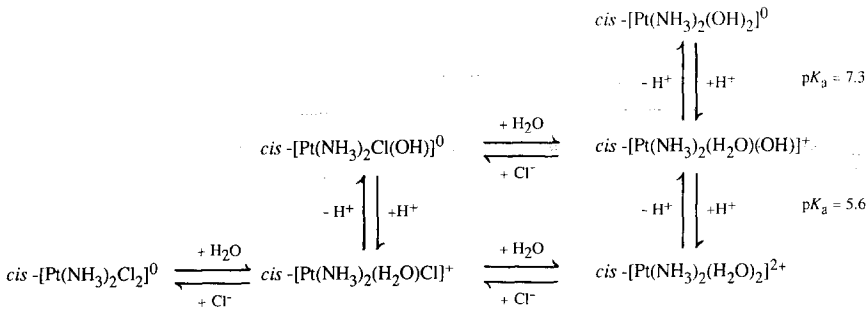

Fig. 1. Hydrolysis equilibria of *cis*-diamminedichloroplatinum(II) (CDDP), showing the formation of mono- and dicationic species. At pH < 5, these are reduced to parent, monoaqua, and diaqua species.

inum(II) (CBDCA or JM-8), and tetrachloro(*trans*-1,2-diaminocyclohexane)platinum(IV) (TCDCP) (Fig. 2). Before LC–ED methods were applied to these drugs, they were investigated by polarography to determine if a suitable potential existed for their reduction at a mercury electrode. ETA-AAS was employed to determine the degree of recovery of total platinum after separation on the column, and to aid identification of platinum-containing peaks, as indicated by the electrochemical detector.

The initial rate at which CDDP is transformed in various media, when incubated *in vitro*, has been reported by several laboratories[14–21]. A discrepancy in these data, reported for loss of CDDP in whole plasma, can be traced to the different

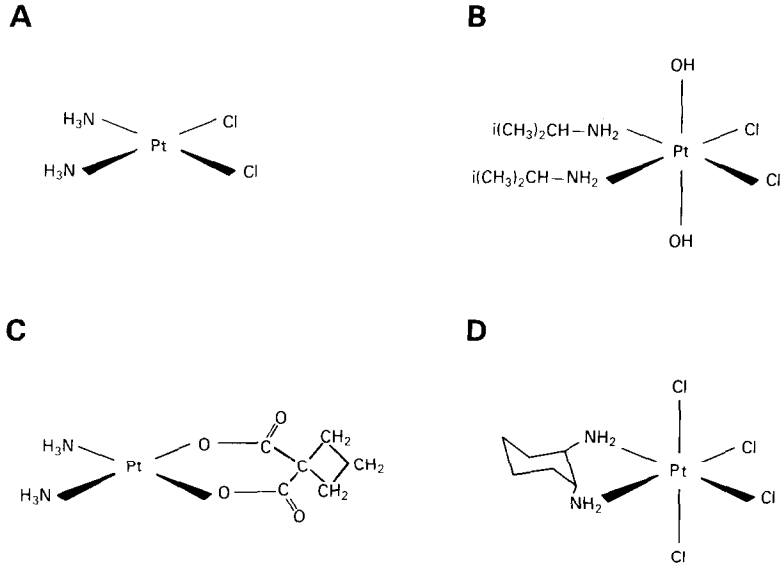

Fig. 2. Structural formulae of platinum antitumour compounds under investigation: (A) *cis*-diamminedichloroplatinum(II) (NSC-119875) (CDDP, cisplatin, platinol, neoplatin, platinex), (B) *cis*-dichloro-*trans*-dihydroxy-*cis*-bis(isopropylamine)platinum(IV) (NSC-256927) (CHIP, JM-9, Iproplatin), (C) diammine(1,1-cyclobutanedicarboxylato)platinum(II) (NSC-241240) (CBDCA, JM-8, Carboplatin), and (D) tetrachloro(*trans*-1,2-diaminocyclohexane)platinum(IV) (NSC-363812) (TCDCP, tetraplatin).

TABLE I

FIRST-ORDER RATE CONSTANTS (k_r) FOR THE DISAPPEARANCE OF CDDP/Pt IN VARIOUS MEDIA

Medium	*Temp (°C)*	*Determination*	k_r (h^{-1})	*Ref.*
Water	37.0	CDDP by LC–ED	0.33	This work
Water	37.0	CDDP by LC–UV and LC–ETA-AAS (off line)	0.32	20
Water	37.0	Estimated from Arrhenius plots of data taken from ref. 14	0.36	17
Water	25.0	LC–UV	0.11	19
Water	Room temperature	UV spectrophotometry/Cl^- titration	0.132	18
Dog plasma	37.0	Total platinum in ultrafiltrate	0.22	15
Human plasma	37.0	Total platinum in ultrafiltrate X-ray fluorescence	0.27	16
Dog plasma	37.0	Total platinum in ultrafiltrate by ETA-AAS	0.25	17
Human plasma	37.0	CDDP in ultrafiltrate by LC–ETA-AAS (off-line)	0.46	20
Human plasma	37.0	CDDP in ultrafiltrate by LC–ED and total platinum in ultra filtrate by ETA-AAS	0.45 0.25	Fig. 9B Fig. 9B
Plasma ultrafiltrate + 100 m*M* sodium chloride	37.0	CDDP in ultrafiltrate by LC–ED and CDDP by LC–ETA-AAS (off-line)	0.32 0.32	This work 20

analytical methods employed (Table I). We have used our LC–ED method to follow the fate of CDDP and the monoaqua species in aqueous solution, whole plasma and plasma ultrafiltrate. The results with the electrochemical detector are compared to those obtained using ETA-AAS to determine the total "free" platinum in ultrafiltrate.

EXPERIMENTAL

Apparatus and materials

Liquid chromatography was carried out with a Model 6000A pump (Waters Assoc., Milford, MA, U.S.A.), modified for reductive ED as described previously[13]. Two columns were used in the investigation: a 15 cm × 4.6 mm I.D. Zorbax® C_8 (5 μm) analytical column, and a 8 cm × 6.2 mm I.D. Zorbax® ODS (3 μm) Golden Series column (Du Pont, Wilmington, DE, U.S.A.). Samples were introduced into a Model U6K universal injector (Waters) with a 25-μl blunt tipped microsyringe (Hamilton, Reno, NV, U.S.A.). The mobile phase was, for the most part, 10 m*M* sodium acetate, buffered at pH 4.60; 5 m*M* heptanesulphonic acid (Kodak Laboratory Chemicals, Rochester, NY, U.S.A.), was added as an ion-pairing reagent to separate the cationic hydrolysis products from the neutral parent complex. Hexanesulphonic acid, obtained as PIC B6 from Waters, was also investigated as an ion-pairing agent.

An EG & G (Princeton Applied Research, Princeton, NJ, U.S.A.) Model 174A polarographic analyzer, interfaced with a Model 303 static mercury drop electrode (SMDE) was used to investigate the platinum complexes by polarography. A Model 7064A *X-Y* plotter (Hewlett-Packard, Palo Alto, CA, U.S.A.) was used, with a full-scale deflection (f.s.d.) of $Y = 10$ V, $X = 1.5$ V, to record the polarograms. An EG

& G Model 310 flow-cell adaptor was used for LC detection with a HMDE, drop size large, held at a fixed potential. All chromatograms were recorded on an Omniscribe chart recorder (Houston Instruments, Austin, TX, U.S.A.) with a f.s.d. of 10 V and a chart speed of 1.27 cm/min (0.5 in./min).

Deoxygenation of the mobile phase for LC, and the solutions used in polarographic experiments, was carried out by purging with helium gas, which had been previously passed through a four-stage scrubbing system. Details of the procedure were reported previously[13]. Fractions of the eluate were collected for off-line determination of platinum by ETA-AAS using a Redirac fraction collector (LKB instruments, Gaithersburg, MD, U.S.A.). ETA-AAS was carried out using an Instrumentation Laboratory Model 951 AA spectrometer and a Model 555 carbon furnace (Allied Analytical Systems, Lexington, MA, U.S.A.).

Sample preparation

The platinum complexes CDDP (NSC 119875), CHIP (NSC 256927), CBDCA (NSC 241240), and TCDCP (NSC 363812) were obtained from the Pharmaceutical Resources Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, U.S.A. Stock solutions (1 m*M*) of CHIP, CBDCA and TCDCP were prepared with triple-distilled deionised water; ten-fold dilutions were made in 100 m*M* sodium chloride, 100 m*M* potassium chloride, and 100 m*M* sodium perchlorate for polarographic studies. These solutions were aged in water at 22°C for 24 h and then analysed by polarography. It was reasoned that hydrolysis would be reduced in 100 m*M* sodium chloride, but promoted in sodium perchlorate to provide a mixture of platinum species. After ten days at 4°C, the solutions were analyzed by LC–ED and then returned to storage at 4°C for a total period of three months, by which time equilibrium was presumed to be complete. Further analyses were carried out by LC–ED to determine the parent complex and hydrolysis products.

Heparinized human plasma (O+), donated by one of us (P.J.P.), was obtained via plasmapheresis in the Department of Transfusion Medicine at the NIH. The rate of disappearance of CDDP in water, whole plasma and plasma ultrafiltrate (*in vitro*) was followed by LC–ED. Plasma (50 ml) was incubated at 37.0 ± 0.01°C with 33.3 μM (10 μg/ml) CDDP, added as a solution of the formulated drug cisplatin; 2-ml aliquots were removed at hourly intervals and filtered to remove plasma proteins by transfer to a Centriflo CF 50A [50 000 molecular weight cut off (MWCO)] ultrafiltration membrane cone (Amicon, Danvers, MA, U.S.A.). The driving force for separation was a Sörvall GLC-2B laboratory centrifuge (Du Pont Instruments). Samples were spun 1 min at 2000 rpm to remove water of hydration, which results from soaking the CF 50A cones in water prior to use; a further 10-min spin at 2500 rpm ($< 1000\ g$) resulted in the accumulation of enough ultrafiltrate volume for analysis by both LC–ED and ETA-AAS. Plasma ultrafiltrate (50 000 MWCO) (50 ml), obtained as described above, was incubated with 10 μg/ml CDDP in the presence of 0.005 mol of added sodium chloride. The fate of CDDP was monitored by LC–ED.

RESULTS AND DISCUSSION

Polarography

The application of reductive ED to metal speciation in liquid chromatography,

depends upon the capacity of the species in question to undergo reduction at a suitable potential, given an appropriate electrode. Many platinum complexes, including CDDP, have been investigated by polarography[22–26]. However, no such studies have been reported for CHIP, CBDCA or TCDCP. It is necessary to carry out preliminary polarographic analyses in order to determine whether a suitable reduction potential exists for each complex, before LC–ED can be evaluated.

The results of current (d.c.) sampled polarographic analysis of CHIP, CBDCA, and TCDCP, aged for 24 h at 22°C in 100 m*M* potassium chloride and in 100 m*M* sodium perchlorate, are shown in Fig. 3. Two weak waves, with half-wave potentials ($E_{1/2}$) at *ca.* −200 mV and −1.0 V *versus* Ag/AgCl, were observed for 100 μM CHIP aged in 100 m*M* potassium chloride. A different polarogram was observed for 100 μM CHIP, aged in 100 m*M* sodium perchlorate; a distinctive maximum, around −1.0 V, and another, more cathodic wave, was probably the reduction of hydrolysis products of CHIP. In comparison, 100 μM CBDCA does not show much polarographic activity, whether aged in potassium chloride or sodium perchlorate. Thus ED would not appear to be a suitable detection method. The polarograms recorded for 100 μM TCDCP in 100 m*M* potassium chloride and 100 m*M* sodium perchlorate are remarkably similar, with two anomalous waves at −0.6 V and −1.3 V. The rise in current of the supporting electrolyte is unusually large for an SMDE; moreover, the background curve has a wave at −1.6 V, which may well be caused by reduction of acidic impurities. On the basis of these polarograms, it appeared that a current-sampled HMDE might be useful as an electrochemical detector for CHIP and

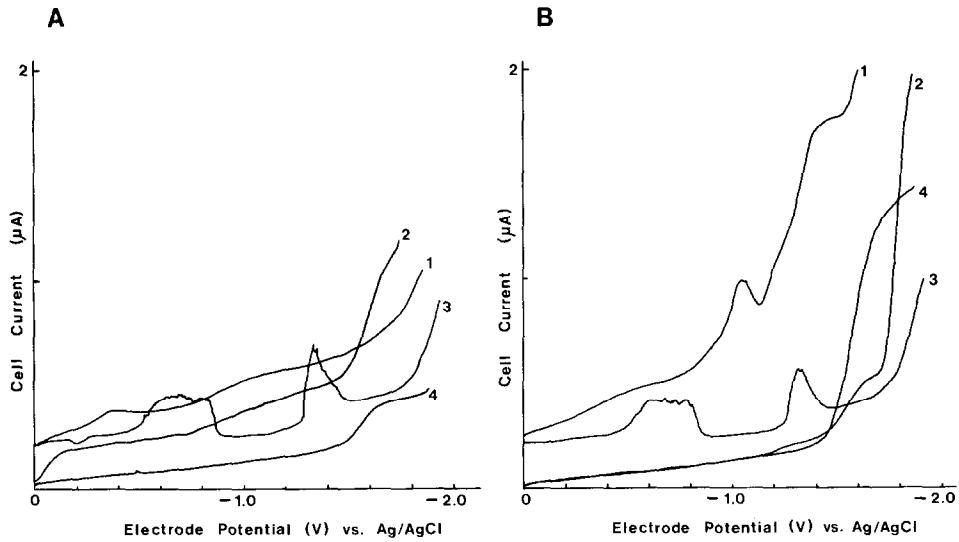

Fig. 3. Current d.c.-sampled polarograms of platinum complexes under evaluation for antitumour activity. EG & G 174A polarographic analyzer with a SMDE, drop size small; clock, 1.0 s; scan range, 0 to −1.9 V *vs.* Ag/AgCl; scan rate, 5 mV/s; scan direction, negative; low-pass filter, off; current range, 2 μA f.s.d. (except curve 3, TCDCP: 5 μA f.s.d.). (A) Complexes aged at 22°C for 24 h in 100 m*M* potassium chloride: curve 1, 100 μM CHIP; curve 2, 100 μM CBDCA; curve 3, 100 μM TCDCP; curve 4, 100 m*M* potassium chloride. (B) Complexes asged at 22°C for 24 h in 100 m*M* sodium perchlorate; curve 1, 100 μM CHIP; curve 2, 100 μM CBDCA; curve 3, 100 μM TCDCP; curve 4, 100 m*M* sodium perchlorate.

TCDCP. The aged solutions were stored at 4°C in the dark for ten days and then investigated by LC–ED.

Liquid chromatography with electrochemical detection

Two stationary phases and several mobile phases, containing alkylsulphonic acid ion-pair reagents, were investigated. Chromatograms of each complex, aged for ten days in water at 4°C, are shown in Fig. 4, where 10 m*M* sodium acetate and 10 m*M* heptanesulphonic acid (pH 4.60) were used with a 15-cm C_8 column. The column was disconnected from the electrochemical detector so that fractions could be collected at 0.4-min intervals to determine the platinum content. Those chromatographic peaks determined to contain platinum are shown in black. After ten days at 4°C an aged CDDP solution contains several species, including the parent complex, mono-

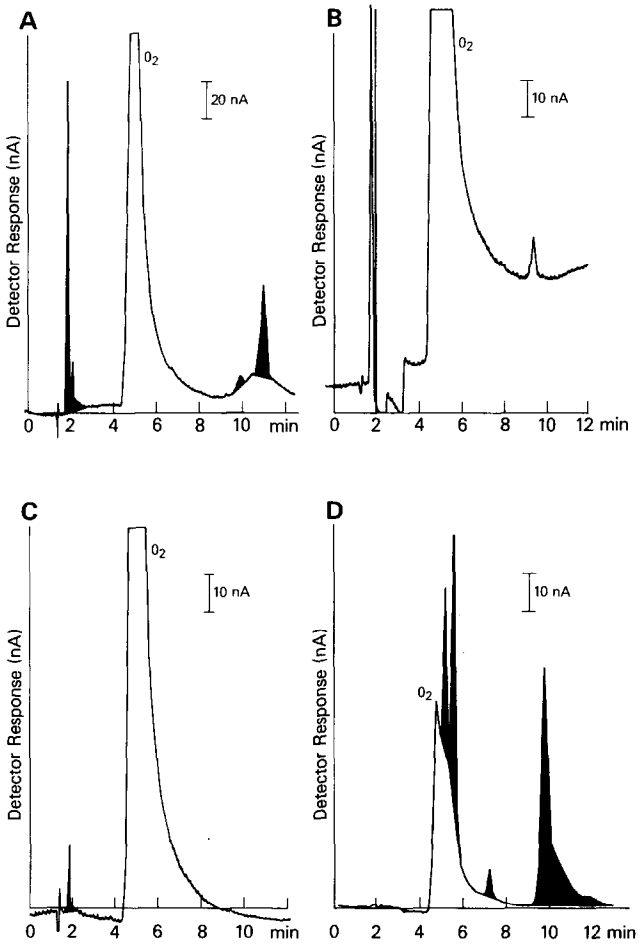

Fig. 4. LC–ED of platinum complexes, after ten days in water at 4°C: column, 15-cm C_8 Zorbax; mobile phase, 10 m*M* sodium acetate and 10 m*M* heptanesulphonic acid (pH 4.60); flow-rate, 1 ml/min; detector, HMDE, $E = -1.0$ V. (A) 333 μM (100 μg/ml) CDDP (recovery 97%), (B) 1 m*M* CHIP (recovery 0%), (C) 1 m*M* CBDCA (recovery 99%), and (D) 1 m*M* TCDCP (recovery 38%).

aqua and diaqua hydrolysis products, all of which are well resolved; 97 ± 5% platinum was recovered (Fig. 4A). CHIP and its hydrolysis products were so strongly retained on the C_8 column with 10 m*M* heptanesulphonic acid, that platinum is not detected in any fraction collected up to 12 min after injection (Fig. 4B). As expected, 1 m*M* CBDCA has a small peak current of only 20 nA, at the potential used, although the recovery of platinum is 99 ± 5% (Fig. 4C). Chromatograms of TCDCP all have four platinum-containing peaks, although the resolution from oxygen is not good on the C_8 column (Fig. 4D). Recovery values for TCDCP, with this system, are 38 ± 3% for 1 m*M* TCDCP aged in water, 97 ± 5% for 100 μM TCDCP aged in sodium chloride (Fig. 5A) and 9 ± 1% for 100 μM TCDCP aged in 100 m*M* sodium per-

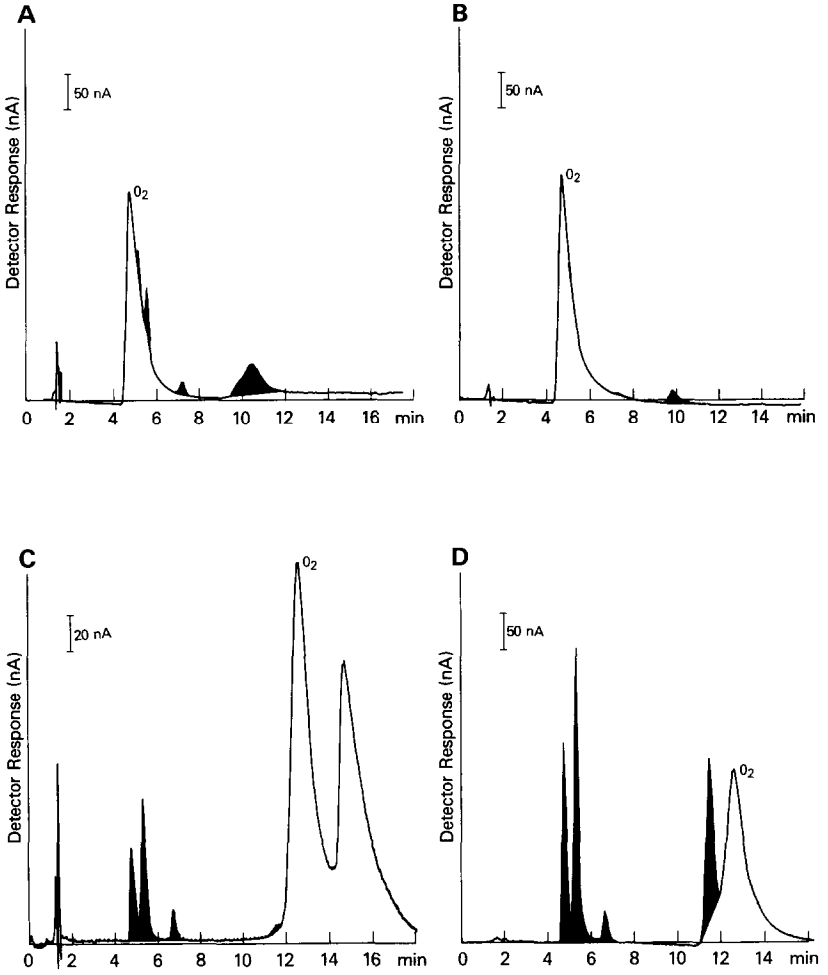

Fig. 5. LC–ED of TCDCP, after ten days at 4°C in various solutions: column: (A and B) 15-cm C_8 Zorbax Analytical, (C and D) 8-cm ODS Zorbax Golden Series; mobile phase, 10 m*M* sodium acetate, 10 m*M* heptanesulphonic acid (pH 4.60); flow-rate, 1 ml/min; detector, HMDE, $E = -1.0$ V. (A) 100 μM TCDCP in 100 m*M* sodium chloride; (B), 100 μM TCDCP in 100 m*M* sodium perchlorate; C, 100 μM TCDCP in 100 m*M* sodium chloride; D, 1 m*M* TCDCP.

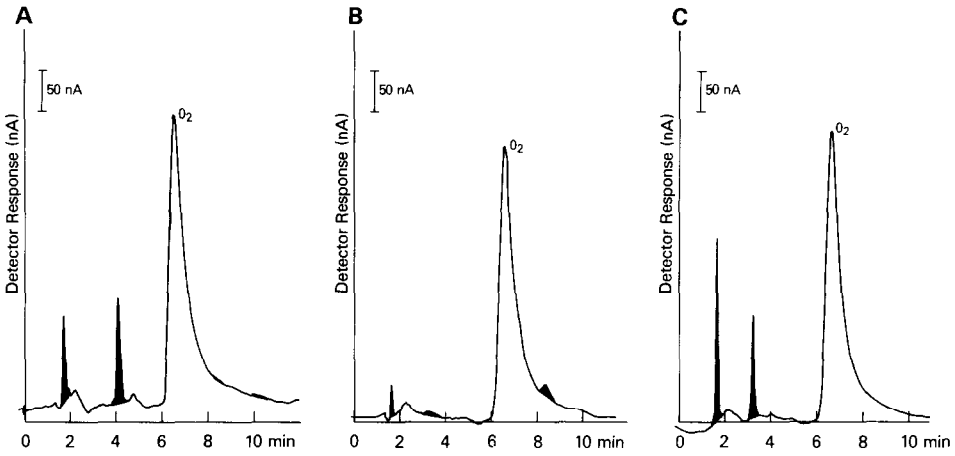

Fig. 6. LC–ED of platinum complexes, after three months in water at 4°C: column, 8-cm ODS Zorbax Golden Series; mobile phase, 10 m*M* sodium acetate, 5 m*M* hexanesulphonic acid (pH 4.60); flow-rate: 1 ml/min; detector, HMDE, $E = -1.0$ V. (A) 333 μM (100 μg/ml) CDDP (recovery 60%), (B) 1 m*M* CHIP (recovery 28%), and (C) 1 m*M* CBDCA (recovery 75%).

chlorate (Fig. 5B). It is apparent that, after ten days at 4°C, even in the presence of 100 m*M* chloride, TCDCP can undergo degradation to form at least four species. In pure water, or in an aqueous solution containing a non-coordinating anion, such as perchlorate, further degradation is apparent, leading to formation of charged species (probably hydrolysis products), which are retained on the column. Resolution of the four dominant platinum species in aged solutions of TCDCP can be improved by using an 8-cm ODS column (Zorbax Golden Series). A comparison between the 15-cm C_8 and 8-cm ODS (C_{18}) column is shown for TCDCP in Fig. 5; the dominant platinum-containing peaks are well resolved both from each other and from the oxygen peak. A fifth peak in Fig. 5C was checked for platinum by ETA-AAS, and platinum has shown to be absent.

After three months storage in the dark at 4°C, the solutions were analysed again by LC–ED. Fig. 6 shows the chromatographic separation of CDDP, CHIP, and CBDCA, from their degradation products, on an 8-cm ODS column with a mobile phase consisting of 5 m*M* hexanesulphonic acid and 10 m*M* sodium acetate at pH 4.60. The recovery data were 60 ± 5% for CDDP, 28 ± 4% for CHIP and 75 ± 5% for CBDCA. The recovery value for CBDCA indicates that some further degradation has taken place. CBDCA was resolved into two species: one eluted near the solvent front at 1.6 min, the other at 3.2 min. These results for CBDCA are in agreement with those reported by Gaver and Deeb[27] who showed that CBDCA had degraded after one week at −25°C. The mobile phase was strengthened by modification with 10% methanol, and by reducing the concentration of heptanesulphonic acid from 10 m*M* to 5 m*M*. The results are shown in Fig. 7. The recovery of platinum was somewhat improved: (A) 105 ± 5% for CDDP; (B) 19 ± 3% for CHIP; (C) 110 ± 5% for CBDCA; (D) 92 ± 5% for TCDCP. The recovery for CHIP and its degradation products is still poor; better recovery is possible for TCDCP, but at the expense of resolution.

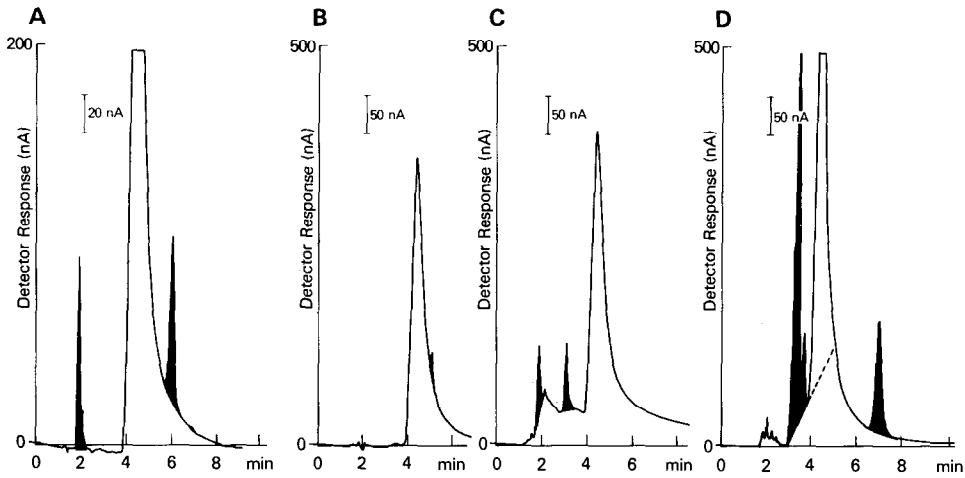

Fig. 7. LC ED of platinum complexes, after three months in water at 4°C: column: 15-cm C_8 Zorbax; mobile phase, 10 m*M* sodium acetate, 5 m*M* heptanesulphonic acid and 10% methanol (pH 4.60); flow-rate, 1 ml/min; detector, HMDE, $E = -1.0$ V. (A) 333 μM (100 μg/ml) CDDP (recovery 105%), (B) 1m*M* CHIP (recovery 19%), (C) 1 m*M* CBDCA (recovery 110%), and (D) 1 m*M* TCDCP (recovery 92%).

Fig. 8 shows how quantitative analysis of the first hydrolysis product of CDDP, the monoaqua species, has been improved. Earlier[13], it was shown that CDDP could be selectively determined by LC–ED with a working detection limit of 62 ng/ml. The optimum working potential for a "large" HMDE was found to be -250 mV *vs.* Ag/AgCl (Fig. 8A). However, it was not necessary to maintain this potential after the detection of CDDP at 1.9 min. It is possible to improve sensitivity for the monoaqua species favourably by changing the working potential from -250 mV to -1.0 V *vs.* Ag/AgCl. However, because sensitivity is decreased for CDDP at -1.0 V, the potential is maintained at -250 mV for the first 2 min of the analysis (Fig. 8B). An off-line determination of platinum was carried out after injection of 25 μl of 333 μM (100 μg/ml) CDDP, aged in water at 37.0°C for 7.8 h. The results are presented in Fig. 8C in the form of a histogram of platinum content as a function of time. The recovery was 108 ± 10%. The peak height of CDDP corresponded to a complex concentration of 23 μg/ml, which was 23% of the original concentration. Looking at the AAS data, 80% of the total platinum recovered was in the monoaqua form, and 20% was CDDP, which is in good agreement with LC–ED; at 7.79 h, the diaqua species had not yet been detected. However, the diaqua species did appear in the chromatogram after *ca.* 30 h of incubation. The sensitivity of electrochemical detectors for complex species depends upon the ligands which are coordinated to the metal atom. Ligand substitution may produce a large change in the electrochemical behaviour, even though there may be no change in the formal oxidation state of the metal.

Rate of loss of CDDP in various media: application of LC–ED methods

The rate at which CDDP disappears in various media has been studied by several methods (Table I). In water, the initial rate follows first-order kinetics (k_r

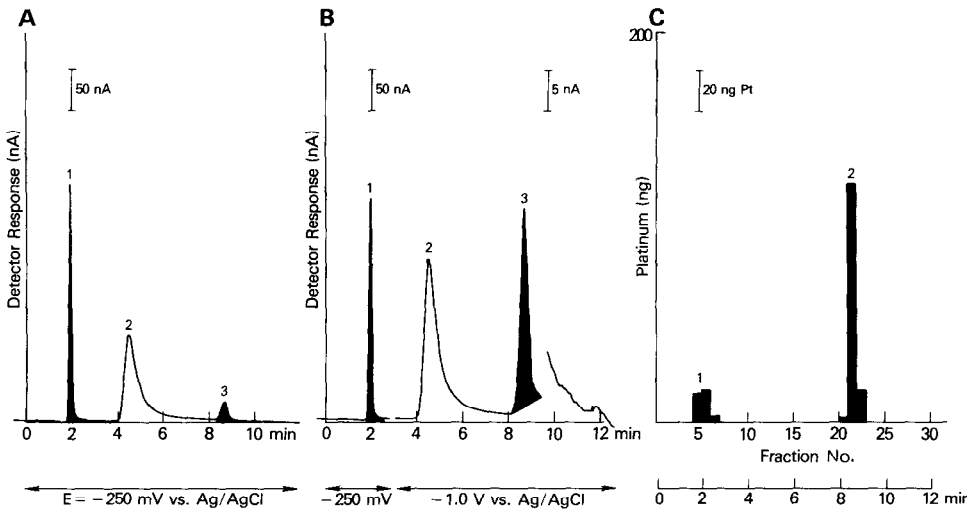

Fig. 8. LC–ED method improvement for CDDP [initial concentration 333 μM (100 μg/ml) complex] and the first hydrolysis product, aged in water at 37.0°C for 7.79–8.39 h; column 15-cm C_8 Zorbax; mobile phase, 10 mM sodium acetate, 5 mM heptanesulphonic acid (pH 4.60); flow-rate: 1 ml/min. (A) t = 7.79 h; electrochemical detector: E = −250 mV *vs.* Ag/AgCl; peaks: 1 = CDDP (24.6 μg/ml), 2 = oxygen and 3 = presumed monoaqua. (B) t = 7.99 h; electrochemical detector: E = −250 mV (0–2.4 min), −1.0 V (2.4–12 min); peaks: 1 = CDDP (23.0 μg/ml), 2 = oxygen and 3 = presumed monoaqua. (C) t = 8.39 h; detection off-line ETA-AAS of fractions collected at 0.4-min intervals: 1 = CDDP (350 ng platinum), 2 = presumed monoaqua (1400 ng); recovery = 108%.

= 0.33 h^{-1} at 37.0°C). The first acid hydrolysis product, *cis*-diammineaquachloroplatinum(II), may undergo further acid hydrolysis, to form *cis*-diamminediaquaplatinum(II), or, depending on the pH, base hydrolysis to form a hydroxo complex, or reverse hydrolysis (anation) via a second-order process to form the parent complex. The first acid hydrolysis step of CDDP was followed, using the LC–ED method described above. Fig. 9A shows the loss of 333 μM (100 μg/ml) CDDP at 37.0°C in water; the appearance of the monoaqua species was recorded 1.5 h after the reaction was started. The early data points were lost because the recorder pen was unexpectedly driven off-scale by the improved sensitivity for this species. In addition, it is apparent that a significant amount of the monoaqua species is rapidly formed during the time taken to dissolve and dilute the parent compound. Analysis of the initial slopes gives a rate constant of 0.33 h^{-1} for CDDP hydrolysis.

A sample of whole plasma was incubated with 10 μg/ml CDDP at 37.0°C *in vitro*. Aliquots were withdrawn at timed intervals, and the plasma proteins were removed by centrifugal ultrafiltration. A 25-μl aliquot of the plasma ultrafiltrate, containing "free platinum", was analysed by LC–ED for parent CDDP. The remaining ultrafiltrate was analysed for total platinum by ETA-AAS. A semi-log plot of the results is presented in Fig. 9B. The difference in slopes, and therefore, the rate constants reported in Table I, can now be explained. ETA-AAS measures total platinum in ultrafiltrate; it cannot distinguish between parent CDDP and the hydrolysis products. LC–ED can distinguish between several platinum species that may be present in plasma ultrafiltrate. The incubation of CDDP in plasma ultrafiltrate, loaded

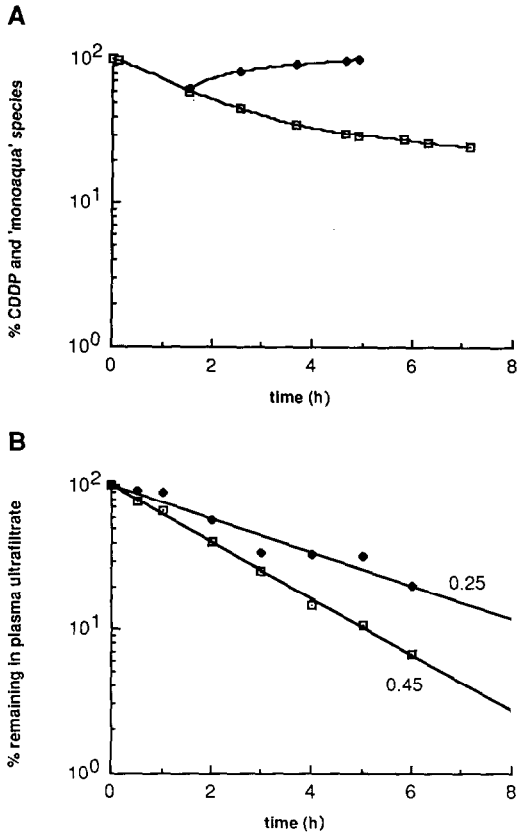

Fig. 9. Rate of loss of CDDP in various media over 8 h at 37.0 ± 0.01°C. (A) LC–ED monitoring of CDDP, initial concentration 333 μM (100 μg/ml), in water, showing appearance of the presumed monoaqua species; k_{CDDP} = 0.33 h^{-1}. %CDDP (⊡) on ordinate refers to percent of initial CDDP remaining in the solution. % monoaqua (◆) is referenced to a different basis and refers to the percent attained of the steady state value observed from 10 to 30 h. (B) 10 μg/ml CDDP incubated with human plasma: determination of CDDP by LC–ED (⊡) (k_{CDDP} = 0.45 h^{-1}); determination of total free platinum by ETA-AAS (◆) (k_{Pt} = 0.25 h^{-1}).

with 100 mM sodium chloride, was carried out for reason of comparison with ref. 20. It was found to follow first order kinetics initially (k_r = 0.32 h^{-1}), which is in excellent agreement with previous studies of ref. 20. In addition, we have detected the monoaqua species less than 1 h after incubating CDDP in plasma ultrafiltrate, even in the presence of 100 mM sodium chloride. This is surprising, because application of the law of mass action indicates that in the presence of high chloride ion concentrations, the equilibria, shown in Fig. 1, should be driven back towards the parent CDDP form.

CONCLUSION

Some platinum anti-cancer drugs now under investigation may be determined by LC–ED. It would seem that CDDP, and its major hydrolysis product, are well

resolved on a 15-cm C_8 column modified with 5 m*M* heptanesulphonic acid and detected electrochemically using a two-stage working potential. TCDCP and its degradation products are also well resolved on an 8-cm ODS column modified with heptanesulphonic acid. Method development for CHIP is continuing, but the detection problems of CBDCA requires a different approach. LC–ED has been compared to ETA-AAS methods for monitoring platinum levels in human plasma ultrafiltrate. The difference between these two methods may have significant implications for the clinical use of CDDP, where it is important to monitor closely the parent drug concentration.

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