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EVALUATION OF REDUCTIVE AMPEROMETRIC DETECTION IN THE LIQUID CHROMATOGRAPHIC DETERMINATION OF ANTINEOPLASTIC PLATINUM COMPLEXES

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SUMMARY

The usefulness of reductive electrochemical detection at mercury drop electrodes has been determined for platinum complexes separated by solvent-generated anion-exchange high-performance liquid chromatography. Both current-sampled dropping mercury and hanging mercury drop electrodes (DME and HMDE) provide significant advantages over UV absorbance and off-line non-flame atomic absorption detection. The effects of chromatographic and polarographic parameters on analytical system performance have been investigated. By raising the detector cell temperature, the detector response to *cis*-dichlorodiammineplatinum(II) (DDP) can be shifted anodically to 0.0 V vs. Ag/AgCl, thereby increasing detector selectivity for this compound. The noise-limited minimum detectable quantities of DDP with DME and HMDE are 1.8 ng and 70 pg injected, respectively. DDP can be determined in untreated urine at levels below 100 ng/ml.

INTRODUCTION

Cisplatin [*cis*-dichlorodiammineplatinum(II); DDP, Fig. 1] is a square planar complex of divalent platinum that is quite active against a variety of solid tumors and is widely used clinically in the treatment of testicular and ovarian cancers [1]. Work in these laboratories has resulted in the development of chemically bonded [2] and solvent-generated anion-exchange [3, 4] (SGAX) chromatographic systems for the separation of platinum complexes differing in ligand composition found in biological samples following cisplatin administration.

Because of the absence of significant molecular electronic absorptivity (λ_{max} = 301 nm; ϵ_{301} = 150) [5], the central platinum ion is the analyte characteristic

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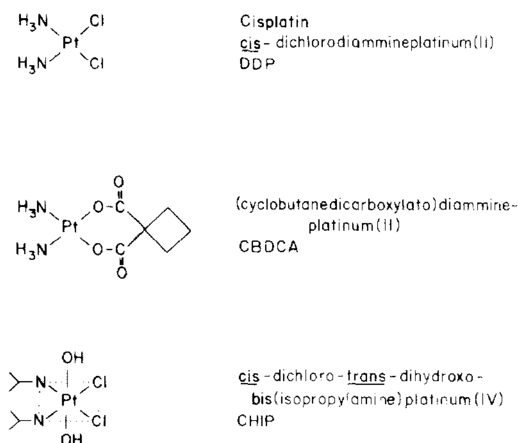

Fig. 1. Antineoplastic platinum complexes investigated.

most readily exploited for the detection of cisplatin and other complexes. Post-column analysis of eluent fractions by non-flame atomic absorption spectrophotometry (NFAA) has been combined with the chromatographic selectivity of column switching in a method capable of determining DDP at levels of 2 μg/ml in urine [6].

The feasibility of electrochemical platinum(II) detection for chromatographic systems has now been investigated to meet the need for a detector at least as sensitive as the described application of NFAA but capable of continuously monitoring the column effluent. With both oxidation and reduction of platinum(II) possible [7], electrochemistry provides numerous electrode and reaction possibilities. Reduction at mercury was chosen for study since, as explained in this paper, the nature of the platinum(II) reduction and the presence of surfactant in the mobile phase makes solid electrodes unattractive.

Platinum(II) complexes are poorly behaved depolarizers, interacting strongly with the electrode surface [7, 8]. The reduction of these compounds involves a two electron transfer to the central atom of an adsorbed molecule [8, 9]. Polarograms of chloro- and chloroammine-platinum(II) complexes include maxima and minima [7, 10] resulting from these surface effects. Platinum metal, adsorbed on the electrode surface as growing crystal nuclei, is produced in the irreversible reduction [11]. The electro-deposited metal alters the advantageous hydrogen overpotential of mercury causing catalytic hydrogen currents at potentials as anodic as −600 mV (saturated calomel electrode) [12]. Electro-oxidations of platinum(II) complexes occur through bridging halides and are quite slow in the absence of free halide [13].

Because of these analytically unsuitable electrode reactions, voltammetric methods have been infrequently used for the determination of platinum. In one reported electrochemical method [14], the catalytic hydrogen current resulting from platinum metal deposition is used quantitatively in a sensitive differential pulse polarographic determination of platinum at mercury.

Electrochemical detectors for high-performance liquid chromatography (HPLC) have been reviewed recently [15—17]. While oxidative amperometry

and coulometry at glassy carbon or carbon paste surfaces are most commonly used, reduction at mercury or solid electrodes has also been frequently applied. Mercury electrodes have been configured as either mercury immobilized on a solid support [18] or as mercury drops [19—21]. This paper describes the application of polarographic HPLC column monitoring to the clinical analysis of cisplatin in urine.

EXPERIMENTAL

High-performance liquid chromatography

The HPLC columns used were obtained from Technicon (Tarrytown, NY, U.S.A.) and were 150 × 4.6 mm packed with C_8-bonded 5-μm spherical porous silica. The column temperature was maintained with a water jacket (Alltech, Arlington Heights, IL, U.S.A.) and thermostated using a circulating pump, type FJ (Haake, Saddle Brook, NJ, U.S.A.). Columns were prepared for use by washing with 200 ml of water followed by 150 ml of 0.5% (w/v) hexadecyltrimethylammonium bromide (HTAB)—water. Deoxygenated mobile phase (500 ml) was pumped through the column prior to use. The mobile phase was not removed from the column for interim storage.

UV detection was with a Model 450 variable-wavelength detector (Waters Assoc., Milford, MA, U.S.A.) set at 301 nm. A 2-sec low-pass filter time constant was used. A Model 210 loop injector (Altex, Berkeley, CA, U.S.A.) with loops of various capacities was used for sample introduction. Mobile phases were pumped with either a Model 6000A reciprocating piston pump (Waters) or a Model 4100 screw-driven syringe pump (Varian, Walnut Creek, CA, U.S.A.). A syringe infusion pump Model 341 (Sage Instrument, Cambridge, MA, U.S.A.) fitted with a 35-ml syringe was used to pump solutions directly onto the electrode and for post-column electrolyte addition. Chromatograms were recorded on an Omniscribe dual-pen recorder (Houston Instruments, Houston, TX, U.S.A.) while polarograms were recorded on a Houston Omnigraphic X-Y recorder.

Electrochemical measurements

Cell potential control and current measurements were performed with a Model 174A Polarographic Analyzer (Princeton Applied Research, Princeton, NJ, U.S.A.). A Model 303 Static Mercury Drop Electrode was used to record quiet solution polarograms and was modified with a Model 310 flow adaptor, both from Princeton Applied Research, for flowing stream measurements. Elevated detector flow cell temperatures were achieved by wrapping half of a 1-m, 140-W heating tape around the cell. An inlet thermostat was added to the flow cell to insure that the stream temperature was that measured in the cell. One meter of stainless-steel tubing (1.6 mm I.D. × 0.23 mm I.D.) was coiled inside the wall of the cell and fitted into the electrode flow adaptor in place of the PTFE capillary tubing originally installed. The opposite end was attached to the column outlet. The temperature was monitored with a calibrated thermistor immersed in the cell and was adjusted by changing the heater voltage.

Oxygen in the inert sparge gas was reduced with a vanadous chloride scrubbing system [22]. Eight grams of ammonium metavanadate (Aldrich, Milwau-

kee, WI, U.S.A.) were boiled in 100 ml of concentrated hydrochloric acid (Fisher, Fairlawn, NJ, U.S.A.) for 10 min. After cooling and dilution to 1 l with water, the solution was added to three gas scrubbing vessels, each containing 15 g of amalgamated zinc granules (prepared from 20-mesh zinc, Fisher). The deoxygenating scrubbers were connected in series and were followed by two similar vessels containing water. Oxygen permeation through the PTFE feed line on a Waters pump was eliminated by enclosing this line in 7.9 mm I.D. PVC tubing through which inert gas was flowing. Configured in this manner, the entire fluid path of the HPLC instrument is sequestered from oxygen, without the use of a cumbersome glove box.

Urine analysis for cisplatin

Urine samples were frozen in dry ice immediately after collection and stored in dry ice until analysis. Sample preparation prior to chromatographic analysis [6] included thawing in a 50°C water bath with constant swirling followed by 30 sec of ultrasonication and filtration through a 3-μm Millipore filter. An aliquot (50 μl) of this material was injected onto a chromatographic system comprised of a dynamic anion exchange column (described above) thermostated at 60°C, a mobile phase of 5 m*M* citrate (pH 6.5) containing 0.1 m*M* HTAB and an electrochemical detector consisting of hanging mercury drop cathode (area = 0.026 cm^2; polarized at 0.00 V vs. Ag/AgCl) in a cell thermostated at 60°C.

Non-flame atomic absorption

Total platinum content of urine samples and of HPLC effluent fractions was determined as previously described [6] using a 175 B atomic absorption spectrophotometer fitted with a CRA-90 electrothermal atomizer (both from Varian).

Materials

Cisplatin was obtained in crystalline form from the National Cancer Institute (Bethesda, MD, U.S.A.) and its solutions were prepared in 0.1 *M* potassium chloride. Citric acid, sodium citrate and potassium chloride were AR grade (Mallinckrodt, St. Louis, MO, U.S.A.). HTAB was from Aldrich. Mercury was triple-distilled instrument grade (D.F. Goldsmith, Evanston, IL, U.S.A.). All water used was distilled in glass after mixed-bed deionization.

RESULTS AND DISCUSSION

Polarographic detectors used in conjunction with HPLC require a mobile phase optimized for both analyte resolution and analyte response, since the column effluent serves as the electrode reaction medium. In addition, potentiostat and electrode parameters must be optimized for the analyte(s) of interest. The development and evaluation of a clinically applicable assay for cisplatin has included these interrelated aspects.

Mobile phase composition

Although DDP and other platinum complexes differing in ligand composition can be resolved by SGAX using a simple aqueous HTAB mobile phase, this

system was found to be incompatible with electrochemical detection because of inadequate supporting electrolyte. Both the HTA cation and bromide are specifically adsorbed to the mercury electrode [23], altering double-layer capacitance [23] and diminishing electrode reactivity [24].

Addition of citrate to the mobile phase offered greater manipulation of DDP retention [4], and also served as a non-reactive supporting electrolyte. Citrate concentrations as low as 3 m*M* are adequate to allow the recording of polarographically detected chromatograms. A mobile phase containing 5 m*M* citrate was used for all subsequent studies.

DDP retention in HTAB—citrate mobile phases is pH dependent. As pH rises and the balance of citrate is shifted toward polyanions, retention increases [4]. Detector signal and noise were not affected by mobile phase pH. Final pH selection was made in conjunction with column temperature selection as described below.

In all instances, samples of cisplatin contained chloride ions. Standards were prepared in 0.1 *M* potassium chloride solutions to avoid chemical degradation of DDP [5] and biological samples similarly contained high levels of endogenous chloride [25]. Chloride causes a baseline disturbance in the polarographic chromatogram just after DDP elution (Fig. 2). Mobile phase citrate concentrations

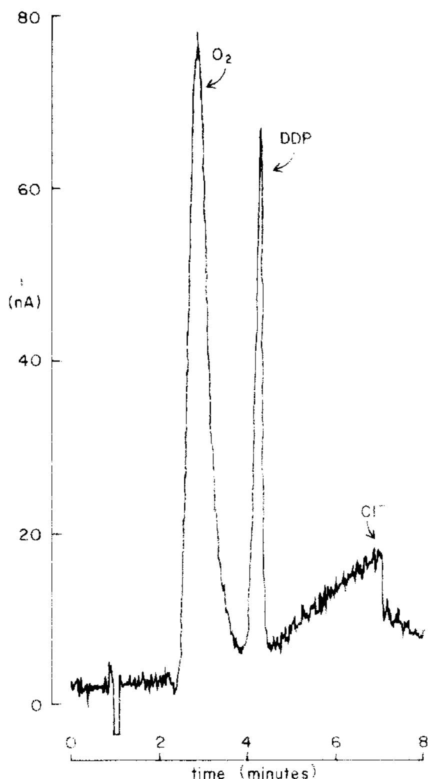

Fig. 2. Effect of injected chloride on polarographic chromatogram. Column: 150 × 4.6 mm, 7 μm C_8; sample: 150 μl of 2.0 μg DDP per ml 0.1 *M* potassium chloride; mobile phase: 10 m*M* citrate—0.1 m*M* HTAB, pH 7.3; flow-rate: 2.0 ml/min; column temperature: 24°C; detector: 0.5 sec DME, 0.026 cm² drop, −100 mV (Ag/AgCl); cell temperature: 24°C.

below 10 m*M* resolve the chloride wave from DDP so that analyte peak measurements are not compromised. Other anions (iodide, bromide and nitrate) did not produce the anomalous wave attributed to chloride.

Effect of column temperature

Column temperature affects the DDP–SGAX system in three ways. As temperature increases: (a) chromatographic efficiency increases; (b) the stability of both eluting DDP and the column itself decrease; and (c) column selectivity, measured for eluite pairs having nonidentical retention mechanisms [26], is altered.

Column temperature was raised to increase column efficiency, and therefore resolution and peak height improve. DDP retention decreased relative to oxygen, a significant interference in polarographically detected HPLC [18]. The k'_{DDP} value decreased from 7.8 to 1.8 (a 333% change) when column temperature was increased from 28°C to 78°C, while this increase in column temperature only resulted in a slight increase in k'_{O_2} from 2.9 to 3.2 (a 10% change).

To compensate for the diminished retention of DDP at higher temperatures, mobile phase pH was raised to maintain DDP elution at $k' = 2$–3, just prior to oxygen, but resolved from the unretained and early-eluting material. Column stability, however, limited the extent to which temperature and pH could be simultaneously raised. Twelve hours at 78°C with citrate mobile phase (pH 7.3, measured at 24°C) was sufficient to destroy column performance. Decreased retention, band broadening and asymmetry, and voiding at the head of the column were observed, indicating hydrolysis of both the C_8-anchoring silyl ether and the silica support [27]. Data defining the time course of this degradation were not collected. Subsequent work at temperatures below 65°C and pH values (measured at 24°C) $\leq$6.5 yielded column lifetimes of ca. 250 h of system operation. Losses in efficiency and retention, degradation of peak symmetry and peak shouldering under these conditions were again symptomatic of hydrolysis of the bonded phase. Repeated urine injections probably contributed to column deterioration. This was confirmed by backpressure elevations and darkening of the packing at the top of the column.

Elevated column temperatures accelerated DDP degradation. Riley et al. [3] estimated that at 25°C, $<$5% loss of DDP occurs on-column and concluded that the predominant reaction in the mobile phase is aquation. Monitoring the change in reduction current in a 64°C incubation of DDP (0.44 m*M*) in mobile phase (0.1 m*M* HTAB–5 m*M* citrate, pH = 6.5 at 24°C) with an immersed dropping mercury electrode (DME) (0 mV vs Ag/AgCl) revealed apparent first-order kinetics for DDP degradation. The rate constant for the process was 0.054 min^{-1}. The current diminished from 5.2 μA initially, to 0.09 μA, consistent with previous reports of the polarographic inactivity of aquated species derived from DDP [28, 29].

DDP degradation during its 4.5-min residence on column corresponds to a calculated loss of 22% of parent drug. This degradation will have a significant effect on sensitivity (units of peak area per unit concentration) but of greater analytical significance is the effect of this loss on precision. The increased rate of DDP aquation with temperature ($\Delta H^{\neq}$ = 20 kcal/mol [5]) is only partially compensated by the decreased reaction time which results from decreased re-

tention. Careful control of column temperature is, therefore, essential for analytical precision.

The selectivity of the polarographic detector for DDP relative to the aquated species formed on-column prevents the loss of peak shape integrity seen in similar systems with UV detection [3]. The lack of polarographic response to the aquated species (at the potential used) and the first-order kinetic behavior prevent on-column degradation from compromising calibration linearity of response to cisplatin.

Effect of flow-rate

When air-saturated mobile phase was pumped across the electrode the oxygen reduction current was distinctly flow dependent. DDP reduction current, measured in a deoxygenated flowing stream, however, was insensitive to flow-rate above 0.5 ml/min (Fig. 3). An apparent inverse relationship between flow and peak-height response was seen under chromatographic conditions (Fig. 4), that did not originate in the detector. The polarographic peak-height trend parallels that of the UV detector above ca. 0.5 ml/min, and arises from increasing column efficiency with decreasing flow-rate. The flow independence of the polarographic DDP response is caused by the slow adsorption step in the DDP electrode process. A flow-rate of 1.0 ml/min was chosen for analyses, taking advantage of the increase in efficiency and sensitivity without making the retention of DDP inconveniently long.

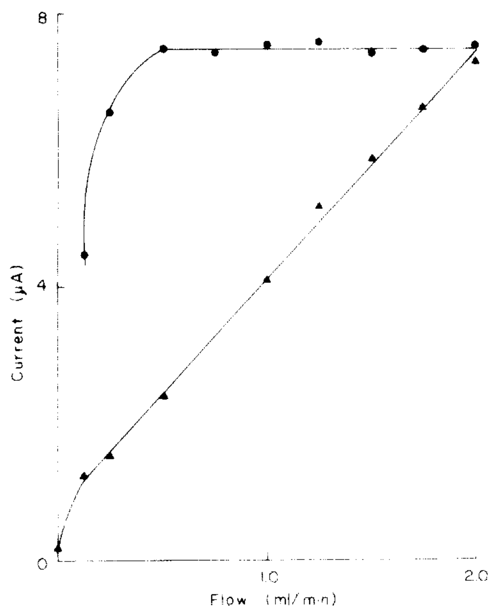

Fig. 3. Effect of flow-rate on DDP and oxygen reduction currents, measured in solution pumped directly into detector cell. O_2 (▲): air equilibrated mobile phase (5 m*M* citrate—0.1 m*M* HTAB, pH 6.5) at −700 mV (Ag/AgCl). DDP (●): 132 μg DDP per ml deoxygenated mobile phase at −410 mV (Ag/AgCl). Detector: 0.5 sec DME, 0.026 cm^2 drop; cell temperature: 24°C.

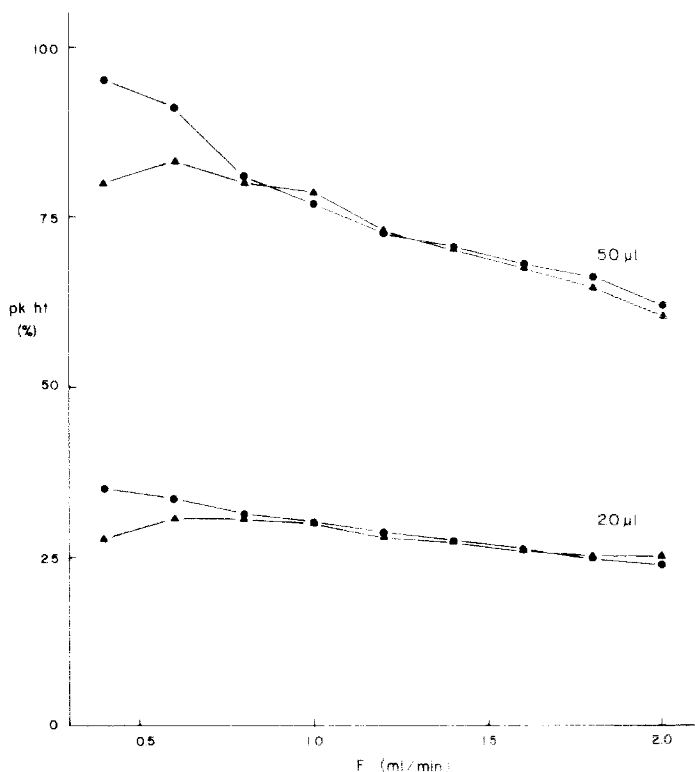

Fig. 4. Effect of flow-rate on chromatographic peak height. Column as in Fig. 2; sample: 20 and 50 μl of 100 μg DDP per ml; mobile phase as in Fig. 3; column temperature: 60°C; detector (DME): 1.0 sec DME, 0.026 cm^2 drop, 0.0 V (Ag/AgCl), 100% = 6.9 μA; detector (UV) 301 nm absorbance, 100% = 0.031 a.u. (●) UV, (▲) DME.

Effect of detector mode

Detection at a dropping mercury electrode was performed in sampled d.c. and normal pulse modes [30]. Because the hanging mercury drop electrode (HMDE) has a constant surface area, continuously monitored d.c. could be used with this electrode configuration, in addition to the other modes. Differential pulse, while offering additional selectivity [18], was not used because instrumental deficiencies in the potentiostat (time constant 5 sec at 1 sec drop time [31]) distorted the chromatographic bands (width at half-height 5—20 sec) [32].

When peak-height sensitivity to DDP (50 μl of 1 μg/ml injected; 55°C column; 5 m*M* citrate—0.1 m*M* HTAB, pH 6.5, at a flow-rate of 1 ml/min) was determined with a DME (24°C cell temperature, 1 sec drop time), sampled d.c. (−120 mV, Ag/AgCl) and normal pulse (0 — 120 mV, Ag/AgCl) both yielded a sensitivity of 0.14 μA/μg injected. The sensitivity advantage for pulse, seen in diffusion-limited systems [18], is not seen in this system, consistent with an adsorption-limited reduction of DDP.

Effect of drop size

The electrode used quickly produces a capillary-suspended drop that is static during most of its lifetime. The nominal masses of the three drop sizes available

are 1.2, 2.5 and 5.4 mg corresponding to surface areas of 0.0096, 0.0156 and 0.0261 cm^2 [33]. Actual drop size is dependent on mercury head pressure (limited to about 5% variation) and capillary and mercury flow-valve condition.

DDP solutions were prepared at 25, 50 and 100 μg/ml and injected with the detector set at each of the drop size selections. The observed increase in peak height response was not directly proportional to the increase in electrode surface area which is probably attributable to adsorption of DDP and HTAB to the drop surface. Baseline noise, however, was independent of drop size, so "large" (0.026 cm^2) drops were used in all subsequent measurements.

Drop time

Drop time effects three components of the analytical signal: (a) faradaic current; (b) capacitive current; and (c) peak representation (since it is recorded as a series of discrete current measurements at a DME).

Detector sensitivity decreased slightly as the drop time increased from 0.5 sec (DME) to several minutes (HMDE) (Fig. 5). Fresh drops were hung with each injection when a HMDE was used but sensitivity to DDP did not change if the drop formation was not synchronized with injection.

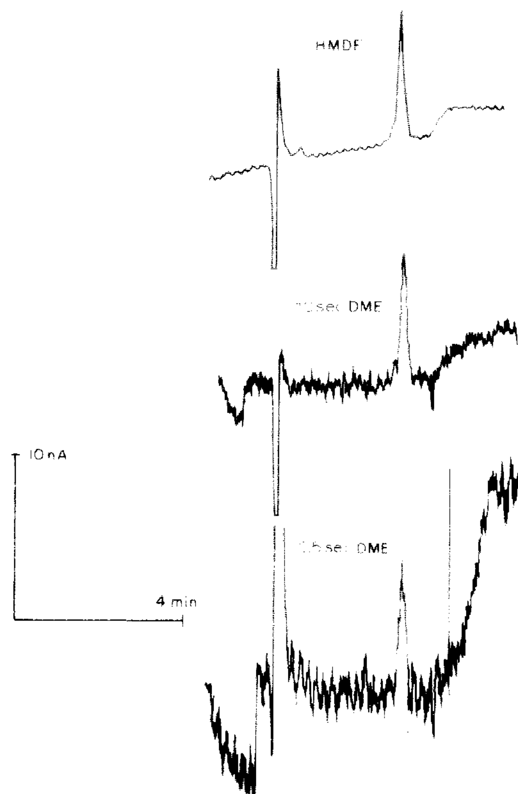

Fig. 5. Effect of drop time on noise and sensitivity. Column as in Fig. 2; sample: 50 μl of 500 ng DDP per ml; mobile phase as in Fig. 3; flow-rate: 1.0 ml/min; column temperature: 60°C; detector: 0.026 cm^2 drop, 0.0 V (Ag/AgCl); cell temperature: 60°C; 0.3 sec low-pass filter; HPLC pump: reciprocating piston.

The slight loss in sensitivity with increasing drop time may be due to loss of reactive surface area due to HTAB adsorption [23, 24]. Adsorption of inactive organic [10] and inorganic [8] substances has been shown to inhibit the reduction of those platinum complexes requiring such intimate electrode contact. Since HTAB is adsorbed at the mercury surface and is not itself reduced (accumulating on the surface until a potential-dependent coverage is reached), the available reactive electrode surface at the time the current is sampled is diminished as the drop time is increased, resulting in a decrease in the reduction current measured. Limiting coverage is apparently complete throughout peak detection in the HMDE mode and sensitivity is decreased from the 0.5- and 1.0-sec DME recordings.

HTAB adsorption on a time scale comparable to drop life (1—10 sec to reach limiting coverage) is consistent with reported surfactant adsorption kinetics [24, 34] and accounts for the drop-time dependence of detector noise (Fig. 5). In the absence of depolarizer, drop growth is accompanied by capacitive current as the charged double layer is expanded. Without complicating adsorption this capacitive current decays exponentially after drop growth ends [33]. When drops were formed and then expanded stepwise during continuous fixed-potential polarization, the decay of charging current was found to be greatly potential dependent. At potentials anodic to −100 mV (Ag/AgCl) the decay was slow. Long-chain alkyltrimethylammonium cations adsorb to the electrode surface in complete monolayers at potentials cathodic to the potential of zero charge (PZC) [23]. Since the detector was always operated at potentials anodic to the PZC (i.e., positive charge on the mercury surface side of the double layer), electrostatic repulsion inhibited the rate of adsorbate approach [35] and decreased the limiting coverage [23]. Slow adsorption of HTAB (limited by mass transfer) alters the double layer and results in current flow in this dynamic capacitor. This prolongation of charging current is responsible for the increase in baseline noise seen with decreasing drop time. Drop-to-drop decay variations cause fluctuations in the current sample taken at the end of each drop. Continuous amperometric monitoring of a hanging mercury drop eliminates drop-variable capacitive noise. An additional contribution to noise at a 0.5-sec DME may be a failure to re-establish drop-sheathing laminar flow after mechanical drop dislodgement [31].

While long drop times reduce noise they also degrade peak representation fidelity. Drop times greater than 1 sec result in signal sampling which is too infrequent for precise peak height measurement (stepped signal). Continuous detection at a hanging drop, in addition to reducing capacitive noise, improves the signal quality by eliminating discrete data sampling entirely.

Sparge gas

Removing oxygen from the mobile phase and preventing its redissolution was most conveniently accomplished by continuous sparging with an inert gas [36]. The mole fraction solubilities (in water at 25°C) of helium, nitrogen and argon are: $0.7 \cdot 10^{-5}$, $1.4 \cdot 10^{-5}$ and $2.5 \cdot 10^{-5}$, respectively [36]. A helium atmosphere, therefore, leaves less dissolved gas in the mobile phase than either nitrogen or argon and was used throughout. Helium lowers the high-frequency noise, seen when the polarographic detector cell is heated. Nitrogen and argon

sparging cause significant bubble formation in the heated cell. Growing on the reference electrode frit, these bubbles produce signal noise apparently by destabilizing the liquid junction. Small bubbles formed at the heated, atmospheric pressure outlet of the column, impinge on the electrode surface causing local flow disturbances and noise.

Effect of cell temperature

Fortuitous, but incompletely understood, is the observation of a change in detector response with an increase in cell temperature (Fig. 6). Two not completely separate components to this change are seen: an increase in current and an anodic shift of the potential of maximum response. Miner [37] has reported HPLC detector current–temperature coefficients of 0.5–5.0%/°C for organic eluites detected amperometrically at a glassy carbon anode. Although the calculation of a single coefficient is inappropriate in the present work, due to the shift in the hydrodynamic polarogram, the large increase in current is consistent with the non-diffusion controlled DDP electrode reaction [37].

The observed shift in peak potential in flowing stream was not expected. The reference electrode contribution is insignificant; the Ag/AgCl reference electrode is relatively temperature insensitive. From 25 to 50°C its potential

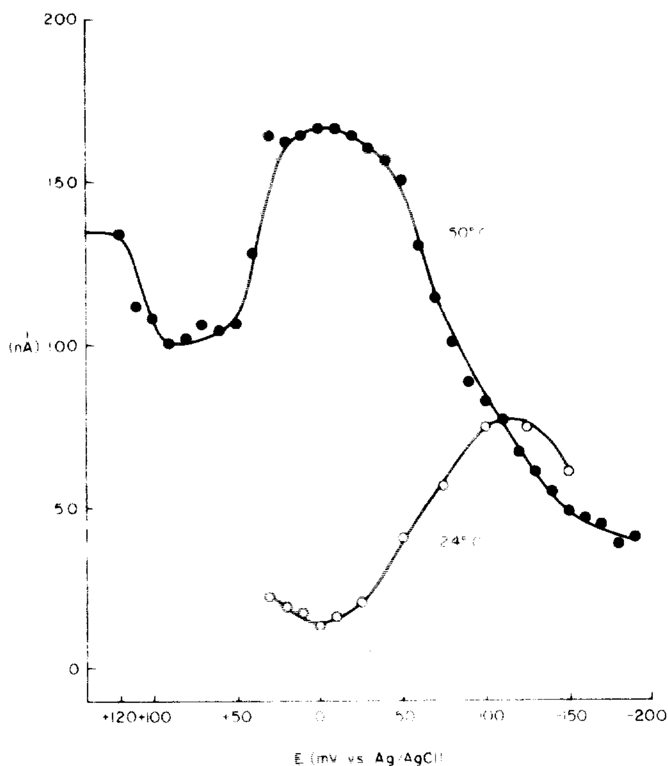

Fig. 6. Effect of cell temperature on the hydrodynamic polarogram. Column as in Fig. 2; sample: 50 μl of 10.5 μg DDP per ml; mobile phase as in Fig. 3; flow-rate: 2 ml/min; column temperature: 50°C; detector 1.0 sec DME, 0.026 cm² drop.

changes only 18 mV, shifting cathodically relative to the hydrogen electrode [38]. The specific adsorption of DDP to the working electrode and its reduction to platinum metal with liberation of all ligands might suggest an entropic explanation [39] for the potential shift. However, the absence of this effect in quiet solution eliminates this simple explanation. The shift in the chromatographically determined DDP polarogram may be kinetic in nature, resulting from altered relative rates of HTAB and DDP adsorption producing a potential-dependent change in the reactive electrode surface area. Alternatively, it may be due to a catalytic component of the measured current (chemical oxidation of mercury by DDP [40]) increasing with temperature.

The temperature-induced detector response changes have great practical significance. The combined cell temperature and potential changes (−120 mV at 24°C to 0 mV at 50°C) increase the DDP response by a factor of 2.1 while increasing the baseline noise by a factor of only 1.1, thereby yielding significant enhancement in DDP detectability.

In addition to the signal-to-noise ratio improvement due to the current increase, there is an improvement in detector selectivity for DDP brought about by the anodic potential shift. Changing the cell potential from −120 mV to 0 mV (Ag/AgCl) virtually eliminates the detector response to oxygen, since 0 mV is anodic to the foot of the first oxygen wave, $E_{1/2} = -50$ mV (Ag/AgCl). The baseline disturbance caused by the injection of chloride is diminished by a factor of 10 by the potential change from −120 to 0 mV. Many of the interferences in urine chromatograms arising from material reduced by the detector are also decreased by the anodic potential shift. Detector selectivity cannot, however, be increased indefinitely by anodic adjustments. Urine contains many easily oxidizable substances (e.g., catecholamines and ascorbic acid) [25] and such materials will generate increasingly intense negative (oxidation) peaks as the potential is made more positive. In addition thiols present in urine (e.g., glutathione and cysteine) [25] will promote the oxidation of the mercury electrode itself at 0 mV (Ag/AgCl) [41], also producing negative peaks.

Determination of DDP in urine

DDP is not extractable from aqueous media and the physical properties of urine (particularly its lack of macromolecular solutes) make this medium more suitable for direct HPLC analysis than other biological fluids. Absorbance detection at 301 nm allows the quantitation of DDP in urine only at levels $\geq$ 100 μg/ml [6].

The described method has been successfully used to determine DDP in urine samples collected during DDP therapy (Figs. 7 and 8). Correlation with a previously applied clinical method [6] was high and is described by the equation ElCD = 0.95 × NFAA +0.31, r = 0.998 (Table I). DDP can be quantitated in urine at levels below 100 ng/ml (Fig. 8) with an interassay precision of ±4%. Replicate injections of individual urine samples resulted in determination of cisplatin with precision of ±2%. Polarographic detector selectivity for DDP obviates the need for dissimilar-phase column switching which was previously used [6] to provide chromatographic selectivity.

The actual minimum detectable quantity is dependent on sample composition and is greater than the detection limit imposed by the system itself (Table

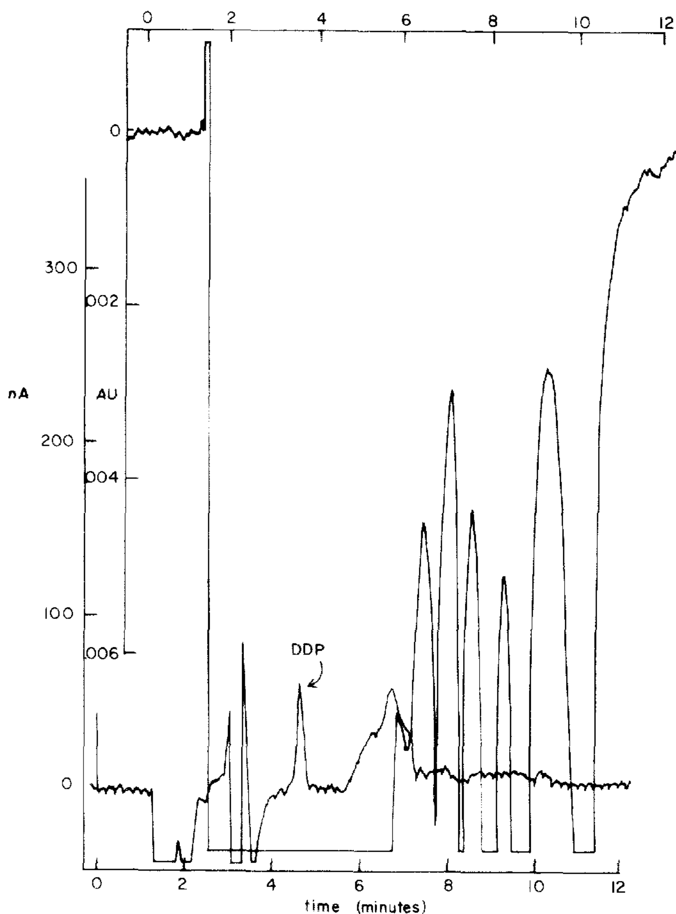

Fig. 7. ElCD and UV chromatograms of patient urine taken 4 min after DDP administration (patient 1). Column as in Fig. 2; sample: 50 μl urine estimated to contain 1.9 μg DDP per ml; mobile phase as in Fig. 3; flow-rate: 1.0 ml/min; column temperature: 60°C; detector (ElCD): 1.0 sec DME, 0.026 cm^2 drop, 0.0 V (Ag/AgCl); cell temperature: 60°C; detector (UV): 301 nm absorbance; HPLC pump: reciprocating piston.

II). Eluites, which are either reduced or oxidized, produce positive or negative recorder peaks, respectively, in the vicinity of DDP and cause the minimum quantity detectable in urine samples to be greater than the 1.4 ng/ml (50 μl injection) best-case detection limit. Thus, in Fig. 8 it can be seen that noise is insignificant relative to DDP peak height, but that the preceeding oxidation peaks would compromise DDP quantitation at much lower levels. It is not reasonable to state a detection limit in urine as a characteristic of system performance because of the variability of interferences. This variability applies to urine samples from a single patient during the course of therapy. Initial samples are clear and nearly colorless as the kidneys remove the fluid of pre-therapy hydration. Urine color and sediment increases within 2 h as the rate of urine production decreases.

Detector potential adjustments have been used to maximize response selectivity, compensating for increased chromatogram complexity in later sam-

ples (i.e., those taken 6 h after dosing). Fig. 8 was recorded at −40 mV (Ag/AgCl) to minimize interfering oxidation peaks. The change in detector sensitivity that accompanies the reinjection of the sample at a different potential is corrected by interpolated two-point recalibration, above and below the apparent sample concentration.

TABLE I

DETERMINATION OF CISPLATIN AND TOTAL PLATINUM IN URINE OF PATIENTS DURING THERAPY

150 mg cisplatin per m² body surface, 1 mg/ml in 0.5% dextrose—half-normal saline, intravenous injection over 15 min.

Time*	Urine volume (ml)	Cisplatin** (μg/ml)	Cisplatin*** (μg/ml)	Total platinum § (μg platinum per ml)
Patient 1				
0 h 0 min	525	44.2	45.6	32.7
0 h 30 min	200	44.6	46.9	31.2
2 h 0 min	15	15.1	12.6	13.5
4 h 0 min	30	1.9	2.6	1.1
8 h 0 min	50	1.5	1.5	6.6
25 h 30 min	125	1.5	1.5	2.8
Patient 2				
§§	30	0	—	0.16
1 h 40 min	375	69.6	—	43.3
3 h 25 min	500	7.9	—	5.81
5 h 5 min	250	1.2	—	2.02
7 h 45 min	500	0.09	—	1.72

*Time after end of drug administration.
**LC-ElCD. Patient 1: first course of DDP; conditions as in Fig. 7; patient 2: second course of DDP; conditions as in Fig. 8.
***LC-NFAA (ref. 6).
§ Direct NFAA total platinum (i.e., platinum concentration irrespective of ligands coordinated to the metal or platinum valency).
§§ Sample before dose.

TABLE II

SUMMARY OF DETECTOR CHARACTERISTICS

Values determined with previously unused, equilibrated column.

	Sensitivity*	Noise**	Detection limit*** (ng)
0.5 sec DME	1.23 nA/ng	2 nA	3.2
1.0 sec DME	1.12 nA/ng	1 nA	1.8
HMDE	1.10 nA/ng	0.04 nA	0.07
301 nm absorbance	$5 \cdot 10^{-6}$ a.u./ng	$2 \cdot 10^{-4}$ a.u.	80

*Slope of peak height vs. mass of DDP injected. Chromatographic conditions as in Fig. 8; detector cell temperature: 60°C; 0.026 cm² drop; 0.0 V (Ag/AgCl).
**Peak-to-peak with 1-sec (ElCD) and 2-sec (UV) low-pass filter.
***Mass injected giving signal which is twice noise.

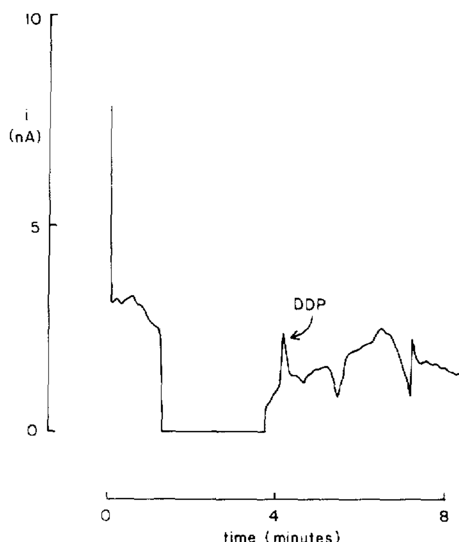

Fig. 8. Chromatogram of patient urine taken 7 h 45 min after DDP administration (patient 2). Column as in Fig. 2; sample: 50 μl urine estimated to contain 91 ng DDP per ml; mobile phase as in Fig. 3; flow-rate: 1 ml/min; column temperature: 60°C; detector: HMDE; 0.026 cm^2 drop, −40 mV (Ag/AgCl), cell temperature: 62°C; 1 sec low-pass filter; HPLC pump: syringe.

Application to the determination of other complexes

A variety of platinum complexes are currently being evaluated for antineoplastic activity and clinical utility [42]. Several have been successfully chromatographed [6] using the SGAX system with UV detection. CBDCA and CHIP (Fig. 1) are both eluted similarly to DDP. CHIP, a tetravalent platinum complex, can be detected polarographically with a sensitivity comparable to that of DDP (Fig. 9). However, no polarographic response to CBDCA (2 m*M*) was seen even in quiet solutions at potentials more cathodic than 1.0 V vs. Ag/AgCl.

The polarography of platinum complexes is highly dependent on the nature of the ligands [28, 29]. Half-wave potentials and electrode mechanisms are similarly changed by ligand substitutions. Detector selectivity will decrease with increasing cathodic potentials necessary for the detection of the less reducible complexes, and sensitivity to different complexes will change with electrode reaction mechanism.

Operating considerations

Operated as either a DME or HMDE, the detector produces a linear peak-height response to injected DDP. These calibration slopes, as well as noise and calculated interference-free detection limits are shown in Table II. Performance parameters determined for DDP detection by UV absorbance at 301 nm are also given for comparison. The sensitivities, and therefore the detection limits, are determined from recorded peak heights and so are a function of the chromatographic system. Because column condition and temperature, mobile phase composition and flow-rate will all affect peak height, these data should not be considered as absolute detector parameters. They are, however, an accurate

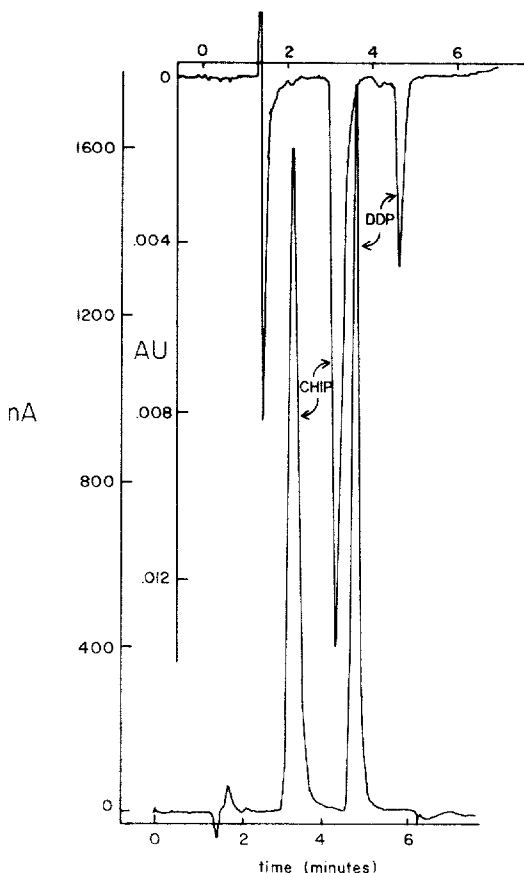

Fig. 9. ElCD and UV chromatograms of CHIP and DDP. Column as in Fig. 2; sample: 6.2 μg CHIP and 4.2 μg DDP in 50 μl; mobile phase as in Fig. 3; flow-rate: 1 ml/min; column temperature: 50°C; detector (ElCD) 1.0 sec DME, 0.026 cm^2 drop, 0.0 V (Ag/AgCl); cell temperature: 50°C; detector (UV): 301 nm absorbance.

comparison of the detectors under the conditions described. Since the sensitivities of the electrochemical detection modes are similar, noise determines the detection limits. The detection limit for the HMDE, with a 50-μl injection is 1.4 ng DDP per ml, comparable to direct total platinum determinations by NFAA in the absence of interferences.

While detection at a HMDE is useful for the determination of trace levels of DDP it is also slightly less convenient than a DME. Twenty to 24 min after initial drop formation, detector response is abruptly lost but is reproducibly restored with a new drop. In addition to hanging a fresh drop with each injection, attention must be given to unexplained but reproducible + 0.4 nA/min drift. At current ranges of 10 nA full scale and less, this drift is significant. The constantly regenerated DME operates at higher platinum levels with less user intervention.

CONCLUSIONS

Electrochemical detection provides the necessary sensitivity and selectivity for chromatographic monitoring of DDP in urine for at least 8 h after drug administration. In the absence of interferences its limit of detection is 1000-fold lower than that of UV absorbance. In addition, its selectivity allows the quantitation of DDP in untreated urine. The renewable mercury drop electrode is useful in the described system because of (a) requisite analyte adsorption prior to reduction, complicated by competing adsorption of mobile-phase surfactant, and (b) accumulation of the reduction product on the electrode. Such cumulative surface effects degrade signal quality and are difficult to overcome with solid electrodes.

Detector selectivity for DDP is improved by the anodic shift in potential of maximum response with increasing temperature. It is not, however, a platinum-specific detector, so that its application to the determination of other platinum complexes formed in vivo after DDP administration must be preceded by identification and electrochemical characterization of these species.

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