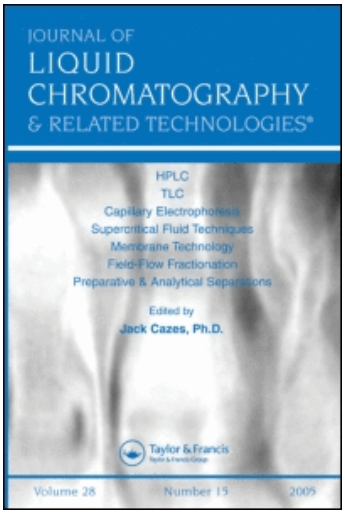

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SENSITIVE DETERMINATION OF CISPLATIN IN BODY FLUIDS WITH HPLC AND ON-LINE REDUCTIVE ELECTROCHEMICAL DETECTION

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ABSTRACT

A sensitive method for the determination of the antitumor agent cisplatin has been developed consisting of solvent-generated anion exchange chromatography and on-line reductive electrochemical detection. CDDP was retained on a hexadecyltrimethylammonium-loaded reversed phase HPLC column using a 5mM citrate-buffered eluent (pH 6.5). Exploiting adsorption of CDDP at a dropping mercury electrode and NH_4^+-enhanced catalytic proton reduction, a detection limit of 10^{-8} M (3 ng/ml) has been achieved. Application of the developed method to the quantification of CDDP in plasma ultrafiltrate and urine is demonstrated and discussed.

INTRODUCTION.

Cisplatin (cis-diamminedichloroplatinum(II), CDDP) is a potent cytostatic drug, active against various types of solid tumours

(1). Nephrotoxicity of CDDP is dose-limiting and the underlying mechanisms are not yet known (2). Renal clearance of non-protein bound platinum exceeds the glomerular filtration rate, indicating tubular secretion of CDDP or CDDP-derived platinum compounds (3). To contribute to the study of the mechanisms behind cisplatin-induced nephrotoxicity, a sensitive analysis of the rapidly eliminated CDDP and its low-molecular weight metabolites is desired. Atomic Absorption Spectroscopy (AAS) has often been used for the analysis of platinum in body fluids (4,5). However, AAS does not discriminate between the various CDDP-derived compounds, thus giving only limited information about the biotransformation of CDDP and the individual renal clearances of CDDP and its metabolites. Discrimination between the compounds becomes possible by the use of high-performance liquid chromatography (HPLC) which makes on line detection of platinum compounds in the eluent desired. Up to now on-line detection using UV-detection (6), electrochemical detection (7-9), post-column derivatisation followed by UV-detection (10), quenched phosphorescence (11) or inductively-coupled plasma atomic emission spectroscopy (12) lacked sensitivity. The often used alternative of off-line AAS detection is although sensitive is very laborious (13-15). Therefore we developed a sensitive on-line reductive electrochemical quantification of CDDP after solvent-generated anion exchange chromatography. The applicability of the method is demonstrated by the determination of CDDP added to drug-free plasma ultrafiltrate and urine.

EXPERIMENTAL.

Chemicals: CDDP (99.9%) was kindly supplied by Bristol Myers Company (Syracuse, NY, USA). All other reagents used were of analytical grade.

HPLC system: a Microspher C18 (3μm particle size, 100 mm x 4.6 mm I.D.) column (Chrompack International, Middelburg, the Netherlands) was loaded at 1ml/min for 1 h with 10 mM hexadecyltrimethyl-ammoniumhydroxide (HTA, Eastman Kodak, Rochester, NY, USA) in 5 mM citrate buffer (pH 6.5) and subsequently equilibrated with eluent consisting of 10^{-4}M HTA in 5 mM citrate buffer (pH 6.5).

Electrochemical detection: a PAR 303 static mercury drop electrode (medium drop size), equipped with a platinum auxiliary electrode and a Ag/AgCl reference electrode, was combined with an Olivetti M240 driven potentiostat (GPES-Autolab, ECO-chemie, Utrecht, The Netherlands). A model 310 flow adaptor (EG&G Princeton Applied Research, Princeton, New Yersey, USA) was used for LC-detection. Ultra pure mercury for polarographic measurements (Johnson Matthey, Roysten, England, GB) was used. Eluent and flow cell were purged and kept under nitrogen (<5 ppm oxygen), purified by a gas clean oxygen filter to less than 1 ppm oxygen (Chrompack International). Stainless steel tubing and gas-tight fittings were used throughout.

The electrochemical behavior of CDDP was investigated by recording the polarograms in eluent as described by Elferink et

al. (16). Electrochemical detection was achieved by applying a pulse step from an initial potential at which CDDP adsorbs at the electrode surface to the potential region of catalytic hydrogen reduction (16).

The HPLC-detection parameters were derived from the polarogram recorded in the eluent of choice. Drop time, pulse duration and current sampling time were optimized. A schematic presentation of these parameters is given in figure 1.

Sample preparation: freshly prepared plasma ultrafiltrate (Centriflow ultrafiltration membrane cones, CF 50A ; Amicon, Oosterhout, The Netherlands) was spiked with CDDP and immediately analysed. Fresh urine was centrifuged for 10 min at 3000 rpm. The supernatant was spiked with CDDP and immediately analysed.

RESULTS AND DISCUSSION.

The electrochemical behavior of CDDP in eluent is shown in figure 2 by a sampled direct current polarogram of 10^{-5}M CDDP in eluent (10^{-4}M HTA in 5mM citrate buffer at pH6.5). At -0.1 V, facilitated reduction of CDDP occurred due to adsorption of CDDP at the mercury drop, mediated by its chloride ligands. The minimum in the reduction wave was due to a gradually decreasing absorptivity of CDDP at lower potentials. At -1.4 V, direct reduction of the central Pt(II) ion took place. At -1.6 V, platinum at the mercury surface reduced the hydrogen

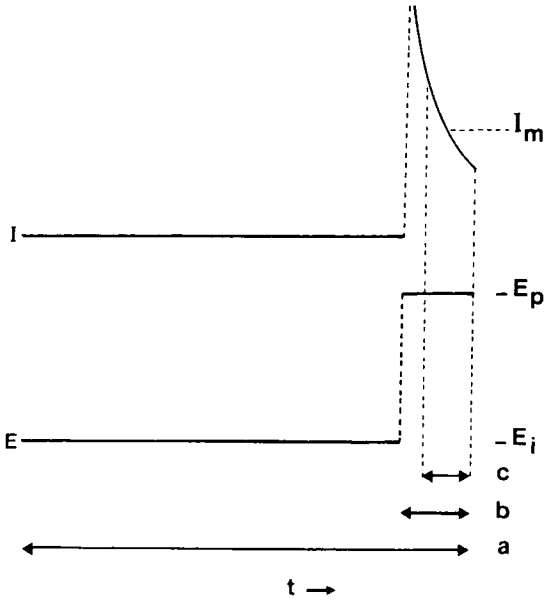

FIGURE 1.
Schematic representation of the electrochemical detection parameters. a = droptime, b = pulse duration, c = current sampling time , E_i = initial potential, E_p = pulse potential, I_m = measured current.

overpotential resulting in catalytic proton reduction. These observations corresponded with our previous reports on the electrochemical behavior of CDDP in NaCl (16). Catalytic proton reduction might be used to increase the detection sensitivity of CDDP. Lowering the pH will not only increase catalytic proton reduction but also the polarographic background. However, when NH_4^+ was used as a proton donor then catalytic proton reduction increased without increasing the polarographic background to the same extent (16). Therefore, we investigated the electrochemical

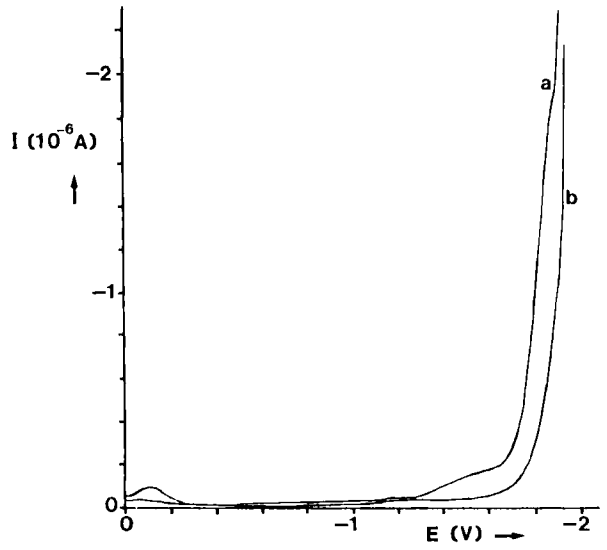

FIGURE 2.
Sampled direct current polarograms of a) 10^{-5}M CDDP in eluent (10^{-4}M HTA in 5mM citrate buffer at pH 6.5) and b) eluent. droptime = 1 s , scanrate = -10 mV/s , current sampling time = 20 ms , current range = 1μA .

behavior of CDDP in eluent supplemented with NH_4^+ in more detail. Figure 3 shows the sampled direct current polarogram of CDDP in eluent supplemented with 0.1M NH_4Cl. The proton reduction wave of the eluent shifted about 100 mV to a less negative potential. The catalytic hydrogen wave of CDDP also shifted to a less negative potential but the signal to background ratio improved. Lack of a distinct plateau in the region of catalytic proton reduction is a disadvantage, however standardization of the electrochemical parameters by our computer assisted potentiostat and our

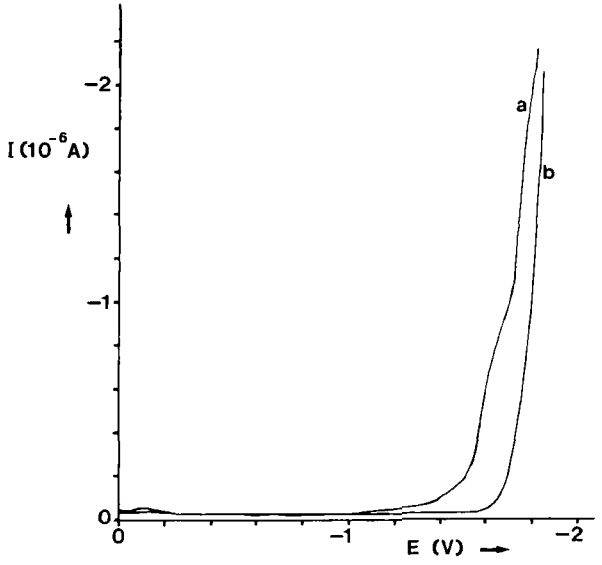

FIGURE 3.
Sampled direct current polarograms of a) 10^{-5}M CDDP in eluent supplemented with 0.1M NH_4Cl and b) eluent supplemented with 0.1M NH_4Cl.
droptime = 1 s , scanrate = -10 mV/s , current sampling time = 20 ms , current range = 1μA .

computerized data processing procedure warrant the applicability for quantitative purposes.

The effect of various anions on the ammonium-enhanced catalytic proton reduction was investigated by recording polarograms of 10^{-4}M CDDP in eluent supplemented with 1 - 90 mM ammonium salt. Normal pulse polarography with 0 V as initial potential and a droptime of 1 s was used to include the effect of adsorption of CDDP at the electrode surface exploited in electrochemical detection.

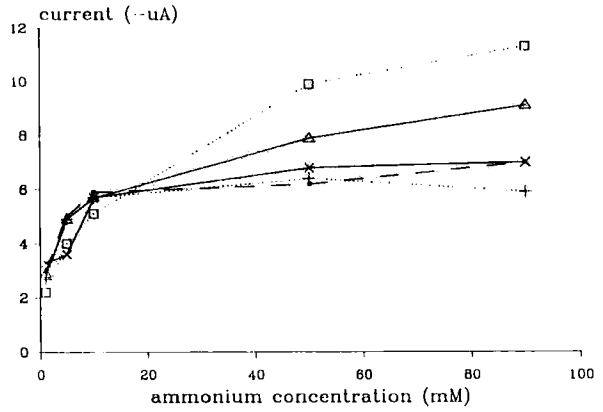

FIGURE 4.
The effect of various ammonium salts and their concentration on catalytic proton reduction of 10^{-4}M CDDP in eluent .
NH_4Ac (—▲—), NH_4Cit (···□···), NH_4Cl (···+···), NH_4NO_3 (—■—), $(NH_4)_2SO_4$ (—✕—).
Normal pulse polarography, initial potential = 0 V, droptime = 1 s , scanrate = -10 mV/s , pulse duration = 30 ms , current sampling time = 20 ms, effective accumulation time = droptime-pulse duration = 970 ms.

A stepwise increase of the ammonium salt concentration from 1 mM to 90 mM, caused an increase of the catalytic current which leveled off at concentrations higher than 50 mM (figure 4). Differences in height between the catalytic hydrogen waves with the ammonium salts investigated were limited. Ammonium citrate was most effective in increasing the catalytic current. Therefore, 0.1M of ammonium citrate was chosen to enhance catalytic proton reduction by CDDP and thus the sensitivity of detection.

Another argument to choose ammonium citrate is its influence on the chromatographic behavior of CDDP. While monovalent anions

in the eluent decrease the chromatographic retention of CDDP, polyvalent anions (especially citrate) cause an increase in retention of CDDP, which is beneficial to the separation of CDDP from early eluting, endogenous compounds in body fluids. This effect of citrate on CDDP retention is maximal at concentrations of 5-10mM (17). Therefore, 0.1M ammonium hydroxide brought to pH 6.5 with citric acid was the best way to introduce 0.1M NH_4^+ for maximum sensitivity of detection with a minimal loss of CDDP retention. However, when using ammonium salts other than ammonium chloride, the chromatographic behavior of CDDP was dramatically disturbed by the presence of Cl^- in the injected sample i.e. 0.1M NaCl present in CDDP standard solutions to avoid hydrolysis of CDDP. Peakshape deteriorated making quantification very difficult. Because plasma ultrafiltrate and urine also contain Cl^- this problem had to be solved. Replacement of 0.1M ammonium hydroxide brought to pH 6.5 with citric acid by 0.1M ammonium chloride in the eluent solved these problems but resulted in a decrease in the capacity factor of CDDP from 2.5 to 1.3 due to the above mentioned effect of monovalent anions on the retention of CDDP. Higher concentrations of HTA in the eluent (0.5 or 1 mM) had only a small positive effect on the retention of CDDP. However, baseline current and noise increased while electrochemical detection sensitivity to CDDP decreased , possibly due to adsorption of this cation onto the negatively charged electrode surface. Therefore, increasing the HTA concentration in the eluent was not a useful way to restore retention of CDDP.

As shown in figure 2, CDDP adsorbs at the mercury electrode at potentials around 0 V. This property might be used to enhance the sensitivity of electrochemical detection by accumulating CDDP at the electrode (at a potential in the region of adsorption) prior to stepping the potential into the region of catalytic proton reduction.

To establish optimal detection conditions, time and potential dependent accumulation of cisplatin on the Hg electrode was investigated in batch in a solution of 10^{-5}M cisplatin in eluent supplemented with 0.1M NH_4Cl. This was done by a) varying the initial potential from 0.1 V to -1.4 V and applying a potential step to the potential region of catalytic H^+ reduction (-1.6 V) and b) varying the accumulation time (drop time minus potential pulse time) at the determined optimal accumulation potential. The adsorptivity of cisplatin at the static mercury drop electrode appeared to be maximal at 0 V (Figure 5). The surface excess of adsorbed cisplatin increased with time, reaching a plateau at 3 s (Figure 6). However, when monitoring a chromatographic effluent, a sufficient number of measurements has to be made during elution of a peak in order to quantify accurately peak height and peak-area. Therefore, as a compromise, a drop time of 1 s, giving 92% of the maximum signal, was chosen.

An optimal signal/noise ratio was obtained with a pulse duration of 30 ms and current sampling during the last 20 ms of pulse application (figure 7).

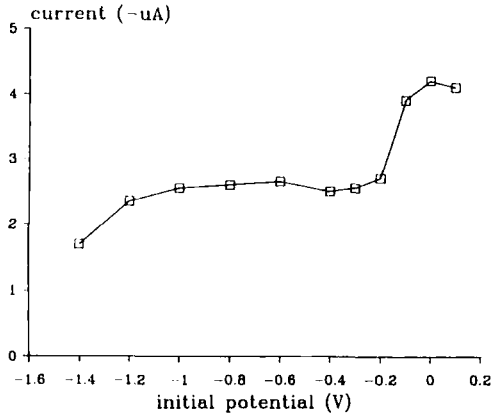

FIGURE 5.
The effect of initial potential on catalytic proton reduction due to 10^{-5}M CDDP in eluent supplemented with 0.1M NH_4Cl.
droptime = 1 s , pulse duration = 30 ms , effective accumulation time = droptime - pulse duration = 970 ms , current sampling time = 20 ms, pulse potential = -1.6 V.

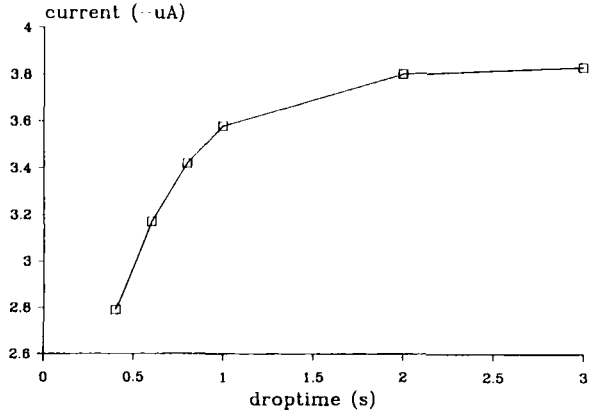

FIGURE 6.
The effect of deposition time on catalytic current due to 10^{-5}M CDDP in eluent supplemented with 0.1M NH_4Cl.
pulse application time = 30 ms , current sampling time = 20ms , effective accumulation time = droptime (t) - 30 ms.

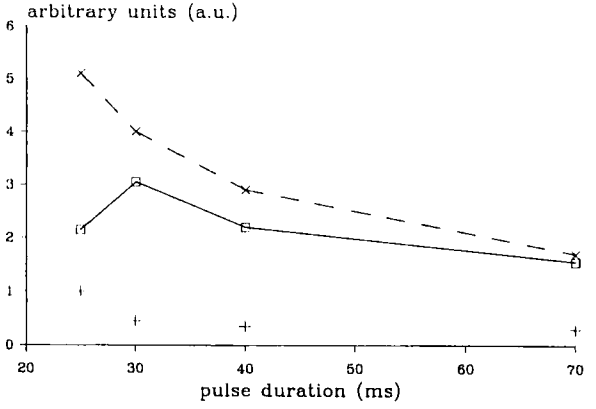

FIGURE 7.
The relation between pulsetime and signal/noise ratio.
Signal (1a.u.=-100nA, —✱—), noise (1a.u.=-4nA, ··+··), signal to noise ratio (1a.u.=15, —⊟—).
Droptime = 1 s, current sampling time = 20 ms, initial potential = 0 V, pulse potential = -1.6 V.
10^{-6}M cisplatin in eluent supplemented with 0.1M NH_4Cl.

Under optimized conditions - 0.1 M NH_4Cl, 10^{-4}M HTA and 5 mM citrate buffer (pH 6.5) at a flow of 1.0 ml/min, a drop time of 1 s, an initial potential of 0 V followed by a 30 ms pulse to -1.6 V with 20 ms current sampling - CDDP could be quantified down to 10^{-8} M (3 ng/ml) with a signal to noise ratio S/N = 3. This limit of detection is below the limits of detection earlier obtained with the polarographic detection of the second generation antineoplastic platinum drugs cisdichloro(1,1-bis-(aminomethyl)cyclohexane)platinum(II) (TNO-1), aqua(1,1-bis-(aminomethyl)cyclohexane)sulphatoplatinum(II) (TNO-6) (18) and diammine(1,1-cyclobutanedicarboxylato)platinum(II) (carboplatin) (19). However, detection sensitivity for these platinum compounds

might improve considerably when ammonium enhanced catalytic proton reduction and, when possible, accumulation of the complex on the mercury drop electrode (e.g. TNO-6 by its Cl^- ligands) appear applicable.

The limit of detection of 10^{-8}M as present in the injected sample implicates that in the column effluent, due to band broadening, CDDP is detected in the nanomolar range. The lowest reported detection limit for graphite furnace AAS is 10^{-8}M Pt in biological samples (4, 20). Supposing this limit of detection is also obtainable in collected fractions from a chromatographic run, then our on-line electrochemical detection is more sensitive than the laborious off-line graphite furnace AAS method. Furthermore, loss in resolution of the chromatographic peaks due to off-line detection in collected fractions is omitted by our on-line detection. Wang et al. (21) reported a sensitive adsorptive stripping polaro-graphic assay for CDDP with a limit of detection of $2x10^{-8}$M. However, direct quantification of CDDP in urine was not possible due to interfering endogenous compounds. The long preconcentration time makes this assay unsuited for on-line quantification of CDDP in body fluids. As an off-line detection method this assay is also unsuited because of its time consuming character. Zhao and Freiser (22) were able to quantitate Pt(II) complexes at the subnanomolar range by derivatizing the Pt(II) complex to a formazone complex and using the strong adsorption of this complex at a mercury electrode together with

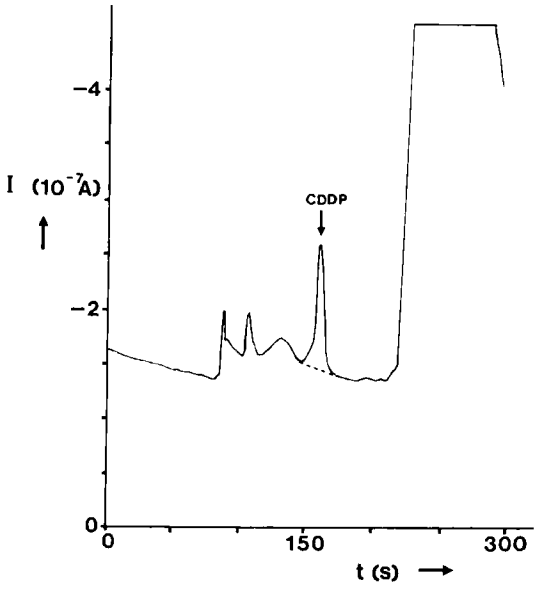

FIGURE 8.
Chromatogram of human plasma ultrafiltrate, freshly spiked with 10^{-7}M CDDP (——) and blanc plasma ultrafiltrate (---).
column : Microspher C18 (3μm, 100x4.6mm), eluent : 0.1M NH_4Cl and 10^{-4}M HTA in 5mM citrate buffer (pH6.5), flow : 1.0 ml/min, injection volume : 20 μl.
Droptime = 1 s , pulse application time = 30 ms , current sampling time = 20 ms. Initial potential = 0 V, pulse potential = -1.6 V.

catalytic proton reduction. Because the formazone complex formation is slow, this assay is also unsuited for on-line derivatisation followed by electrochemical detection. As an off-line detection method it would render a very sensitive assay but it would take several hours to analyse one single HPLC run. Furthermore, the interference of competing ligands like Cl^- and interferences due to surfactants, as observed by Zhao and

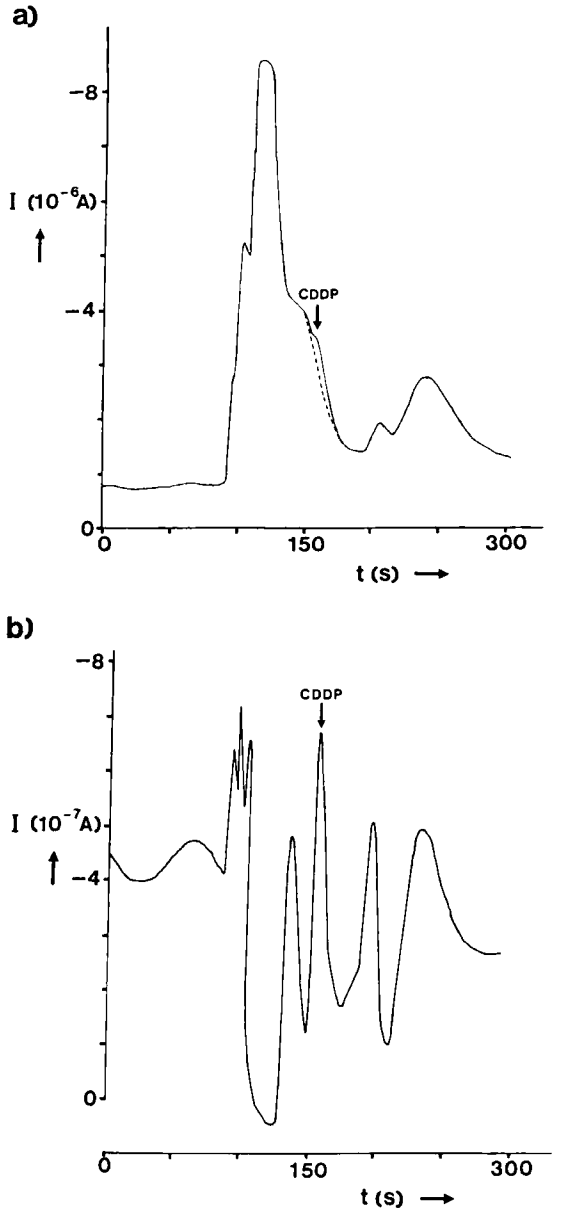

FIGURE 9.
a. Chromatogram of urine, freshly spiked with 10^{-6}M CDDP (——) and blanc urine (---).
Conditions as in FIGURE 8.
b. Chromatogram obtained after the blanc subtract procedure on the chromatograms shown in FIGURE 9a.

Freiser, could cause problems in the analysis of CDDP in body fluids.

In our assay, endogenous compounds did not interfere in the determination of CDDP in plasma ultrafiltrate. Figure 8 shows a typical chromatogram of human plasma ultrafiltrate freshly spiked with 10^{-7}M cisplatin. However, endogenous compounds complicated the quantification of cisplatin in freshly spiked urine (Figure 9a). Only when a representative blanc urine sample was present, quantification down to 10^{-7}M was possible by the use of blanc subtract (figure 9b). In clinical situations however there might be a problem to obtain a representative blanc urine. The best solution to this problem is to look for alternative HPLC conditions providing separation of endogenous compounds from CDDP by a selective increase in retention of the latter.

It can be concluded that we developed a very sensitive polarographic procedure to detect CDDP on-line in HPLC effluents. The use of ammonium-enhanced catalytic hydrogen reduction and, if applicable, accumulation of the platinum compound at the electrode might also be promising to lower the limit of detection for other platinum drugs and metabolites as well. Therefore, the developed on-line polarographic detection is a powerful analytical tool for the selective analysis of platinum complexes in body fluids.

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REFERENCES.

1. Einhorn, L.H., Cancer Treat. Rep., 63, 1659, 1979.

2. Safirstein, R., Winston, J., Goldstein, M., Moel, D., Dikman, S. and Guttenplan, J., Am. J. Kidney Dis., 8(5), 356, 1986.

3. Jacobs, C., Coleman, C.N., Rich, L., Hirst, K. and Weiner, M.W., Cancer Res., 44, 3632, 1984.

4. Smeyers-Verbeke, J., Detaevernier, M.R., Denis, L. and Massart, D.L., Clin. Chim. Acta, 113, 329, 1981.

5. Sharma, R.P. and Edwards, I.R., Ther. Drug Monitor., 5, 367, 1983.

6. Kizu, R., Higashi, S-I. and Myazaki, M., Chem. Pharm. Bull., 33, 4614, 1985.

7. Parsons, P.J. and Leroy, A.F., J. Chromatogr., 378, 395, 1986.

8. Richmond, W.N. and Baldwin, R.P., Anal. Chim. Acta, 154, 133, 1983.

9. Krull, I.S., Ding, X-D, Braverman, S., Selavka, D., Hochberg, F. and Sternson, L.A., J. Chromatogr. Sci., 21, 166, 1983.

10. Marsh, K.C., Sternson, L.A. and Repta, A.J., Anal. Chem., 56, 491, 1984.

11. Gooijer, C., Veltkamp, A.C., Baumann, R.A., Veldhorst, N.H. and Frei, R.W., J. Chromatogr., 312, 337, 1984.

12. de Waal, W.A.J., Maessen, F.J.M.J. and Kraak, J.C., J. Chromat., 378, 253, 1987.

13. Riley, C.M., Sternson, L.A., Repta, A.J. and Bannister, S.J., J. Pharm. Pharmacol., 34, 826, 1982.

14. Daley-Yates, P.T. and McBrien, D.C.H., Biochem. Pharmacol., 33(19), 3063, 1984.

15. Repta, A.J. and D.F. Long, D.F., Cisplatin current status and new developments, Prestayko, A.W., Crooke, S.T. and Carter, S.K., eds., Academic Press 1980, p. 285.

16. Elferink, F., Leeuwenkamp, O.R., Pinedo, H.M and van der Vijgh, W.J.F., J. Electroanal. Chem., 239, 297, 1987.

17. Riley, C.M., Sternson, L.A. and Repta, A.J., J. Chromatogr., 219, 235, 1981.

18. van der Vijgh, W.J.F., van der Lee, H.B.J., Postma, G.J. and Pinedo, H.M., Chromatographia, 17(6), 333, 1983.

19. Elferink, F., van der Vijgh, W.J.F. and Pinedo, H.M., Anal. Chem., 58, 2293, 1986.

20. Reed, E., Sauerhoff, S., and Poirier, M.C., Atomic Spectroscopy, 9(3), 93, 1988.

21. Wang, J., Peng, T. and Lin, M-S., Bioelectrochem. Bioenerg., 16, 395, 1986.

22. Zhao, Z. and Freiser, H., Anal. Chem., 58, 1498, 1986.