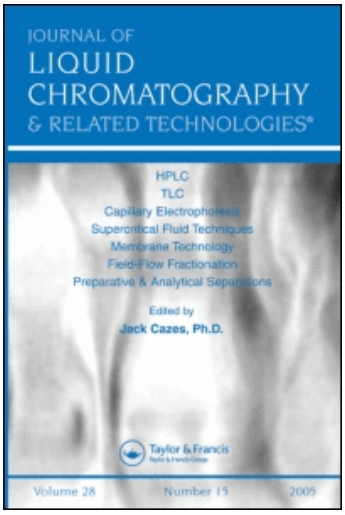


Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Dual Electrode Liquid Chromatography-Electrochemical Detection (LCEC) for Platinum-Derived Cancer Chemotherapy Agents

X. D. Ding[a]; I. S. Krull[a]

[a] Institute of Chemical Analysis Northeastern University, Boston, Massachusetts, USA

To cite this Article Ding, X. D. and Krull, I. S.(1983) 'Dual Electrode Liquid Chromatography-Electrochemical Detection (LCEC) for Platinum-Derived Cancer Chemotherapy Agents', Journal of Liquid Chromatography & Related Technologies, 6: 12, 2173 — 2194

To link to this Article: DOI: 10.1080/01483918308064903

URL: http://dx.doi.org/10.1080/01483918308064903

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(12), 2173-2194 (1983)

DUAL ELECTRODE LIQUID CHROMATOGRAPHY-ELECTROCHEMICAL DETECTION (LCEC) FOR PLATINUM-DERIVED CANCER CHEMOTHERAPY AGENTS

X.-D. Ding and I.S. Krull*

Institute of Chemical Analysis
Northeastern University
360 Huntington Avenue
Boston, Massachusetts 02115 USA

ABSTRACT

Trace methods of analysis and speciation have now been developed for a number of platinum derived anti-cancer chemotherapeutic agents, drugs such as: cis-dichloro diammine platinum (CDDP),cis-diammine-1,1-cyclobutane dicarboxylate platinum (CBDCA), and cis-dichloro-trans-dihydroxy diisopropylamine platinum (CHIP). It is possible to utilize parallel dual electrode operations for all three of these Pt derivatives, with overall improved analyte specificity and identification. At the same time, these approaches provide calibration plots of detector sensitivity as a function of the particular working electrode potentials in use via dual electrode LCEC. These response ratios as a function of the applied potentials then become quite unique for individual Pt compounds. By suitably selecting the operating electrode potentials in parallel operation, it is possible to alter the detectability of individual Pt analytes and to drastically vary the resultant LCEC chromatograms. The overall analyte selectivity possible via dual electrode LCEC surpasses that thus far possible via LC-polarographic reduction or single electrode LCEC operations. Glassy carbon as well as gold/mercury electrodes can readily be used for some of these Pt derivatives. These overall trace methods of analysis and speciation for the Pt anti-cancer agents have also been applied to plasma samples spiked with known levels of each drug. It is also possible to utilize these single or dual electrode approaches for the analysis of each of these Pt derivatives in cancer patients undergoing chemotherapy treatment.

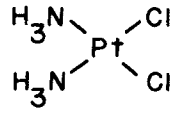
*Author to whom correspondence and reprint requests should be addressed.

0148-3919/83/0612-2173$3.50/0

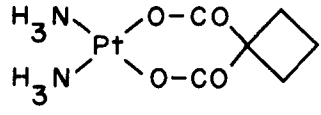
INTRODUCTION

A relatively large number of platinum derivatives have exhibited varying degrees of anti-tumor activity, and several of these have reached clinical trials within the past decade or two (1, 2). Synthetic efforts continue to produce more advantageous Pt derived cancerostatic/chemotherapeutic agents, which would hopefully then find their way into the clinical setting. To some extent, pharmacokinetics, pharmacology, and clinical studies have been hampered by a general lack of sensitive and truly specific methods of trace analysis for any of the Pt derivatives and their metabolites or breakdown products (3). Sternson and colleagues have described a number of HPLC based methods for a variety of these Pt derivatives, including the use of graphite furnace atomic absorption spectroscopy (GFAA), ultraviolet detection (UV), and most recently, dropping mercury electrode polarographic reduction (DME) (1, 4-6). Recently, Krull et al. have described the utilization of single electrode liquid chromatography-electrochemical detection (LCEC) for the trace analysis and speciation of three distinct platinum derived chemotherapeutic agents (3). Figure 1 indicates the structures of these three compounds, which are the same derivatives of interest in this current dual electrode LCEC study. These three platinum compounds are: cis-dichloro diammine platinum (II)(CDDP, cis-Pt), cis-diammine-1,1-cyclobutane dicarboxylate platinum (II)(CBDCA), and cis-dichloro-trans-dihydroxy diisopropylamine platinum (VI)(CHIP). Ideally, any trace analysis and speciation method should offer a number of advantages, including: 1) parts-per-billion (ppb) or sub-ppb detection limits; 2) high degree of analyte specificity leading to unambiguous compound identification; 3) minimum amount of sample preparation compatible with analytical instrumentation; 4) ease of instrumental operation; 5) high reproducibility of analysis and high precision for repeat analyses on same sample; and 6) inexpensive overall instrumentation, materials, supplies, support items. Although most analytical laboratories today possess at least one complete HPLC system, relatively few of these same labs have the ability and/or inclination to interface this with GFAA in order to perform Pt compound speciation analyses (5). At the same time, those laboratories with HPLC have rarely utilized the dropping mercury electrode (DME) polarographic detector in order to perform reductive LC-DME type studies (4). Although some Pt derivatives are UV absorbing, HPLC-UV cannot provide suitably low detection limits for routine application in clinical or research settings (4-6). Of the more commonly used HPLC detectors, including UV, FL (fluorescence), RI (refractive index), and EC (electrochemical), perhaps only EC meets all of the above suggested criteria for a trace method of analysis and speciation for these and other Pt derivatives (7, 8).

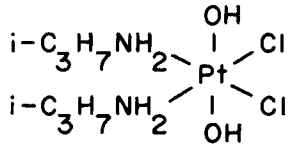
Although single electrode LCEC, utilizing the thin-layer flow cell, has been available for about the past decade, it is only within the past few years that dual electrode LCEC has gained popularity and interest (9-12).



cis-DICHLORO DIAMMINE
PLATINUM (II) (CDDP) (cis-Pt)



cis-DIAMMINE-1, 1-CYCLOBUTANE
DICARBOXYLATE PLATINUM (II) (CBDCA)



cis-DICHLORO-trans-DIHYDROXY
DIISOPROPYLAMINE PLATINUM (IV) (CHIP)

Figure 1. Cis-Platinum derivatives detected via liquid chromatography-electrochemical detection approaches (LCEC).

Although coulometric dual electrode type detectors for HPLC are now commercially available, it would appear that almost all of the basic research, system optimization, and applications have involved the amperometric type dual electrode approach (13). In view of the rather significant advantages that single electrode LCEC approaches have now been shown to possess for Pt compound analyses, it seemed natural to develop and demonstrate any additional analytical capabilities that dual electrode LCEC might provide (3). All three of the Pt derived anti-cancer agents indicated in Figure 1 have now been studied via these newer approaches, in order to improve detection limits and overall compound/analyte specificity. At the same time, these studies have been applied to these same drugs in human plasma samples. The current report summarizes all of this information.

EXPERIMENTAL

Reagents

Cisplatin (CDDP) was obtained from a number of sources: 1) pure CDDP from Strem Chemicals, Inc. (Newburyport, Mass.); 2) cis-Platinol from The Massachusetts General Hospital/Harvard Medical School, formulated and marketed by Bristol Laboratories, Inc. (Syracuse, New York); and 3) pure CDDP from Johnson-Matthey, Inc. (West Chester, Penna.). HPLC mobile phase water and that used for sample solution preparations was HPLC grade from Fisher Scientific Co. (Medford, Mass.). HPLC grade methanol (MeOH) used for the mobile phase was obtained from MCB Manufacturing Chemists, Inc. (Cincinatti, Ohio), as their Omnisolv brand solvent. Hexadecyltrimethylammonium bromide (HTAB), used as the ion-pairing reagent in the HPLC mobile phase, was obtained from Eastman Kodak Co. (Rochester, N.Y.). Sodium acetate for the HPLC buffer was obtained from J.T. Baker Chemical Co. (Phillipsburg, N.J.) as their trihydrate crystal of HPLC grade purity.

Apparatus

The cyclic voltammogram of CDDP was obtained on a Bioanalytical Systems (BAS) Model CV-1B unit (Bioanalytical Systems, Inc., West Lafayette, Ind.), using a supporting electrolyte of 50 mm, pH 3.5 phosphate buffer plus 10% MeOH, at a scan rate of 200 mV/sec, with a glassy carbon working electrode and an Ag/AgCl reference electrode (4). The CVs were obtained by plotting applied working potential vs current generated, in the conventional manner.

The HPLC instrumentation consisted of the following items: 1) a Laboratory Data Control (LDC) Model 709 pulse dampened solvent delivery system (Laboratory Data Control, Riviera Beach, Florida); 2) a Rheodyne Model 7010 syringe loaded HPLC injection valve (20ul loop attached) (Rheodyne Corp., Berkeley, Calif.); 3) an Alltech reversed phase, C_{18}, 10 um, 25-cm x 4.6-mm i.d., stainless steel HPLC analytical column (Alltech Associates, Inc., Deerfield, Ill.), or a Biophase, C_{18}, 10 um, 25-cm x 4.6-mm i.d., HPLC analytical column (Bioanalytical Systems, Inc.); 4) a Bioanalytical Systems (BAS) Model LC-4B amperometric detector for electrochemical detection (Bioanalytical Systems, Inc.); 5) a BAS dual electrode LCEC cell with two Au/Hg or two glassy carbon working electrodes and a Ag/AgCl reference electrode ($[Cl^-]$ = 3.0 M) (Bioanalytical Systems, Inc.); and 6) a Honeywell dual pen strip chart recorder, 10 mV (Honeywell Corp., Minneapolis, Minn.). All HPLC injections were performed with a 25 ul or 50 ul flat-tipped micro-syringe made by Hamilton Corp. (Reno, Nev.). The nitrogen gas used for degassing the HPLC mobile phase in reductive LCEC work was obtained from Yankee Oxygen, Inc. (Boston, Mass.).

Methods

The optimum potentials eventually used in these LCEC studies were determined either by an initial cyclic voltammetry (CV) study, as with CDDP

(3), or by linear hydrodynamic voltammetry, using either flow injection or LCEC approaches. Those oxidative or reductive potentials, with a given working electrode surface, which provided for the maximum current response (peak heights) for a given amount of analyte injected, with minimum electrode fouling after prolonged use, were then used for the final LCEC determinations. An actual CV for CDDP has been presented elsewhere, using a glassy carbon working electrode, as above (3).

Most of the HPLC separations described below involved a mobile phase consisting of 0.01M sodium acetate buffer at pH 4.60 and 0.15 mM hexadecyltrimethylammonium bromide (HTAB), all at a flow rate of 1.0 ml/min. Specific pH values for the various mobile phases are indicated below (Results and Discussion). In some cases, capacity factors were adjusted by the addition of methanol (MeOH) in a fixed ratio to the above aqueous phase. Specific electrochemical detector working potentials are indicated for the individual experiments described below. In the reductive operating mode, oxygen was continuously removed from the HPLC mobile phase by degassing under nitrogen, as recommended by the supplier of the EC detectors (10-12). Sample solutions used for injections in either the oxidative or reductive modes were not initially degassed, due to the small volumes often available and the satisfactory HPLC resolution of the oxygen peak from the analytes. This is the reason for the often large oxygen peak evident in many of the reductive LCEC chromatograms. Retention times of the Pt derivatives were measured directly from the final chromatograms or with an electrical timer off-line. Quantitation and minimum detection limits (MDLs) were determined using peak heights rather than peak areas, with a signal-to-noise ratio of at least 3:1 for MDL determinations. Plasma was obtained from pooled, whole blood by centrifuging fresh blood samples at 2,000 rpm for about 10 mins, and then carefully separating the plasma from the separated red blood cells. This plasma was then immediately used for the analytical work-up and LCEC studies with individual Pt drugs or mixtures thereof. LCEC analyses of stability solutions or blood/plasma samples were done at least in duplicate, alongside multiple injections of freshly prepared standards, separated by at least one injection of blank mobile phase, infusion solution, blood, or plasma alone.

The final analysis of CDDP from plasma involved the spiking of plasma at known concentration levels, a simple filtration of this solution, and then direct injection onto the LCEC system. In the case of CHIP from plasma, these solutions were initially diluted with an equal volume of MeOH, centrifuged, filtered, and then injected onto the LCEC. The analysis for CBDCA in these dual electrode studies did not involve the derivatization to CDDP described earlier in the single electrode approaches (3). Rather, CBDCA was analyzed directly in these current studies, and its determination in human plasma was not studied via dual electrode methods. Recoveries of all three derivatives from whole blood required an initial separation of the plasma from the whole cells, followed by a sample work-up as described above. Another approach for whole blood analysis involved the addition of an equal volume of acetonitrile (ACN), shaking for a

few minutes to lyse the cells present, centrifugation at 2,000 rpm for about 5-10 mins to remove solid matter, filtration of the supernatant aqueous:ACN portion, and final injection onto LCEC.

In the analysis for CDDP from actual cancer patient blood samples, it was shown that the presence of both ethylenediamine tetraacetic acid (EDTA) and heparin, added to the patient blood at the hospital to stabilize and prevent clotting, did not interfere in the final LCEC analysis for the Pt drug of interest. Once the patient blood was treated as above, it was then spun down at 1,500 rpm for 5-10 mins, and the plasma was separated from the heavier red blood cells. To 5 ml of this plasma was then added 0.5 ml of 5.0 M saline solution (NaCl), both solutions were thoroughly mixed, and this final sample was placed on Dry-Ice for shipment from the hospital to the analytical laboratory at the University. Analysis of CDDP infusion solutions simply involved an addition of 0.5 ml of the 5.0 M saline solution to 5.0 ml of the infusate, mixing, and storage as above. When received at the University, trichloroacetic acid (TCA) in a 10% aqueous solution was added to the plasma in a 1/1 (v/v) ratio, and this mixture was vigorously shaken in order to precipitate all proteins present. This solution was then spun down in a centrifuge at 2,000 rpm for about 10 mins, and the supernatant was removed and filtered through a BAS sample filtration kit (micro filter) with centrifugation (Bioanalytical Systems, Inc.). The filtered liquid was then used for direct LCEC injections. Overall percent recoveries of CDDP spiked to human (non-patient) plasma at the 20 ppm level using the above methods were 89.0 ± 1.3% (average ± standard deviation) (n=3).

Optimization of the basic LCEC operating conditions involved aqueous solutions of the Pt derivatives prepared in HPLC grade water or saline solutions. These were simply filtered and then injected onto the LCEC. All sample filtrations were performed with a 0.45 um sample filtration kit for HPLC (Waters Assocs., Inc., Millipore Corp., Bedford, Mass.). HPLC mobile phases prior to degassing were filtered through a 0.45 um solvent filtration kit for HPLC (Waters/Millipore Corp.).

The methods of performing reductive LCEC analyses with dual electrode detection, using either glassy carbon or gold/mercury surfaces, were essentially those suggested by the manufacturer of the electrochemical detector for LC (Bioanalytical Systems, Inc.), in their various technical publications. Additional technical information and guidance is available in certain recent scientific/technical publications (10-12). The parallel dual electrode LCEC methods used in these studies have been based, in part, on earlier literature reports and/or scientific presentations (9-12). Application of such techniques and instrumentation to this class of Pt derived anti-cancer agents is described here for the first time (3).

RESULTS AND DISCUSSION

The results described here for the trace analysis and speciation of three important Pt anti-cancer agents, Figure 1, have entirely utilized dual

electrode detection in HPLC (LCEC). All of the results presented here have utilized dual electrodes oriented in the parallel adjacent mode, although the use of both parallel and series has been advocated elsewhere in the literature (10-12). At least in our own experience, dual electrode LCEC is more reproducible and internally consistent, intra- and inter-day, in the parallel adjacent orientation. Most dual electrode work thus far reported has utilized the glassy carbon type electrodes, with much less being described for the gold/mercury type. It has been our experience that the glassy carbon surfaces can be made more reproducible from day-to-day than the gold/mercury type, and that the former are perhaps more amenable to series operation than the latter. In any case, since this work has been directed towards improving the overall specificity (speciation) of the analysis for Pt derivatives, the parallel adjacent mode is ideally suited for such goals. Improved detection limits may sometimes be obtained via the series orientation, but since our initial single electrode results provided satisfactory minimum detection limits (MDLs) for these compounds (10 ppb for CDDP), there was no need to further improve them via the dual electrode techniques available with series operation (3). Whereas the earlier LCEC results utilized only the gold/mercury electrode surface for reductive operations, it has now proven possible to utilize the glassy carbon type for both oxidation and reduction of certain Pt derivatives, as well as the Au/Hg electrodes. A dual electrode cell with a single glassy carbon and a single Au/Hg surface might provide additional capabilities over those already available for these studies. The final, overall LCEC approaches described below have now been shown more than adequate, qualitatively and quantitatively, for the direct analysis of residual, intact CDDP in patient infusion solutions and blood plasma samples, the latter obtained at the end of a 2 hr infusion period.

Parallel Dual Electrode LCEC Calibration Plot Ratios for Improved Specificity. Application of Overall LCEC Dual Electrode Methods to Actual Samples.

The utilization of dual electrode LCEC in the parallel mode can provide significantly improved analyte identification (speciation) by plotting the EC detector response (peak height/peak area) vs concentration injected as a function of the working potential of each electrode (10, 12). We have now applied these methods for improved LCEC analyte identification with both the glassy carbon and Au/Hg type dual electrodes, utilizing a wide variety of applied working potentials (vs Ag/AgCl). These are, in essence, two calibration plots for each Pt derivative, wherein each calibration plot varies according to the working potential applied. The overall data at each concentration point on such plots can then be ratioed, similar to wavelength ratioing in multiple wavelength UV detection in HPLC. In addition, the EC detector responses obtained at the two different potentials can be subtracted, in order to provide another data point specific for that particular analyte. Both the ratio of EC detector responses and the differences of these same two EC detector responses can then be utilized to confirm or deny the presence of a suspected

analyte in a complex sample matrix (10, 12). These dual electrode LCEC approaches are best utilized for a suspected analyte in a sample matrix wherein the known standard is analyzed under the same LCEC conditions on the same working day. Working curves at the dual potentials of interest must be obtained for both the suspected standard and the analyte in the sample matrix at the same time or thereabouts. Identical EC detector ratios or differences in EC detector responses for the known standard and the suspected analyte can then provide significant qualitative confirmation to the overall LCEC analysis. It is very important to recognize at the start that dual electrode EC responses will vary from day-to-day, and that working calibration curves obtained on one day cannot be accurately or reliably utilized to confirm the presence of that analyte in a sample analyzed on another day. However, this is no different, in practice or principle, from the well accepted practice of determining calibration plots for standards on the same day as the samples are being analyzed via any detection method in HPLC, GC, or direct instrumental analysis. Instrumental response variability is a very common occurrence, whether one works with separation-detection or direct-detection methods of analysis and instrumentation.

Figure 2 illustrates a set of calibration plots (linear) obtained for CDDP using glassy carbon dual electrode LCEC at working potentials of +1.05 V and + 1.00 V, over the concentration range of 5-40 ppm. The HPLC conditions utilized here involved a reversed phase C_{18} column with a mobile phase of 0.01 M acetate buffer, pH 4.60, plus 0.15 mM hexadecyltrimethyl-ammonium bromide (HTAB), at a flow rate of 1.0 ml/min. Figure 3 is the same study performed under the identical conditions as in Figure 2, but on another day, with somewhat different results obtained. Clearly, different surfaces on one or both of the glassy carbon or gold/mercury electrodes would provide different EC detector responses from day-to-day. This is the same as the observation that UV lamp intensities for an HPLC-UV detector will and often do vary from day-to-day as well. We have now obtained a large number of similar dual electrode response ratios for both glassy carbon and Au/Hg surfaces, for CDDP, CBDCA, and CHIP, as a function of applied, operating potentials. The final ratios of these detector responses have been summarized in Table 1, using HPLC conditions as indicated above (Experimental Section) or below in various Figures. All of these results using glassy carbon electrodes were obtained on the same working day, as were those utilizing the Au/Hg surfaces, but these were two different days.

Our previous efforts in utilizing single electrode LCEC with these same Pt drugs made no attempt to speciate for one or more of these drugs when all three were present in the same injection solution (3). In cases where more than a single Pt derivative is used simultaneously in cancer chemotherapy, this could be of interest. Ideally, an analyst would like to be able to vary the selectivity possible via dual electrode LCEC, for one or more Pt derivatives, and obtain final conditions selective for one, two, or more such compounds. It

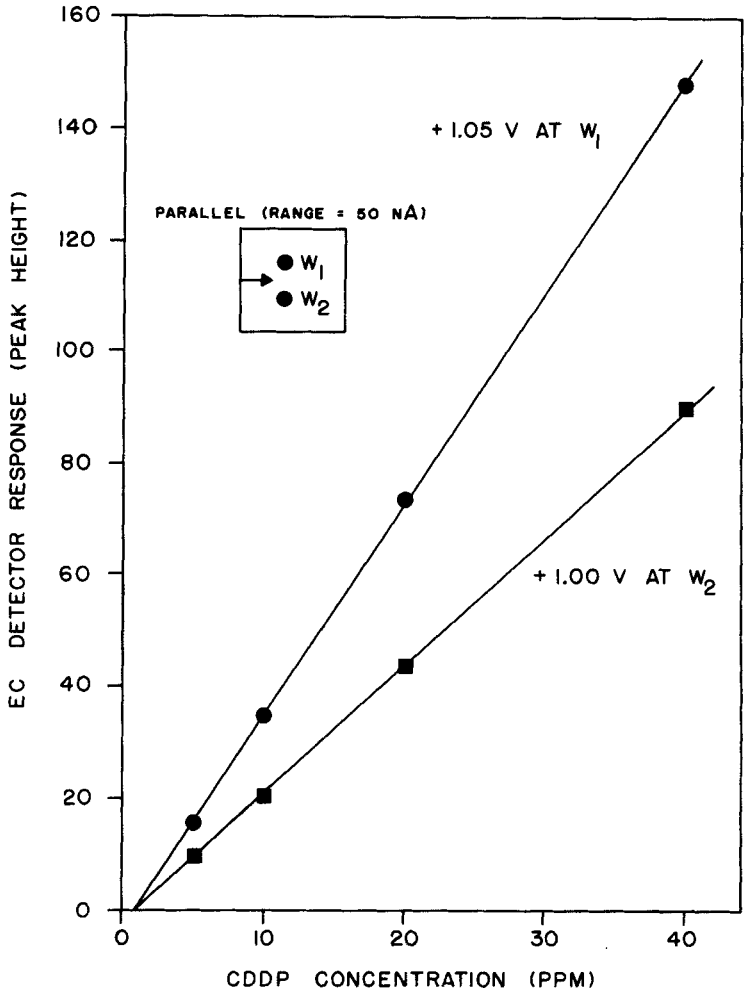


Figure 2. Plot of [CDDP] vs oxidative EC peak heights at two different working potentials with dual glassy carbon electrodes in parallel orientation to HPLC effluent.

should be entirely feasible to vary the LCEC detector parameters appropriately, and thereby have one of the three compounds present appear on the LCEC chromatogram, change the EC conditions somewhat, make another one appear and the first disappear or remain apparent, etc. Of course, in the final HPLC eluent, all three analytes of interest would always be present, but the dual

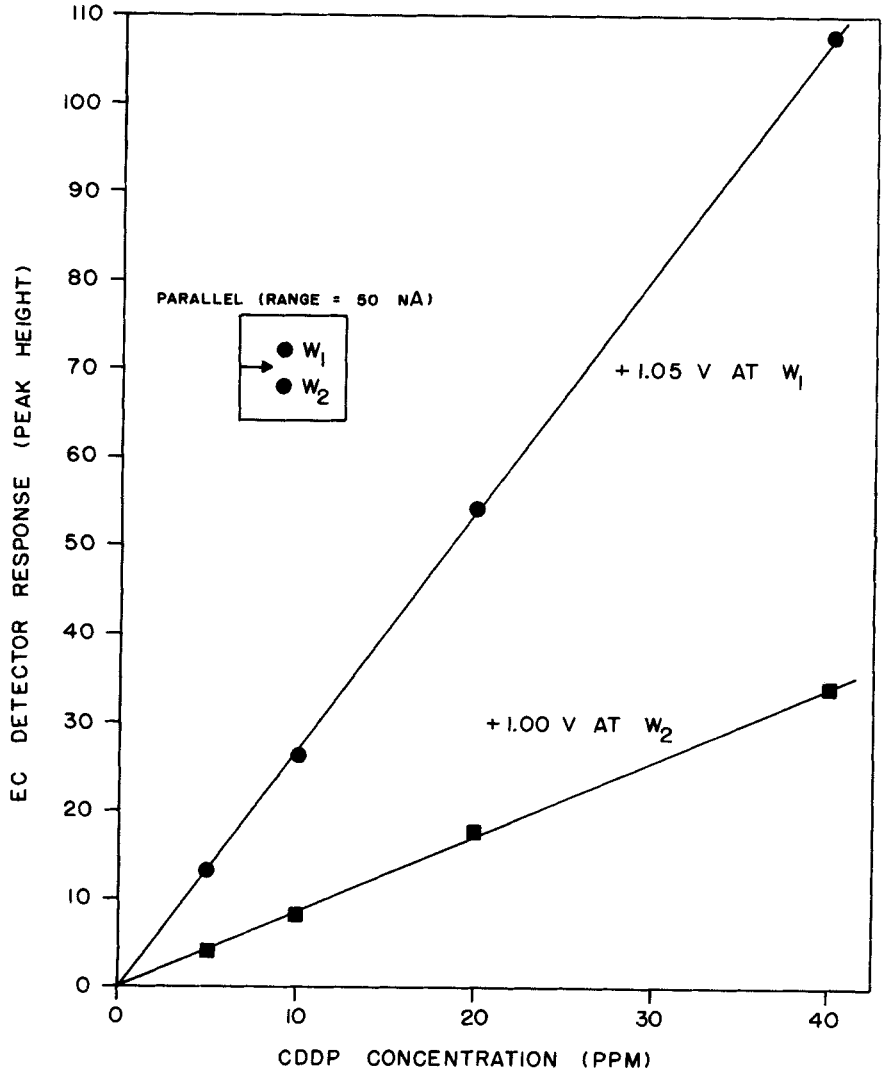


Figure 3. Plot of [CDDP] vs oxidative EC peak heights at two different working potentials with dual glassy carbon electrodes in parallel orientation to HPLC effluent.

TABLE 1. SUMMARY OF DUAL ELECTRODE RESPONSE RATIOS FOR CDDP, CBDCA, AND CHIP AT VARIOUS WORKING POTENTIALS

COMPOUND STUDIED	ELECTRODE SURFACE	WORKING POTENTIALS	DETECTOR RESPONSE RATIOS
CDDP	GLASSY CARBON	+1.05V/+1.00V	3.0
CDDP	GLASSY CARBON	-0.50V/+1.05V	2.6
CHIP	GLASSY CARBON	-0.50V/-0.45V	2.0
CBDCA	GLASSY CARBON	+1.24V/+1.18V	6.4
CDDP	GOLD/MERCURY	-0.01V/+0.01V	1.9
CHIP	GOLD/MERCURY	-0.01V/+0.01V	2.6

electrode detector, depending on the particular conditions used, would only detect one, another, or another. We have now been able to obtain just these desirable sets of operating conditions, as illustrated for one such set in Figure 4. The specific LCEC conditions are indicated in this Figure, with one glassy carbon electrode operated at +1.15V and the other at -0.40V. The top chromatogram illustrates the presence of both CBDCA and CDDP, while the bottom chromatogram indicates the presence of only CHIP, at these particular working potentials. By varying these potentials, or by holding one constant and varying the other, it is indeed possible to make one or more of these three LCEC peaks completely or partially disappear. Indeed, at these same concentration levels injected, by simply varying the potentials applied, any of the three peaks present can be made to increase, decrease, or completely disappear from the final chromatograms. This is indeed true analyte specificity in LCEC, and it now provides a new method of performing Pt compound speciation in the absence of an element selective detector, such as the GFAA (7, 8).

An alternative set of LCEC conditions for improved Pt analyte speciation via dual electrode approaches is indicated in Figure 5, with the specific conditions as indicated. Again, using parallel orientation of the two electrodes, it is possible to analyze for both CBDCA and CDDP present together using oxidative/reductive modes. In the oxidative mode, Figure 5 (top), both CBDCA and CDDP are apparent, but at different relative sensitivities. These relative sensitivities for these two Pt compounds, at +1.20V should be compared with the analogous responses obtained at a slightly lower working potential, viz., +1.15V, Figure 4 (top). In the reductive mode of detection, Figure 5 (bottom), only the CDDP is apparent at a working potential of -0.46V, together with the oxygen dissolved in the sample solution injected. The ratio of these two CDDP peak heights at this particular level injected with these two working potentials again becomes an identifying trait for this Pt derivative. There is an abnormal amount of apparent peak tailing for the CDDP peak in the reductive mode in Figure 5, but since this is an analytical standard injected here, it would appear not due to an interferent co-eluting with CDDP under these HPLC conditions. We prefer to believe that at this particular reductive potential, prolonged use of the glassy carbon electrode with HTAB present in the mobile phase causes some type of electrode fouling. This may be the cause of the apparent peak tailing observed here, but additional work would be needed to conclusively prove this point. At much lower reductive working potentials, -0.01V with a Au/Hg electrode, there is no apparent peak tailing, Figures 6-8.

Two other pertinent studies remain to be described here, especially with regard to the utilization of these methods for Pt drugs in human plasma samples. Indeed, Figure 6 illustrates the analysis of spiked plasma samples at two different concentration levels, as indicated. Specific conditions for the work-up and preparation of plasma samples for CDDP determinations has been

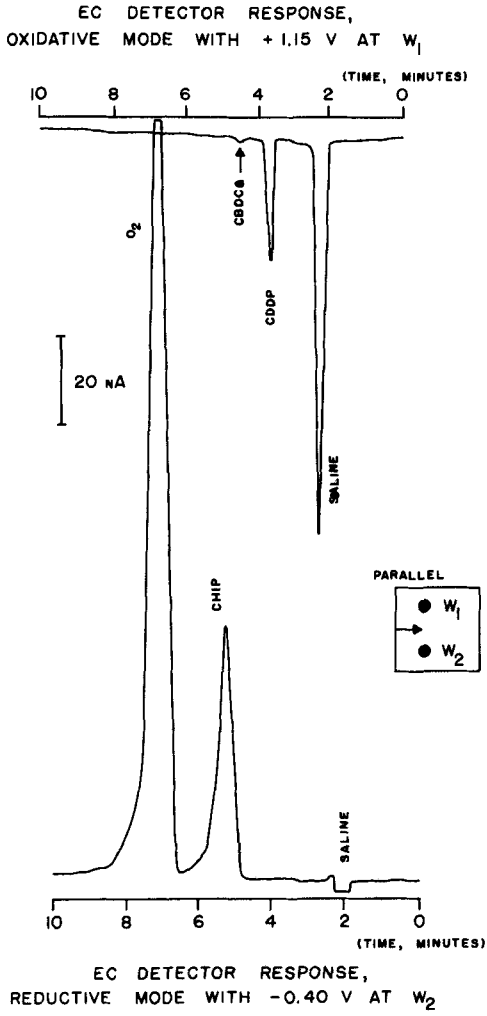


Figure 4. Dual electrode LCEC chromatograms of CDDP, CBDCA, and CHIP injected together onto a C_{18} RP column with a mobile phase of 95% 0.01M acetate buffer, pH 4.60, 0.15 mM HTAB, plus 5% MeOH, flow rate 1.0 ml/min. BAS dual glassy carbon electrodes in paralle orientation. CDDP (40 ppm), CBDCA (80 ppm), CHIP (80 ppm), all in 0.9% saline solution.

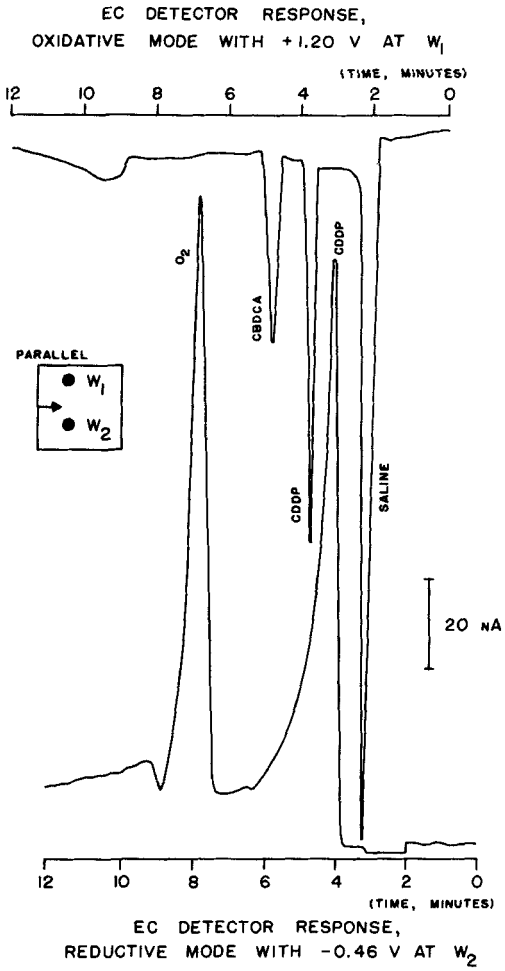


Figure 5. Dual electrode LCEC chromatograms of CDDP and CBDCA injected together onto a C_{18} RP column with a mobile phase of 0.01M acetate buffer, pH 4.60, 0.15 mM HTAB, flow rate of 1.0 ml/min. BAS dual glassy carbon electrodes operated in the parallel orientation. CDDP (20 ppm) and CBDCA (40 ppm) in 0.9% saline solution.

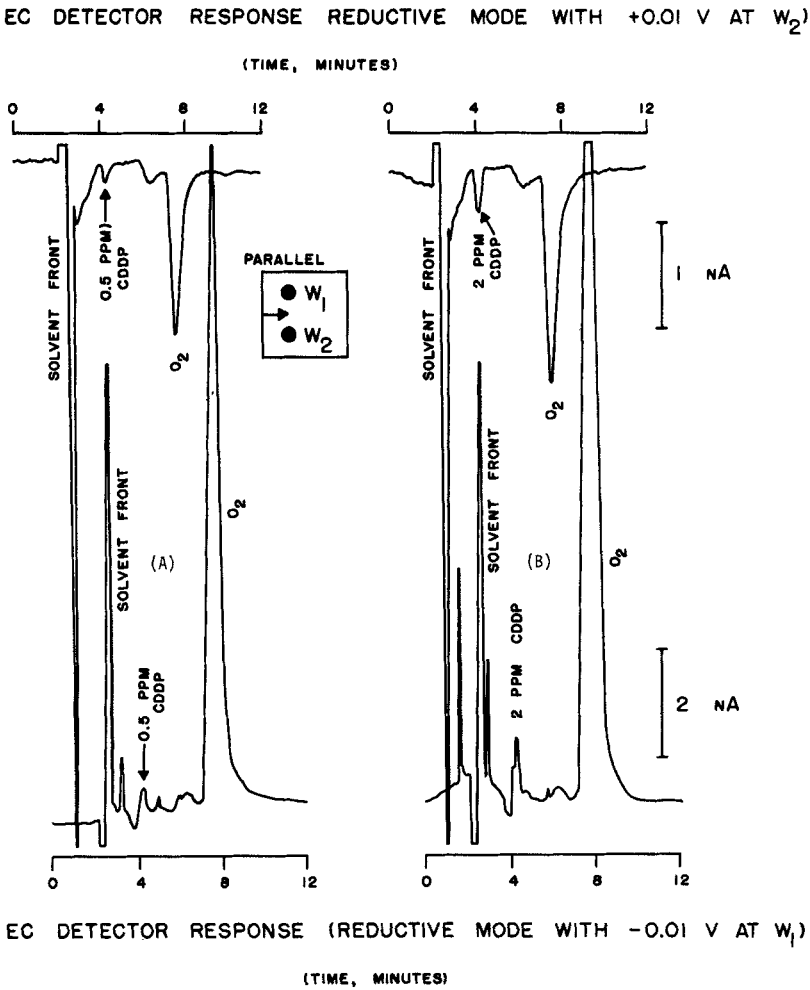


Figure 6. Parallel dual electrode (reductive/reductive) LCEC chromatograms of CDDP in human plasma: (A) 0.5 ppm; (B) 2.0 ppm. LCEC conditions used a C_{18} RP column with a mobile phase of 0.01M NaCl + 0.01M acetate buffer, pH 4.60, 0.15 mM HTAB, flow rate 1.0 ml/min, Au/Hg working electrodes operated in the parallel orientation (BAS).

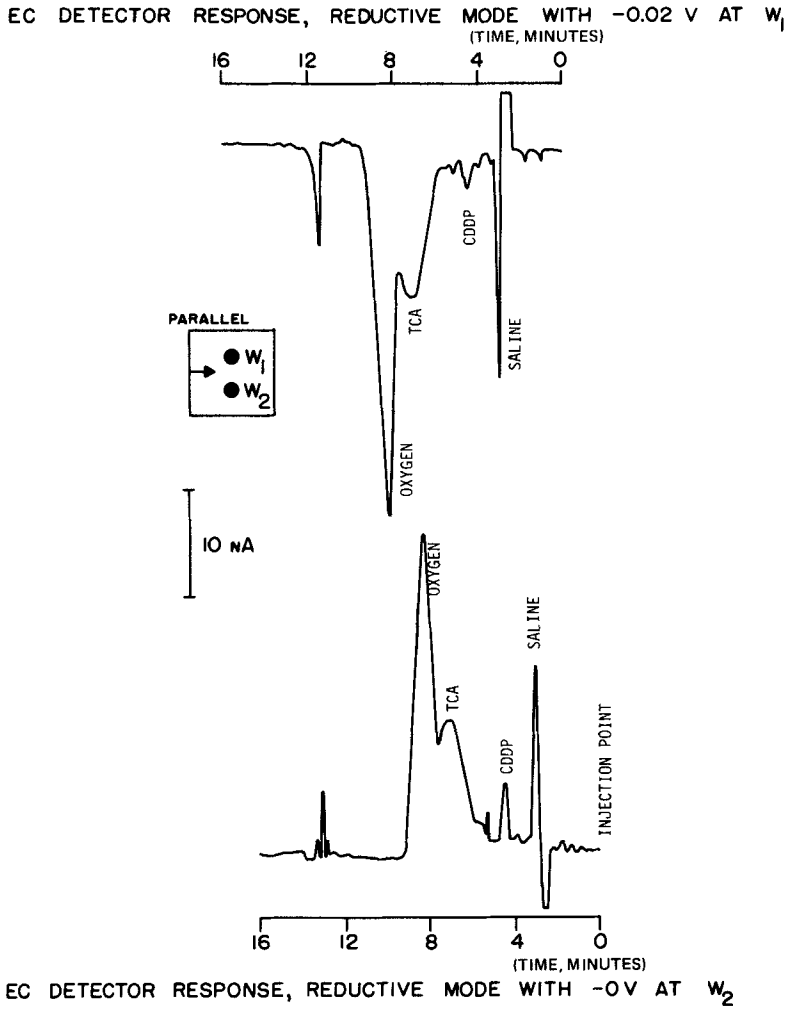


Figure 7. Dual electrode LCEC (reductive/reductive) chromatograms of cancer patient plasma sample after two hour infusion with CDDP, showing the presence of intact CDDP, work-up with TCA. HPLC used RP C_{18} column with mobile phase of 0.01M NaCl + 0.01M acetate buffer, pH 4.60, + 0.15 mM HTAB, 1.0 ml/min flow rate. BAS dual Au/Hg electrodes operated in the parallel orientation.

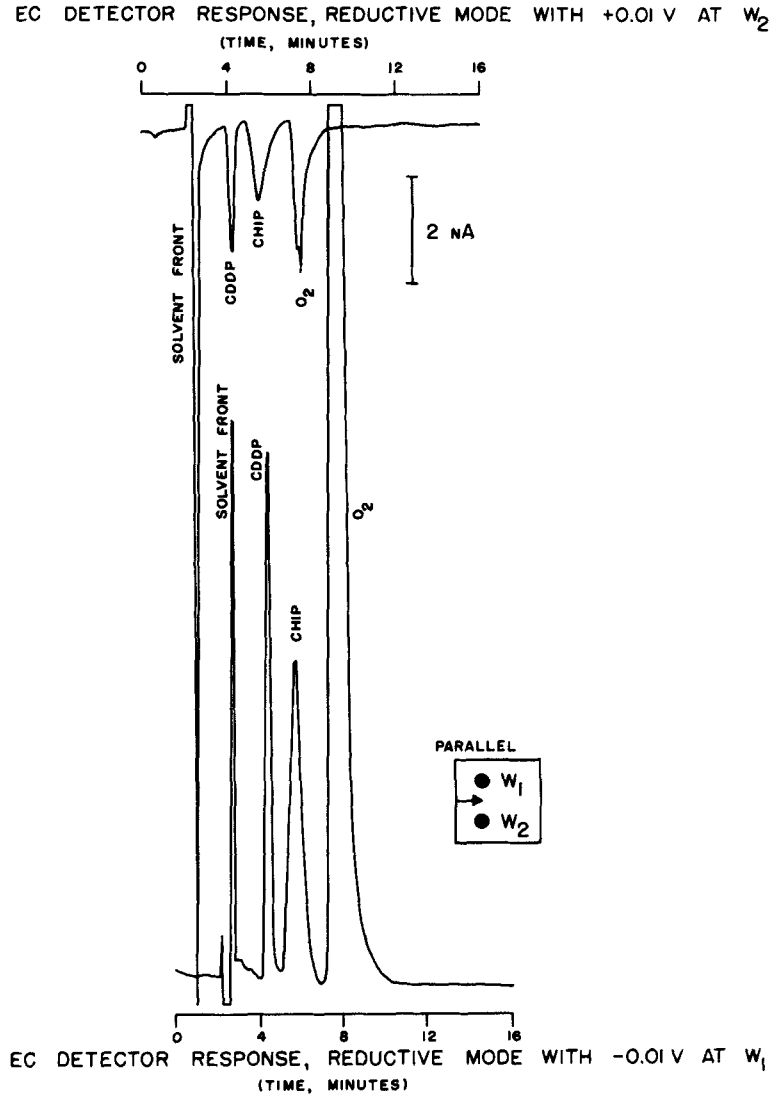


Figure 8. Parallel dual electrode (reductive/reductive) LCEC chromatograms of CDDP and CHIP at the 5.0 ppm levels. LCEC conditions used a RP C_{18} column with a mobile phase of 0.01M acetate buffer + 0.01M NaCl, pH 4.60, 0.15 mM HTAB, flow rate 1.0 ml/min. BAS dual Au/Hg working electrodes operated in the parallel orientation.

presented above (Experimental Section). In this particular study, two Au/Hg working electrodes were utilized, for both the oxidative and reductive EC detection of CDDP in plasma. Although Figure 6A suggests that 0.5 ppm (500 ppb) may be the detection limit for CDDP in plasma; in subsequent studies with actual cancer patient plasma samples, it has now been possible to detect as little as 0.1 to 0.2 ppm (100 to 200 ppb) of CDDP at the end of a 2 hr infusion period. These detection limits for CDDP in cancer patient plasma samples are more than adequate for determining actual levels of CDDP in such samples at the end of a conventional 2 hr infusion treatment, Table 2. Table 2 summarizes the levels of CDDP actually measured in patient infusion solutions, plasma just before infusion started, and plasma taken at the very end of the infusion (2 hrs). These particular patients were receiving the customary CDDP infusion levels at The Sidney Farber Cancer Center, Boston, Mass. Figure 7 is a typical dual electrode LCEC study of a cancer patient plasma sample taken at the very end of a two hour infusion period with CDDP, showing the presence of intact CDDP with specific conditions as indicated. In this case, sample work-up involved denaturation of the plasma sample with trichloroacetic acid (TCA), as discussed previously (Experimental Section). At the two reductive potentials used in this study, Figure 7, viz., -0.02V and -0.00V, the peak for CDDP represents a concentration of about 4.42 ppm in the original plasma itself.

Our earlier studies with single electrode LCEC for CDDP in plasma also suggested a detection limit of about 100 ppb or thereabouts. One would not expect parallel dual electrode LCEC to provide improved detection limits, as discussed already by others (10, 12). Clearly, the dual electrode approaches described here provide at least as useful detection limits for these Pt derivatives in human plasma, but now combined with greatly improved and enhanced analyte identification and specificity. We have indeed been able to successfully apply these dual electrode methods to actual cancer patient infusion and plasma samples containing unknown levels of intact CDDP, as above, Table 2. We have chosen in these patient studies to use the Au/Hg electrodes with relatively low operating potentials of -0.02V/-0.00V, although other suitable operating conditions would also be feasible. Our detection limits with these conditions have been more than adequate for the actual levels of CDDP present in real world patient samples, and thus there has been no need to utilize higher oxidative/reductive working potentials, as suggested by others. Indeed, the use of much higher working potentials for plasma samples might only lead to decreased analyte specificity because of matrix interferences, without providing us with significantly improved detection limits that are unnecessary in any case.

Finally, Figure 8 illustrates the use of dual Au/Hg electrodes with a mixture of CDDP and CHIP, both at the 5 ppm levels in saline solution, wherein both Pt derivatives can be detected simultaneously using two different

TABLE 2. QUANTITATIVE DETERMINATIONS FOR CDDP IN PATIENT INFUSION SOLUTIONS AND PLASMA SAMPLES

SAMPLE TYPE/NUMBER[1]	LEVELS CDDP DETERMINED BY LCEC[2] (AVERAGE ± STD. DEV.)	LEVEL CDDP AS PREPARED FOR[3] INFUSION TO PATIENT
INFUSION SOLN. #1	153 ± 2.0 ppm (n=4)	171 ppm
PATIENT PLASMA BEFORE INFUSION #1	ND[4]	----
PATIENT PLASMA AT END INFUSION #1	4.42 ± 0.9 ppm (n=4)	----
INFUSION SOLN. #2	72.8 ± 5.29 ppm (n=4)	75 ppm
PATIENT PLASMA BEFORE INFUSION #2	ND[4]	----
PATIENT PLASMA AT END INFUSION #2	0.748 ± 0.093 ppm (n=4)	----

1. These results represent chemotherapeutic treatments for two separate cancer patients on two separate days, using drug infusions over a 2 hr period, i.v., sample treatment and work-up conditions as indicated elsewhere (Experimental Section).
2. Dual electrode (reductive/reductive) LCEC approaches used for these quantitative analyses.
3. Infusion solutions prepared in hospital by qualified personnel using standard methods, levels of CDDP in initial infusion solutions as recorded at time of preparation.
4. ND = not detectible with the minimum detection limits inherent in LCEC approach (100 ppb). There should be no CDDP at all in these plasma samples before infusion began.

reductive potentials in LCEC. Again, it is clear that other EC working parameters would be feasible for such improved specificity of both analytes present in the same infusion or plasma solutions/samples.

SUMMARY

In the past, most practical methods of trace analysis and speciation for Pt derivatives utilized element selective detection via graphite furnace AA or related techniques (8). It may yet prove feasible to apply HPLC-inductively coupled plasma (ICP) emission spectroscopy or direct current plasma (DCP) emission spectroscopy for these and related Pt derivatives, but this will depend on final detection limits possible via such approaches (7). We have tried to demonstrate that the dual electrode LCEC approaches now possible with these Pt derivatives can indeed provide sensitivity and selectivity practical for real world sample analyses. Our ability to apply these methods to actual plasma samples spiked with Pt drugs or to actual cancer patient samples containing CDDP initially infused, clearly demonstrates that these newer methods of Pt analysis and speciation are indeed of practical utility and immediate applicability. Although it is very difficult to demonstrate specificity unequivocally, or to compare the LCEC specificity with the HPLC-GFAA specificity for these same compounds, it would at least appear as if these dual electrode LCEC methods will provide as much analyte specificity as any other existing method of metal speciation (8). At the same time, these newer methods of metal analysis and speciation can be readily applied with currently available instrumentation that costs considerably less, overall, than either GFAA, ICP, or DCP instruments. It is also the case that the LCEC interfacing is much easier to accomplish and maintain than almost any other metal speciation approach involving HPLC separations.

ACKNOWLEDGEMENTS

We are very grateful to Ron Shoup and Peter Kissinger of Bioanalytical Systems, Inc., for providing us with valuable information and guidance in the initial phases of this work. Karl Bratin, of Chas. Pfizer & Co., also provided valuable technical assistance and suggestions, which proved helpful in obtaining the final, successful results. Blood samples for the studies with cis-Pt drugs in human plasma, non-patient, were provided by The Massachusetts General Hospital via Fred Hochberg. Authentic samples of all three Pt derivatives were provided by Bristol Laboratories, Inc. and Johnson-Matthey, Inc. The sample of Platin, the commercial formulation of cis-Pt, was provided by The Massachusetts General Hospital, Boston, Mass. Samples of cis-Pt infusion solutions, as well as cancer patient blood/plasma samples taken before and after infusion, were provided by Dave Henner of The Sidney Farber Cancer Institute, The Children's Hospital, Boston, Mass.

This work was supported, in part, by a grant from the NIH Biomedical Sciences Research Support Grant, No. RR07143, Department of Health and Human Resources, to Northeastern University. Mr. X-D. Ding was supported in these studies via a Visiting Chinese Scholar Fellowship from the Government of China (1982). We are indeed grateful for these various sources of financial assistance.

This is contribution number 157 from the Institute of Chemical Analysis at Northeastern University.

REFERENCES

1. Abbreviations used: HPLC = high performance liquid chromatography; EC = electrochemical detection; LCEC = liquid chromatography-electrochemical detection; MDL = minimum detection limits; ppb = parts-per-billion; ppm = parts-per-million; Au/Hg = gold/mercury amalgam; cis-Pt = CDDP = cis-dichloro diammine platinum (II); CBDCA = cis-diammine-1,1-cyclobutane dicarboxylate platinum (II); CHIP = cis-dichloro-trans-dihydroxy diisopropylamine platinum (VI); UV = ultraviolet detection; FL = fluorescence detection; GFAA = graphite furnace atomic absorption spectroscopy. MeOH = methanol; ACN = acetonitrile; TCA = trichloroacetic acid.
2. A.W. Prestakyo, S.T. Crooke, and S.K. Carter, eds., Cisplatin: Current Status and New Developments. Academic Press, New York, 1980.
3. I.S. Krull, X-D. Ding, S. Braverman, C. Selavka, F. Hochberg, and L.A. Sternson. Trace analysis for cis-platinum anti-cancer drugs via liquid chromatography-electrochemical detection (LCEC). J. Chromatogr. Sci., 21: 166-173 (1983).
4. S.T. Bannister, L.A. Sternson, and A.J. Repta. Evaluation of polarographic detection in the liquid chromatographic determination of anti-neoplastic Platinum complexes. Anal. Chim. Acta. In press (1983).
5. C.M. Riley, L.A. Sternson, and A.J. Repta. Assessment of cisplatin reactivity with peptides and proteins using reverse-phase high performance liquid chromatography and flameless atomic absorption spectroscopy. Anal. Biochem. 124: 167-179 (1982).
6. Y. Chang, L.A. Sternson, and A.J. Repta. Development of a specific analytical method for cis-dichlorodiammineplatinum (II) in plasma. Anal. Lett. B11(6): 449-459 (1978).
7. I.S. Krull and S. Jordan. Interfacing of GC and HPLC with plasma emission spectroscopy. Amer. Lab. 21-33 (October, 1980).
8. I.S. Krull, Trace Metal Analysis by High Performance Liquid Chromatography. In Liquid Chromatography in Environmental Analysis. J.F. Lawrence, ed., The Humana Press, Inc., Clifton, New Jersey. In press, 1983. Chapter 5.
9. LCEC Bibliography: Recent Reports in Liquid Chromatography/Electrochemistry. R.E. Shoup, ed. BAS Press, Inc., Bioanalytical Systems, Inc., West Lafayette, Indiana, 1982.

10. I.S. Krull, K. Bratin, R.E. Shoup, P.T. Kissinger, and C.L. Blank. Liquid chromatography/electrochemistry for trace analysis: Recent advances in instrumentation, methods, and applications. Amer. Lab. 57-65 (February, 1983).
11. K. Bratin and P.T. Kissinger. Reductive LCEC of organic compounds. J. Liquid Chromatogr. 4(Suppl. 2): 321-357 (1981).
12. D.A. Roston, R.E. Shoup, and P.T. Kissinger. Liquid chromatography/electrochemistry: thin-layer multiple electrode detection. Anal. Chem. 54: 1417A-1434A (1982).
13. R.W. Andrews, C. Schubert, J. Morrison, E.W. Zink, and W.R. Matson. Dual electrode cells for LCEC: recent developments. Amer. Lab. 140-151 (October, 1982).
14. I.S. Krull and X-D. Ding. Unpublished results (1983).