Direct Analysis of Platinum in Plasma and Urine by Electrothermal Atomic Absorption Spectrophotometry*

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ABSTRACT

An improved technique is described for analysis of platinum (Pt) concentrations in plasma and urine by electrothermal atomic absorption spectrophotometry (EAAS). The method is intended for use in therapeutic monitoring of cancer patients treated with platinum-containing antitumor drugs. Samples (0.1 ml) of plasma, urine, or Pt standards are diluted to two ml with a matrix solution that contains diammonium edetate, ammonium dihydrogen phosphate, ammonium hydroxide, and octoxynol detergent. Concentrations of Pt in the diluted samples are determined directly by EAAS analysis with Zeeman background correction. Standard additions are unnecessary; Pt concentrations are read from a calibration chart of peak heights, which is linear up to 1.6 mg per liter. The detection limit is 0.02 mg of Pt per liter. Day-to-day precision (coefficient of variation, based on 21 consecutive runs) ranges from 4.2 to 11.7 percent, depending upon the Pt concentration in the plasma and urine specimens. Recovery of Pt added to plasma and urine specimens averages 103 ± 8 and 99 ± 6 percent, respectively. Concentrations of Pt are stable in plasma and urine specimens stored at 4°C or −20°C for four weeks. Analyses of Pt concentrations in serial plasma and urine specimens from cancer patients receiving cisplatin chemotherapy demonstrate the clinical utility of the technique.

Introduction

Measurements of platinum concentrations in plasma and urine are used for therapeutic monitoring of cancer patients treated with cis-diamminedi-chloroplatinum(II) ('cisplatin') and cis-
diammine(1,1-cyclobutanedicarboxylato)-platinum(II) ("carboplatin").\textsuperscript{4,9,13,17} Methods for analysis of Pt in biological materials by electrothermal atomic absorption spectrophotometry (EAAS) have been reported by Delves and Shuttler,\textsuperscript{3} El-Yasigi and Al-Saleh,\textsuperscript{4} LeRoy et al,\textsuperscript{9} McGahan and Tyczkowska,\textsuperscript{10} and Priesner et al.\textsuperscript{14} Based upon trials of these procedures in our laboratory, the techniques that involve direct sample injection into the graphite furnace yield variable recoveries of added Pt because of matrix interference, while those that require preliminary acid digestion or dry ashing are cumbersome and time consuming. These difficulties have been overcome by the direct EAAS assay described in this paper, which incorporates matrix modification by additions of diammonium edetate, ammonium dihydrogen phosphate, and ammonium hydroxide, as suggested by Delves and Shuttler,\textsuperscript{3} and octoxynol ("Triton X-100"), as suggested by El-Yasigi and Al-Saleh\textsuperscript{4} and Priesner et al.\textsuperscript{14} This modified procedure is being used in the authors' laboratory to study platinum pharmacokinetics in cancer patients treated with various chemotherapeutic regimens.

Method

Principle

Serum or urine samples are diluted with a matrix modifier solution that contains diammonium edetate, ammonium dihydrogen phosphate, ammonium hydroxide, and octoxynol detergent. Platinum concentrations in the diluted samples are determined directly by EAAS analysis with Zeeman background correction.

Reagents

1. **Matrix modifier solution.** Into a one-liter volumetric flask are transferred 60 mg of edetate diammonium ((NH\textsubscript{4})\textsubscript{2}-EDTA)* and 175 mg of ammonium dihydrogen phosphate (NH\textsubscript{4}H\textsubscript{2}PO\textsubscript{4}).\textsuperscript{†} The chemicals are dissolved in approximately 800 ml of distilled water, 0.3 ml of octoxynol ("Triton X-100")\textsuperscript{‡} and three ml of ammonium hydroxide solution (NH\textsubscript{4}OH, 7.7 mol per liter)\textsuperscript{††} are added, and the contents of the flask are diluted to the mark with distilled water.

2. **Dilute nitric acid solution** (approximately 0.1 mol per liter). In a 0.5-liter volumetric flask, 3.2 ml of nitric acid reagent (15.7 mol per liter)\textsuperscript{§} are diluted to the mark with distilled water.

3. **Platinum intermediate standard solution** (10 mg Pt per liter). One ml of platinum spectrophotometric standard solution (10 g per liter)\textsuperscript{||} and four ml of nitric acid reagent (15.7 mol per liter) are placed in a one-liter volumetric flask. The contents are diluted to the mark with distilled water. This solution is stable indefinitely in a polyethylene bottle at room temperature.

4. **Platinum working standard solutions.** Into five 25-ml volumetric flasks are pipetted 0.5, 1, 2, 3, and 4 ml of platinum intermediate standard solution. Dilute nitric acid solution (5 ml) is added to each flask and the contents are diluted to the mark with distilled water. These working standards contain, respectively, 0.2, 0.4, 0.8, 1.2, and 1.6 mg Pt per liter. In another 25 ml volumetric flask, five ml of dilute nitric acid is diluted to the mark with distilled water, to serve

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† Baker Chemical Co., Phillipsburg, NJ.
‡ Sigma Chemical Co., St. Louis, MO.
§ Fisher Scientific Co., Fairlawn, NJ.
|| Standard Reference Material #2128-4, National Bureau of Standards, Gaithersburg, MD.
as a blank sample (0 mg Pt per liter). The working standard solutions are stable for at least three months when stored in polyethylene bottles at room temperature.

**Special Apparatus**

1. **Plasticware:** Polypropylene sample cups (two ml) with caps† can be used without preliminary washing.

2. **Electrothermal atomic absorption spectrophotometer:** The method is intended for use with a model 5000-Z electrothermal atomic absorption spectrophotometer** fitted with (a) platinum hollow cathode lamp, (b) automatic sampling system, (c) heated graphite atomizer, (d) pyrocoated graphite tubes, and (e) Zeeman background correction system. The inert gas is ultrapure argon.‡‡

**Procedure**

1. **Sample collection and dilution:** Serum or heparinized plasma are equally satisfactory for analysis of Pt, but use of heparinized plasma avoids the delay for blood clotting. Urine specimens from cancer patients following chemotherapy with cisplatin are routinely diluted one to 20 (v:v) with distilled water, so that the concentrations of urine Pt generally fall in the linear portion of the calibration chart (<1.6 mg per L).

2. **Preparation of standards and samples:** Into duplicate sets of sample cups are pipetted 0.1 ml of (a) working blank and standard solutions that contain 0, 0.2, 0.4, 0.8, 1.2, and 1.6 mg of Pt per liter, (b) heparinized plasma samples, or (c) diluted urine samples. Matrix modifier solution (1.9 ml) is added, the cups are capped, and the contents are mixed by inversion.

3. **EAAS analysis:** The sample cups are placed in the automatic sampler tray and the EAAS analyzer is adjusted to the following settings: (a) sample volume, 40 µl; (b) wave length, 265.9 nm; (c) spectral band width, 0.7 nm; (d) lamp current, 30 mA; (e) integration time, five sec; (f) recorder mode, peak height; and (g) recorder time constant, 0.3 sec. The temperature program is listed in table I. The samples are analyzed automatically, testing one set of standards before the unknown samples and the other set after the samples, to verify that analytical sensitivity is constant during the run.

4. **Computation:** Concentrations of Pt in the plasma or urine samples are read from a calibration chart of peak heights versus Pt concentrations in the standard samples. Computations by the method of standard additions are not routinely required, but may be performed periodically to verify that the standard additions line is parallel to the calibration line. When 24-hr collections of urine are tested, excretion of Pt in urine is reported as mg of Pt per day; when 'spot' urine specimens are col-

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** Perkin-Elmer Co., Norwalk, CT.
†† Linde Division, Union Carbide Corp., New York, NY.

*Using the Perkin-Elmer Model 5000-Z Analyzer.*
lected at specific intervals after treatment with Pt-containing drugs, concentrations of urine Pt are reported as mg of Pt per g creatinine.

**Notes**

1. If the concentration of Pt in a plasma or diluted urine sample exceeds 1.6 mg per liter, the analysis is repeated after the original sample has been diluted with water to adjust the concentration to the linear portion of the calibration chart.

2. If the concentration of Pt in a 1:20 dilution of a urine sample is less than 0.05 mg per liter, the analysis is repeated using an undiluted sample or one that has been diluted one to five with distilled water.

3. If a plasma sample is hemolyzed or if a urine sample shows hematuria, the concentration of Pt should be determined by the standard additions technique, in order to avoid possible interference by hemoglobin iron.

4. Sets of tightly capped tubes containing aliquots (~0.3 ml) of urine and pooled plasma from patients treated with cisplatin are stored at −20°C; one tube from each set is thawed prior to an analytical run and the samples are assayed as quality assurance specimens.

**Statistical Methods**

Statistical tests (standard deviation, coefficient of variation (CV), paired and non-paired t-test, and correlation coefficient) were performed according to Sachs.\(^\text{16}\) Statistical significance was defined as \(p < 0.05\) by a two-tailed t-test.

**Results**

**Experiments to Optimize the Procedure**

Optimization studies involved 10 weeks of daily analytical runs in which Pt (usually 0.8 mg per L) was added to several plasma or urine specimens and the recovery of the added Pt was determined while one or another ingredient of the matrix modifier solution was adjusted systematically to three or more concentrations. Each step of the EAAS temperature program was also optimized. As indicated in the next paragraph, quantitative recovery of added Pt was achieved with the matrix modifier solution and the temperature program that are specified for the present procedure. The same matrix modifier solution and temperature program proved to be satisfactory for plasma and urine samples. Under the specified conditions, the linear portion of the calibration curve extended to 1.6 mg Pt per liter.

**Recovery and Dilution Studies**

Recovery studies were performed by additions of Pt in final concentrations of 0.4, 0.8, and 1.2 mg per liter to nine plasma and 11 urine specimens that contained less than 0.4 mg Pt per liter. With the specified procedure, the standard addition lines were consistently parallel to the calibration line, with slope ratios of \(103 \pm 8\) percent for plasma and \(99 \pm 6\) percent for urine. Statistical comparisons by the paired-sample t-test showed that concentrations of Pt computed from the calibration line did not differ significantly from those computed by the method of standard additions. No significant differences in concentrations of Pt or recovery values were noted when the computations were based on peak areas or peak heights.
The relationship between sample volume and observed concentration of Pt was determined by diluting 0.05, 0.10, 0.15, and 0.20 ml of five plasma and five urine samples to two ml in matrix modifier solution, prior to EAAS analysis. The correlation coefficients \( r \) of sample volumes versus Pt concentrations were 0.998 for the plasma specimens and 1.000 for the urine specimens.

**Sensitivity and Repeatability**

The detection limit for Pt by the present technique, expressed as three times the standard deviation of the reagent blank, was 0.02 mg per liter. Based upon 40 pairs of duplicate analyses of plasma and urine specimens, within-run CV values were 4.2 percent for plasma and 2.3 percent for urine, when the concentrations of Pt were computed from peak heights. Slightly higher values for within-run CV were obtained when the concentrations of Pt in these specimens were computed from peak areas, averaging 5.5 percent for plasma and 4.0 percent for urine \( (p < 0.05 \text{ versus corresponding values computed from peak heights}) \). Therefore, computation based on peak heights was chosen for routine use in the procedure.

When concentrations of Pt were in the upper half of the linear portion of the calibration chart, the run-to-run CV values were 5.7 percent for plasma and 4.2 percent for urine, based upon replicate analyses of specimens of plasma (Sample A, mean concentration of Pt = 1.2 mg per liter) and urine (Sample B, mean concentration of Pt = 17.7 mg per liter; diluted 1:20) in 21 consecutive runs. When concentrations of Pt were in the lower half of the analytical range, the run-to-run CV values were 11.4 percent for plasma and 9.9 percent for urine, based upon replicate analyses of specimens of plasma (Sample C, mean concentration of Pt = 0.32 mg per liter) and urine (Sample D, mean concentration of Pt = 6.0 mg per liter, diluted 1:20) in 21 consecutive runs.

**Stability Studies**

The stability of concentrations of Pt during refrigerated storage of body fluids was tested by replicate analyses of plasma and urine specimens obtained from six patients after chemotherapy with cisplatin. The specimens were aliquoted and analyzed within 24 hours post-collection and after storage at 4°C and at −20°C. Following refrigeration at 4°C or −20°C for 1, 2, or 4 weeks, no significant changes of concentrations of Pt were observed in the plasma or urine specimens, based upon paired comparisons with the results of the initial set of analyses.

**Tests for Interference**

Analytical interference was tested by additions of albumin, glucose, sodium chloride, and human erythrocyte hemolysate to specimens of plasma and urine obtained from five patients after chemotherapy with cisplatin. Additions of glucose (up to 50 g per liter), bovine serum albumin (up to 40 g per liter), or NaCl (up to 800 mmol per liter) did not significantly \( (p > 0.05) \) affect the observed concentrations of Pt. Additions of hemolysate at a hemoglobin concentration of 10 g per liter to the plasma and urine specimens caused artefactual diminution (10 to 15 percent) of the observed concentrations of Pt suggesting that hemoglobin iron may interfere in the analysis of Pt.

**Reference Values**

Specimens of heparinized plasma and morning specimens of urine were
obtained from 18 healthy adult volunteers (10 men, 8 women; age 21 to 59 years; physicians, clinical scientists, technologists, and clerks), who denied any current illness or exposure to platinum compounds. The concentrations of Pt in plasma and urine specimens from these subjects were consistently below the detection limit of the analytical method (0.02 mg per liter).

Concentrations of Pt in Specimens from Patients Treated With Cisplatin

Blood samples were obtained from six cancer patients at various intervals (10 min to 4 days) following an i.v. infusion of cisplatin (60 or 100 mg per m² of body surface area) over one to two hours. Venous blood was collected into one plain tube and the one heparinized tube. No significant difference was found between the concentrations of Pt in 18 paired serum and plasma specimens. Concentrations of plasma Pt at 10 min post-infusion were 3.3 ± 1.1 mg per liter at the 100 mg per m² dosage (N = 3) and 2.2 ± 0.5 mg per liter at the 60 mg per m² dosage (N = 3). In figure 1 are shown the concentrations of plasma Pt in serial specimens from two patients ('A' and 'B') who received the 100 mg per m² dose of cisplatin. The urinary excretion of Pt in patients 'A' and 'B' amounted to 20.2 and 20.9 mg, respectively, during the first day post-infusion, and diminished to 2.1 and 1.9 mg, respectively, during the fourth day post-infusion (figure 2). Concentration of plasma Pt and excretion of urine Pt were measured in two patients 30 days after cisplatin therapy. In a patient who had received 100 mg per m², concentration of plasma Pt on day 30 was 0.37 mg per liter and excretion of urine Pt was 1.1 mg per day; in a patient who had received 60 mg per m², concentration of plasma Pt on day 30 was 0.13 mg per liter and excretion of

![Figure 1. Concentrations of plasma Pt in two cancer patients at intervals from 10 min to 96 hr after i.v. infusion of cisplatin (100 mg per m² of body surface area) over two hours. Patient 'A' (solid circles) was a 72 year old man with squamous cell carcinoma of the tongue; patient 'B' (open circles) was a 55 year old man with squamous cell carcinoma of the cervical lymph nodes, metastatic from an unidentified primary site. In patient 'A', the regression equation for concentration of plasma Pt (mg per liter) versus interval post-infusion (hr) was y = 3.41 - 0.89 \cdot \log(x), and the correlation coefficient (r) was 0.989. In patient 'B', the corresponding regression equation was y = 2.38 - 0.79 \cdot \log(x), and the correlation coefficient was 0.992.](image-url)
urine Pt was 0.33 mg per day. These data demonstrate that the analytical range and sensitivity of the EAAS procedure are adequate to determine Pt concentrations in plasma and urine of cancer patients following chemotherapy with cisplatin.

Discussion

For background information on the pharmacology and pharmacokinetics of cisplatin and carboplatin, readers may consult recent articles and reviews.1,6,11,12,13,17 Four different approaches have been employed to monitor cancer chemotherapy with platinum-containing antitumor drugs: (a) analysis of Pt in body fluids by electrothermal atomic absorption spectrophotometry (EAAS),3,4,9,10,14 (b) analysis of the drugs (e.g., cisplatin, carboplatin) and drug metabolites in body fluids by high-performance liquid chromatography (HPLC) with ultraviolet spectrophotometric detection,5,8,12 (c) analysis of Pt in body fluids by inductively coupled plasma-source mass spectrometry (ICP-MS),2 and (d) in vivo determination of Pt in organs and tissues by X-ray fluorescence (XRF).7 Large dosages of platinum-containing drugs are usually administered to cancer patients, so analytical sensitivity is rarely a limiting factor in the clinical application of these techniques. Since platinum is seldom encountered in the laboratory environment, sample contamination is also not a significant problem. Determination of Pt by EAAS, as described in the present paper, has the advantages of speed, convenience, and sample stability during prolonged storage; the disadvantages of EAAS are the fairly expensive instrumentation that is required and the scarcity of EAAS equipment in clinical pharmacology and drug assay laboratories. The HPLC equipment is more readily available in such laboratories, but the HPLC techniques for measurement of cisplatin and carboplatin in body fluids are relatively difficult and, owing to drug instability, prompt analysis is required. Determination of Pt by ICP-MS seems ideal from the viewpoints of analytical speed, specificity, and precision, but the
equipment is extraordinarily costly and is currently available in very few laboratories throughout the world. Determination of contents of organ Pt by in vivo XRF analysis is difficult to standardize and, owing to the highly specialized nature of the apparatus, is likely to remain a research technique. On the basis of these considerations, the present authors selected analysis of Pt by EAAS as the basis for the pharmacokinetic studies of Pt-containing anti-tumor drugs that are currently underway in our laboratory. The direct EAAS method that is described in this paper reduces matrix interference, requires minimal sample volumes, and avoids time-consuming laboratory. The direct EAAS method that is likely to remain a research technique. On the basis of these considerations, the present authors selected analysis of Pt by EAAS as the basis for the pharmacokinetic studies of Pt-containing anti-tumor drugs that are currently underway in our laboratory. The direct EAAS method that is described in this paper reduces matrix interference, requires minimal sample volumes, and avoids time-consuming internal standardization by the standard additions technique. In the authors' experience, a skilled technologist can perform duplicate analyses of concentrations of Pt in approximately 20 samples of plasma or urine within a working day.

Acknowledgments

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References