OH); mass spectrum: parent ion *m/z* 223.0849 (63.7%) (calc. for $C_{11}H_{13}NO_4$: 223.0843), 224 (M + 1) (9.8), 195 (6.4), 167 (33.5), 139 (7.4), 126 (45.0), 111 (100), 83 (61.8), and 67 (23.0).

3-Amino-5,5-dimethyl-1-oxo-2-cyclohexene - 2 - carboxamide (XXV)—Compound XXIV (223 mg, 1 mmole) was dissolved in 5 ml of saturated ammoniacal methanol. After standing at room temperature for 24 hr, the solvent was evaporated *in vacuo*. The residue was dissolved in acetone, from which colorless crystals appeared (115 mg, 63%), mp 188–189° dec.; UV: λ_{max} (ethanol) 260 (log ϵ 64.6) nm; IR (KBr): 3400, 3300, 3200, 3140, 2950, 2930, 2860, 1630 (intense), 1600–1580 (broad, intense), 1470, 1390, 1385, 1380, 1370, 1330, 1280, 1080, 760, and 635 cm^{-1}; NMR (CD_3COCD_3): δ 1.04 (s, 6H, *gem*-dimethyl groups), 2.40 (s, 4H, methylene groups), 3.07 (s, 2H, NH_2), 6.77 (broad s, 1H, CONH), and 10.57 (broad s, 1H, CONH); mass spectrum: parent ion *m/z* 182.1049 (100%) (calc. for $C_9H_{14}N_2O_2$: 182.1054), 183 (M + 1) (10.4), 167 (8.3), 154 (17.4), 139 (6.3), 126 (32.0), 113 (13.2), 98 (17.6), 85 (93.9), 84 (71.7), 71 (8.4), 70 (27.5), 68 (26.0), 57 (10.4), 56 (17.2), and 55 (21.7).

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Determination of Platinum in Serum and Ultrafiltrate by Flameless Atomic Absorption Spectrophotometry

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Abstract □ A graphite furnace atomic absorption spectrophotometric assay, capable of accurately determining nanogram amounts of platinum in serum and ultrafiltrate, was developed. A sample serum or ultrafiltrate was acidified with nitric acid and heated to destroy the protein–platinum bond. A measured excess of ammonium 1-pyrrolidinedithiocarbamate was added, and the platinum complex was extracted into isopropylacetone. The extract was injected into the graphite furnace. The sample was dried, charred, and atomized using optimal conditions. The resulting absorbance was used to determine the platinum content.

Keyphrases □ Platinum—determination in serum and ultrafiltrate, flameless atomic absorption spectrophotometry □ Atomic absorption spectrophotometry, flameless—determination of platinum in serum and ultrafiltrate □ Analytical techniques—determination of platinum in serum and ultrafiltrate by flameless atomic absorption spectrophotometry

A procedure to determine platinum levels in serums and ultrafiltrates of patients receiving *cis*-diamminedichloroplatinum(II)[1] [$PtCl_2(NH_3)_2$] was investigated. Platinum levels were monitored during a course of treatment using both serum and ultrafiltrate samples.

[1] Platinol.

BACKGROUND

Several analytical techniques to determine platinum in biological samples have been reported, such as neutron activation analysis (1), X-ray fluorescence (2), radioisotope dilution (3), flameless atomic absorption spectrophotometry (4–7), and high-performance liquid chromatography (8). Atomic absorption spectrophotometry has facilitated the determination of minute concentrations of metals in biological fluids. Flameless atomic absorption spectrophotometry was chosen for this study.

Previous procedures for platinum estimation in biological fluids using this technique involved wet-ashing with nitric acid–perchloric acid for sample preparation prior to injection into the graphite furnace. In the present study, two parameters had to be considered, the limited volume of each serum sample and ultrafiltrate and the number of samples to be assayed for platinum content. Wet-ashing was not suitable for the present study. Attempts to determine platinum levels in serum and ultrafiltrate by direct injection into the graphite furnace with no prior sample treatment were unsuccessful. The results obtained by direct injection were highly variable due to sample splatter within the graphite furnace and onto the quartz end-windows during charring.

The present report describes a technique that involves the formation

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of an organic solvent-extractable platinum complex to overcome sample splatter during charring. The procedure has the sensitivity, specificity, and ease of operation required for the routine analysis of platinum in serum and ultrafiltrate.

EXPERIMENTAL

Reagents and Materials—Distilled, deionized water was passed through a 0.45-μm hydrophobic filter[2]. Isopropylacetone[3], 1-pyrrolidinedithiocarbamate[4], nitric acid[3], hydrochloric acid[3], and *cis*-diamminedichloroplatinum(II)[5] were used as received.

Instrumentation—The atomic absorption spectrometer[6] was equipped with a graphite furnace and ramp accessory[7] and an autosampler system[8]. A platinum, hollow cathode lamp[9] was the light source. The graphite furnace was supplied with pyrolytic-coated graphite tubes. Atomization peak heights of platinum were recorded on a strip-chart recorder[10] at a chart speed of 10 mm/min and recorder current of 10 mv.

Operating Conditions—The optimum parameters for platinum determination in the test solutions were: drying cycle, 120° for 15 sec with a temperature ramp of 10 sec; charring cycle, 1100° for 15 sec with a temperature ramp of 15 sec; and atomization cycle, 2700° for 8 sec with no ramping. The ramp setting permitted a gradual rise in the furnace temperature during each run. The carrier gas was argon at a flow rate of 40 ml/min. Atomization peak heights were recorded at 265.9 nm, using a 3× scale expansion.

Instrument Calibration—When 20 μl of the platinum standard solution (1 μg/ml) is injected into the furnace, the resulting signal should not be <0.6 absorbance unit.

Preparation of Standard Solutions—*Platinum Stock Standard Solution—cis*-Diamminedichloroplatinum(II) (0.1541 g, 99.8% purity)[11] was weighed accurately into a 1-liter volumetric flask. Distilled, deionized water (50 ml) and 20 ml of concentrated hydrochloric acid were added, and the mixture was heated gently on a hot plate until dissolution. The solution was cooled to room temperature and diluted to volume with distilled, deionized water. Each milliliter is equivalent to 100 μg of platinum.

Platinum Working Standard Solution—One milliliter of the platinum stock standard solution was diluted to 100.0 ml with distilled, deionized water. Each milliliter is equivalent to 1.0 μg of platinum. This solution was prepared fresh each working day.

Ammonium 1-Pyrrolidinedithiocarbamate Solution, 1% in Distilled, Deionized Water—If the solution was not clear, it was filtered[12]. This solution was prepared fresh each working day.

Isopropylacetone was saturated with distilled, deionized water.

Platinum-Free Serum and Ultrafiltrate[13]—The ultrafiltrate was prepared by filtering serum through ultrafiltrate cones[14], which filtered out molecules of >50,000 daltons.

Preparation of Standard Curve—One milliliter of the platinum-free serum or ultrafiltrate was pipetted as needed into each of five 16 × 125-mm screw-capped test tubes. Then 0, 25, 50, 100, and 250 μl of the platinum working standard was added to each tube, and the solutions were allowed to stand 5 min. Then 0.5 ml of concentrated nitric acid was added to each tube and mixed well. The tubes were capped and placed in a boiling water bath for 5 min. They then were removed from the bath and cooled to room temperature. Distilled, deionized water (5 ml) was added to each tube and mixed; then 5.0 ml of 1% ammonium 1-pyrrolidinedithiocarbamate solution was added and mixed vigorously for 1 min. Then 1.0 ml of isopropylacetone saturated with distilled, deionized water was added and mixed vigorously for 2 min.

The solution was centrifuged for 3 min at 1000×*g*. With a Pasteur pipet, the isopropylacetone (upper) layer was transferred carefully to the polyethylene sample cups to avoid trapping water droplets in the transfer. The instrument was optimized and the sampler was set to 3. The instrument recorded the absorbance of the blank (zero) and each sample. The instrument was autozeroed after each blank reading. The three absorbance readings of each standard were averaged, and a working curve of absorbance *versus* concentration of platinum was plotted.

Sample Preparation—One milliliter of serum or ultrafiltrate was pipetted into a 16 × 125-mm screw-capped test tube. Then 0.5 ml of concentrated nitric acid was added, and sample preparation proceeded as already described. The three absorbance readings were averaged, and the concentration of platinum was obtained from the working curve. If the sample absorbance was greater than the absorbance of the highest standard, the sample was diluted with platinum-free serum or ultrafiltrate and the determination was repeated.

If there were more than six samples per run, an additional blank was placed at the end of the run and the 100-ng/ml platinum standard was rerun to observe any drift in instrument conditions.

Table I—Absorbance Data for Standard Platinum Solutions

Solution	Platinum, ng/ml	Absorbance 1	2	3	$\bar{X}$
Serum					
1	25	0.035	0.036	0.036	0.036
2	50	0.078	0.066	0.069	0.071
3	100	0.145	0.131	0.140	0.139
4	250	0.309	0.337	0.341	0.329
Ultrafiltrate					
1	25	0.028	0.033	0.035	0.033
2	50	0.063	0.062	0.064	0.063
3	100	0.147	0.134	0.140	0.140
4	250	0.333	0.357	0.344	0.345

Table II—Recovery Data for Standard Platinum in Serum and Ultrafiltrate *versus* Standard Platinum in Water

Platinum Added, ng/ml	Platinum Found, ng/ml Serum	Ultrafiltrate	Recovery, %[a] Serum	Ultrafiltrate
50	44.1	39.7	88.2	79.4
100	92.2	87.5	92.2	87.5
200	182.6	182.6	91.3	91.6

[a] Recovery values are an average of three values.

RESULTS AND DISCUSSION

Initial work on the determination of platinum in serum used whole serum injected directly into the furnace without prior treatment. During drying, either ramped or unramped, an audible cracking or frying sound was heard; at times, a cinder of dried sample was physically ejected from the furnace. During charring, ramped or unramped, the sample crusted over. As the temperature increased to ~350°, the sample literally exploded due to improper drying and spattered the quartz end-windows with charred sample. Because of these and other problems in direct sample injection, another procedure was investigated.

Ammonium 1-pyrrolidinedithiocarbamate forms organic-soluble complexes with over 30 elements, including platinum (9). The platinum complex forms in the pH 1–14 range and is extracted quantitatively into isopropylacetone in the pH 1–10 range. By heating the sample with nitric acid to destroy the protein, the platinum–ammonium 1-pyrrolidinedithiocarbamate complex can be extracted into isopropylacetone to eliminate matrix effects from the untreated serum or ultrafiltrate. Standard curves of platinum in serum or ultrafiltrate gave a linear response over the 25–250-ng of platinum/ml range when run against platinum in distilled, deionized water, but platinum recoveries were low. This low recovery can be improved by preparing the standards in either serum or ultrafiltrate as required.

Table III—Recovery Data for Standard Platinum in Serum and Ultrafiltrate Using Sample Matrix

Platinum Added, ng/ml	Platinum Found, ng/ml Serum	Ultrafiltrate	Recovery, %[a] Serum	Ultrafiltrate
50	47.6	47.1	95.2	94.2
100	96.6	96.5	96.6	96.5
200	195.0	194.8	97.5	97.4

[a] Recovery values are an average of three values.

[2] Millipore Corp., Bedford, MA 01730.
[3] ACS grade, Fisher Scientific Co., Pittsburgh, PA 15219.
[4] White Label, Eastman Organic Chemicals, Rochester, NY 14650.
[5] Bristol Laboratories, Division of Bristol-Myers Co., Syracuse, NY 13201.
[6] Model 460, Perkin-Elmer Corp., Norwalk, CT 06856.
[7] Model HGA2200, Perkin-Elmer Corp., Norwalk, CT 06856.
[8] Model AS-1, Perkin-Elmer Corp., Norwalk, CT 06856.
[9] Intensitron 303-6501, Perkin-Elmer Corp., Norwalk, CT 06856.
[10] Model 156, Perkin-Elmer Corp., Norwalk, CT 06856.
[11] Purity was established by nonaqueous titration with perchloric acid in acetic acid.
[12] Whatman No. 30 filter paper.
[13] Supplied by Upstate Medical Center, Syracuse, NY 13210.
[14] Centriflo membrane cones, CF50A, Amicon Corp., Lexington, MA 02173.

Table IV—Day-To-Day Variability Data

Day	Concentration of Platinum, ng/ml	Average Absorbance	
		Serum	Ultrafiltrate
1	50	0.081	0.079
	100	0.160	0.160
	250	0.390	0.389
2	50	0.073	0.064
	100	0.151	0.140
	250	0.349	0.324
3	50	0.075	0.071
	100	0.148	0.140
	250	0.369	0.352

Table I contains the absorbance data obtained for a typical standard curve of platinum in serum and in ultrafiltrate; the relationship of platinum to absorbance was linear in the 0–250-ng/ml range. Table II contains the recovery data for serum and ultrafiltrate with known amounts of standard platinum as compared to similarly treated pure solutions of platinum in distilled, deionized water. The recoveries ranged from 79.4 to 92.2%. This result demonstrates that a standard curve of platinum cannot be prepared from distilled, deionized water. Recoveries of platinum from serum and ultrafiltrates using the respective matrix to prepare the standard curve are shown in Table III.

The day-to-day variability data in Table IV indicate that a standard working curve must be run each time for significant results. Placement of a standard at the end of each sample run is used to monitor the drift in instrument conditions during the run. The sampler trays[8] hold 30 polyethylene cups, giving a maximum run of four standards and 24 samples. The pyrolytic-coated graphite furnaces were replaced routinely at ~200 injections. Studies were not run to determine the maximum number of injections possible with these furnaces. It was necessary to optimize the optical alignment and the furnace alignment each day before beginning a run.

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Solubility and Partitioning IV: Aqueous Solubility and Octanol–Water Partition Coefficients of Liquid Nonelectrolytes

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Abstract □ The aqueous solubility and octanol–water partition coefficient of over 100 nonelectrolyte organic liquid solutes are related by the simple equation $\log S_w = -1.016 \log PC + 0.515$, where S_w is the molar solubility of liquid solutes in water and PC is the experimental partition coefficient of the solutes in the octanol–water system. The liquids studied represent a wide variety of organic compounds including aliphatic and aromatic hydrocarbons, alcohols, esters, ethers, aldehydes, and ketones. This finding is in agreement with that reported by Hansch and coworkers. However, these results are signficant because only the experimental values for the aqueous solubilities and octanol–water partition coefficients are included, as opposed to the calculated partition coefficients used by Hansch. This relationship is extremely useful in understanding the overall solubility and partitioning phenomenon for organic liquids and provides a basis for studying crystalline solids and gases.

Keyphrases □ Aqueous solubility—aliphatic and aromatic hydrocarbons, liquid nonelectrolytes, experimental values compared with calculated values □ Partitioning—octanol–water partition coefficients, experimental values compared with calculated values, aliphatic and aromatic hydrocarbons, liquid nonelectrolytes □ Hydrocarbons, aliphatic and aromatic—aqueous solubility and partition coefficients obtained experimentally compared with calculated values □ Liquid nonelectrolytes—aliphatic and aromatic hydrocarbons, aqueous solubility and partition coefficients obtained experimentally compared with calculated values

The aqueous solubility and partition coefficient of a drug are key parameters in determining its biological activity. The partition coefficient frequently is used in quantitative structure–activity studies. Its usefulness in the assessment of transport properties of drugs through biological membranes, extraction of solutes in aqueous–organic liquid systems, measurement of equilibria, and design of controlled-release drug delivery systems is well documented (1–4).

The aqueous solubility of a drug influences the dissolution rate and thus the rate and extent of absorption through biological membranes. The efficiency or biological performance of drugs from these formulations depends on the release and transfer of drug molecules to the systemic circulation. The release and transport of drugs are determined by solubility and the partition coefficient. The combined effects of aqueous solubility and the membrane–water partition coefficient on absorption were quantitatively described by Yalkowsky and coworkers (5–8).

This paper is part of a series dealing with the relationship between solubility and partitioning and deals exclusively with liquid nonelectrolyte solutes in water and octanol–water partitioning systems. In subsequent contributions, nonelectrolyte crystalline solids as well as weak acids and bases will be investigated.

There is a direct quantitative relationship between aqueous solubility and partitioning. However, due to a lack of reliable solubility and partitioning data, attempts to quantitate this relationship have met with only limited success (9).

This report demonstrates that there is a simple, nearly

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