DETERMINATION OF PLATINUM IN BIOLOGICAL MATERIAL BY DIFFERENTIAL PULSE POLAROGRAPHY: ANALYSIS IN URINE, PLASMA AND TISSUE FOLLOWING SAMPLE COMBUSTION

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A method is described for determining total platinum content in urine, blood plasma and tissues of patients or experimental animals receiveing *cis*-dichlorodiammineplatinum(II). The method is based on drying and combustion of the biological material in a muffle furnace. The product of the combustion is dissolved successively in *aqua regia*, hydrochloric acid and ethylenediamine. The resulting platinum–ethylenediamine complex yields a catalytic current at a dropping mercury electrode allowing to determine platinum by differential pulse polarography. Platinum levels of *c.* 50–1 000 ng per ml of the biological fluid or per 0·5 g of a tissue can readily be analyzed with a linear calibration.

Recently we described a clinically useful analytical method for monitoring species derived from the antineoplastic agent *cis*-dichlorodiammineplatinum(II) (*cis*-DDP) in urine[1]. The analysis was based upon derivatization of the platinum species by a reaction with sodium diethyldithiocarbamate and in the final step upon determination by differential pulse polarography (d.p.p.). The procedure exhibited a high degree of specificity and high sensitivity provided by d.p.p. and it circumvented the matrix effects.

Our further work was aimed at finding a method for the analysis of platinum in other biological materials. This report describes a method for platinum analysis based also in its final step upon estimation with the aid of d.p.p. In contrast to the method described in our previous report[1] the new procedure makes it possible to estimate total platinum not only in urine but also in blood plasma and tissues.

EXPERIMENTAL

Instrumentation

D.p.p. was performed on a polarographic analyzer PA 2 (Laboratory Instruments, Czechoslovakia). A three-electrode system was used, comprising a dropping mercury electrode (d.m.e.), a spectroscopic graphite counter electrode and a saturated calomel reference electrode. D.m.e. had a mercury flow rate of 0·927 mg/s. Polarograms were obtained with a pulse amplitude of −50 mV and a sweep rate of 2 mV/s. Drop time control of the PA 2 analyzer was set at 2·0 s. Initial potential was −1·3 V. Samples were combusted in a Model L100 (VEB Electro Bad Frankenhause GDR) muffle furnace.

Materials

cis-DDP was obtained from Lachema (Czechoslovakia) and was used without further purification. Platinum standard solution, each mililitre of which was equivalent to 40 μg of platinum was prepared fresh each working day by dissolving *cis*-DDP in distilled water. If not stated otherwise the amounts of platinum reported in this paper are related to elementary platinum. Ethylenediamine was purchased from Fluka (Switzerland). Human urine and blood plasma were obtained from volunteers not undergoing *cis*-DDP therapy. Fresh heparinized bovine blood plasma taken from a cow not receiving *cis*-DDP was obtained by courtesy of Dr B. Hofírek. Fresh heparinized rat blood plasma was taken from female rats (body mass *c.* 300 g), strain Wistar. Liver tissue was taken from male mice (body mass *c.* 30 g), strain (CBA C57BL/10)F1, or from female rats (strain Wistar) immediately after the sacrifice.

Preparation of Standard Curve

2 ml of a platinum-free biological liquid (urine or blood plasma) or 0·5 g of a tissue are placed into each of six new porcelain crucibles (35 mm in diameter and 30 mm high). Then 0, 5, 10, 20, 50 and 80 μl of the platinum standard solution is added and the mixtures are allowed to stand for 30 minutes. The crucibles are placed in a sand bath heated to *c.* 120°C and the samples are dried for 1 hour until complete dehydration is achieved. If blood plasma is analyzed, the residues are further dissolved in 1 ml of *aqua regia* and again evaporated to dryness in the sand bath. The crucibles are then covered with lids, loaded into a muffle furnace and heated according to the following schedule: 60 minutes at 200°C, 30 minutes at 250°C, 60 minutes at 350°C, 30 minutes at 425°C and finally 90 minutes at 700°C. After combustion of the samples, the crucibles are cooled to room temperature, the residue dissolved in 2 ml of freshly prepared *aqua regia* and allowed to stand overnight. Each crucible content is quantitatively transferred into a new 50 ml glass beaker (40 mm in diameter and 55 mm high). The inner surface of the crucible and the lid are rinsed with 1 ml of *aqua regia*, which is also added to the pertinent beaker. The content of the beaker is evaporated to dryness on a steam bath and the residue is dissolved (always after evaporation to dryness on a steam bath) successively in 2 ml of concentrated hydrochloric acid, 2 ml of 0·025 mol/l ethylenediamine and 2 ml of 0·01 mol/l ethylenediamine. After final evaporation the residue is redissolved in 2 ml of a supporting electrolyte consisting of 0·1 mol/l potassium chloride and 0·1 mol/l ethylenediamine. After *c.* 10—60 minute standing the sample is deoxygenated for 5 minutes with nitrogen and analyzed by d.p.p. The height of the d.p.p. peak (I) is measured at −1·65 V (Fig. 1) and a working curve of I *versus* concentration of platinum is plotted.

Sample Preparation

2 ml of the biological liquid (urine or blood plasma) or 0·5 g of a tissue are placed into a new porcelain crucible. Then it is dried in a sand bath, loaded into the muffle furnace and sample preparation proceeds as described above. The concentration of platinum is determined from the working curve. If the d.p.p. peak is markedly higher than that of the standard with the highest platinum concentration, the sample is diluted with the solution obtained by taking the pertinent platinum-free biological material through the analysis scheme and the determination is repeated.

RESULTS AND DISCUSSION

D.p.p. estimation of platinum according to the procedure described in this report is based on the catalytic electrode reaction[2,3] during which platinum–ethylenediamine

complex is reduced to a lower oxidation state, which is highly reactive and reduces protons to hydrogen. However, we have shown in our recent report[1] that platinum analysis in biological samples, based on the latter electrochemical activity, is complicated by the organic matrix. To overcome this obstacle initial efforts were directed at treating urine, plasma and tissue (including its homogenate) in mixtures of acids and hydrogen peroxide as recommended for wet digestion of an organic material whose inorganic component is to be analyzed. Wet digestion together with d.p.p. analysis yielded inconsistent and poor recovery of platinum from all biological material investigated in this work even when several known polarographic activities of platinum species[2-5] were examined.

Chemical derivatization of platinum in a biological liquid aimed at yielding an extractable product, used as an approach to eliminate matrix effects[6,7], was also examined. This procedure in connection with d.p.p. analysis appeared to be useful only for platinum determination in urine, as shown in our recent report[1]. Analyses of plasma and homogenized tissue thus treated exhibited high variability in d.p.p. responses, even when this material was first subjected to wet digestion. Thus, in our experience wet mineralization of biological material did not prove suitable for platinum analysis by means of d.p.p.

Quite recently a method has been described for determination of total platinum in tissues by atomic absorption spectroscopy (a.s.s.) based also on total combustion of the tissue[8]. It has been shown that, contrary to wet digestion, mineralization by combustion of the tissue eliminates matrix effects to such an extent that it repre-

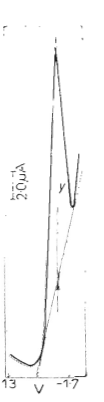

FIG. 1

Differential pulse polarogram of platinum ethylenediamine complex in 0·1 mol/l potassium chloride with 0·1 mol/l ethylenediamine. The complex was formed from *cis*-dichlorodiammineplatinum(II) in bovine blood plasma treated by the procedure outlined. Conc. of Pt in plasma 585 ng/ml. The value of *y* was taken to represent the height of this peak

sents a means for a precise and reproducible analysis of a variety of tissues by means of a.a.s. We have, therefore, directed out further efforts at using combustion as a pretreatment for urine, plasma and tissues samples in the expectation that d.p.p. analysis of the dissolved ashes will provide consistent and satisfactory recovery of platinum from the biological materials tested in the present study.

Table I contains the d.p.p. peak height values obtained for a typical standard curve for platinum in human urine, bovine and human blood plasma, and mouse liver, if the d.p.p. activity of platinum–ethylenediamine complex[1,2] was exploited; potassium chloride–ethylenediamine electrolyte gave a linear calibration for platinum in the concentration range of *c.* 50 – 1 600 ng/ml in the case of biological liquids and for platinum content on 0·5 g of a tissue ranging from *c.* 100 – 3 200 ng. Construction of these standard curves resulted in generation of the lines with a zero-order correlation coefficient of 0·99. The upper limit of analytical utility of d.p.p. for these analyses is given by irregularities in mercury drop dislodging at higher platinum concentrations. These irregularities are probably connected with small bubbles formed at the capillary orifice as a consequence of the catalytic process yielded at the d.m.e. by platinum–ethylenediamine complex[2].

D.p.p. results for biological materials containing known amounts of platinum (800 – 1 600 ng) taken through the analysis scheme were compared with those obtained for similarly treated pure solutions of *cis*-DDP in distilled water or in some simple electrolytes. The recovery ranged from 115 – 138%. This result, along with the fact that various biological materials containing platinum yielded after treatment by the procedure described d.p.p. peaks of different height, indicates that the standard curve for platinum must be made using the pertinent sample matrix. Thus in the case

TABLE I

D.p.p. peak height values for platinum standard in different biological materials

Amount of Pt added[a] ng	Peak height[b], μA			
	human urine	human blood plasma	bovine blood plasma	mouse liver
200	3·55	2·57	3·00	3·06
400	6·40	5·50	5·57	5·96
800	13·28	10·24	11·73	11·03
2 000	33·86	29·09	28·56	30·41
3 200	50·42	44·61	47·92	44·23

[a] *cis*-DDP was added to 2 ml of the biological fluid or to 0·5 g of the tissue. [b] Average value for analysis of three samples.

of analysis of urine or blood plasma the standard curve is made with the same kind of platinum-free fluid taken from the patient or the experimental animal before *cis*-DDP administration. If it is impossible to take the amount of the platinum-free liquid sufficient for making the standard curve from the same experimental animal whose biological liquid shall be analyzed, satisfactory results can be obtained if platinum-free liquids are taken from the control animals (Table II). Similarly, in the case of analysis of a tissue of an experimental animal, the standard curve for platinum is made with the same kind of the platinum-free tissue taken from the control experimental animal (Table II).

Time and temperature of pyrolysis are important factors influencing the precision of estimation[8]. For important results it was necessary to use porcelain crucibles only once because the products of combustion attacked the glaze, which probably resulted in trapping of platinum in the glaze matrix. As for the accuracy of the platinum estimation the best results were obtained if the samples for the standard curve were dried and combusted simultaneously with the samples containing unknown amounts of platinum

The precision of the d.p.p. estimation of platinum by the procedure described in this report was determined on the basis of measurement of ten identical samples of human urine and blood plasma, containing platinum at a concentration of 800 ng/ml. In the supporting electrolyte used throughout the study, a precision of 8% was obtained

Interference studies were carried out, too. As for the effect of metal ions on the platinum d.p.p. peak exploited in this work, results were obtained which were basically identical to those of Alexander and coworkers, who analyzed platinum in ores[2,3]. Moreover, the addition of small amounts of some low-or high-molecular weight organic compounds to the biological samples resulted in most cases in an increase of the d.p.p. peak. The data contained in Table I along with the results

TABLE II

D.p.p. peak height values for Pt standard in rat blood plasma and rat liver (rats No 1—4 of identical strain and of the same age). *cis*-DDP added to 2 ml of plasma or to 0·5 g of the tissue; amount of Pt added: 1 170 ng

Sample	Peak height, μA			
	No 1	No 2	No 3	No 4
Plasma	16·27	17·22	16·93	16·81
Liver	17·62	17·02	17·85	18·12

obtained with *cis*-DDP dissolved in distilled water (mentioned in this report earlier) indicate that the combustion was probably incomplete. Nevertheless, it destroyed the organic matric to such an extent that d.p.p. could be used for the reproducible quantitative analysis of platinum in most biological materials.

The utility of the method was tested by administering *cis*-DDP to two female rats at a dose 5 mg of *cis*-DDP/kg body mass intravenously through the tail vein. Two hours after dosing, animals were sacrificed and liver tissue harvested. Platinum level found in this tissue was (3·6 ± 0·1) and (3·4 ± 0·1) μg Pt per gram of the tissue (average ± standard deviation for determination of three samples obtained from each liver). This platinum level agrees very well with previously reported values obtained by means of a.a.s[8] (4·1 and 3·4 μg Pt/g of rat liver tissue).

In conclusion, this paper describes the method for platinum determination in biological material by d.p.p., which has good precision and sensitivity comparable to platinum analysis by a.a.s.[8,9]. The procedure described here makes it possible to determine total platinum independently of valency or ligands attached to the metal. It is, therefore, unnecessary to consider possible derivatization of *cis*-DDP which has already been observed[10] both *in vivo* and *in vitro*. The method described in this report thus represents an alternative of the methods so far used in platinum analysis. Moreover, d.p.p. instrumentation is relatively simple so that the method can become widely used even in clinical laboratories.

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