Chemical modifiers in electrothermal atomic absorption determination of Platinum and Palladium containing preparations in blood serum

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Abstract : The biological liquids matrixes influence on the characteristic masses and repeatability of Pt and Pd electrothermal atomic absorption spectroscopy (ETAAS) determination was studied. The chemical modifiers dimethylglyoxime and ascorbic acid for matrix interferences elimination and ETAAS results repeatability improvement were proposed while bioliquids ETAAS analysis, and their action mechanism was discussed.

Keywords: electrothermal atomic absorption spectroscopy; platinum, palladium; chemical modifiers; bioliquids.

Introduction

Platinum- and palladium-based preparations like carboplatin, ephazole, etc. are widely used in medicine due to their efficient antiradiation and antitumor activity.

An urgent task is investigation of these preparations (by Pt and Pd content) distribution in the subcellular structures of tumor cells and blood corpuscles of patients.

However determination of Pt and Pd content in bioliquids (whole blood and blood serum, urine, lymph, ascitic fluid, etc) by atomic spectroscopy techniques involves sample acid rupture, organic matrix destruction, and preliminary separation and preconcentration of Pt and Pd [1-10].

While electrothermal atomic absorption spectroscopy (ETAAS) technique features the sensitivity required to determination of Pt and Pd trace amount in human bioliquids, there is some sacrifice of measurements accuracy due to significant matrix interferences [11, 12].

The goal of the present study is a search of efficient chemical modifiers for matrix effects elimination in Pt and Pd determination in bioliquids and development of the direct procedure (without preliminary sample preparation) for their determination in blood serum.

Experimental Section

Apparatus

Platinum and palladium determination was carried out with atomic absorption spectrophotometer Saturn 3 equipped with electrothermal atomizer Grafit-2 and deuterium

background corrector. Sampling was performed with Unipipette P 200 device with an accuracy within ±0.4%. Elements absorbance was monitored at the resonance wavelengths 265.9 nm (Pt) and 247.9 nm (Pd). The solution tested were sampled out on the pyrolytic coated graphite platforms produced by OKBA (Severodonetsk, Ukraine). The source of resonance emission was hollow-cathode lamps from CORTEC Ltd. (Moscow, Russia). Heating of the furnace was performed by a 4-step program after preliminary temperature optimization at the pyrolysis and atomization stages. Inert gas was "highest-grade" argon.

Platinum(IV) complexes thermal destruction studies were performed with Q-1500D derivatograph Paulik-Paulik-Ergey system (operating conditions: dynamic mode, air atmosphere, temperature range 20-800°C) in the covered alundum crucibles. Chemical composition of the platinum dimethylglyoximate pyrolysis products was determined using x-ray phase difractometer Dron 2.

Reagents and standard solutions

All reagents were of analytical or higher grade. Standard solutions of H_2PtCl_6 and H_2PdCl_4 were prepared from the certified reference materials that were donated by Phisico-Chemical Institute of National Academy of Science (Odessa, Ukraine). Commercial Carboplatin from Ebewe Pharma (Austria) was used without additional purification.

Procedure

Standard solution of Pt(IV) (252 µg/L), Pd(II) (25 µg/L) or Carboplatin was sampled into the volumetric test tube of 10 mL capacity and the ethanolic solutions (dimethylglyoxime and diphenylcarbazone) or aqueous solutions (chromazurole S, ascorbic acid, hydrazine hydrochloride or diantipyrylmethane) of the modifiers were then added. Pig blood serum (1.0 mL) prepared by blood centrifugation at 3000 rps/min during 15 min was added to the mixture, diluted with water to the total volume of 3.0 mL and stirred. A portion of the resulting solution (20 µL) was sampled out on L'vov platform into the graphite furnace followed by the atomizer heating under optimum conditions. The modifies efficiency was judged from characteristic masses m_0, i.e., the analyte masses producing an integrated absorbance of 0.0044, and relative standard deviation S_r of absorbance data.

The characteristic masses (m_0) were calculated by the formula:

$$m_0 = \frac{C \cdot V \cdot 0.0044}{A},$$

where C − analyte solution concentration; V − solution volume; A − integrated absorbance value.

Results and Discussion

Selection of optimal time-temperature heating program

Optimum temperature-time heating regime in platinum and palladium ETAAS determination was established by simulation of thermodynamic parameters of high-temperature evaporation, pyrolysis and free atoms formation processes of the platinum(IV)

and palladium(II) compounds in the graphite furnaces using program package HSC CHEMISTRY 4.0.

Data of the computer simulation were summarized as the temperature dependencies of metals different forms (in kmol) in the condensed and gas phases of the analytical zone – graphite tube of the electrothermal atomizer.

Figures 1 and 2 give the simulation results for standard solutions of H_2PtCl_6 and H_2PdCl_4, and medical preparations – carboplatin $C_6H_{12}N_2O_4Pt$ and ephazole $C_{20}H_{32}N_2O_2PdCl_4$.
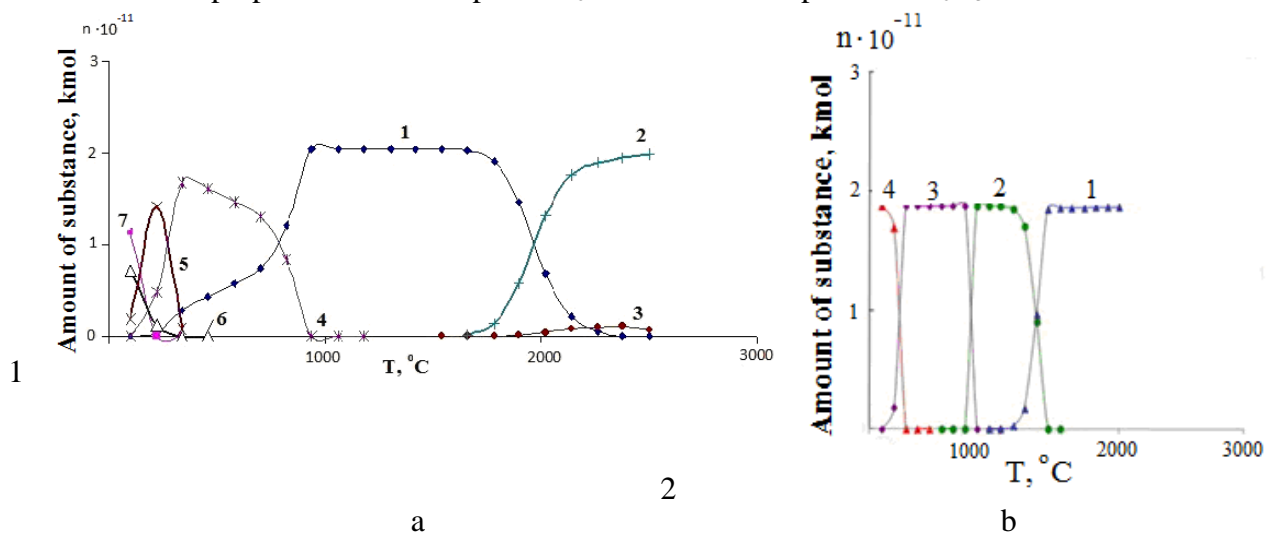

Figure 1. Simulation of atomization of platinum (a) $1 – Pt_c$; $2 – Pt_g$; $3 – PtC_g$; $4 – PtCl_c$; $5 – PtCl_{2c}$; $6 – PtCl_{3c}$; $7 – PtCl_{4c}$; and palladium (b) $1 – Pd_g$; $2 – Pd_c$; $3 – PdO_c$; $4 – PdCl_{2c}$ compounds. Subscript c – condensed phase; *g* denotes the gas phase.
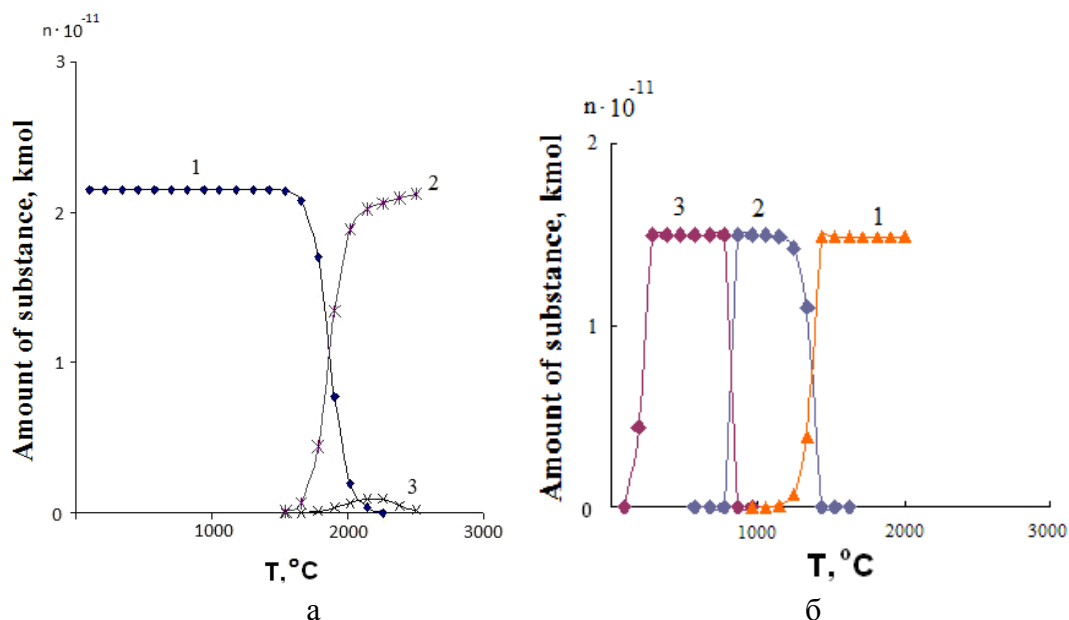

Figure 2. Simulation of carboplatin (a) $1 – Pt_c$; $2 – Pt_g$; $3 – PtC_g$ and ephazole (b) $1 – Pd_g$; $2 – Pd_c$; $3 – PdO_c$ atomization. Subscript c – condensed phase; *g* – gas phase.

The data of fig. 1a and 2a show that the thermostable platinum carbide PtC with decomposition temperature more than 2600°C may form at the temperature range 1800-2500°C in graphite furnace gaseous phase. This process may cause the platinum free atoms

1   amounts decreasing and may be one of the reasons of depressing matrix influence while
2   analysis of samples containing great carbon amounts, for example, bioliquids. In the case of
3   the palladium the carbide compound is not formed under these conditions.
4   Figs. 1 and 2 revealed that the Pt and Pd free atoms formation in the standard solutions of
5   Pt- and Pd-containing compounds differ insignificantly. Platinum atomic vapor is formed in
6   evaporation of the condensed reduced platinum. In the case of palladium, PdO is first formed
7   in the condensed phase followed by its reduction to free Pd and subsequent evaporation of the
8   latter. The pyrolysis maximum allowable temperature in standard solutions of platinum and
9   palladium evaporation amounted to 1800°C and 1400°C, respectively, whereas those for
10 carboplatin and ephazole were somewhat lower (1500°C).
11   On this basis the drying step was carried out at 110°C during 30 s, atomization step was
12 carried out at 2800°C at a "gas-stop" mode of operation during 5 s, and the furnace high-
13 temperature cleaning was carried out at 2900°C within 3 s. Pyrolysis was carried out with
14 stepwise increasing the temperature to 1800°C (Pt) and 1400°C (Pd) during 80 s.
15
16 **Bioliquids matrix effects on platinum and palladium ETAAS determination**
17
18   Table 1 gives the experimental characteristic masses m_0 and relative standard deviations S_r
19 for Pt and Pd absorbance in "non-matrix" solutions and bioliquids under optimum conditions.
20 The standard solutions characteristic masses are close to those available from the literature:
21 10 pg for palladium [13] and 56 pg for platinum [14].
22   The bioliquids matrixes decrease the sensitivity in platinum and palladium ETAAS
23 determination by the factor of 1.5-2 and 1.5-4 respectively. Moreover, a considerable
24 repeatability decrease is observed for both elements.
25
26 Table 1. Effect of bioliquid matrixes on Pt and Pd ETAAS determination

	Metrological characteristics			
Sample	Pt		Pd	
	m_0, pg	S_r (n=3; P= 0.95)	m_0, pg	S_r (n= 4; P= 0.95)
"No-matrix" solution	65	0.07	10	0.12
Blood serum	96	0.23	18	0.07
Ascitic liquid	106	0.14	16	0.09
Lymphocytes	131	0.29	39	0.20

27
28   The use of stabilized temperature furnace with a platform eliminates or essentially reduces
29 matrix interferences providing that chemical modification is an essential part of the procedure.
30
31 **Chemical modifiers in Pt and Pd ETAAS determination in blood serum**
32
33   ETAAS determination of Pt and Pd in the biological samples conventionally involves such
34 chemical modifiers as $(NH_4)_2H_2EDTA+NH_3+NH_4H_2PO_4$ [15], ammonia [16], copper sulfate
35 [17].
36   In solving a problem of Pt and Pd atomization processes modification an efficiency of
37 organic reagents which form stable complex compounds with Pt(IV) and Pd(II) ions in
38 aqueous solutions – dimethylglyoxime, chromazurol S, diphenylcarbazone and diantipyril
39 methane – were used. Water solubility of sparingly soluble modifiers was increased by

1   addition of 1.3 % (by volume) of ethanol. The modifiers efficiency was evaluated by their
2   ability to increase the sensitivity and repeatability of ETAAS technique.
3   Table II gives the results as the ratios of characteristic masses and relative standard
4   deviations of Pt and Pd experimental analytical signal in pig blood serum samples with and
5   without modifiers (m_0 and S_r) and (m_0^M and S_r^M).
6
7   Table II. Efficiency of chemical modifiers in Pt and Pd ETAAS determination in pig blood
8   serum**

Modifier (0.001 mol/L)	m_0/m_0^M	m_0^{st}/m_0^M	S_r/S_r^M (n = 3; P= 0,95)
Platinum			
Dimethylglyoxime *	1.0	1.0	1.5
Diphenylcarbazone *	0.98	0.69	0.73
Chromazurol S	0.98	0.69	0.48
Palladium			
Ascorbic acid	1.5	1.0	2.0
Hydrazine hydrochloride	0.97	0.71	0.80
Diphenylcarbazone *	0.86	0.69	0.90
Dimethylglyoxime*	1.2	1.0	0.98
Chromazurol S	1.0	0.83	0.88
Diantipyrylmethane *	0.91	0.80	0.63

9   * in water with 1.3% C_2H_5OH added (by volume)
10   ** three-fold diluted with water
11
12   Biological samples matrixes decrease the Pt and Pd ETAAS determination sensitivity in
13   comparison with standard solutions, which don't contain biological matrixes. The degree of
14   this influence elimination was estimated by Pt and Pd characteristic masses ratio in standard
15   solutions (without biological matrix) m_0^{st} and in pig blood serum solutions containing matrix
16   modifier m_0^M. The complete elimination of matrix influence (the ratio m_0^{st}/m_0^M is close to 1)
17   and 1.5-fold and 2-fold relative standard deviation decrease for platinum and palladium,
18   correspondently, are observed only for dimethylglyoxime and ascorbic acid.
19   At temperatures below 470 K in the crystallization water melts the interaction between
20   palladium and ascorbic acid takes place that leads to Pd(II) exothermic reduction to metal
21   [18]. Besides, in the ascorbic acid presence a precipitate forms, so analyte compounds
22   pyrolysis is faster and more complete. Developed surface of the sediment probably leads to
23   elements evaporation rate increasing. The presence of ascorbic acid in the sample solution
24   reduces the diffusion of analytes to the peripheral zones of the furnace at the pyrolysis stage
25   [19].
26   Effect of dimethylglyoxime is due to change in the mechanism of free atoms formation,
27   prevention of analyte low-temperature losses and change on character of platinum and/or its
28   compounds distribution at the surface of the graphite platform. Derivatography studies Pt(IV)
29   dimethylglyoximate pyrolysis prepared by the standard procedure [20] support this suggestion
30   (Fig. 3).
31   Complex pyrolysis proceeds by a stepwise mechanism and is completed at a temperature
32   as low as 300°C. X-ray diffraction pattern of the ash residue showed that the main reaction
33   product of the platinum dimethylglyoximate complete pyrolysis is reduced platinum.
34   Studies   on   the   pyrolysis   intermediate   products   (230°C)   of   platinum(IV)
35   dimethylglyoximate by x-ray phase analysis support the stepwise pyrolysis nature of the

platinum complex. At the first stage of the pyrolysis such possible phases as $[(NH_3)_2PtOH)]_2(NO_3)_2$, $[Pt(NH_3)_2](NO_3)_2$, $C_2H_{12}N_4O_4Pt$, $C_4H_{12}N_4O_8Pt_2$, and PtO_2 were detected. It is known that the radical pyrolysis of the complexes which modifier forms with analyte, leads to the analyte chemisorption at the graphite surface [21]. This prevents its diffusion to the cold periphery ends of the platform, and result in the better repeatability in atomization and improvement of the precision of ETAAS determinations.
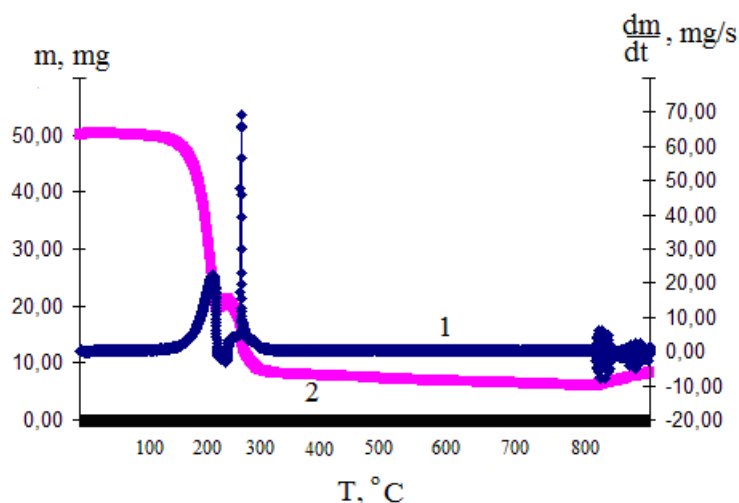
Figure 3. Derivatography data on Pt(IV) dimethylglyoximate pyrolysis: 1 − differential curve of the mass loss; 2 − integral curve of the mass loss

Pt and Pd ETAAS determination in pig blood serum

Dimethylglyoxime and ascorbic acid were used as modifiers in direct (without samples acid rupture) Pt and Pd ETAAS determination in the pig blood serum.

Blood serum (2.0 mL) was sampled into the volumetric test tube of volume 10 mL, solution of dimethylglyoxime (0.8 mL, 0.0075 mol/L for Pt) or ascorbic acid (0.5 mL, 0.3 mol/L for Pd) was added, diluted with distilled water to the total volume of 6.0 mL and stirred. The portions (20 μL) of the resulting solutions were sampled out on the graphite platform and integral absorbance was measured under optimum temperature conditions of the atomizer. Pt and Pd content was calculated by the method of standard calibration plot. Calibrating solutions were prepared by dilution of the Pt(IV) and Pd(II) standard solutions. Lineal range of calibration was from 50 to 400 μg/L for Pt(IV) and from 10 to 50 μg/L for Pd(II). The blank was made in all experiments.

The validity of the results was verified by "added-found" method. The results are presented in Table III.

Table III. Platinum and palladium determination in pig blood serum (n=5; P=0.95)

Element	Modifier	Added (in conversion to metal, μg/L)	Found	
			$C \pm \delta$, μg/L	S_r
Platinum	Dimethylglyoxime	252*	266±15	0.044
Palladium	Ascorbic acid	25	23±2	0.035

* added as Carboplatin

The recovery is + 106% for Pt and 92% for Pd, respectively. Detection limit was found to be 20 μg/L for platinum and 5 μg/L for palladium

Conclusion

To summarize, the direct (without preliminary sample acid rupture) procedure of platinum and palladium determination in the blood serum with the use of dimethylglyoxime and ascorbic acid as modifiers was developed. Relative standard deviation does not exceed 0.05.

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