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Preconcentration and determination of ultra-traces of platinum in human serum using the combined electrodeposition–electrothermal atomic absorption spectroscopy (ED–ETAAS) and chemometric method

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ABSTRACT

Platinum compounds, including *cis*-dichlorodiaminoplatinum(II) or cisplatin, are an important class of anti-cancer drugs, which should be carefully monitored in the biological fluids. In this study, electrodeposition coupled with electrothermal atomic absorption spectrometry (ETAAS) was used for determination of Pt concentration in the human serum samples. The chemometric techniques were also used to verify the probable interactions among the important and effective parameters in the atomization process. Using response surfaces obtained by two factorial design techniques, the experimental design was applied for three effective parameters namely ashing temperature, atomizing temperature and modifier concentration as effective parameters on the atomization of Pt. The in situ digestions of serum samples, as well as the separation of the ultra-traces of Pt from concomitant in these samples were performed by using the in situ electrodeposition (ED) technique prior to the measurement by ETAAS. Six plasma samples of a patient who was administered parenteral cisplatin were analyzed using the proposed ED–ETAAS technique. The results showed the pharmacokinetic parameters of cisplatin in serum in accordance to the well-established data. A relatively good reproducibility %RSD = 2.44, low limit of detection LOD = 2.54 μg/L and promising characteristic mass m_o = 91.30 pg were obtained using this technique.

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1. Introduction

Platinum compounds, including cisplatin (*cis*-diaminedichloroplatinum(II)) are among the effective anti-cancer drugs currently administered for the treatment of patients. This platinum coordination compound alters the natural time course of several human malignancies such as, testicular, ovarian, bladder, lung, gastric, head and neck cancer with a high cure rate [1–3]. It is an inorganic complex based on platinum. Its antitumor activity was first revealed by Rosenberg et al. [4]. Only the *cis*-form of the molecule has significant clinical activity. It is proposed that in the cell, cisplatin undergoes a hydrolysis reaction, and its hydrolysis products react with nuclear targets, i.e. DNA [5]. Dosages of drugs must be adjusted carefully in order to assess their effectiveness and avoid unnecessary side effects during chemotherapy. Changes in the concentrations of platinum in the body fluids, mainly blood and urine, have played an important role in establishing pharmacokinetics and therefore, dosage regime of the drugs [6]. However, in spite of its strong anti-cancer potency, chemotherapy with cisplatin causes many serious side effects such as nephrotoxicity, orthotoxicity, nausea, vomiting, neuropathy and allergy [7–9]. The nursing and medical staffs are evidently concerned about the risks of hazardous exposure to this compound such as increased urine mutagenicity, chromosome aberrations and increased hair loss [10]. It is therefore necessary to determine the platinum concentration in the human body after treatment by cisplatin. Several instrumental methods of analysis have been used extensively in many laboratories for these purposes. However, ETAAS remains superior among the most common analytical techniques for determination of trace metal concentrations in the biological samples. However, the direct determination of trace elements in biological samples is still difficult by using conventional ETAAS, because not only their concentrations are near or below the detection limit of this technique, but also the matrix of these samples may cause serious interferences. Preconcentration and separation of analyte from interfering concomitant by digestion and chemical pretreatment are the regular solution for these problems. The *ex situ* wet digestion of the samples achieving a complete charring of the organic material may introduce some contamination and dry ash which can lead to the loss of volatile species. Therefore, it has been required to develop a proper digestion method with minimal sample pretreatment and manual manipulations of the sample to separate the traces of analyte from concomitant.

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The author and coworkers in our laboratory developed a combination technique of electrodeposition with some atomic spectroscopy method for separation of analytes from their interfering contaminations prior to determination by AAS technique [11–18]. The application of reported techniques are investigated in this work, for separation of platinum from the problematic matrices of blood samples in order to fulfill the requirements for these analyses, to overcome interferences and to minimize sample pretreatment. The electrolysis takes place inside a sample drop introduced into the graphite tube just before drying, pyrolysis and atomization steps. The ED–ETAAS method is applicable for determination of all elements, which deposited on the surface of atomizer tube by electrochemical reduction or oxidation at a proper voltage [13–18]. As the analyte deposited in a pure form onto the electrode surface, the majority of interferences are overcome [15], and better sensitivity and detection limit should be achieved. On the other hand, the overall process of analysis is much more time saving due to a very few and much less number of steps than the regular procedures for these purpose [13–18]. This technique is convenient namely for the analysis of samples with difficult matrices, such as sea water, urine, blood serum, plasma and waste water [13–18].

The major aim of this work is concerned with combining the use of a chemical modifier and in situ electrodeposition for proper platinum separation from the complex blood plasma matrix prior to the measurement. It would result in elaboration of standard operational procedure for platinum determination in blood samples without *ex situ* sample decomposition. In addition, a chemometric technique is employed to verify the probable interactions among the effective parameters in the atomization process.

2. Materials and methods

2.1. Instrumentation

A Perkin–Elmer 503 atomic absorption spectrometer with a deuterium lamp for background correction, equipped with a HGA-2100 furnace controller, has been used in this research. Data were evaluated using peak height absorbance. A 8 cm Ir rod as anode and the furnace as cathode were connected to a DC power supply (0–12) via an ammeter (0–150 mA) indicating the deposition current. Thermal program for the HGA-2100 furnace applied after each steps of electrodeposition.

2.2. Reagents

Ultra-pure water was obtained by passing distilled water through mili-Q exchange and membrane filtration system. Titrasol solution (1000 mg/L Pt stock standard solution) was prepared from Serva Company (Heidelberg, Germany). Cisplatin drug (0.5 mg/mL) was purchased from Ebeve Company (San Francisco, USA). All acids used, namely, HNO_3, HCl, H_2SO_4, $HClO_4$ are of Merck (Darmstadt, Germany) ultra-pure quality, and all diluted by mili-Q water. All stock solutions were stored in the high density polyethylene containers previously soaked in a 10% HNO_3 acid solution over night.

2.3. Sample preparation

All standard and sample solutions were prepared in 0.7% $HClO_4$. The standard solutions were prepared in the concentrations of 10, 20, 30 and 40 μg/L. The blank solution was also prepared in the same condition.

2.4. ED–ETAAS procedure

The experiments involved both conventional and electrodeposition sample introduction into the graphite furnace. In the conventional way, the aliquots of 30 μL of the 10–40 μg/L sample or standards injected into the atomizer, the thermal program followed. The electrodeposition parameters, such as the pH of solutions, the deposition potential and the time length of deposition, should be optimized. In the ED–ETAAS, the aliquots of 30 μL of the 10–40 μg/L sample or standard solutions electrolyzed at 0.5–5 V for 20–100 s and the pH ranges 0.5–2 inside the graphite tube. After removing the Ir-electrode from the furnace, the spent electrolytes removed to separate the deposited element from the matrix. The deposited metal was dried and a thermal program followed to measure the analyte content. The calibration was performed using acidified (0.7% $HClO_4$) aqueous standard solutions containing up to 40 μg/L of platinum.

3. Result and discussion

3.1. ETAAS conditions

To achieve the optimized conditions for measurement of Pt by ETAAS, a full factorial experiment design is used. By the full factorial design methodology, main and interaction effects can be easily evaluated.

In this approach, some of parameters have to be optimized in order to obtain maximum signal absorbance. In fact, the type of modifier and its concentration, ashing and atomization temperatures generally considered as the most important factors. Chemical modifiers are known as compounds, which are introduced into the graphite atomizer simultaneously with the test samples, for which it is expected to decrease the matrix effects. This can be achieved by one of the following mechanisms, depending on the type of the analytes: (a) by the conversion of the matrix compounds into volatile compounds or by decrease in the volatility of volatile analyte compounds, (b) by increase in the volatility of refractive carbide forming compounds of analyte. In this work, as Pt is in the same group as Pd which is known as universal modifier for stabilizing the volatile compound [19], Pt–C interaction seems to form refractive species for which a volatilizing compound as a modifier should be used. Halogen injecting process can be used for reduction of memory effects due to refractory carbides forming compounds [20]. Four types of acids such as: H_2SO_4, HNO_3, HCl and $HClO_4$ were chosen to investigate their ionic strength as a proper electrolyte in ED step as well as their oxidizing power for digestion of biological sample, and also as the suitable volatilizing modifiers for Pt compounds. $HClO_4$ shows a higher performance, due to its high oxidizing power and releasing halogen assisted volatilization [20]. The effect of different $HClO_4$ concentrations from 0.02 to 0.30 M on the absorbance of atomized platinum is shown in Fig. 1. The absorption signals decreased in the low concentrations of $HClO_4$, perhaps due to the formation of refractory metallic carbide and incomplete analyte releasing. As it can be derived from results shown in Fig. 1, the absorbance increased because of the increase of volatilization of Pt compound and complete atomization of Pt compound by increasing the concentration of $HClO_4$ up to 0.05 M.

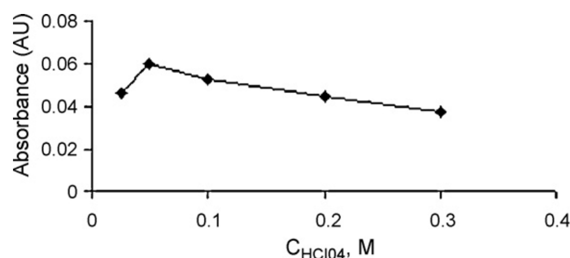

Fig. 1. The effect of different concentrations of modifier on absorbance signal of electrodeposited Pt, prior to measurement by ETAAS.

Table 1
The optimum temperature program for the graphite furnace used for the determination of electrodeposited platinum from standard solution.

Step	Temperature (K)	Time (s)
Dry	323–423	30
Ash	1273	20
Atom	2823	4
Clean	2923	5

However, it decreased again with a further increase of the $HClO_4$ concentration above 0.05 M because of losing the volatile Pt compounds in the temperatures below the atomization temperature. The heating of sample should be performed with most caution, in order to achieve in situ digestion and decomposition of residual of the sample in the oxidizing atmosphere of $HClO_4$, such that there would be no analyte loss prior to the atomization. The ashing temperature varied from 1173 to 1573 K, with and without using modifier, respectively. Determination of the optimum atomization temperature carried out by testing different atomization temperatures between 2773 and 3023 K, with and without using modifier. Different positions of the effective atomization temperature on the background and the absorption signal, with and without the presence of the chemical modifiers, confirmed the expected effect of this modifier. It was found that, the optimum 1273 K ash temperature and 2823 K atomization temperatures could be obtained by using $HClO_4$ as the chemical modifier. While, without using a modifier these temperatures increased to 1400 and 2920 K, respectively. The results also showed that, by using this modifier at the specified concentration, not only the ashing temperature decreases, but also the atomization step could be carried out at the lower temperature, which could improve the life time of the atomizer. The time of the ashing and atomization steps were determined experimentally to provide optimum conditions. The optimum of 1273 K ash temperature and 2823 K atomization temperatures by using $HClO_4$ as the chemical modifier at 0.05 M, are obtained for the optimum temperature program, which is applied to the graphite furnace used in the determination of the electrodeposited Pt, as shown in Table 1.

4. Electrodeposition–electrothermal AAS (ED–ETAAS)

The most important variable for the deposition is the applied potential to the electrolysis cell. The higher applied voltage causes more current and hence more gas evolution through the sample drop that causes more agitation, enhancing the decomposition of the biological samples in oxidizing electrolyte, which in turn should improve the efficiency of deposition. The voltage higher than the threshold for maximum deposition could also produce more hydrogen gas. The electrolysis of an aliquots 30 μL sample injected into the graphite furnace through a micropipette was carried out at the cell potentials of 0.50–5.00 V. Up to the value of 4.00 V, the signal grew rapidly and then decreased (Fig. 2). At the higher voltage values, violent gas expansion occurred in the solution resulting in the sample splash through the atomizer's dosage opening. These effects

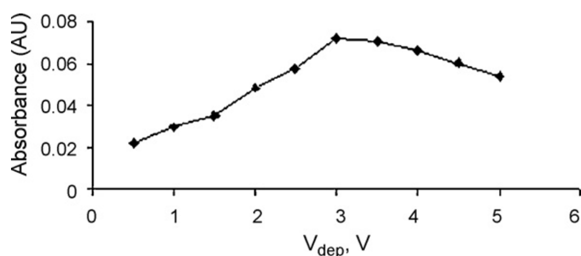

Fig. 2. Plot of AA signal versus applied voltage for 1.2 ng Pt, electrodeposited from 30 μL solution containing, 40 μg/L Pt in 0.7% $HClO_4$.

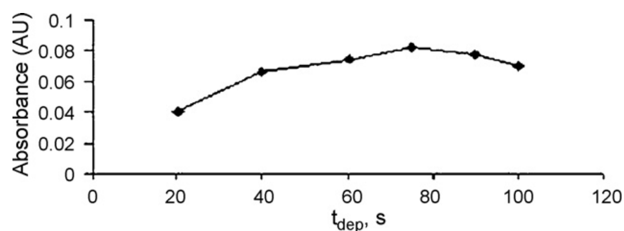

Fig. 3. Plot of AA signal versus time length of deposition for 1.2 ng Pt, electrodeposited from 30 μL solution containing, 40 μg/L Pt in 0.7% $HClO_4$.

caused the analyte losses or at least deterioration of the atomization peaks. The effect of the electrolysis voltage value on the electrodeposition signal of platinum is shown in Fig. 2. Another important factor that controls the degree of deposition of reduced metal is the duration of the deposition. The dependence of the AA signal for electrodeposited Pt to the time of the deposition is shown in Fig. 3. The electrodeposition voltage equal to 4 V and the electrodeposition time of 75 s were chosen for the element. Under these conditions, the analyte quantitatively deposited, within a short time. The pH of the sample also has an important effect on the deposition efficiency in that it influences the degree of hydrogen evolution. Hydrogen evolution could increase the deposition efficiency of metals by contributing to the stirring action, and hence the diffusion layer thickness and improving the efficiency. The effect of pH on the efficiency of the deposition for platinum investigated with the solution containing 1.2 ng of platinum at pH values varying between 0.5 and 2.0. The best pH value for an efficient deposition is within the range of 1.0–1.5 that is used for all of the experiments by buffering the electrolytes with potassium chloride in 0.01 M solution of hydrochloric acid.

5. Analytical figures of merit

Calibration curves obtained under the optimum determined conditions in ranges of 10–40 μg/L for Pt in solution of 0.7% $HClO_4$. The limits of 12 detection (LOD) and the characteristic masses (m_o) for the measurement of Pt by conv-ETAAS compared with those by ED–ETAAS. The detection limit was determined as ($3\,S_b/m$), measured in 11 analytical blanks. The S_b and m are standard deviation of the blank and the calibration curve slope, respectively. The detection limit of ED–ETAAS is better than that of conv-ETAAS method. This ascribed to an improvement in the analyte signal, due to the lack of losing element, as the result of more effective binding to the furnace surface and a lag time for analyte release to the higher temperatures. The ED–ETAAS technique also showed an improvement in the slope of the calibration curves, because the analyte separated by deposition onto the graphite furnace from the interfering matrix. The slopes of the obtained calibration curves and the results for measurements of m_o and LOD, by both techniques, are shown in Table 2. The reproducibility of Pt measurements by ED–ETAAS was investigated and compared to that by conv-ETAAS. The percent of relative standard deviations (%RSD) for conv-ETAAS and ED–ETAAS were 3.86% and 2.44% for 6 measurements, respectively. For evaluation of the performance of the ED–ETAAS technique in the measurement of traces of Pt, the reliability of the analysis further assessed through recovery studies. Ultra-traces of Pt spiked to

Table 2
Comparison of limit of detection (LOD), characteristic mass (m_o) and precision (RSD %) in measurement of Pt in standard solution with conv-ETAAS and ED–ETAAS.

Technique	LOD (μg/L)	m_o (pg)	%RSD (n = 6)
Conv-ETAAS	3.45	94.3	3.86
ED–ETAAS	2.54	93.1	2.44

Table 3
Results of recovery test for Pt measurements, of spiked to blank and blood plasma samples, by ED–ETAAS technique.

Added standard	Blank		Blood plasma	
Conc. (μg/L)	Abs.	Recovery (%)	Abs.	Recovery (%)
0	–	–	0.054	–
10	0.016	–	0.071	–
20	0.035	109.4	0.090	105.9
30	0.054	102.8	0.110	103.4
40	0.075	104.2	0.128	99.2

Table 4
The Results of tracing Pt in blood plasma of patient treated by cisplatin.

Sample	The period of time after using cisplatin (h)	Conc. of Pt in cisplatin in plasma (μg/L)
1	72	187
2	120	103
3	190	47
4	240	26
5	260	12
6	280	<LOD[a]

[a] LOD: limit of detection (μg/L).

the aliquots of a blood plasma sample, electrodeposited under the conditions of electrodeposition specific to Pt. The results were close to 100% average recovery (99–105%) for added analytes, nearly the same as the results of recovery of spiked to the interference-free blank samples, as shown in Table 3.

6. Tracing the cisplatin in human serum

The technique of electrodeposition was applied to measurement of Pt in the plasma samples. The amount of cisplatin in the plasma samples from different patients who were treated by cisplatin after administration in different times are measured by using the proposed technique of in situ ED–ETAAS at optimum conditions. After preparation of standard solutions of Pt in a buffer of potassium chloride and hydrochloric acid, the calibration curves of standards, for both of the external standards and standard addition method, constructed in the range of 10–50 μg/L. The slopes of the analytical calibration curves, for both Pt in an interference-free solution and that of cisplatin added to a blood plasma sample, were the same within the experimental error. This indicates the capability of the ED–ETAAS technique to separate the Pt from the bulk of the interfering matrix in a blood plasma sample that performs an interference-free determination with full recovery of added analyte. For in situ digestion of blood samples, magnesium nitrate as an ashing aid used in addition to $HClO_4$, which itself acts as oxidizing reagents as well as modifier function. This compound applied as an ashig aid because it simplifies the ashing of organic complex matrix of biological samples, as reported in literature [21–23]. Table 4 shows the results obtained for tracing the cisplatin in blood plasma of patients whom treated with this drug, by using the proposed developed method.

7. Conclusion

It is demonstrated in this investigation, that employ the combined electrodeposition technique with ETAAS has progressed substantial towards the ultimate goal of ETAAS of direct, interference-free measurements of ultra-trace elements in complex samples, with remarkable improvement in analytical performance. In a complex sample, such as blood sample, the direct measurement of Pt by conv-ETAAS required some prestep procedures for digestion and separation of interfering matrix prior to the measurements. Whereas, the proposed in situ ED–ETAAS has capability of performing the analyses at minimum manual manipulations of samples, and prevents the contamination of samples, as well as improvement in the performance of the results of the trace analysis in these samples. Therefore, a precise, accurate and interference-free ETAAS based methodology, for these purposes, is developed and proposed by the in situ ED–ETAAS. It could be concluded, from this brief evaluation, that the proposed technique is quite promising, in order to provide full analytical confidence to the clinical studies.

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