Determination of Platinum and Palladium in Chelated Systems by GFAAS

Application Note

Atomic Absorption

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Introduction

With the discovery of the anti-tumour properties of cis-dichlorodiammineplatinum(II) (cisplatin) [1], intensive research has been completed which examines the role of platinum in biological systems. One major field of interest has been the interaction of cisplatin and related compounds with biological systems, especially with DNA and its constituents. The interactions of palladium complexes and DNA have also been examined. Principally, this is due to the labile nature of palladium. Platinum substitution reactions are known to be slow. Palladium proves to be an ideal model because it has similar coordination properties and substitution reactions occur generally in the order of 10^5 times faster when compared with platinum.

The technique of atomic absorption spectrometry (AAS) has been employed in this area of research to measure levels of platinum. It has been used to measure the distribution of Pt in biological fluids and systems [2] as well as used to study platinum coordination reactions [3,4].

It has been found that the species active toward the tumour is diaquodiammineplatinum(II). Generally, it is synthesized by the removal of the chloride ions from the complex using silver nitrate [5]. This allows the aquated form to be present in solution. Though this process is almost always quantitative, there are circumstances when accurate levels of platinum (and complex) need to be known. One such case is the determination of the stability constants for Pt-DNA and Pd-DNA complexes. In this case, it is necessary to use graphite furnace AAS [6]. This paper presents two methods which are suitable for the determination of platinum and palladium in systems such as cisplatin and related compounds.

Agilent Technologies

Experimental

Method

An Agilent SpectrAA 400 Atomic Absorption Spectrometer and GTA96 Graphite Tube Atomizer were employed for the measurements.

Platinum

A platinum SpectrAA hollow cathode lamp was used and the samples dispensed into pyrolytically coated graphite tubes. The Pt resonance line at 265.0 nm was used with a slit width of 0.2 nm. The lamp current setting was 5 mA.

A 1000 mg/L Pt solution (BDH Ltd, Spectrosol grade) was used as the stock standard solution. An intermediate solution of 10 mg/L Pt was prepared by serial dilution using 0.1% v/v HNO_3. Working standards were also prepared by serial dilution from the intermediate solution using 0.1% v/v HNO_3.

Palladium

A palladium SpectrAA hollow cathode lamp was used with pyrolytically coated tubes. The Pd resonance line at 244.8 nm was used with a slit width of 0.3 nm. The lamp current setting was 5 mA.

A 1000 mg/L Pd (Aldrich, USA) was used as the stock standard solution. An intermediate solution of 10 mg/L Pd as well as the working standards were prepared as for platinum.

Results and Discussion

Ashing and atomization studies were performed in order to determine the optimal furnace parameters. A Pt solution was made in the presence of KNO_3 and ethylenediamine for this purpose. The results are shown in Figure 1. It is clear from Figure 1 that the best region for ashing lies between 800–1000 °C and a temperature of 2700 °C was chosen as the atomization temperature. The furnace parameters used for the analysis are shown in Table 1.

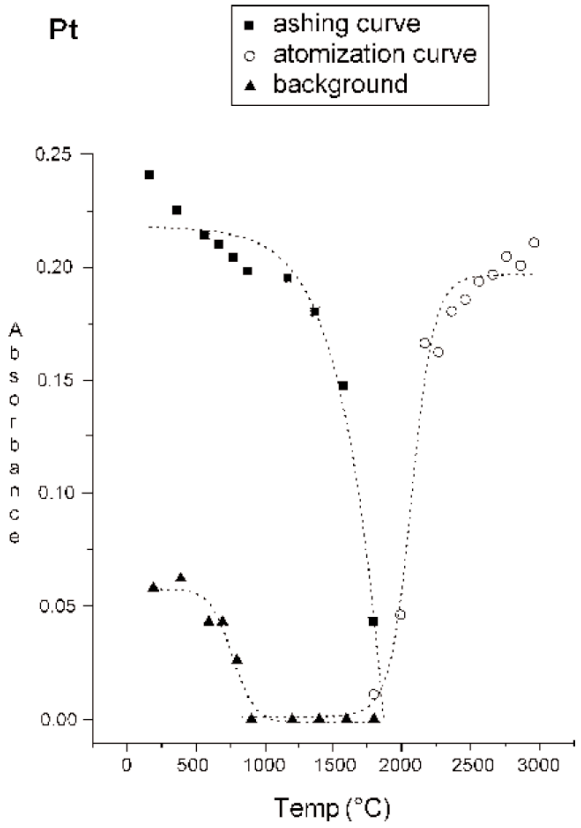

Figure 1. Optimal furnace parameter results for Pt.

Table 1. Furnace Parameters

Step no	Temperature (°C)	Time (sec)	Gas flow (L/min)	Gas type	Read command
1	85	5.0	3.0	Normal	No
2	95	40.0	3.0	Normal	No
3	120	10.0	3.0	Normal	No
4	700	5.0	3.0	Normal	No
5	700	2.0	3.0	Normal	No
6	800	5.0	3.0	Normal	No
7	800	2.0	3.0	Normal	No
8	800	2.0	0.0	Normal	No
9	2700	1.3	0.0	Normal	Yes
10	2700	2.0	0.0	Normal	Yes
11	2700	2.0	3.0	Normal	No

A comparison was performed on the use of premixed standards and those made using the automix facility of the autosampler. The results are shown in Table 2. It can be seen that the automix facility provides a calibration graph which is comparable with that from the premixed standards. This is a useful feature as it helps to minimize operator error as well as reduce consumption of the stock solution. The autosampler parameters are shown in Table 3. A 100 µg/L Pt solution was used as the standard solution. Figure 2 shows a representative calibration graph.

The precision of the determination was established by analyzing a series of five solutions containing approximately 20 mg of K_2PtCl_4 in 100 mL. After serial dilution, the Pt concentration was calculated. Table 4 shows these results and it can be seen that the recovery is very good.

Table 2. Comparison of Standard Solutions

Conc (mg/L)	Abs Premixed	Automixed
0.0	0.000	0.011
50.0	0.044 (7.7)	0.044 (3.3)
100.0	0.092 (5.2)	0.089 (1.0)
150.0	0.142 (1.0)	0.135 (1.1)

The values in parentheses are the relative standard deviations for 3 replicates

Table 3. Sampler Parameters

	Volumes (fL) Solution	Blank	Modifier
Blank	–	20	
Standard 1	5	15	
Standard 2	10	10	
Standard 3	15	5	
Sample	10	10	
Recalibration rage	0		
Reslope rate	0		
Multiple inject No	Hot Inject No		Pre Inject No

Table 4. Platinum Recovery

Solution	Expected result (mg/L)	Found result (mg/L)	%
1	94.5	89.8	95.0
2	93.5	91.5	97.9
3	98.7	97.4	98.7
4	96.8	93.7	96.8
5	99.6	99.9	99.4

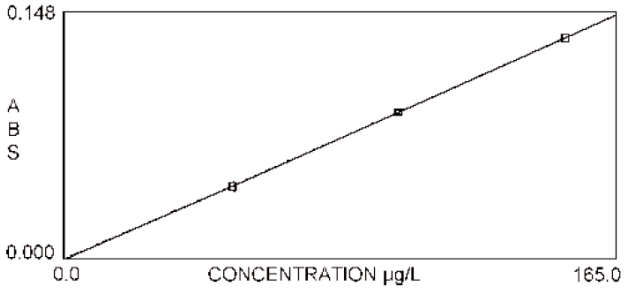

Figure 2. Representative calibration for Pt.

Similar experiments were carried out for palladium. Figure 3 shows the ashing and atomization curves and Table 5 shows the furnace parameters used for the analysis.

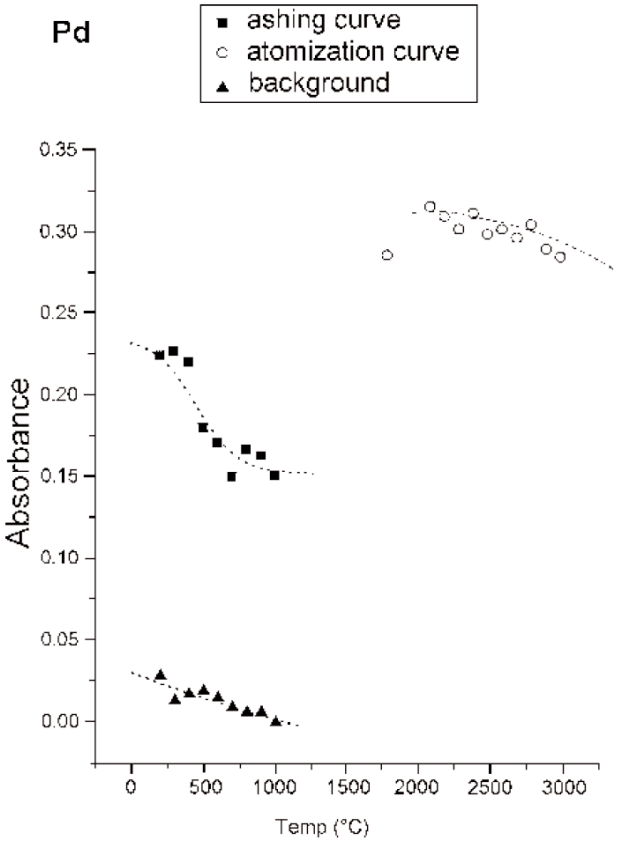

Figure 3. Ashing and atomization curves for Pd.

Table 5. Furnace Parameters

Step no	Temperature (°C)	Time (sec)	Gas flow (L/min)	Gas type	Read command
1	85	5.0	3.0	Normal	No
2	95	40.0	3.0	Normal	No
3	120	10.0	3.0	Normal	No
4	800	5.0	3.0	Normal	No
5	800	1.0	3.0	Normal	No
6	800	2.0	0.0	Normal	No
7	2600	1.0	0.0	Normal	Yes
8	2600	2.0	0.0	Normal	Yes
9	2600	2.0	3.0	Normal	No

A comparison was again made on the use of the automix facility for preparing the calibration curve, and, as for platinum, the graph compared very favourably with that obtained from premixed standards. Table 6 shows the autosampler parameters and a representative curve is shown in Figure 4. A 100 µg/L Pd solution was used as the standard solution. The precision of the analysis was also found to be comparable with that obtained for platinum.

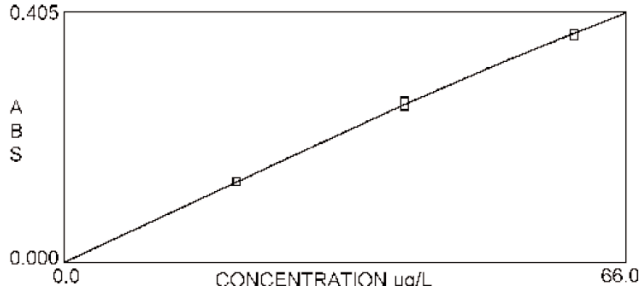

Figure 4. Representative calibration for Pd.

Table 6. Sampler Parameters

	Volumes (fL)		
	Solution	Blank	Modifier
Blank	–	20	
Standard 1	2	18	
Standard 2	4	16	
Standard 3	6	14	
Sample	10	10	
Recalibration rage	0		
Reslope rate	0		
Multiple inject No	Hot inject No		Pre inject No

Our work has been involved with the determination of formation constants for Pt/Pd complexes of bidentate ligands. For this task, it is therefore important to have a reliable means of accurately measuring the concentrations of each of the reaction components.

The systems which have been examined are complexes of Pt and Pd with bidentate ligands. Tables 7 and 8 show some results of the analyses. Some of this work has been presented elsewhere [7].

Table 7. Results of Analysis

Complex	Expected result (g/L)	Experimental result (g/L)	% Yield	Solvent
Pd(bmpe)2+	17.5	9.9	57	water
Pd(en)2+	17.8	17.5	98	water
Pd(bmpe)2+	9.9	8.3	84	DMSO
Pd(en)2+	10.0	8.1	81	DMSO

The constituents of DNA shows a wide variation in their solubilities in a number of solvents. It was therefore important to establish the efficiency of the removal of chloride from the complex and, consequently, the yield of the solvated species. Table 7 shows the results for two complexes which were dissolved in water and dimethylsulfoxide. Once established, proton NMR spectroscopy can be used to study the interactions between the metal complexes and DNA. A number of other ligands were also investigated and these results are shown in Table 8.

Table 8. Results of Analysis

Complex	Expected result (g/L)	Experimental result (g/L)	Percentage yield
Pd $(TMED)^{2+}$	2.8	2.4	86
Pd $(dmp)^{2+}$	6.36	6.36	100
	2.2	1.7	77
Pd2(bispep)$^{4+}$	4.4	1.2	27
Pd $(bpe)^{2+}$	3.9	2.7	70
Pd $(Ie)^{2+}$	7.0	5.62	80
Pt $(NH_3)_2^{2+}$	9.95	8.58	86
Pt $(en)^{2+}$	7.22	4.1	57

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Key to Ligand Abbreviations

en = ethylenediamine

TMED = tetramethylethylenediamine

bmpe = 1,2-bis(6-methylpyridin-2-yl)ethane

bispep = 1,2-di-(4-methyl-1-piperazinyl)ethane

dmp = 1,4-dimethylpiperazine

Ie = 1,2-bis(2-imidazolin-2-yl)ethane

bpe = 1,2-bis(pyridin-2-yl)ethane

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