Application Note

The Determination of Pt for Cancer Studies

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1 Introduction

Platinum complexes, such as cisplatin, carboplatin, and oxaliplatin, have been used since the early 1970s to treat various solid tumors (testicles, ovaries, head, neck and, recently, colon). It was found that platinum is fixed in a covalent way to the DNA, forming various types of adducts. The tumor treatment properties of platinum complexes are directly related to the fixation of platinum on DNA.

The influence of platinum on DNA is dependent on the time the injection was done in an organism. This is also true for the toxicity of platinum complexes on other organs, which is determined by analyzing solutions coming from extraction of target organs over time. The quantity of solutions is around 1 milliliter.

Phosphorus and sulfur were also determined. Phosphorus allows direct determination of the proportion of platinum to nucleotide, knowing that there is one atom of phosphorus per nucleotide of DNA.

2 Principle

2.1 Technique used

The elemental analysis of solutions was undertaken by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). The sample is nebulized then transferred to an argon plasma. It is decomposed, atomized and ionized whereby the atoms and ions are excited. We measure the intensity of the light emitted when the atoms or ions return to lower levels of energy. Each element emits light at characteristic wavelengths and these lines can be used for quantitative analysis after a calibration.

2.2 Wavelength choice

The choice of the wavelength in a given matrix can be made using the "profile" function, or by using Win-IMAGE, which is rapid semi-quantitative analysis mode using multiple wavelengths. The principle is the same in either case: record the scans of analytes at low concentration, and of the matrix. By superimposing the spectra, we see possible interferences.

2.3 Limits of detection estimation

The limits of detection are calculated using the following formula:

$$LOD = k \times BEC \times RSD_0$$

With:
LOD = limits of detection,
k= 3 for the normal 3-sigma values,
BEC = Background equivalent concentration,
RSD_0 = relative standard deviation of the blank.

To calculate the LOD, a calibration curve is constructed using two points, 0 ppm and 5 ppm, or some concentration where the calibration is linear; this gives the BEC. The RSD_0 is evaluated by running the blank ten times.

3 Sample preparation

Samples were obtained by extraction from the spleen, liver, kidney, intestine and brain of mice. For the kidney, the samples were diluted by a factor of 6 (0.5 mL in 3 mL). For the brain and intestine a dilution of 2X was made.

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4 Instrument specification

The work was done on a ULTIMA.The specifications of this instrument are listed below in Table 1 and 2.

Table 1: Specification of spectrometer

Parameters	Specifications
Mounting	Czerny Turner
Focal length	1 m
Nitrogen purge	Yes
Variable resolution	Yes
Grating number of grooves	2400 gr/mm
1st order resolution	0.010 nm
2nd order resolution	0.005 nm
Order	2nd order

Table 2: Specification of RF Generator

Parameters	Specifications
Type of generator	Solid state
Observation	Radial
Frequency	40.68 MHz
Control of gas flowrate	by computer
Control of pump flow	by computer

5 Operating conditions

The operating conditions are listed in Table 3 below.

Table 3: Operating conditions

Parameter	Condition
RF Generator power	1200 W
Plasma gas flowrate	16 L/min
Auxiliary gas flowrate	0 L/min
Sheath gas flowrate	0.2 L/min
Nebulizer flowrate	0.16 bars
Sample uptake	1 mL/min
Type of nebulizer	Ultrasonic
Injector tube diameter	3.0 mm

6 Wavelength selection and analytical conditions

The line with the highest sensitivity was used for analysis of all analytes, as there were no problems with interferences. The analysis conditions were the same for all elements.

Table 4: Analytical conditions

Element	Slits µm	Analysis Mode	Integration Time (sec)
P	20 x 15	Gaussian	0.2
Pt	20 x 15	Three points on peak	3
S	20 x 15	Gaussian	0.2

7 Discussion

7.1 Limit of Detection

The limit of detection for platinum of 0.1 µg/mL was calculated using the formula in paragraph 2.3.

7.2 Results

The samples were measured using an autosampler. Concentrations are in mg/L for S and P and in µg/L for Pt.

Table 5: Intestine sample results

	J30-	J1-	J2-	J4-	J8-	J12-	J24-
S	10.88 ± 1.27	0.66 ± 0.0510.46 ± 0.41		11.52 ± 1.14	9.59 ± 1.506.66 ± 1.466.26 ± 1.05		
Pt	74.83 ± 9.20	5.73 ± 0.7681.59 ± 1.30		70.09 ± 2.94	29.21 ± 1.84	83.16 ± 5.00	30.78 ± 2.86
P	530.16 ± 47.71	47.86 ± 2.58	368.37 ± 25.42	260.88 ± 14.87	317.52 ± 63.25	477.81 ± 30.10	264.97 ± 23.05

	J30+	J1+	J4+	J8+	J12+	J24+	J48+
S	5.38 ± 0.154.83 ± 0.236.37 ± 0.526.11 ± 0.336.54 ± 0.270.75 ± 0.083.47 ± 0.21						
Pt	59.67 ± 2.33	45.37 ± 5.17	40.57 ± 2.07	83.63 ± 6.02	67.76 ± 1.32	8.13 ± 0.5657.48 ± 3.10	
P	260.98 ± 12.53	284.39 ± 10.24	408.10 ± 15.92	312.57 ± 20.63	367.46 ± 26.46	50.91 ± 1.30	189.82 ± 9.57

Table 6: Brain sample results

	C30-	C1-	C2-	C4-	C8-	C12-	C24-	C48-
S	5.57 ± 0.24	3.41 ± 0.42	4.79 ± 0.15	4.63 ± 0.60	4.87 ± 0.69	3.97 ± 0.93	3.94 ± 0.48	3.98 ± 0.51
Pt	0.276 ± 0.288	0.471 ± 0.183	0.564 ± 0.072	0.561 ± 0.075	0.187 ± 0.348	0.379 ± 0.420	0.212 ± 0.228	0.202 ± 0.363
P	77.23 ± 4.68	62.01 ± 2.76	86.40 ± 6.54	90.56 ± 2.52	87.71 ± 6.75	72.86 ± 22.06	76.33 ± 5.58	79.80 ± 11.64

	C30+	C1+	C2+	C4+	C8+	C12+	C24+	C48+
S	3.79 ± 0.28	3.65 ± 7.4	4.13 ± 0.72	4.06 ± 0.45	3.45 ± 0.84	2.66 ± 1.29	2.62 ± 0.06	3.23 ± 0.42
Pt	0.492 ± 0.177	0.511 ± 0.417	0.416 ± 0.507	0.314 ± 0.207	0.344 ± 0.495	0.323 ± 0.279	0.225 ± 0.204	0.547 ± 0.306
P	83.69 ± 3.9	93.04 ± 10.08	83.48 ± 1.67	89.16 ± 9.63	81.29 ± 8.88	81.40 ± 5.61	56.21 ± 3.6	81.44 ± 19.17

Table 7: Liver sample results

	Pt (µg/L)	RSD (%)	P (mg/L)	RSD (%)	S (mg/L)	RSD (%)
0a	7.8	18	341.4	3	9.12	5.6
0b	5.4	11	318.7	2.9	10.8	5.5
0c	9	12	345	5.7	13.9	10
8a	6.6	8.9	401.4	5.5	10.2	5.2
8b	5.4	7.8	326.4	4	7.5	3.3
8c	4.2	12	325.8	3.5	7.8	1
16a	7.2	2.2	246.6	4.8	12	1.9
16b	6.6	18	247.8	1.5	7.8	5.9
16c	9	13	237	3.3	7.8	5.5
0a-	166.8	3.2	343.2	2.5	18	1.7
0b-	208.8	1.9	348.6	8.5	15.6	5.2
0c-	256.8	1.7	367.8	3.1	12	3.5
8a-	153	3.3	278.4	0.8	7.8	1.2
8b-	190.8	1.4.	346.2	3.1	8.4	1.9
8c-	100.8	1.7	299.4	5.8	7.2	4.5
16a-	172.8	3.4	313.2	4.4	9.6	4.6
16b-	191.4	2	273	3.1	10.2	3
16c-	121.8	5.7	288.6	7	12.6	0.69
0a+	134.4	1.4	282.6	9.8	10.8	10
0b+	157.8	1.4	278.4	4.5	8.4	2.4
0c+	118.2	3.4	285	6.1	9	6.1
8a+	181.8	2.2	361.8	6	8.4	6.1
8b+	111	1.7	243	4.2	5.4	4.2
8c+	180	0.37	316.8	4.9	7.4	1.2
16a+	130.2	0.31	287.4	5.1	6.4	3
16b+	104.4	1.9	250.8	7	4.8	5.6
16c+	91.2	4.6	269.4	2	4.8	4.1

Table 8: Kidney sample results

	Pt (µg/L)	RSD (%)	P (mg/L)	RSD (%)	S (mg/L)	RSD (%)
0a	6.7	10	56.9	3.7	1.6	3.2
0b	11.7	10	48.6	1.9	1.5	7.6
0c	13.4	5.9	47.4	8.1	1.2	12
8c	9.1	6.7	47.4	5	1.2	5.4
16c	5.4	19	7.62	2		
0b-	81.7	0.8	38.2	10	4	8
0c-	43.5	6.4	53.7	6.9	1.3	2.2
8a-	15.5	4.7	14.4	2.2	0.7	5.5
8b-	11.5	7.4	6.7	15		
8c-	32.3	4.1	53.4	1.7	1.3	4.3
16b-	10.4	8.8	7	8		
16c-	27.2	1.7	65.5	4.6	1.7	6
0a+	38.8	0.37	66.3	5.2	1.7	15
0b+					2.6	6
0c+	22.7	7.9	44.97	4	1.62	3.3
8b+	39.7	2				
8c+	10.9	9.7	0.93	8		
16a+	36.1	4.1	67.8	5.8	1.9	6
16b+	10.8	9.2	2.6	20		
16c+	28.5	2.5	45.9	5	1.3	7

8 Summary

The results in this Application Note demonstrate that it is possible to detect very low levels of an analyte using the ultrasonic nebulizer, even with a very small amount of sample (one to two millimeters). Applications of ICP-AES in the medical field are not very well developed, but the work done here shows the feasibility in cancer treatments.

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