A New Sensitive Method for Quantitative Determination of Cisplatin in Biological Samples by Kinetic Spectrophotometry

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This study describes a kinetic spectrophotometric method for accurate, sensitive and rapid determination of cisplatin in biofluids. The developed method is based on the inhibitory effect of cisplatin on the oxidation of Janus Green by bromate in acidic media. The change in absorbance as the criteria of the oxidation reaction was followed spectrophotometrically. To obtain the highest rate of sensitivity, efficient reaction parameters were optimized. Under optimum experimental conditions, a calibration graph was obtained linearly over the range 10.0 – 5750.0 $\mu\text{g L}^{-1}$ and the limit of detection ($3s_b/m$) was 4.2 $\mu\text{g L}^{-1}$ of cisplatin. The interfering effect of diverse species was investigated. The developed method was used for the quantification of cisplatin in bio fluids of patients treated with cisplatin, spiked bio fluids and pharmaceutical samples and yielded satisfactory results.

Keywords Kinetic spectrophotometry, cisplatin, biological sample, cancer patient

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Introduction

Studies have demonstrated that cancer is one of the leading causes of disease throughout the world. Annually, tens of millions of humans are diagnosed with cancer worldwide, and more than one-half of the patients ultimately die as a result of the cancer.¹ Cancer in several countries is the second most prevalent cause of mortality after cardio-vascular illnesses. Chemotherapy is one of the cancer treatment options. In chemotherapy, anticancer agents that kill the cancer cells in the body are used.^{2,3} The aim of chemotherapy is to treat cancer in several ways: either to reduce tumor volume before surgery or irradiation, or eradicate cancer cells that may be dispersed in the surrounding tissues or organs after tumor surgery (*i.e.* preventive chemotherapy), or relieve cancer symptoms (such as pain), or to control the tumor growth if the tumor has already spread (palliative therapy). Each chemotherapy drug has its own internationally defined dose of administration, which is calculated by taking into account body mass. The dose of the drug is calculated individually for each patient depending on body weight and height.^{4,6} The patient's general conditions and co-morbidity are also considered. Individuals respond differently to chemotherapy and this response may have significant clinical importance. Calculation of a definite dose of chemotherapy reduces the possible toxic reactions of drugs.^{7,8} There are five basic conditions that determine treatment tactics, *i.e.*, the right medication, right patient, right dosage, right administration route and right timing.⁹ This helps a physician to make therapy more rational and avoid therapeutic errors, as a too small dose of medication will have no therapeutic effects which will affect overall survival and the length of the disease-

free period, while a too high dose increases the toxic effects in the body that could possibly lead to death.¹⁰⁻¹²

Cisplatin [*cis*-dichlorodiamino-platinum(II)] is a platinum-containing drug used for chemotherapy of cancer patients. It can alter the natural duration of multiple human malignancies, like ovarian, testicular, lungs, bladder, head, gastric, and neck cancers, with an increased healing rate.¹³⁻¹⁵ In fact, cisplatin reacts with nuclear target DNA. Despite the powerful anticancer potency of cisplatin, chemotherapy with cisplatin can also lead to potential severe complications, including nephron-toxicity, nausea, orthotoxicity, neuropathy, vomiting, allergy and increased hair loss.¹⁶⁻¹⁸ It is therefore considered necessary to monitor the platinum concentration in the human body following cisplatin dosing. Actually, the detection of platinum in biological fluids at trace levels continues to hold analytical interest. The analytical methods reported are based on the determination of intact cisplatin¹⁹⁻²¹ and elemental platinum from biological samples. The methods are based on the measurement of platinum and include inductively coupled plasma-mass spectrometry,²² atomic absorption spectrometry,²³ electrochemical methods,²⁴ polarography,²⁵ spectrophotometry,²⁶ neutron activation analysis²⁷ and high performance liquid chromatography.²⁸

In recent years, electrochemical methods using a modified electrode have become the most common technique for analysis of various specimens, including drugs with different matrices. But, the methods have disadvantages, such as low repeatability, difficult procedures for the preparation of the electrode and time-consuming processes.²⁹⁻³³ Especially, chromatographic methods are expensive and entail complex operations.³⁴ As spectrophotometric methods offer sufficient accuracy, selectivity and rapidity along with simplicity of operation, such methods are commonly used in laboratories and a great number of specimens have been quantified with such techniques.³⁵⁻³⁸

With respect to the scientific reports, numerous kinetic spectrophotometric techniques were found to approximate the

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active pharmaceuticals. Certainly, multiple benefits could be observed while applying such techniques for analyzing medicines. The benefits include greater selectivity as a result of measuring the enhanced absorbance values over the reaction time rather than the measurement of a concentrate absorbance value.³⁶⁻³⁹ Actually, such advantages would resolve additive interferences that have a probable influence on other procedures, such as the volumetric titration and direct spectrophotometric techniques.⁴⁰⁻⁴² Additionally, all interferences caused by tinted or further ingredients in the pharmaceutical formulations could be avoided to the extent that they do not contribute to the reactions introduced for developing the kinetic technique.^{43,44}

Thus, this study was performed for designing an easy, fast, highly sensitive, strongly selective and affordable kinetic spectrophotometric procedure for quantification of cisplatin in the human serum and urine during the chemotherapy. Also, spiked biological samples and pharmaceutical samples were analyzed. However, there have been little analytical procedures in the literature to analyze cisplatin in real samples with different matrices.

Experimental

Apparatus and chemicals

According to the research design, a double beam UV-Vis spectrophotometer Agilent (8435, USA) with 1 cm matched glass cell was utilized to record the absorption spectra and absorbance measurements. Each solution was stored in a thermostatic water bath at $25.0 \pm 0.1^\circ\text{C}$. In addition, a watch stop was employed to record the reaction time.

In addition, analytical reagent grade chemicals and doubly distilled water were utilized. Cisplatin (Sigma-Aldrich) stock solution ($100.0 \mu\text{g mL}^{-1}$) was procured just prior to use. A proper volume of the solution was applied to obtain the working solution. A Janus Green solution ($4.45 \times 10^{-4} \text{ mol L}^{-1}$) was prepared by dissolving 0.4549 g of Janus Green (Merck) in the water and diluting to 2 L. Sulfuric acid solution (2.0 mol L^{-1}) was obtained by suitably diluting the concentrated sulfuric acid (Merck). A 0.05 mol L^{-1} of potassium bromate solution was obtained by dissolving 16.7011 g of KBrO_3 (Merck) in the water and diluting to 2 L in a calibrated flask.

General procedure

Notably, the inhibited reaction was followed spectrophotometrically by monitoring the change in absorbance of the reaction mixture at 618 nm (λ_{max}). For establishing thermal equilibrium, the reagent solutions were fixed at $25.0 \pm 0.1^\circ\text{C}$ for 30 min in the thermostated water bath. Then, 0.55 mL of $4.45 \times 10^{-4} \text{ mol L}^{-1}$ of the Janus Green solution, 1.2 mL of 2.0 mol L^{-1} sulfuric acid solution and standard solution consisting of 0.2 mL of $10.0 \mu\text{g mL}^{-1}$ of cisplatin were added to a series of 10 mL volumetric flasks. After that, the solution was mixed and water was used to dilute it to 8 mL . For the next stage, 0.85 mL of 0.05 mol L^{-1} bromate solution was poured and diluted to the mark. Measurement of the time was started just after the addition of the last drop of the bromate solution. The solution was completely mixed and a certain volume of the solution was transferred to a glass cell. Then, the change in absorbance in the presence of cisplatin, named as inhibited reaction (ΔA_s), was evaluated *versus* water at λ_{max} and 25°C for the time interval of 30 – 240 s. The same procedure was carried out in the absence of cisplatin was iterated at the same conditions for obtaining the values for the uninhibited reaction (ΔA_b). The difference in absorbance changes was regarded as the response

Fig. 1 Absorption spectra of the uninhibited reaction. (Conditions: Janus Green, $26.7 \mu\text{mol L}^{-1}$; sulfuric acid, $2.4 \times 10^{-1} \text{ mol L}^{-1}$; bromate, $3.5 \times 10^{-3} \text{ mol L}^{-1}$; 25°C and 420 s). Inset shows the absorption spectra of the inhibited reaction (in presence of $200.0 \mu\text{g L}^{-1}$ of cisplatin).

($\Delta A = \Delta A_b - \Delta A_s$). The calibration graph was created by drawing the responses against the cisplatin concentration.

Preparation of real samples

Preparation of serum and urine samples of patient treated with cisplatin. The serum sample was collected after 2 and 8 h from a larynx cancer patient (age, 38; sex, male) after infusion of 60 mg of cisplatin. For purification, 5 mL of $10\% \text{ CCl}_3\text{CO}_2\text{H}$ was added to 5 mL of the serum, shaken and centrifuged for 5 min at 5000 rpm. An appropriate amount of the serum sample was treated just after preparing according to the recommended procedure.

The urine sample was collected after 8 and 16 h from a larynx cancer patient (age, 38; sex, male) after infusion of 60 mg of cisplatin. Then, centrifugation of the sample was performed for 15 min at 2000 rpm. Afterwards, a $0.45\text{-}\mu\text{m}$ Whatman filter was used to filter the supernatant. In addition, the solid phase extraction procedure using C_{18} cartridge (Supelco Inc., 10 mL) was applied to treat and preconcentrate cisplatin from the sample.⁴⁵ According to the procedure, the extracted cisplatin was quantified.

Preparation of real samples using the standard addition method. Human serum and urine were used as biological samples for the determination of cisplatin. Real samples were prepared from the person who had not taken cisplatin. They were spiked with various amounts of cisplatin (over the range of calibration graph) and solid phase extraction technique with C_{18} cartridge (Supelco Inc., 10 mL) was used for purification and preconcentration of cisplatin from the samples.⁴⁴ The extracted cisplatin was determined by the developed method.

Pharmaceutical sample preparation

Dilution of the cisplatin injection solution (1.0 mg/mL) was carried out in a 0.5-L volumetric flask. Then, appropriate amounts of the solution were analyzed by the recommended procedure.

Results and Discussion

Absorption spectra

Figure 1 and its inset present the absorption spectra of the

Fig. 2 Effect of Janus Green concentration on the rate of uninhibited (ΔA_b), inhibited (ΔA_s) reactions and response (ΔA). (Conditions: sulfuric acid, $2.4 \times 10^{-1} \text{ mol L}^{-1}$; cisplatin, $200.0 \mu\text{g L}^{-1}$; bromate, $3.5 \times 10^{-3} \text{ mol L}^{-1}$; 25°C and 420 s).

uninhibited and inhibited reaction mixtures for 420 s with time intervals 30 s. As can be seen, the changes in absorbance are reduced in the presence of low levels of cisplatin. Hence, it is possible to use the sensitive suggested reaction mechanism for the detection of trace amounts of cisplatin.

Mechanism of the reaction

Review of the literature presented the principle of the kinetics methods for quantitative determination of various species. To sum up, selection of a reaction mixture including an indicator that was used for kinetic determination of a catalyst/inhibitor is on the basis of the redox reaction where the catalyst/inhibitor changes the rate of oxidation during a cyclic process. It is notable that since the catalyst/inhibitor concentration is in direct proportion to the reaction time, it is possible to apply the change in the reaction rate to quantify the catalyst/inhibitor.⁴¹ Finally, the reaction system for the Janus Green-bromate mechanism can be expressed as follow:

In the presence of cisplatin, decolorizing of Janus Green was inhibited. Cisplatin can be reacted with bromine based on the reaction as below so that suppressive impact is applied on the Janus Green decolorization by bromine:

where Red represents the reduced format and Ox refers to the oxidized form of reactant.

Optimization of reaction parameters

Optimization of the reaction variables through the presented general process was carried out. That is, the explored parameters were varied while others were fixed.

The effect of the Janus Green concentration on the rate of the uninhibited and inhibited reactions were studied over a range from 22.25 – 31.15 $\mu\text{mol L}^{-1}$. According to Fig. 2, the sensitivity increased up to 24.48 $\mu\text{mol L}^{-1}$ of Janus Green concentration. However, at higher concentrations, the reaction rate declined and this could be ascribed to the dye aggregation. Hence, 24.48 $\mu\text{mol L}^{-1}$ of the Janus Green was chosen as the

Fig. 3 Effect of sulfuric acid concentration on the rate of uninhibited (ΔA_b), inhibited (ΔA_s) reactions and response (ΔA). (Conditions: Janus Green, $24.48 \mu\text{mol L}^{-1}$; cisplatin, $200.0 \mu\text{g L}^{-1}$; bromate, $3.5 \times 10^{-3} \text{ mol L}^{-1}$; 25°C and 420 s).

Fig. 4 Effect of bromate concentration on the rate of uninhibited (ΔA_b), inhibited (ΔA_s) reactions and response (ΔA). (Conditions: Janus Green, $24.48 \mu\text{mol L}^{-1}$; sulfuric acid, $2.4 \times 10^{-1} \text{ mol L}^{-1}$; cisplatin, $200.0 \mu\text{g L}^{-1}$; 25°C and 420 s).

optimal concentration for further investigations.

In addition, the effect of sulfuric acid concentration was investigated on inhibited and uninhibited reactions in the concentration range of 0.20 and 0.28 mol L^{-1} (Fig. 3). As can be seen, the highest sensitivity was observed at 0.24 mol L^{-1} , while the sensitivity diminished at higher concentrations. However, the protonation of Janus Green at higher acid concentrations, which resulted in highly hard oxidation, led to dysfunctions. Thus, 0.24 mol L^{-1} of sulfuric acid was utilized for additional investigations.

The effect of bromate concentration on the reaction rate was explored over the range 3.0 and 5.0 mmol L^{-1} . According to Fig. 4, the net reaction rate increased up to 4.25 mmol L^{-1} and thus was chosen as the optimal concentration of oxidant. Thus, 4.25 mmol L^{-1} of bromate was utilized for additional investigations.

Under optimum experimental conditions, the effect of the temperature on the reaction rate was studied in a range between 15 and 30°C . However, increasing the temperature to 25°C resulted in increased sensitivity, while it declined at higher temperatures. Finally, 25°C was chosen as the optimal temperature.

According to Fig. 5, the optimal time was observed *via* measurement of the changes in the absorbance during 30 – 540 s. As can be seen, the reaction rate increased up to 240 s. Therefore, 240 s was selected for further study.

Calibration graph and limit of detection

The calibration graph (Fig. 6) was obtained made by plotting

Fig. 5 Effect of time on the rate of uninhibited (ΔA_b), inhibited (ΔA_s) reactions and response (ΔA). (Conditions: Janus Green, $24.48 \mu\text{mol L}^{-1}$; sulfuric acid, $2.4 \times 10^{-1} \text{ mol L}^{-1}$; cisplatin, $200.0 \mu\text{g L}^{-1}$; bromate, $4.25 \times 10^{-3} \text{ mol L}^{-1}$ and 25°C).

Fig. 6 Calibration graph: Janus Green, $24.48 \mu\text{mol L}^{-1}$; sulfuric acid, $2.4 \times 10^{-1} \text{ mol L}^{-1}$; bromate, $4.25 \times 10^{-3} \text{ mol L}^{-1}$, 25°C and 240 s. Inset shows the calibration graph in the range $10.0 - 500.0 \mu\text{g L}^{-1}$ of cisplatin.

Table 1 Comparison of some of the analytical parameters of spectrophotometric methods used for the determination of cisplatin

Reagent	Linear range/ $\mu\text{g L}^{-1}$	Sensitivity/ $\mu\text{g L}^{-1}$	Detection limit/ $\mu\text{g L}^{-1}$	RSD, %	Application media	Ref.
<i>ortho</i> -Phenylenediamine	420.0 – 3160.0	0.29	420.0	1.3	Spiked biological sample (urine)	46
<i>ortho</i> -Phenylenediamine	400.0 – 1400.0	0.21	380.0	1.26	Pharmaceutical sample (tablet)	47
1,10-Phenanthroline	250.0 – 6000.0	0.072	120.0	1.10	Pharmaceutical sample (injection solution)	48
Janus Green	10.0 – 2000.0	2.0×10^{-4}	4.2	1.86 – 1.11	Real biological sample (serum and urine)	This work
	2000.0 – 5750.0	2.0×10^{-5}			Spiked biological sample (serum and urine) Pharmaceutical sample (injection solution)	

the response *versus* cisplatin concentration. Applying the presented process and under the optimum conditions discussed earlier, the calibration graph was linear in a range of $10.0 - 5750.0 \mu\text{g L}^{-1}$ of cisplatin, including two linear segments of $10.0 - 2000.0$ and $2000.0 - 5750.0 \mu\text{g L}^{-1}$ with regression equations as Eqs. (5) and (6), respectively.

$$\Delta A = 2.0 \times 10^{-4}[\text{cisplatin}] + 0.01 \quad (R^2 = 0.9992) \quad (5)$$

$$\Delta A = 2.0 \times 10^{-5}[\text{cisplatin}] + 0.4249 \quad (R^2 = 0.9994) \quad (6)$$

In the above ΔA represents the difference in absorbance between the blank and the sample, [cisplatin] stands for the cisplatin concentration in $\mu\text{g L}^{-1}$ and R^2 is the correlation coefficient. The inset of Fig. 6 shows a part of the calibration graph in a concentration range $10.0 - 500.0 \mu\text{g L}^{-1}$. The regression equation ($\Delta A = 2.0 \times 10^{-4}[\text{cisplatin}] + 0.0136$; $R^2 = 0.9993$) shows the linearity at trace levels of cisplatin. LOD ($3s_b/m$; s_b indicates the standard deviation (SD) of the blank signal and m refers to the slope of the calibration graph) was $4.2 \mu\text{g L}^{-1}$ of cisplatin for 10 replicate determinations.

Precision

The precision of the developed method was investigated at three concentration levels in the defined range of the test concentration that have been done three times. It was found that the relative standard deviations were 1.86, 1.20 and 1.11% for 50.0, 500.0, and 5000.0 $\mu\text{g L}^{-1}$ of cisplatin. Based on the findings, the observed relative SDs for each concentration level were lower than 2.0%. The above result reveals reasonable applicability of the presented method. In Table 1, some of the analytical characteristics obtained in this work were compared with those that reported in the literature previously.⁴⁶⁻⁴⁸ It can be

seen that the methods suffer from limitations, such as low

Table 2 Tolerance limit for foreign species on the determination of $500.0 \mu\text{g L}^{-1}$ of cisplatin

Foreign species	Tolerance limit	Normal range in human blood ³³	
		Men	Women
Triglyceride	2.2 g L ⁻¹	<2.0 g L ⁻¹	<2.0 g L ⁻¹
Urea	15.0 mmol L ⁻¹	3.0 – 9.2 mmol L ⁻¹	5.2 – 7.2 mmol L ⁻¹
Certain	0.56 mmol L ⁻¹	0.06 – 0.12 mmol L ⁻¹	0.05 – 0.12 mmol L ⁻¹
Glucose	7.0 mmol L ⁻¹	3.9 – 6.4 mmol L ⁻¹	3.9 – 6.4 mmol L ⁻¹
Cholesterol	2.0 mmol L ⁻¹	<5.2 mmol L ⁻¹	<5.2 mmol L ⁻¹
Phosphate	1.6 mmol L ⁻¹	0.8 – 1.4 mmol L ⁻¹	0.8 – 1.4 mmol L ⁻¹
Na ⁺ , K ⁺ , Ca ²⁺	1000	—	—

sensitivity, small linear dynamic range and high limit of detection in all cases.

Interference investigation

The interfering effect of the foreign species on quantifying $500.0 \mu\text{g L}^{-1}$ of cisplatin was examined. Notably, the tolerance limit was described as the concentration of the added samples, which resulted in an error higher than $\pm 5\%$ on analytical signal. Table 2 presents the outputs. According to the results, interferences from triglyceride, urea, certain, glucose, cholesterol and phosphate were not found. Also, the interfering limit of the substances was less than the normal range found in human blood. Moreover, Na⁺, K⁺ and Ca²⁺ cations did not show any interfering effect. Thus, analytical variables confirmed the potential of the introduced technique as one of the alternatives to quantitatively detecting cisplatin.

Table 3 Determination of cisplatin in human serum and urine samples of a larynx cancer patient (age, 36; sex, male) after injection of 60 mg cisplatin

Sample	Sampling time after infusion/h	Proposed method		Inductively coupled plasma		Statistical test	
		Found ^a /μg L ⁻¹	RSD, %	Found ^a /μg L ⁻¹	RSD, %	<i>t</i> -test ^b	<i>F</i> -test ^c
Human serum	2	301.7 ± 3.2	1.060	302.4 ± 3.1	1.025	0.272	1.065
	8	221.1 ± 2.4	1.085	220.2 ± 2.1	0.953	0.495	1.306
Human urine	8	39.1 ± 0.41	1.048	39.6 ± 0.33	0.833	1.645	1.541
	16	63.8 ± 0.62	0.972	63.4 ± 0.61	0.962	0.797	1.033

a. Mean ± standard deviation ($n = 3$). b. Tabulated *t*-value for four degrees of freedom at (0.95) is 2.776. c. Tabulated *F*-value for three degrees of freedom at *P* (0.95) is 19.00.

Table 4 Determination of cisplatin in spiked human serum and urine samples

Sample	Added/μg L ⁻¹	Found ^a /μg L ⁻¹	RSD, %	Recovery, %
Human serum	100.0	101.1 ± 1.1	1.09	101.1
	200.0	198.8 ± 1.8	0.90	99.4
	1000.0	1002.1 ± 11.1	1.11	100.2
	2000.0	2019.3 ± 19.8	0.98	101.0
Human urine	100.0	99.1 ± 1.1	1.10	99.1
	200.0	208.8 ± 2.1	1.00	104.2
	1000.0	1012.1 ± 11.7	1.16	101.2
	2000.0	1991.6 ± 19.9	1.00	99.6

a. Mean ± standard deviation ($n = 3$).

Real sample analysis

For evaluating reliability and analytical utility of the introduced technique, it was utilized to determine cisplatin in biological and pharmaceutical samples.

Serum and urine samples of a patient treated with cisplatin were purified as discussed previously. Then, a certain volume of each sample have been analyzed by the presented process and inductively coupled plasma as an alternative technique. Table 3 reports the outputs of three replicate determinations. As it can be seen, the results of cisplatin determination by two methods are in good agreement together and statistical tests confirm the reliability (*t*-test) and precision (*F*-test) of them. Moreover, the biological specimens were prepared from a person who had not taken cisplatin and sample preparation was treated as discussed previously. Quantification was performed using the recommended procedure. Outputs are given in Table 4. The recovery rate values and relative standard deviations (RSD%) confirm the applicability of the presented technique to detect cisplatin in spiked biological specimens. The obtained results show that the designed procedure has no interfering impact of the matrix effect.

Pharmaceutical sample preparation was conducted as discussed previously. Then, an appropriate amount of the sample was analyzed by the introduced procedure. Table 5 reports outputs of three replicate determinations of cisplatin. The precision (RSD%) was almost 1%, confirming the repeatability of the developed method. The reliability of the method was assessed by statistical *t*-test. It was found that the experimental value (3.46) differs from the critical value (4.30, 95% confidence level, and 2 degrees of freedom). Based on the differences between critical and experimental values, the systematic error to detect cisplatin in the pharmaceutical specimens via the presented procedure could be neglected.

Table 5 Determination of cisplatin in cisplatin injection solution 1.0 mg mL⁻¹ using the developed procedure

Sample	Found ^a /mg mL ⁻¹	RSD, %	Statistical <i>t</i> -test ^b	Pharmaceutical Co.
Injection solution	0.98 ± 0.01	1.02	3.46	PCH Pharmachemi

a. Mean ± standard deviation ($n = 3$).

b. Tabulated *t*-value for two degrees of freedom at (0.95) is 4.30.

Conclusions

A convenient, reliable, selective, sensitive, and affordable kinetic spectrophotometric procedure was described and validated for the analysis of cisplatin. Moreover, the method was not be dependent on the utilization of the expensive advanced tools. It should be mentioned that the presented technique has been applied to approximate cisplatin in distinct real samples with no interference from the traditional excipients. Also, the above benefits would enhance probable implementation of the presented technique in quality control laboratories in order to estimate the mentioned medicines either in their bulk or dose form formulations.

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