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

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Abstract

This study describes a kinetic spectrophotometric method for accurate, sensitive and rapid determination of cisplatin in biofluids. The developed method is based up on inhibitory effect of cisplatin on oxidizing of Janus Green by bromate in the acidic media. The change in absorbance as criteria of the oxidation reaction is followed spectrophotometrically. To obtain the highest rate of sensitivity, efficient reaction parameters have been optimized. Under optimum experimental conditions, calibration graph has been observed linearly over the range 10.0 - 5750.0 µg L⁻¹ and the limit of detection (3σ/m) was 4.2 µg L⁻¹ of cisplatin. The interfering effect of diverse species was investigated. The developed method was used for quantification of cisplatin in biofluids of patients who treated with cisplatin, spiked biofluids and pharmaceutical samples satisfactorily.

Keywords: Kinetic spectrophotometry, Cisplatin, Biological sample, Cancer patient.
Introduction

Studies demonstrated that cancer is one of the main burdens of disease throughout the world. Annually, tens of millions of humans would be diagnosed with the cancer worldwide, and above 1/2 of the patients would ultimately experience death resulting from cancer.\(^1\) Cancer in several countries is the second most popular cause of mortality after cardio-vascular illnesses. Chemotherapy is one of 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 square meters. Dose of drug is calculated individually for each patient depending on body weight and height.\(^4-6\) The patient’s general conditions and co-morbidity is also considered. Individuals respond differently to received 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 too small doses of medication have no therapeutic effect and that affects overall survival and disease-free period, while too high dose increases the toxic effects in the body for up to a possible death.\(^10-12\)

Cisplatin [cis-dichlorodiamino-platinum (II)] is a platinum-containing drug used for chemotherapy of cancer patients. It would alter natural duration for multiple human malignancies like ovarian, testicular, lungs, bladder, head, gastric, and neck cancers with the increased healing rate.\(^13-15\) In fact, Cisplatin reacts with nuclear target DNA. Despite the powerful anticancer potency of Cisplatin, chemotherapy with cisplatin would cause a lot of severe complications, including nephron-toxicity, nausea, orthotoxicity, neuropathy, vomiting, allergy and increased hair loss.\(^16-18\) It is therefore considered for monitoring the platinum concentration in the humans' bodies following dosing cisplatin. Actually, detecting platinum in the biological fluids at trace level continues to remain of analytical interest. The analytical methods reported are based on the determination of intact cisplatin\(^19-21\) and elemental platinum from biological samples. The methods
are based on the measurement of platinum including inductively coupled plasma-mass spectrometry\textsuperscript{22}, atomic absorption spectrometry\textsuperscript{23}, electrochemical methods\textsuperscript{24}, polarography\textsuperscript{25}, spectrophotometry\textsuperscript{26}, neutron activation analysis\textsuperscript{27} and high performance liquid chromatography.\textsuperscript{28}

In recent years, electrochemical methods using a modified electrode are the most common technique for analysis of various specimens including drugs with different matrices. But, the methods have disadvantages such as low repeatability, hard operation in preparation of electrode and time consuming.\textsuperscript{29-33} Especially, chromatographic methods are expensive, high cost and hard operation.\textsuperscript{34} As spectrophotometric methods have sufficient accuracy, selectivity and rapidity along with the simplicity of operation, it commonly used in laboratories and a great number of specimens were quantified using it.\textsuperscript{35-38}

With respect to the scientific reports, numerous kinetic spectrophotometric techniques were found to approximate the active pharmaceuticals. Certainly, multiple benefits would be observed while applying such techniques for analyzing the 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.\textsuperscript{36-39} Actually, such advantages would resolve additive interferences that have a probable influence on other procedures like the volumetric titration and direct spectrophotometric techniques.\textsuperscript{40-42} Additionally, they would avoid all interferences caused by the tinted or further ingredients in the pharmaceutical formulations till they are not contributed to the reactions introduced for developing the kinetic technique.\textsuperscript{43, 44}

Thus, this article has been performed for designing an easy, fast, generable, 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 sample were analyzed. However, there have been little analytical procedures in the literature to analyze the 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 has been utilized to record the absorption spectra and absorbance measurements. Each solution has been stored in a thermostatic water bath at 25.0 ± 0.1 °C. In addition, a stop watch has been employed to record the reaction time.
In addition, the analytical reagent grade chemicals and doubly distilled water have been utilized. Cisplatin (Sigma-Aldrich) stock solution 100.0 μg mL\(^{-1}\) has been procured just prior to using it. A proper volume of the solution has been applied to procure the working solution. A Janus Green solution (4.45 × 10\(^{-4}\) mol L\(^{-1}\)) has been prepared via dissolving 0.4549 g of Janus Green (Merck) in the water and diluting to 2 L. Sulfuric acid solution (2.0 mol L\(^{-1}\)) has been procured via suitably diluting the concentrated sulfuric acid (Merck). A 0.05 mol L\(^{-1}\) of potassium bromate solution has been procured via 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 has been followed spectrophotometrically via monitoring the change in absorbance of the reaction mixture at 618 nm (\(\lambda_{\text{max}}\)). For establishing thermal equilibrium, the reagent solutions has been fixed at 25.0 ± 0.1 °C for 30 min in the thermostated water bath. Then, 0.55 mL of 4.45 ×10\(^{-4}\) 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 μg mL\(^{-1}\) of cisplatin to a series of 10 mL volumetric flasks was added. After that, the solution has been mixed and water has been used to dilute it to 8 mL. For the next stage, 0.85 mL of 0.05 mol L\(^{-1}\) bromate solution has been poured and diluted to the mark. Measuring the time was began just following the addition of the last drop of the bromate solution. The solution has been completely mixed and some volumes of the solution has been transferred to a glass cell. Then, change in absorbance in presence of cisplatin, named as inhibited reaction (\(\Delta A_s\)), has been evaluated versus water at \(\lambda_{\text{max}}\) and 25 °C for the time interval of 30-240 s. The same procedure was done in the absence of cisplatin has been iterated at the same conditions for obtaining the values for the uninhibited reaction (\(\Delta A_b\)). The difference in absorbance changes have been regarded as the response (\(\Delta A = \Delta A_b - \Delta A_s\)). The calibration graph has been created via 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 larynx cancer patient (age:38; sex: male) after infusion of 60 mg of cisplatin. For purification, 5mL of 10% CCl\(_3\)CO\(_2\)H was added to 5mL of the serum, shacked and centrifuged for 5 min at 5000 rpm. 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 larynx cancer patient (age: 38; sex: male) after infusion of 60 mg of cisplatin. Then, centrifugation of the sample has been performed for 15 minutes at 2000 rpm. Afterwards, a 0.45 µm Whatman filter has been used to filter the supernatant. In addition, the solid phase extraction procedure using C\textsubscript{18} cartridge (Supelco Inc., 10 mL) has been applied to treat and pre-concentrate of cisplatin from the sample.\textsuperscript{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\textsubscript{18} cartridge (Supelco Inc., 10 mL) was used for purification and pre-concentration of cisplatin from the samples.\textsuperscript{44} The extracted cisplatin was determined by the developed method.

**Pharmaceutical sample preparation**

Dilution of the cisplatin injection solution (1.0 mg/mL) has been done in a 0.5 L volumetric flask. Then, appropriate amounts of the solution were analysed by the recommended procedure.

**Result and discussion**

**Absorption Spectra**

Fig. 1 and its inset present the absorption spectra of uninhibited and inhibited reactions mixture for 420 s with time interval 30 s. As it can be seen, the changes in absorbance is reduced in the presence of low levels of cisplatin. Hence, it is possible to use the sensitive suggested reaction mechanism to trace amounts detection of cisplatin.

**Mechanism of the reaction**

Review of the literature presented the principle of the kinetics methods in order to 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 a direct proportionate to the reaction time, it is possible to apply change in the reaction rate to quantify the catalyst/inhibitor. Finally, the reaction system for the Janus Green-bromate mechanism can be expressed as follow:

\[
\begin{align*}
JG_{(Red)} + BrO_3^- + 6 H^+ & \rightarrow JG_{(Ox)} + Br^- + 3 H_2O \\
5 Br^-+ BrO_3^- + 6 H^+ & \rightarrow 3 Br_2 + 3 H_2O \\
Br_2 + H^+ + JG_{(Red)} & \rightarrow 2 Br^- + JG_{(Ox)}
\end{align*}
\]

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:

\[
\text{Cisplatin}_{(Red)} + Br_2 + H^+ \rightarrow \text{Cisplatin}_{(Ox)} + 2 Br^- 
\]

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

**Optimization of reaction parameters**

Optimizing of the reaction variables through the presented general process has been done. In fact, the explored parameters has been variable so that others have been fixed.

The effect of the Janus Green concentration on the rate of on the uninhibited and inhibited reactions has been studied over a range from 22.25- 31.15 µmol L\(^{-1}\). According to Fig. 2, the sensitivity has been enhanced up to 24.48 µmol L\(^{-1}\) of Janus Green concentration. However, at higher concentrations, the reaction rate declined that this could be ascribed to the dye aggregation. Hence, 24.48 µmol L\(^{-1}\) of the Janus Green as the optimal concentration has been chosen for additional investigation.

In addition, the effect of sulfuric acid concentration has been investigated on inhibited and uninhibited reactions in concentration range 0.20 and 0.28 mol L\(^{-1}\) (Fig. 3). As it can be seen, the highest sensitivity has been 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 has been utilized for additional investigations.
The effect of bromate concentration on the reaction rate has been explored over the range 3.0 and 5.0 mmol L\(^{-1}\). According to Figure 4, the net reaction rate increased up to 4.25 mmol L\(^{-1}\) and thus has been chosen as the optimal concentration of oxidant. It may be attributed to noticeably reducing the Janus Green concentration up to 4.25 mmol L\(^{-1}\) of bromate and therefore, the Janus Green oxidation rate was reduced.

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

According to Figure 5, optimal time has been observed via measurement of the changes in the absorbance during 30-540 s. As it can be seen, the reaction rate enhanced to 240 s. Therefore, 240 s was selected for further study.

*Calibration graph and limit of detection*

Calibration graph (Figure 6) has been made by plotting the response versus cisplatin concentration. Applying the presented process and under optimum conditions discussed earlier, the calibration graph has been linear in a range of 10.0-5750.0 µg L\(^{-1}\) of cisplatin, including two linear segments of 10.0-2000.0 and 2000.0-5750.0 µg L\(^{-1}\) with regression equations as equations 1 and 2, respectively.

\[
\Delta A = 2.0 \times 10^{-4} \text{[cisplatin]} + 0.01 \quad (R^2 = 0.9992) \\
\Delta A = 2.0 \times 10^{-5} \text{[cisplatin]} + 0.4249 \quad (R^2 = 0.9994)
\]  
(Equation 1)  
(Equation 2)

So that \(\Delta A\) represents the difference in absorbance between the blank and the sample, [cisplatin] stands for the cisplatin concentration in µg L\(^{-1}\) and \(R^2\) is the correlation coefficient. The inset of Figure 6 shows a part of calibration graph in concentration range 10.0-500.0 µg 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 (3\(s_b/m\); \(s_b\) indicates the standard deviation (SD) of the blank signal and \(m\) refers to the slope of calibration graph) has been 4.2 µg L\(^{-1}\) of cisplatin for ten replicate determinations.
Precision

The precision of the developed method has been investigated at three concentration levels in the defined range like 100% of the test concentration that have been done three times. It has been found that the relative standard deviations are 1.86, 1.20 and 1.11% for 50.0, 500.0, and 5000.0 µg L\(^{-1}\) of cisplatin. Based on the findings, the observed relative SDs for each concentration level have been 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, the methods suffer from limitations such as low 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 µg L\(^{-1}\) of cisplatin has been examined. Notably, tolerance limit has been 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 are less than the normal range of them in human blood. Moreover, Na\(^+\), K\(^+\) and Ca\(^{+2}\) cations were not shown interfering effect. Thus, analytical variables confirmed the potentials of the introduced technique as one of the alternatives to quantitatively detecting cisplatin.

Real sample analysis

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

Serum and urine samples of a patient who treated with cisplatin were purified as discussed previously. Then, a proper content of each sample have been analysed 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 of them was done using 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 has been conducted as discussed previously. Then, an appropriate amount of the sample was analysed by the introduced procedure. Table 5 reports outputs of three replicate determinations of cisplatin. The precision (RSD%) has been almost 1% that confirms the repeatability of the developed method. Reliability of the method has been assessed by statistical $t$-test. It has been found that 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.

**Conclusions**

A convenient, reliable, selective, generable, sensitive, and affordable kinetic spectrophotometric procedure was described and validated for the analysis of cisplatin. Moreover, the method would not be dependent on the utilization of the expensive advanced tools. It should be mentioned that the presented technique has been substantially applied to approximate of cisplatin in their distinct real samples with no interference from the traditional excipients. Also, the above benefits would enhance probable implementation of the presented technique in the quality control laboratories in order to estimate the mentioned medicines either in their bulk or dose form formulations.
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Table 1. Comparison of some of analytical parameters of spectrophotometric methods used for the determination of cisplatin.

| Reagent                  | Linear range (µg L\(^{-1}\)) | Sensitivity (µg L\(^{-1}\)) | Detection limit (µg L\(^{-1}\)) | RSD (%) | Application media                                      | Ref.       |
|--------------------------|-------------------------------|-------------------------------|----------------------------------|---------|-------------------------------------------------------|------------|
| Ortho-phenylenediamine   | 420.0-3160.0                  | 0.29                          | 420.0                            | 1.3     | Spiked biological sample (urine)                      | [46]       |
| Ortho-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)            |            |
**Table 2.** Tolerance limit for foreign species on the determination of 500.0 µg 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               | -                               | -                              |
Table 3. Determination of cisplatin in human serum and urine samples of 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\(^{-1}\)) | R.S.D (%)                  | found\(^a\) (µg L\(^{-1}\)) | R.S.D (%) | t-test \(^b\) | F-test \(^c\) |
| Human   | 2                               | 301.7±3.2            | 1.060                     | 302.4±3.1       | 1.025 | 0.272 | 1.065 |
| serum   | 8                               | 221.1±2.4            | 1.085                     | 220.2±2.1       | 0.953 | 0.495 | 1.306 |
| Human   | 8                               | 39.1±0.41            | 1.048                     | 39.6±0.33       | 0.833 | 1.645 | 1.541 |
| urine   | 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 \(P\)(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) | Found \( ^a \) (µg/L) | RSD (%) | Recovery (%) |
|-----------|--------------|-------------------------|--------|--------------|
| Human     | 100.0        | 101.1±1.1               | 1.09   | 101.1        |
| serum     | 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     | 100.0        | 99.1±1.1                | 1.10   | 99.1         |
| urine     | 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 \((n = 3)\).
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 t-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 $P(0.95)$ is 4.30.
Legend for the figures:

**Figure 1.** Absorption spectra of the uninhibited reaction. (Conditions: Janus Green, 26.7 µmol L\(^{-1}\); sulfuric acid, 2.4×10\(^{-1}\) mol L\(^{-1}\); bromate, 3.5×10\(^{-3}\) mol L\(^{-1}\); 25°C and 420 s). A: Inset shows the absorption spectra of the inhibited reaction (in presence of 200.0 µg L\(^{-1}\) of cisplatin).

**Figure 2.** Effect of Janus Green concentration on the rate of uninhibited (ΔA\(_b\)), inhibited (ΔA\(_s\)) reactions and response (ΔA). (Conditions: sulfuric acid, 2.4×10\(^{-1}\) mol L\(^{-1}\); cisplatin, 200.0 µg L\(^{-1}\); bromate, 3.5×10\(^{-3}\) mol L\(^{-1}\); 25°C and 420 s).

**Figure 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 µmol L\(^{-1}\); cisplatin, 200.0 µg L\(^{-1}\); bromate, 3.5×10\(^{-3}\) mol L\(^{-1}\); 25°C and 420 s).

**Figure 4.** Effect of bromate concentration on the rate of uninhibited (ΔA\(_b\)), inhibited (ΔA\(_s\)) reactions and response (ΔA). (Conditions: Janus Green, 24.48 µmol L\(^{-1}\); sulfuric acid; 2.4×10\(^{-1}\) mol L\(^{-1}\); cisplatin, 200.0 µg L\(^{-1}\); 25°C and 420 s).

**Figure 5.** Effect of time on the rate of uninhibited (ΔA\(_b\)), inhibited (ΔA\(_s\)) reactions and response (ΔA). (Conditions: Janus Green, 24.48 µmol L\(^{-1}\); sulfuric acid, 2.4×10\(^{-1}\) mol L\(^{-1}\); cisplatin, 200.0 µg L\(^{-1}\); bromate, 4.25×10\(^{-3}\) mol L\(^{-1}\) and 25°C).

**Figure 6.** Calibration graph: Janus Green, 24.48 µmol L\(^{-1}\); sulfuric acid, 2.4×10\(^{-1}\) mol L\(^{-1}\); bromate, 4.25×10\(^{-3}\) mol L\(^{-1}\), 25°C and time; 240 s. Inset shows the calibration graph in the range 10.0-500.0 µg L\(^{-1}\) of cisplatin.
Fig. 1
Fig. 2
Fig. 3
Fig. 4
Fig. 5
Fig. 6

\[ y = 0.0002x + 0.01 \]
\[ R^2 = 0.9992 \]

\[ y = 2 \times 10^{-5}x + 0.4249 \]
\[ R^2 = 0.9994 \]
Graphical abstract