

A simple and selective spectrophotometric flow injection determination of trace amounts of ruthenium by catalytic oxidation of safranin-O

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Abstract

In this work, a simple, selective and rapid flow injection method has been developed for determination of ruthenium. The method is based on its catalytic effect on the oxidation of safranin-O by metaperiodate. The reaction was monitored spectrophotometrically by measuring safranin-O absorbance at $\lambda_{\max} = 521$. The reagents and manifold variables, which have influences on the sensitivity, were investigated and the optimum conditions were established. The optimized conditions made it possible to determine ruthenium in the ranges of 0.4–20.0 ng/mL ($\Delta A = 0.2819C_{\text{Ru}} + 1.1840$) and 20.0–100.0 ng/mL ($\Delta A = 0.0984C_{\text{Ru}} + 7.9391$) with a detection limit of 0.095 ng/mL and a sample rate of 30 ± 5 samples/h. Relative standard deviation for the five replicate measurements was less than 1.84%. The proposed method has been successfully applied for analysis of ultra trace amounts of ruthenium in real samples.

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Keywords: Ruthenium; FIA; Spectrophotometry; Safranin-O; Catalytic determination

1. Introduction

Different studies have been carried out regarding the application of materials containing Ru compounds. Due to their electrochemical and thermoelectric properties, such types of materials have shown potential catalytic, pharmaceutical, analytical and theoretical applications. [1,2]. The success of *cis*-platin as an anticancer agent has stimulated the search for other organometallic cytotoxic compounds with more acceptable toxicity profile and, if possible, an increase of antitumor activity. In the last three decades, a wide range of ruthenium agents have been synthesized and tested for antitumor properties. Despite of their low cytotoxic potential *in vitro*, many ruthenium compounds increase the life time expectancy of tumor-bearing hosts. Thus, the increasing importance of the use of ruthenium in widely different fields, particularly in pharmacology [3–6], metallurgy [7,8] and in high technology components [9,10], had made it necessary to develop simple, inexpensive and selective methods for determination of traces of ruthenium in various samples.

Different methods have been reported for determination of this metal such as voltammetry [12], atomic absorption spectrometry [13], fluorimetry [14] and spectrophotometry [15–18]. The availability of spectrophotometric instruments and the simplicity of analytical procedure make the procedure very attractive for a wide range of applications. Flow injection analysis served analytical advantages such as rapid response, automation, low cost, simple instruments, high reproducibility and high selectivity that make it possible to determine trace levels of ruthenium in real samples [19–24]. This article describes an economical, selective and rapid flow injection method for determination of ruthenium in the river and drinking water and human serum. The influence of variables has been studied, and the developed procedure has been employed for the determination of ruthenium in the real samples with satisfactory results.

2. Experimental

2.1. Reagents

Safranin-O solution of 1.0×10^{-2} mol L⁻¹ was prepared by dissolving 0.351 g of dimethyl safranin-O chloride (Merck) in water and diluting it in a 100-mL volumetric flask.

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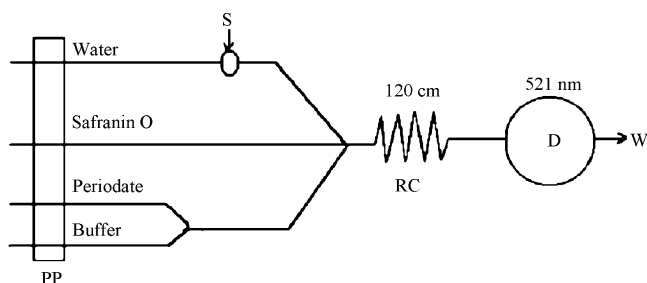


Fig. 1. Schematic diagram of the flow system. (flow rate of 34 mL/h for each channel, sample loop volume of 140 μ L, reaction coil length of 120 cm, absorbance wavelengths: $\lambda_{\text{max}} = 521$ nm); S, injection valve; RC, reaction coil; temperature, 30 $^{\circ}$ C; D, spectrophotometer; PP, peristaltic pump.

Metaperiodate stock solution of 0.01 mol L $^{-1}$ was prepared by dissolving 0.5750 g potassium metaperiodate (Merck) in water and diluting it in a 250-mL volumetric flask.

A 100.0 μ g/mL stock solution of Ru(III) was prepared by dissolving 0.0128 g of RuCl $_3$ (Lobachem) in ethanol in a 100 mL volumetric flask.

All other reagents used were of analytical reagent grade, and doubly distilled water was used throughout. All glassware were cleaned with a detergent solution, rinsed with tap water, soaked in dilute HNO $_3$ solution (2%, v/v), rinsed with water and then were dried.

2.2. Flow injection system

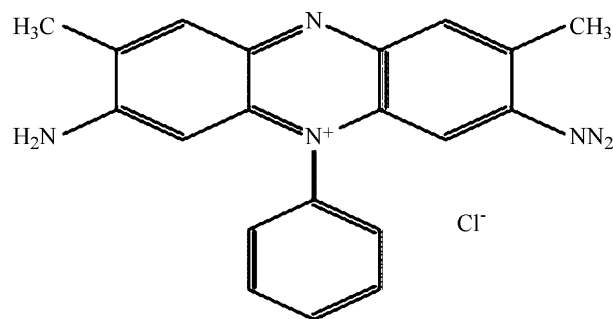
Fig. 1 shows the schematic diagram of the flow system. Silicon rubber tube with 1.0 mm i.d was used for delivery of the solutions. The 12-channel peristaltic pump (Desaga, PLG, 220 V, 70 W) was fitted for pumping solutions. A mixed solution of metaperiodate, buffer, safranin-O and water as a carrier stream was delivered through silicon rubber tubing (at 30 $^{\circ}$ C). The thermostatic water bath (Gallenkamp, BGL) was used at a given temperature of 30 \pm 0.1 $^{\circ}$ C. The standard solution of Ru(III) was injected into a carrier stream with a sample injector (Rhedyne, model 9125). An UV-Visible spectrophotometer (Shimadzu, model 6AV) equipped with a flow through cell with 10-mm path length connected to recorder (Shimadzu C-R4AX Chromatopac) was used for monitoring the variation in the absorbance spectrum.

2.3. Determination of ruthenium in human serum

Mineralization of 2.0 mL of the samples was carried out for 1.0 h at 100 $^{\circ}$ C with the addition of 4 mL of concentrated nitric acid [18]. Then samples were analyzed directly after dilution with doubly distilled water to a suitable volume.

2.4. Determination of ruthenium in ruthenium red and rock samples

A 5.0 mL sample solution was digested and then was treated and subjected to ion exchange separation to eliminate the base metals from ruthenium [24]. After chloride removal by evaporation with H $_2$ SO $_4$ the solution was diluted with water to 25 mL.



Scheme 1. Safranin-O structure.

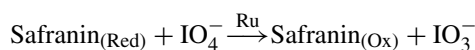
Aliquots of the solution were taken for ruthenium analysis according to the proposed procedure.

3. Results and discussion

Safranin-O (Scheme 1) can be oxidized by metaperiodate slowly. But this reaction rate can be increased by addition of the ultra trace amounts of ruthenium as a catalyst.

This reaction is followed spectrophotometrically (Fig. 2) by controlling absorbance change of the dye at 521 nm by a flow injection method.

Safranin-O and Nil blue have fused heteroaromatic rings structure that has the same oxidation site of amine group. Therefore, catalytic oxidation mechanism of safranin O–IO $_4^-$ –Ru(III) may be similar to Nil blue–IO $_4^-$ –Ru(III) catalytic oxidation system [25]. The total catalytic reaction can be expressed as follows:



The mechanism led to the rate law as:

$$\text{Rate} \propto \text{signal} = -\partial[\text{safranin}]/\partial t = k[\text{Ru}][\text{safranin}][\text{periodate}]$$

Assuming that safranin-O and metaperiodate were present in such excess that their concentration can be regarded as constant,

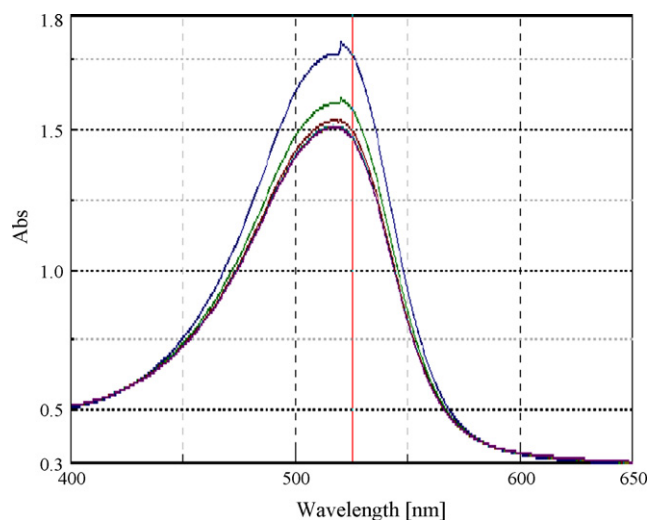


Fig. 2. Variation of the safranin–IO $_4^-$ –Ru(III) system with time. Condition: pH, 4.0; safranin 5.7 \times 10 $^{-5}$ mol L $^{-1}$; IO $_4^-$ 4.0 \times 10 $^{-4}$ mol L $^{-1}$; Ru(III), 40.0 ng/mL; temperature, 30 $^{\circ}$ C; time interval for each scan, 60 s.

the rate equation will be simplified as:

$$\text{Rate} \propto \text{signal} = k'[\text{Ru}]$$

This equation is the quantitative basis for the determination of ruthenium by the proposed method. To have more favorite sensitivity the effect of reagent concentrations and manifold variables on the sensitivity was studied.

3.1. Effect of chemical variables

Experiment was done at different pH values. The results show the maximum peak height with the best baseline is at pH 3.0. While higher pH values decrease the signal. This phenomenon can be due to the fact that at $\text{pH} < 3$, safranin-O is protonated and thus the rate of reaction rate decreases. On the other hand, at higher pH values ($\text{pH} > 3.0$), the ability of metaperiodate to oxidize safranin-O increases with increasing hydroxide ions, thus, decreasing the catalytic role of Ru(III). Therefore, a pH of 3.0 was selected as the optimum pH value.

The effect of safranin-O concentration on the sensitivity was studied at Ru(III) concentration of 20.0 ng/mL and the optimum pH. The results show that by increasing safranin-O concentration up to $1.0 \times 10^{-4} \text{ mol L}^{-1}$, the reaction rate increases, whereas greater amounts of safranin-O concentration cause decrease in sensitivity. This may be due to the aggregation of the dye at higher concentrations. Thus, safranin-O concentration of $1.0 \times 10^{-4} \text{ mol L}^{-1}$ was selected.

The influence of metaperiodate concentration on the sensitivity was studied in the presence of 20.0 ng/mL Ru(III), $1.0 \times 10^{-4} \text{ mol L}^{-1}$ safranin-O, at pH 3.0. The results indicate that the best concentration for metaperiodate is $2.0 \times 10^{-4} \text{ mol L}^{-1}$. By increasing the metaperiodate concentration more than this level, the rate of the uncatalyzed reaction increases to a greater extent than the catalyzed reaction and the difference between catalyzed and uncatalyzed reaction (peak height) diminishes at higher metaperiodate concentration. Therefore, the optimum metaperiodate concentration was selected to be $2.0 \times 10^{-4} \text{ mol L}^{-1}$.

The influence of temperature on the maximum signal (ΔA) was studied for the range of 10–50 °C, under above conditions otherwise as previously described. The results show that by increasing temperature up to 30 °C, signal or the rate of reaction increases. This means that the rate of the uncatalyzed reaction increases with temperature to a greater extent than the rate of the catalyzed reaction, and the difference between the rate of the catalyzed and uncatalyzed reaction ($\Delta A = \Delta A_s - \Delta A_b$) diminished at high temperatures. So temperature was fixed at 30 °C.

The effect of ionic strength on the sensitivity was studied. The sensitivity was slightly changed with increasing ionic strength of the reaction mixture.

3.2. Effect of manifold variables

Influence of variables such as flow rate, reaction coil and injected volume on the sensitivity was studied.

The peak height depends on the residence time of the sample in the system that is affected by flow rate and reaction coil

Table 1
Interferences effect on the determination of 5.0 ng/mL, Ru(III)

Species	Tolerance limit ($W_{\text{ion}}/W_{\text{Ru(III)}}$)
Na ⁺ , K ⁺ , Ca ²⁺ , Mg ²⁺ , Ag ⁺ , Rb ⁺ , Pb(II), Ni(II), Zn(II), Cu(II), Br ⁻ , Cl ⁻ , C ₂ O ₄ ²⁻ , S ₂ O ₈ ²⁻ , HSO ₄ ⁻ , ClO ₃ ⁻ , CO ₃ ²⁻ , NO ₃ ⁻ tatarate, borate	1000
Co ²⁺ , Mn ²⁺ , Hg ²⁺ , Pd ²⁺	800
Os ⁸⁺ , Rh ³⁺	400
Te ⁴⁺ , Se ⁴⁺ , SCN ⁻	200
Fe ²⁺	50
I ⁻	30

length. The effect of flow rate was tested under optimum chemical conditions. The results show that the best pump flow rate (the maximum peak height and minimum dispersion) will be obtained in 34.0 mL/h for each channel. At lower flow rates the dispersion will be high whereas at greater flow rates the reaction may be incomplete.

The results during the investigation of the effect of reaction coil length shows that by increasing the reaction coil length up to 120 cm, the sensitivity increases. At shorter distances there is not enough time for reagents to be mixed and above this reactor length, increased dispersion will decrease the peak height.

The influence of sample volume was tested under optimum conditions; in the range of 20.0–200 μL . The results show that the peak height rose by increasing the volume of sample loop volume, but the injection of a large amount of sample results in peak broadening and tailing. Thus, a sample volume of 140 μL was selected.

4. Analytical characteristics of the method

Under obtained optimized conditions, ruthenium concentration can be determined in the ranges of 0.1–20.0 ng/mL ($r = 0.9948$) and 20.0–100.0 ng/mL ($r = 0.9950$). The experimental limit of detection is 0.095 ng/mL ($S/N_{p-p} = 3$). The relative standard deviation (R.S.D. %) for 10 replicate measurements of 0.7, 7.0 and 15.0 ng/mL of Ru(III) were 1.84, 1.12 and 0.87%, respectively.

Table 2
Comparison of some methods for determination of ruthenium with proposed method

Method	DL (ng/mL)	LDR (ng/mL)	Reference no.
Spectrophotometry	0.25	0.8–100	16
	30	100–2500	18
	0.33	1.12–300	17
Voltammetry	1000	2000–60000	13
Fluorimetry	0.6	1.0–400	15
Atomic absorption	6	Up to 500	14
Flow injection	2.1	5–200	21
	30	40–600	22
	0.7	1–100	19
	2	5–500	20
Proposed method	0.095	0.4–100	–

Table 3
Determination of Ru(III) in the real samples

Sample	Added (ng/mL)	Found (ng/mL)	Recovery (%)
Human serum	0.0	–	–
	3.0	2.91 ± 0.018	97.0
	5.0	5.21 ± 0.033	104.2
	7.0	6.88 ± 0.028	98.3
	9.0	9.35 ± 0.027	103.9
	0.0	–	–
River water	3	3.05 ± 0.018	101.7
	5.0	5.18 ± 0.035	103.6
	7.0	7.24 ± 0.044	103.4
	9.0	8.84 ± 0.054	98.2
	0.0	–	–
	3.0	3.11 ± 0.024	103.7
Drinking water	5.0	4.88 ± 0.029	97.6
	7.0	7.23 ± 0.049	103.3
	9.0	8.85 ± 0.045	95.0

5. Influence of foreign ions

In this stage the influence of contaminant species presented in various samples on the determination of Ru(III), 20.0 ng/mL, was investigated. The tolerance limit was defined as the concentration of added ions causing a relative error less than 3%. (Table 1). As the results show the developed method is very selective.

6. Response characteristics

In Table 2, response characteristics of the proposed method are compared with recently reported methods. Results in Table 2 show that proposed method has a good linear dynamic range and an excellent detection limit. Though, the method is simple, fast, high reproducible and relatively cheap, its selectivity and sensitivity are also better than the other methods listed in Table 2.

7. Analytical application

The proposed method has been successfully applied to determine Ru(III) in the river, drinking water and human serum samples. The results in the Table 3 represent that good recoveries in all samples were obtained. In order to evaluate the validity of the proposed method for the determination of Ru(III) in the real samples, the ruthenium red and rock samples were analyzed by the proposed method and compared with those obtained with inductively coupled plasma atomic emission spectrometry (Table 4).

Table 4
Analysis of ruthenium in rock and ruthenium red samples

Sample	Ruthenium ($\mu\text{g mL}^{-1}$)	
	I.C.P.AES method	Proposed method
Rock 1 (sulphide ore of copper and nickel)	1.32 ± 0.02	1.35 ± 0.04
Rock 2 (cremate ore)	4.75 ± 0.03	4.82 ± 0.07
Ruthenium red	5.12 ± 0.02	5.07 ± 0.05

8. Conclusion

A simple, rapid and selective flow injection catalytic procedure is developed for the determination of Ru(III) with spectrophotometric detection. This method can be used for the determination of nano amounts of ruthenium with a sample rate of 30 ± 5 samples/h. The main advantages of the method are its simplicity and its large dynamic range which make it possible to determine ruthenium in the real samples with satisfactory results.

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