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HPLC determination of oxalic acid using tris(1,10-phenanthroline)ruthenium(II) chemiluminescence—application to the analysis of spinach

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Abstract

The chemiluminescence (CL) of $Ru(phen)_3^{2+}$ was applied to HPLC determination of oxalic acid. Oxalic acid was separated by a C_{18} reverse-phase column with a mobile phase of 0.02 mol/l NH_4Ac. The eluted oxalic acid was mixed with 0.25 mol/l $Ru(phen)_3^{2+}$ and 2.0 mmol/l $Ce(SO_4)_2$ in 0.08 mol/l H_2SO_4, and then pass through a modified luminometer used as a detector. The reaction of Ce(IV) oxidized $Ru(phen)_3^{2+}$ and oxalic acid emitted light. The detection limit was 6.2×10^{-6} mol/l for oxalic acid at a S/N ratio of 3, the relative standard deviation for 5 replicate injections of 1×10^{-3} mol/l oxalic acid standard was calculated as 5.6%, and the linear calibration range was 1×10^{-5} to 4×10^{-3} mol/l. The method was successfully applied to determination of oxalic acid in spinach.

Keywords: Chemiluminescence; Oxalic acid; Spinach; $Ru(phen)_3^{2+}$; HPLC

1. Introduction

The analysis of oxalate is of great importance in food because of its effect on the human body. High oxalate concentration in the blood or urine accompanies a number of maladies including renal failure, vitamin deficiencies. It has also been implicated in the formation of kidney stones, in this case the precipitation of calcium oxalate within kidney occurs and this can cause renal tissue damage. Vegetables generally contain wide variations in oxalic acid, so that selective and precise methods for the determination of oxalic acid are very important.

Recently a number of methods have been reported for the determination of oxalic acid in various matrices. These include the use of a C8 column (Libert, 1981), a porous graphitic carbon column (Dutton, Rastall, & Evans, 1991) to perform the separation of oxalate from other extracted components. Ion chromatography has been reported for the analysis of oxalate in vegetables (Ishii, 1991), in tea (Duan, Li, & Tu, 1995) and oxalate in alumina process liquors (Barnett, Bowser, & Russell, 1995). Derivatization gas chromatography (Chen, Li, Yang, & Zhang, 1989) and capillary electrophoresis (Trevaskis & Trenerry, 1995) have been reported for the analysis of oxalate in vegetables. Electrogenerated chemiluminescence was used for the determination of oxalate (Rubinstein, Martin, & Bard, 1983). Amperometric enzyme electrode was used for the determination of oxalate in urine (Reddy, Higson, & Vadgama, 1997). The CL method with $Ru(phen)_3^{2+}/Ru(bipy)_3^{2+}$ was used for the determination of oxalate (He, Ma, Luo, Yu, & Zeng, 1996; He, Gao, Yuan, Luo, & Zeng, 1997). CL has high sensitivity and simplicity compared to other techniques and coupled with liquid chromatography provides a reliable means for oxalate determinations. The reaction of $Ru(phen)_3^{2+}$ and acidified cerium(IV) produce luminescence (He et al., 1996, 1997). which was enhanced by the presence of oxalate. The CL intensity is related linearly to the concentration of oxalate.

This work presents the studies on the analysis of oxalic acid in spinach using HPLC coupled with post column CL detection.

2. Experimental

2.1. Reagents

Analytical reagent grade chemicals were used throughout. Solutions were prepared with doubly distilled water.

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$Ru(phen)_3^{2+}$ was prepared in our laboratory (He, Ma, Luo, Yu, & Zeng, 1996). The eluent was 0.02 mol/l ammonium acetate. The optimum post column reagents were 0.25 and 1.0 mmol/l $Ru(phen)_3^{2+}$, 2.0 mmol/l Ce(IV) in 0.08 mol/l H_2SO_4 respectively. Standards were prepared by serial dilution of 0.1 mol/l oxalic acid stock solutions.

2.2. Instrumentation

HPLC was performed using a Waters (Milford, MA 01757, USA) liquid chromatograph equipped with a pump (Waters M510), a sample injector (Waters U6K), a C_{18} reverse phase column (5 μm i.d. 4.6 mm×15 cm Waters). Two pump (China) for post column reagents, a modified luminometer described below and a chart recorder were used.

A schematic representation of the system employed was shown in Fig. 1. $Ru(phen)_3^{2+}$ mixed with eluent at outlet of column by a T-piece, and $Ce(SO_4)_2$ mixed with eluent + $Ru(phen)_3^{2+}$ by a Y-piece of glass tube placed in the measuring chamber of the luminometer, which was used as a flow cell of detector. The eluent flow rate was 1.0 ml/min and that at each post column flow rate was 0.7 ml/min.

2.3. Procedure

After filtering with a membrane (0.45 μm Millipore Co.), the sample and the spiked sample solutions were injected into HPLC with reverse-phase C_{18} column. The concentration of oxalic acid in the sample solution was determined by measuring the peak height from the chart recorder and comparison against a calibration curve. The calibration curve and the operation condition was listed in Table 1 (No.2). The detection limit was measured under the condition No. 1 in Table 1.

3. Results and discussion

3.1. The design and manufacture of the flow cell

Previous CL reactions with $Ru(phen)_3^{2+}$ have shown (He et al., 1997) the reaction kinetics to be rapid. The time required to reach maximum intensity is about 2 s. As a consequence, the mixing of the cerium(IV) solution should be placed at the optimum distance before the PMT. A Y-piece of glass tube with 1.5 mm i.d. was used as a flow cell, which was placed in front of the PMT (Fig. 1). The CL response was optimized by operating the system without a column and measuring the response of a 1×10^{-3} mol/l oxalic acid standard solution (20 μl injection) against a variety of post column reagents flow rates. The eluent flow rate through the column was 1.0 ml/min to avoid over-pressuring the system. Maximum CL intensity was obtained with the post column flow rate between 0.5 and 1.0 ml/min. To minimize reagent consumption the flow rate was standarized at 0.7 ml/min.

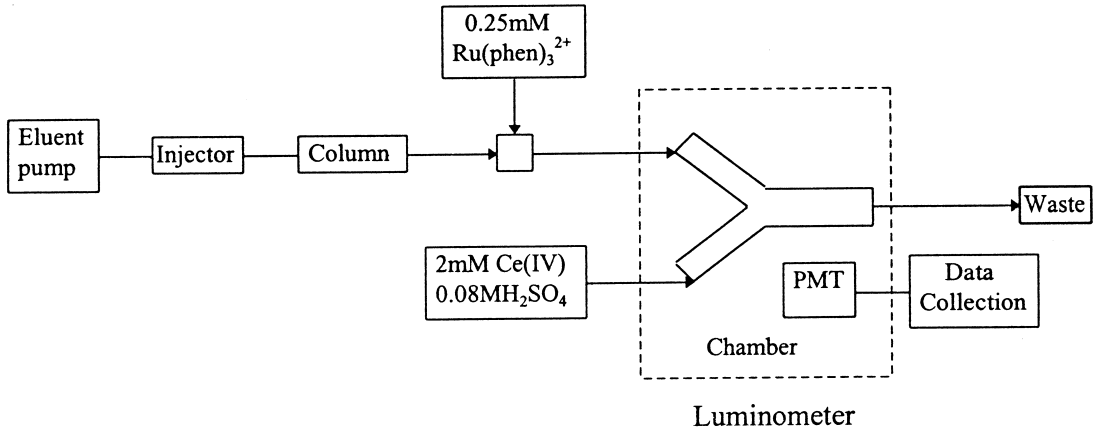

Fig. 1. Post column HPLC with CL detection.

Table 1
Calibration curve and operation condition

No.	Oxalate conc. range (mol/l)	Equation	n	r	$Ru(phen)_3^{2+}$ (mmol/l)	Ce(IV) (mmol/l)	H_2SO_4 (mol/l)	Input range of recorder (mV)
1	1×10^{-5}–4×10^{-4}	$I=2.244+1.212\times10^5C$	6	0.9992	1.0	2.0	0.08	10
2	4×10^{-4}–4×10^{-3}	$I=0.367+1.566\times10^4C$	4	0.9994	0.25	2.0	0.08	20

The length of the flow cell was chosen as 1.0 cm as a compromise to collect the maximum of CL signal without excessive dispersion of longer cells.

3.2. Effect of the concentration of $Ru(phen)_3^{2+}$

The study was carried out with solutions containing various amounts of $Ru(phen)_3^{2+}$. The response of detector increases with increasing concentration of $Ru(phen)_3^{2+}$ as shown in Fig. 2. To minimize reagent consumption 0.25 mmol/l $Ru(phen)_3^{2+}$ was used to measure samples and 1.0 mmol/l $Ru(phen)_3^{2+}$ was used to measure the detection limit.

3.3. Effect of the concentration of Ce(IV) and sulfuric acid

The response of the detector depends on the concentration of Ce(IV), and a study was made in the range 0.5–4.0 mmol/l under standard condition given above. The maximum response was obtained at 2.0 mmol/l Ce(IV) in 0.08 mol/l H_2SO_4, as was shown in Fig. 3.

The response of detector also depends on the concentration of sulfur acid. It was studies in the range 0.04–0.12 mol/l under standard conditions given above. The maximum response was obtained at 0.08 mol/l sulfuric acid [2.0 mmol/l Ce(IV)], as was shown in Fig. 4.

3.4. The determination of oxalic acid in spinach

The spinach sample was treated using the combination of references (Chen et al., 1989; Duan et al., 1995; Ishii, 1991). It was washed with tap water and distilled water followed by drying in oven at 80°C for 2 h, and then ground to powder with mortar box. 0.2 g of spinach powder and 100 ml boiling distilled water were transferred into a 250 ml flask. The pH was adjusted to 1–2 with 2 mol/l HCl. After half an hour, it was put into boiling bath and kept the bath at 100°C for 90 min, and then cooled to room temperature. The sample solution was filtered into 100 ml volumetric flask with filter paper, washed with water, and diluted to volume. The final concentration of solution should be in the range of the calibration.

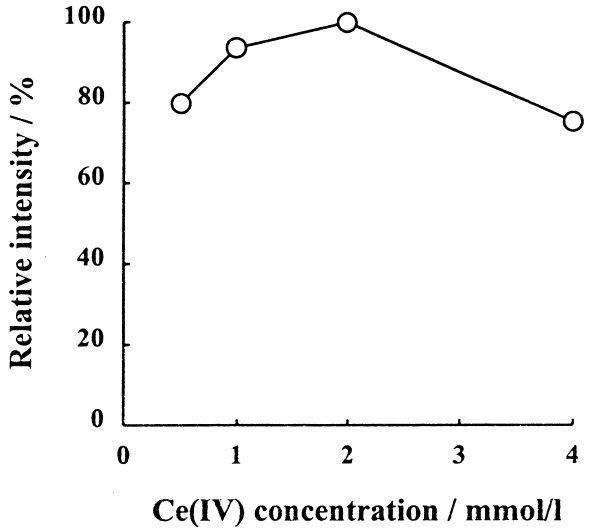

Fig. 3. Effect of the concentration of Ce(IV) in 0.08 mol/l H_2SO_4 on the emission intensity from 1×10^{-3} mol/l oxalic acid in the presence of 0.25 mmol/l $Ru(phen)_3^{2+}$.

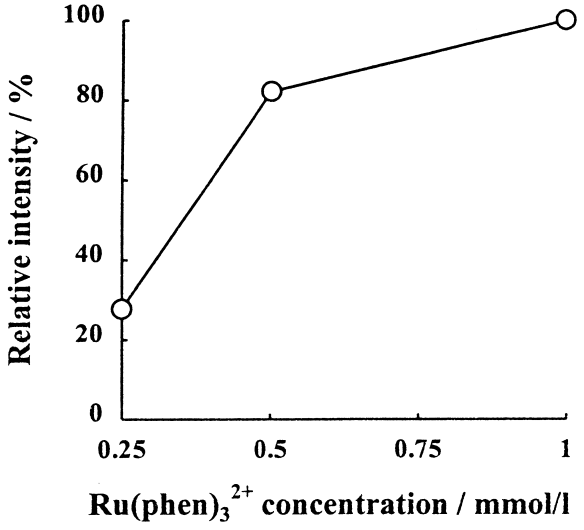

Fig. 2. Effect of the concentration of $Ru(phen)_3^{2+}$ in 2 mmol/l Ce(IV) and 0.08 mol/l H_2SO_4 on the emission intensity from 1×10^{-3} mol/l oxalic acid.

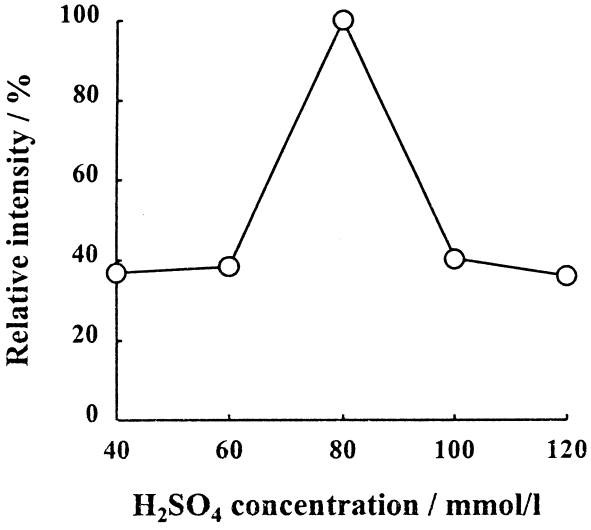

Fig. 4. Effect of the of sulfuric acid in 2 mmol/l Ce(IV) on the emission intensity from 1×10^{-3} mol/l oxalic acid in the presence of 0.25 mmol/l $Ru(phen)_3^{2+}$.

Spiked samples were prepared by adding 0.5, 1.0 ml of 0.1 mol/l oxalic acid into samples and treated as samples.

The calibration curves were obtained for standards solution as was shown in Table 1. The detection limit at signal to noise ratio of 3 was 6.2×10^{-6} mol/l. The relative standard deviation for five replicate injections of 1×10^{-3} mol/l oxalic acid standard was calculated as 5.6%. The recoveries for two levels are 92–102% (5×10^{-4} mol/l added) and 95–102% (1×10^{-3} mol/l added). Oxalic acid content in spinach is 4.2 mg/g, which is consistent with the result reported by Chen et al. (1989).

4. Conclusion

HPLC with post column CL detection has been successfully demonstrated as a technique for the determination of oxalic acid in spinach. It has higher sensitivity; the recoveries were good enough for practical use.

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