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High-Performance Liquid Chromatographic Determination of Oxalic Acid in Tea Using Tris(1,10-phenanthroline)-ruthenium(II) Chemiluminescence

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The chemiluminescence (CL) of $Ru(phen)_3^{2+}$ was applied to HPLC determination of oxalic acid, which was separated by a C_{18} reverse-phase column with a mobile phase of 0.02 mol/l NH_4Ac, mixed with 0.25 mol/l $Ru(phen)_3^{2+}$ and 2.0 mmol/l $Ce(SO_4)_2$ (0.08 mol/l H_2SO_4), and then passed through a homemade CL detector. The reaction of Ce(IV) oxidized $Ru(phen)_3^{2+}$ and the 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 tea.

Keywords Oxalic acid, chemiluminescence, $Ru(phen)_3^{2+}$, high-performance liquid chromatography, tea

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 and vitamin deficiencies. Tea is one of the most popular beverages in the world.[1] The concentration of oxalic acid in tea is high. So over-drinking of tea is a key factor in the formation of kidney stones; thus selective and precise methods for the determination of oxalic acid are very important. A number of methods for the determination of oxalic acid, for example, the electrogenerated CL[2], derivatization gas chromatography[3], HPLC[4,5], ion chromatography[6-8], capillary electrophoresis[9], amperometric enzyme electrode[10], as well as the CL method with $Ru(phen)_3^{2+}$/$Ru(bipy)_3^{2+}$ and cerium(IV) have been reported.[11,12]

Most of the methods require a preliminary separation of oxalic acid from the biological matrix, or lack of the sensitivity, selectivity or simplicity. CL has high sensitivity and simplicity. The coupling of CL with liquid chromatography provides a sensitive and selective means of detection. $Ru(phen)_3^{2+}$ is also a useful CL reagent.[12] Compared with $Ru(bipy)_3^{2+}$, it has higher enhancement and sensitivity.[13] This work presents some studies on the analysis of oxalic acid in tea using HPLC coupling with postcolumn CL detection.

Experimental

Reagents and chemicals

Analytical reagent grade chemicals were used throughout. Solutions were prepared with doubly distilled water. $Ru(phen)_3^{2+}$ was prepared in our laboratory.[11] The eluent was 0.02 mol/l NH_4Ac. The postcolumn reagents were 0.25 and 1.0 mmol/l $Ru(phen)_3^{2+}$ and 2.0 mmol/l Ce(IV) (0.08 mol/l H_2SO_4). Standards were prepared by serial dilution of 0.1 mol/l oxalic acid stock solutions.

Apparatus

HPLC was performed using a Waters 209 liquid chromatograph equipped with a pump (Waters M510), a U6K sample injector (Waters) and a C18 reverse phase column (5 μm i.d. 4.6 mm×15 cm). A modified luminometer and a pen recorder were used.

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

Procedure

First 0.6 g of ground tea and 200 ml of boiling distilled water were transferred into 250 ml flask. The pH

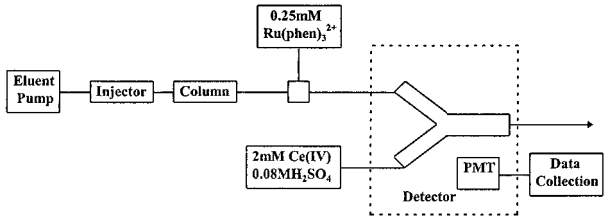

Fig. 1 Postcolumn HPLC with CL detection.

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was adjusted to 1 – 2 with 2 mol/l HCl. After half an hour, the mixture was put into a boiling bath and kept boiling for 90 min, and then cooled to room temperature.[7,8] The solution was filtered, and diluted to 250 ml.

Spiked samples were prepared by adding 0.5 or 1.0 ml of 0.1 mol/l oxalic acid into a sample flask. They were injected into HPLC combined with a C18 reverse phase column after filtering with a membrane (0.45 μm Millipore Co.). The concentration of oxalic acid in the sample solution was determined by measuring the peak height of the liquid chromatogram of oxalic acid using a calibration curve.

Results and Discussion

The designation and manufacture of CL detector

Previous CL reactions with $Ru(phen)_3^{2+}$ have shown[11] the reaction kinetics to be rapid. The time required to reach maximum intensity is about 2 s. As a consequence, the mixing point should be placed at the minimum distance from 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 (see 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 postcolumn reagent flow rates. The eluent flow rate was maintained at 1.0 ml/min as it was generally a suitable flow rate. Maximum CL intensity was obtained with the postcolumn flow rate between 0.5 – 1.0 ml/min. To minimize reagent consumption the flow rate was standarized at 0.7 ml/min.

The length of detection area on the flow cell was set at 1.0 cm. The maximum of CL intensity will flow out of the detecting area if the length is too short. The peak will be wide if the length is too long.

The retention time of oxalic acid is 2 min. The theoretical plate number of the column is about 1000.

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 the detector increases with increasing concentration of $Ru(phen)_3^{2+}$; as is 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.

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

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

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

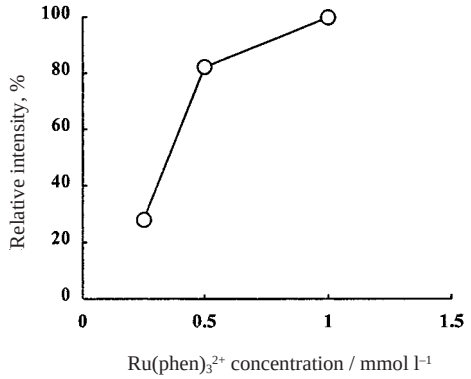

Fig. 2 Effect of the concentration of $Ru(phen)_3^{2+}$ in 2 mmol/l Ce(IV) and 0.08 mol/l H_2SO_4 on the emission intensity from 1×10^{-3} mol/l oxalic acid.

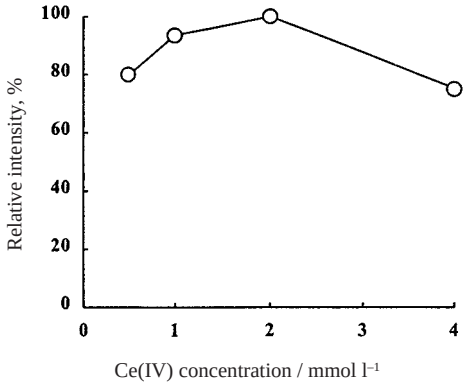

Fig. 3 Effect of the concentration of Ce(IV) in 0.08 mol/l H_2SO_4 on the emission intensity from 1×10^{-3} mol/l oxalic acid in the presence of 0.25 mmol/l $Ru(phen)_3^{2+}$.

Effect of foreign compounds

The other organic acids, for example, citric acid, tartaric acid, or ascorbic acid have no effect on the determination of oxalic acid, because they have lower enhancement compared with oxalic acid[12] and are separated by HPLC.

Calibration and detection limit

Calibration curves were obtained for standards ranging in concentration from C_1: 1×10^{-5} to 4×10^{-4} mol/l, and C_2: 4×10^{-4} to 4×10^{-3} mol/l. The curves were linear with the equations $I_1=2.244+1.212\times10^5C_1$, $r=0.9992$, $n=6$, $Ru(phen)_3^{2+}=1.0$ mmol/l, $[Ce(IV)]=2.0$ mmol/l, $[H_2SO_4]=0.08$ mol/l, the input range of the recorder=10 mV; $I_2=0.3671+1.566\times10^4C_2$, $r=0.9994$, $n=4$, $Ru(phen)_3^{2+}=0.25$ mmol/l, $[Ce(IV)]=2.0$ mmol/l, $[H_2SO_4]=0.08$ mol/l, the input range of the recorder=20 mV. The detection limit at signal to noise ratio of 3 was 6.2×10^{-6} mol/l. The relative standard deviation for 5 replicate injections of 1×10^{-3} mol/l oxalic acid standard was calculated as 5.6%. The recoveries were

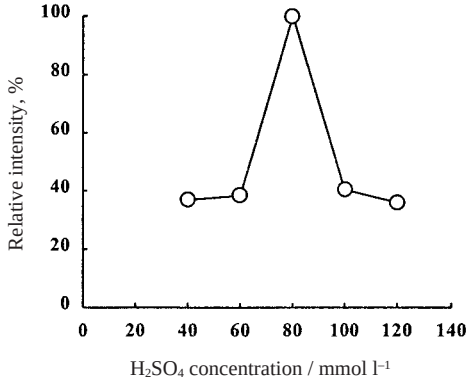

Fig. 4 Effect of the of sulfuric acid in 2 mmol/l Ce(IV) on the emission intensity from 1×10^{-3} mol/l oxalic acid in the presence of 0.25 mmol/l $Ru(phen)_3^{2+}$.

good enough for practical use; all of the determination results are listed in Table 1. Oxalic acid content in tea is 18.23 mg/g.

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Table 1 Determination of oxalic acid in tea

Tea solution contents/10^{-4}mol l^{-1}	Added/ 10^{-4}mol l^{-1}	Found/ 10^{-4}mol l^{-1}	Recovery, %
3.47	4.0	7.81	108.5
		7.68	105.2
		7.37	97.5
	8.0	11.39	99.0
		11.01	94.2
		11.84	104.6

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