DETERMINATION OF ASCORBIC ACID BY FLOW INJECTION CHEMILUMINESCENCE METHOD WITH NOVEL RHODANINE

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INTRODUCTION

Ascorbic acid is important in nutrition and is provided by the diet. Overdose of ascorbic acid will cause abdominal pain, diarrhea, even diabetes and kidney stones. Many methods based on different principles have been used for the determination of ascorbic acid including iodometry,1 spectrophotometry,2 spectrofluorimetry,3 HPLC,4 and electrochemical methods.5 However, they often suffer from a variety of limitations. As a new analytical technique, flow injection chemiluminescence has aroused extensive interest, because of its wide linear range, high sensitivity, and convenient application. Rhodanine and its derivatives are important organic reagents, which were mainly used in spectrophotometry.2 Recent studies have shown that rhodanine and its derivatives are good fluorescent reagents.6 Articles about rhodanine derivatives for chemiluminescence reaction are rarely reported.

In this present work, it was found 2NRASP could be used as a chemiluminescent reagent in the 2NRASP- KMnO4-HCl system, and the CL intensity decreased when ascorbic acid was added to the mixture. Based on this, a new, simple and rapid method was established for the determination of ascorbic acid. The mechanism of the CL reaction is proposed. Furthermore, the method has been used for the determination of ascorbic acid in tablets and fruit with satisfactory results.

MATERIALS AND METHODS

All chemicals were of analytical reagent grade and were used without further purification. All solutions were prepared with distilled deionised water. 2NRASP was synthesized by our own laboratory. The stock solution of 2NRASP (2.000×10⁻⁴ mol/L) was obtained by dissolving 2NRASP in dehydrated alcohol (Laiyang Reagent Ltd., China). The standard solutions were prepared by diluting stock solution with water appropriately. The stock solution of ascorbic acid (Tianjin North Tianyi Chemical Reagent Company, China) (100 μg/mL) was stored in a
refrigerator. Working standard solutions were prepared daily from the stock solution by appropriate dilution immediately before used. Potassium permanganate solution was prepared by dissolving an appropriate amount of potassium permanganate (Yao Shun Import & Export Co, Ltd. China) in water. The hydrochloric acid was purchased from Shanghai Lianshi Reagent Ltd., China. The flow system used in this work is shown in Fig. 1. The fluorescence spectrophotometer (P.E Company, U.S) and UV–Vis spectrophotometer TU-1901 (Beijing Puxi instrument Ltd., China) are used for the mechanism study.

![Diagram of Flow-Injection CL system]

**Fig. 1.** Scheme of Flow-Injection CL system.

(a) Sample or blank solution; (b) 2NRASP; (c) Potassium permanganate; (d) hydrochloric acid; (P) Peristaltic pump; (V) injection valve; (F) CL flowcell; (PMT) photomultiplier tube; (AMP) ultra-weak chemiluminescence analyzer; (PC) personal computer; (NHV) negative high voltage.

**Procedure.** As Fig.1 shows, channel (a) and (b) was used to deliver ascorbic acid sample or blank solution and rhodanine solution respectively at 4.5 mL/min; while channel (c) and (d) was used to deliver potassium permanganate solution and HCl solution respectively at 4.5 mL/min. The negative high voltage was ~400 mv. The measuring chamber was kept at a constant temperature of 25°C.

**RESULTS AND DISCUSSION**

**CL intensity spectrum.** According to experimental technique, chemiluminescence dynamic curve of various systems was determined. As shown in Fig.2, after sampling 3.5s, chemiluminescence signal is produced and 2.8s later, chemiluminescence signal peaks. Then after 4.2s, the signal returns to baseline values. This illustrates that the chemiluminescence reaction is a rapid process. Potassium permanganate in hydrochloric acid cannot produce a CL signal. After adding ascorbic acid, a weak CL signal can be detected. The system 2NRASP-KMnO₄-HCl can produce a very strong signal. However, the CL signal becomes much weaker when ascorbic acid is added. With increasing amounts of ascorbic
acid, the CL signal continues to be weak. So a new kind flow injection chemiluminescence method for determining ascorbic acid was established

![Graph](image)

**Fig. 2** The change of the CL intensity

1: HCl+KMnO₄ 2: HCl+KMnO₄+Vc (10μg/mL) 3: 2NRASP+HCl+KMnO₄+Vc (10 μg/mL) 4: 2NRASP+HCl+KMnO₄+Vc (5 μg/mL) 5: 2NRASP+HCl+KMnO₄

**Optimum conditions.** In this study, the following oxidants were tested in 1.0 mol/L HCl: KMnO₄ (2.5 x 10⁻⁴ mol/L), Ce(SO₄)₂ (0.01 mol/L), K₂S₂O₈ (0.01 mol/L), and K₃Fe(CN)₆ (0.01 mol/L) was tested in 0.1 mol/L NaOH. KMnO₄ (HCl) was chosen for subsequent use. The concentration of HCl was investigated from 1.2 to 3.0 mol/L in the flow injection system. 1.5 mol/L was selected for further experiments. The effect of KMnO₄ concentration on the CL intensity was studied from 1.0 x 10⁻¹ to 2.5 x 10⁻¹ mol/L. 2.0 x 10⁻¹ mol/L was selected. By varying 2NRASP concentration range, it was observed that with the increase of 2NRASP concentration the luminescence intensity increased until 6.0 x 10⁻⁴ mol/L. 6.0 x 10⁻⁴ mol/L was selected for the further experiments. The effect of flow rate was tested in the range of 2.5-6.0 mL/min for each stream. It was discovered that the best signals were obtained at 4.5 mL/min flow rate of each stream.

**Method performance.** Under the optimum conditions as described above, a linear calibration curve, within the range 0.2-10 μg/mL, was obtained. This data is described by the equation: \( \Delta I = -315 + 206 \rho \) (ρ:μg/mL), giving a correlation coefficient of 0.995 (n = 5). The detection limit (3σ) is 0.02898 μg/mL. And the relative standard deviation \((\sigma = 11)\) for 5 μg/mL ascorbic acid standard is 1.5%.

**Interference study.** The influences of foreign species were examined by analyzing a standard solution of 1μg/mL ascorbic acid to which increasing amounts of interfering species were added. The tolerable concentration ratios for interference at 5% level were over 1000 for sucrose, lactose, K⁺, Na⁺, Cl⁻, F⁻, NO₃⁻; 500 for
glucose, starch, cyclodextrin, Cd\textsuperscript{2+}, 300 for fructose, 40 for Ca\textsuperscript{2+}, Co\textsuperscript{2+}, 30 for Cs\textsuperscript{+}, Al\textsuperscript{3+}, tartaric acid, respectively.

**Applications.** Sample Yinqiao Vitamin C tablet used for this work was purchased from Guizhou Bailing Ltd, China. A total of 20 tablets of Yinqiao Vitamin C were accurately weighed individually, grounded and mixed well. Weighed 0.0500 g powder and dissolved in water. After filtering, the filtrate was diluted to a 500ml volumetric flask. The proposed method was applied to determine ascorbic acid in the sample. The recovery rates of the method is 96.6\% and 96.3 \%; RSD was $\pm 3.00\%$ which indicated the results were satisfactory. As we can conclude that the recoveries of added ascorbic acid can be quantitative and \( t \)-tests assumes there was no significant differences between recovery efficiency and 100 \% at confidence level of 95 \%.

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**REFERENCES**