Bull. Chem. Soc. Ethiop. **2009**, 23(1), 113-116. ISSN 1011-3924
Printed in Ethiopia

SHORT COMMUNICATION

DETERMINATION OF IRON IN CHINESE HERBAL MEDICINE BASED ON THE FLUORESCENCE QUENCHING OF RHODAMINE 6G

Yilin Wang*, Jianping Lu and Hong Yang

College of Chemistry and Chemical Engineering, Guangxi University, Nanning 530004, China

(Received August 01, 2008; revised December 4, 2008)

ABSTRACT. A fluorescence quenching method was developed for determination of Fe^{3+} with rhodamine 6G as fluorescent reagent. Fe^{3+} reacting with KI in diluted sulfuric acid produced I_3^- anion which reacted subsequently with rhodamine 6G to form a non-fluorescence compound. As a result, the compound quenched the fluorescence intensity of the system. The fluorescence intensity decreased linearly with the Fe^{3+} concentration in the range from 20 to 200 μg/L. The detection limit was 8.3 μg/L. This method has been applied to determine the iron in Chinese herbal medicine with satisfactory results.

KEY WORDS: Rhodamine 6G, Fluorescence quenching, Iron

INTRODUCTION

Iron is one of essential elements for living beings, and the determination of trace iron in real samples is very important. There are several techniques for iron determination, for example, spectrophotometry [1, 2], atomic spectroscopy [3, 4] and chemiluminescence method [5, 6] are some of them. Rhodamine 6G (R6G) is one type of good analytical reagents that have been used for determination of Cr^{3+} and IO_3^- [7, 8]. However, there has been no report on determination of Fe^{3+} using R6G so far. In this paper, a novel fluorescence analytical method for determination of Fe^{3+} has been established based upon the investigation of the system of Fe^{3+}-KI-R6G. This method has some advantages such as simplicity, high sensitivity and good selectivity, and it has been applied to determine the iron in Chinese herbal medicine with satisfactory results.

EXPERIMENTAL

Apparatus and reagents. A 960CRT spectrofluorophotometer (Shanghai Precision & Scientific Instrument Co., Ltd, China) was used for fluorescence measurements. Analytical reagent grade Fe_2O_3 (Tianjin Damao Chemical Reagent Factory, China), rhodamine 6G (Guangzhou Chemical Reagent Factory, China), KI (Shanghai Shenbo Chemical, Co., China), H_2SO_4 (Guangdong Guanghua Chemical Factory Co., China), polyvinyl alcohol-124 (Guangdong Guanghua Chemical Factory Co., China) were used as received. Doubly distilled water was used. *Angelica*, *codonopsis pilosula* and *astragalus* were bought from a local drugstore.

Procedure. A 1.00 mL of 0.1 M KI solution, certain volume of 1.00 mg/L Fe^{3+} solution were added to a 25 mL standard volumetric flask, the solution was diluted to 12.5 mL with doubly distilled water, then 2.00 mL of 0.1 M sulfuric acid was added, and the solution was mixed thoroughly. After 10 min, 2.00 mL of 5×10^{-5} M rhodamine 6G and 1.00 mL of 0.1 % polyvinyl alcohol-124 was added successively, and then the solution was diluted to the mark. The fluorescence intensity of the solution was measured at 553 nm with excitation at 365 nm.

*Corresponding author. E-mail: ylinwang2002@yahoo.com.cn

RESULTS AND DISCUSSION

Emission spectra. The fluorescence spectra of the system in the presence of Fe^{3+} and in the absence of Fe^{3+} are shown in Figure 1. As can be seen, the maximum emission wavelength of R6G was at 553 nm, however, in the presence of Fe^{3+}, the fluorescence intensity decreased but the maximum emission peak remained unchanged, and the higher the Fe^{3+} concentration, the lower the fluorescence intensity. Based on this, the concentration of Fe^{3+} could be measured, and 553 nm was therefore chosen as operating wavelength.

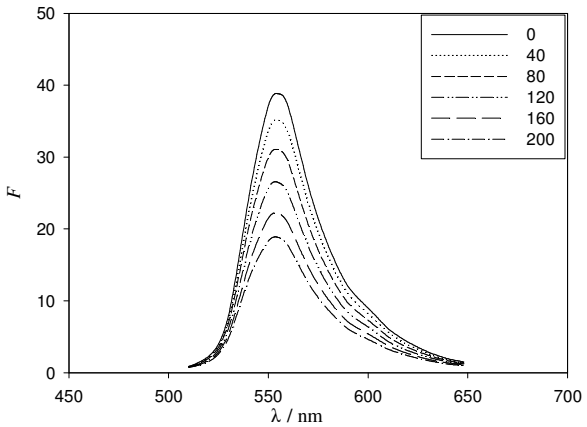


Figure 1. Fluorescence emission spectra of rhodamine 6G (from top to bottom, the concentrations of Fe^{3+} were: 0, 40, 80, 120, 160, 200 μg/L, respectively).

Effect of acidity. The acidity of the solution affected the Δ*F* values. The effect of the sulfuric acid concentration was examined by varying the content of acid in the final solution. When the volume of H_2SO_4 was 2.00 mL, the Δ*F* value changed greatly. All subsequent investigations were carried out in 25 mL of the final solution with 2.00 mL of 0.1 M H_2SO_4.

Effect of KI volume. When 0.00, 0.50, 1.00, 1.50, 2.00 and 2.50 mL of 0.1 M KI were added, respectively, the influence of KI concentration on Δ*F* was also studied. The results indicated that the Δ*F* value of the systems gradually increased with the increase of KI concentration at the rang from 0.50 to 1.00 mL, and when the volume of KI exceeded 1.00 mL, Δ*F* value kept relatively stable with the increase of KI volume. Hence, 1.00 mL of 0.1 M KI was chosen for further studied.

Effect of PVA-124 volume. Polyvinyl alcohol-124 is a common surface-active agent in rhodamine photometric analysis [9]. The effect of PVA-124 on Δ*F* was studied and the results obtained indicated that the Δ*F* value increased with the addition of PVA-124, and in the range of 0.50-1.50 mL, the Δ*F* remained constant, So 1.00 mL of 0.1 % PVA-124 was chosen.

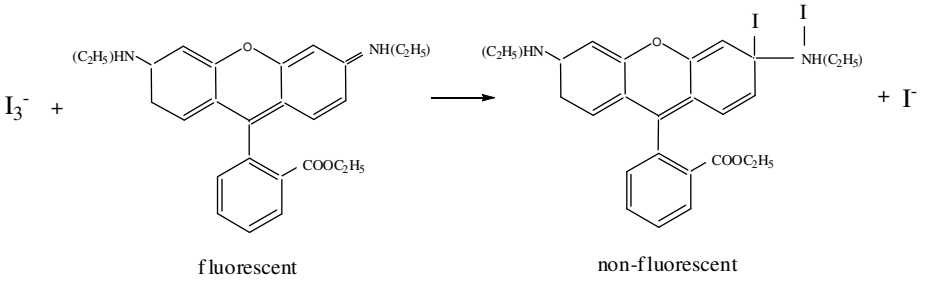
Effect of reaction time. The influence of reaction time on fluorescence quenching value was investigated. After all reagents had been added, fluorescence measurement was performed every 5 min. It was found that the fluorescence quenching value reached the maximum after 5 min, and it remained stable within 30 min. If the system was placed for longer than half hour,

precipitate occurred. Therefore, after placing the system for 10 min its fluorescence intensity was measured.

Effect of R6G concentration. The influence of R6G concentration on the degree of quenching was studied. The results obtained indicated that, when the volume of R6G was less than 1.50 mL, the degree of quenching increases gradually as R6G increases. However, if R6G volume is more than 2.00 mL, the degree of quenching decreases gradually. When R6G volume is in the range of 1.50-2.00 mL, the degree of quenching reached its maximum. Therefore, 2.00 mL of R6G was chosen for the study.

Reaction mechanism. Fluorescence quenching refers to any process which decreases the fluorescence intensity of a sample. A variety of factors can result in quenching. These include inner-filter effect of fluorescence [10], fluorescence resonance energy transfer [11] and charge transfer [12]. We think the quenching mechanism of R6G in the system of Fe^{3+}-KI-R6G is charge transfer. Our experiments indicated that in diluted sulfuric acid medium, Fe^{3+} could hardly quench the fluorescence of R6G, KI could quench it a little. However, in the same condition, I_3^- could quench the fluorescence of R6G greatly. Therefore, the quenching mechanism in this paper could be described by following reactions:

$$2Fe^{3+} + 3I^- = 2Fe^{2+} + I_3^-$$



fluorescent non-fluorescent

An addition reaction occurs between I_3^- anion and R6G at the C=N double bond, because of the heavy atom effect from iodine atom [13], the fluorescence of R6G is quenched.

Calibration graph and precision. The calibration graph for the determination of Fe^{3+} was constructed under the selected conditions. The fluorescence quenching value was proportional to the Fe^{3+} concentration in the range from 20 to 200 μg/L. The linear regression equation was $\Delta F = 0.1251C + 0.4814$, where ΔF is the fluorescence quenching and C represents the concentration of Fe^{3+} (the unit of C is μg/L), the correlation coefficient was 0.9983. The detection limit, based on three times the standard deviation of the blank, was 8.3 μg/L. The relative standard deviation for six replicate determinations was 1.0 % for 40 μg/L Fe^{3+}.

Effects of coexistence ions. According to the procedure, when the relative error was less than ± 5%, the tolerance limit of some coexistence ions for the determination of 40 μg/L Fe^{3+} were examined. The results showed that 500 folds of Ca^{2+}, NH_4^+; 300 folds of Mg^{2+}; 100 folds of Al^{3+}, Zn^{2+}, Co^{2+}; 50 folds of Ba^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and 10 folds of Cu^{2+} had no interference on the determination. From the results, it implies that this method has a good selectivity.

Analytical application. Commercially available Chinese herbal medicine *angelica*, *codonopsis pilosula* and *astragalus* were dried and grinded, each was sampled of 0.5000 g, then, it was

transferred into a crucible. Being carbonized on hot plate, the sample was incinerated in Muffle furnace at 620 ^{o}C for 4 hours. When the sample was cooled to room temperature, 2.00 mL of 6 M HCl and a few drops of H_2O_2 were added, the mixture was evaporated to dry, then 2.00 mL of 6 M HCl was added again, the solution was transferred into a 100 mL volumetric flask and diluted to the mark.

A suitable volume of sample solution was added to a 25 mL tube, the content of Fe^{3+} was determined according to the proposed technique. The results are given in Table 1. It can be seen that the results acquired in fluorescence method are in good agreement with those obtained by inductive coupled plasma atomic emission spectrometry (ICP-AES) performed for comparison. So it is proposed that this technique could be applied to the practical determination of iron in real samples.

Table 1. Analytical results of samples (n = 5).

Samples	Found (μg/g)	Average (μg/g)	RSD (%)	ICP-AES (μg/g)
Angelica	205.6, 198.1, 203.7, 205.9, 199.2	202.5	1.8	207.8
Astragalus	183.7, 181.2, 178.4, 182.3, 179.6	181.0	1.2	176.3
Codonopsi spilosula	165.6, 164.3, 158.8, 165.9, 157.7	162.5	2.6	159.7

AKNOWLEDGEMENTS

Financial support from the scientific research fund of Guangxi University (No. X081057) is gratefully acknowledged.

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