SIMPLE AND SENSITIVE DETECTION FOR TRACE CHROMIUM (VI) USING ALBUMIN AS FLUORESCENCE PROBES

HUI-YAN KUANG, XIAO-YUN YUAN, LEI FENG, HAO-WEN HUANG, CHUN-RAN TANG, KE-QIN DENG, YUN-LONG ZENG*

(School of Chemistry and Chemical Engineering, Hunan University of Science and Technology, Key Laboratory of Theoretical Chemistry and Molecular Simulation of Ministry of Education of China, Xiangtan 411201, PR China)

ABSTRACT

We have developed a novel method for fast determination of chromium(VI) using egg albumin(EAB) as fluorescence probe. The influence factors, including pH, reaction time, concentration of EAB, were investigated on the performance of the method and the optimum experimental conditions were determined. Under the optimum conditions, the relative fluorescence intensity of albumin was linearly proportional to Cr(VI) over a concentration range from 1.0×10^{-7} to 6.0×10^{-5} mol L⁻¹ with a correlation coefficient of 0.9936 and a detection limit of 5.2 nmol L⁻¹. This is a fast, simple, high selective and environment-friendly method, which has been employed to detect trace Cr(VI) in drinking water samples with satisfactory. The infrared spectral information indicated that the possible mechanism of fluorescence quenched may be the change of the secondary structures of EAB in the presence of Cr(VI).

Key words: Trace chromium(VI); fast analysis; egg albumin; fluorescence probe; drinking water

1. INTRODUCTION

In recent years, significant progress has been realized on the impact of pollution on the environment and human health. Water, food and medicine are the possible sources of contaminants such as chromium. Chromium exists in two oxidation states, Cr(III) and Cr(VI) in solution, which have contrasting physiological effects. Cr(III) is known as an essential trace element for maintaining normal physiological function. However, Cr(VI) is highly carcinogenic and mutagenic [1-3], and can cause various of clinical problems, such as immediate cardiovascular shock, negative effects on kidney, liver, and other organs [4]. Hence, the research has drawn much attention in detection of trace Cr(VI) in drinking water recently. Many new methods to detect trace Cr(VI) have been developed, including spectrophotometric method [5,6], fluorescence probe method[7-9], electrochemical method [10,11], ion chromatography [12], inductively coupled plasma mass spectrometry [13], inductively coupled plasma-optical emission spectrometry [14], atomic absorption spectrometry [15]. These methods need either expensive instruments or long analysis time, or consumption of a great deal of reagents [16]. Therefore, developing simple and environment-friendly method for selective, sensitive and rapid determination of trace chromium is still a great challenge.

Protein is a biodegradable and environment-friendly reagent and has widely used in biochemistry and analytical chemistry [17-19]. Egg albumin (EAB) is a cheap, environment-friendly, and egg resources are very rich (available in abundance in egg). But the research of this reagent in analytical chemistry was seldom reported. In this work, we find that EAB shows high selective for Cr(VI), and then have developed a novel method for fast determination of trace chromium(VI) based on the fluorescence quenched of EAB by Cr(VI).

2. EXPERIMENTAL

2.1 Apparatus

Fluorescence measurements were performed using Hitachi F-4500 fluorescence spectrophotometer (Tokyo, Japan) with a xenon discharge lamp, 1cm quartz cells at room temperature. Infrared spectra and UV-visible spectra were recorded using Spectrum One Spectrophotometer and Lamaba 35 UV-Vis Spectrophotometer (Perkin-Elmer, American), respectively.

2.2 Reagents

Egg albumin and K₂Cr₂O₇ (Standard Reagent) were purchased from China Pharmaceutical Group (Shanghai, China). Stock standard aqueous solution of K₂Cr₂O₇ was prepared at concentration of 0.0050 M, and the other concentration of K₂Cr₂O₇ was prepared by diluting the stock standard solution with double distilled water. All other chemicals used were of analytical grade without further purification. Doubly distilled water was used for all chemical procedures.

2.3 Detection of chromium(VI)

Chromium(VI) determination was carried out by successively adding 100 ml varying concentration of Cr(VI) or sample solution into 10 ml 0.40 mg EAB KH₂PO₄-Na₂HPO₄ buffer solution(PBS) solution (pH 9.18). The fluorescence

intensity of the solution was recorded at the peak wavelength (about 345 nm). All measurements were made under room temperature.

3. RESULTS AND DISCUSSION

3.1 Selection of excitation wavelength

Emission and excitation spectra of EAB (a,b) and UV-vis absorption spectra of EAB and K₂Cr₂O₇ (c,d) are shown in figure 1, respectively. There is continuous excitation spectrum of EAB (see Fig. 1 curve b) with a maximum peak locates at 295 nm. When the excitation is set at 295 nm, the albumin has a broad, strong and symmetric emission (curve a in Fig.1) with the maximum fluorescence at 345 nm. As known, potassium dichromate absorbs UV light, which may cause the inner filter and can appear error. Hence, the characteristic of UV-vis absorption spectrum of potassium dichromate was investigated. As seen from Fig.1 (curve d), there are UV-vis absorption peaks of potassium dichromate locating at about 255 nm and 354 nm. Fortunately, the absorption is very weak at the wavelength range from 295 nm to 315 nm. So the fluorescence emission at 345 nm was used for quantitative analysis, and the excitation was set at 295 nm in the following experiments.

Fig. 1. Emission and excitation spectra of EAB (a,b) and UV-vis absorption spectra of EAB and K₂Cr₂O₇ (c,d).

Concentration, egg albumin: 50 mg L⁻¹, potassium dichromate: 3.0 mg L⁻¹.

3.2 Influence of ions on the fluorescence of the albumin

The effect of ions on the fluorescence intensity of EAB was studied and

the results were shown in figure 2. It can be seen that 100 mmol L⁻¹ of these relevant ions (including Ba²⁺, Mg²⁺, Pb²⁺, Mn²⁺, Zn²⁺, Co²⁺, Ag⁺, Fe³⁺, Al³⁺, Fe²⁺, Ni²⁺, Cu²⁺ and Cr³⁺) do not produce noticeable effect on the fluorescence emission (345 nm) of albumin except Cu²⁺ ion. So EAB fluorescence probe is very suitable for Cr(VI).

Fig. 2 Effect of ions on the fluorescence intensity of EAB, the concentrations of the cations: 100 μmol L⁻¹.

3.3 Influence of pH

Buffers such as Tris-HCl, KH₂PO₄-Na₂B₄O₇, and KH₂PO₄-Na₂HPO₄ (PBS) were used to adjust the pH value of the solution, and a satisfactory result for the detection of Cr(VI) was obtained in PBS.

The influence of the solution pH on the fluorescence intensity of EAB was investigated in the presence of Cr(VI) in pH range of 6–10. When the solution of pH value is 9.18, the method has a wider linear concentration range and lower detection limit. Hence, pH 9.18 PBS was used to adjust the pH of the solution in the following experiments.

3.4 Selection of the concentration of egg albumin

The influence of albumin concentration on the fluorescence intensity of the solution was investigated in the presence of 50 mmol L⁻¹ Cr(VI). When the concentration of EAB was 40 mg L⁻¹, the change of fluorescence intensity of the solution reached the maximum (see Fig.3), which indicated that there was high sensitive to Cr(VI) assay in this albumin concentration. So this concentration of EAB was used in the following experiments.

Fig.3 Effect of concentration of EAB on the quenching of the fluorescence at 50 μmol L⁻¹ of Cr(VI).

3.5 Influence of reaction time

The effect of reaction time on the relative fluorescence intensity of the albumin in the presence of Cr(VI) (0, 10, 50, and 100 μmol/L respectively) was

investigated. As shown in Fig.4, the fluorescence intensity of EAB decreases rapidly and when Cr(VI) was added into the albumin solution. The fluorescence intensity reaches the lowest immediately and keeps unchanged more than 50 min. So the reaction time was fixed at 5 min.

Fig. 4 Effect of reacting time on fluorescence intensity of EAB, the concentrations of Cr(VI), (a) 0 (b) 10 (c) 50 and (d) 100 μmol L⁻¹.

3.6 Calibration curve and detection limit

Under optimum conditions, a linearity was observed (insert of Fig. 5) in the Cr(VI) concentration range from 1.0×10^{-7} to 6.0×10^{-5} mol L⁻¹. The linear regression equation was $F_0 - F = 16.4 C + 105.8$, where F_0 and F are the fluorescence intensity of EAB at free and a given Cr(VI) concentration solution, respectively. And C represents the concentration of Cr(VI) (the unit of C is μmol L⁻¹), the correlation coefficient was 0.9936. The detection limit (LOD) is defined by the equation $LOD = 3 S_0/K$, where S_0 is the standard deviation of blank measurements (nine measurements, $n = 11$), K is the slope of calibration graph. The LOD is 5.2×10^{-9} mol L⁻¹ and the relative standard deviation is 2.9 % for the determination of 1.0×10^{-6} mol L⁻¹ Cr(VI) ($n = 11$).

Fig.5 The fluorescence spectra of EAB in the presence of chromium (VI) at various concentrations: a) 0, b) 0.1, c) 1, d) 10, e) 20, f) 30, g) 40, h) 50, i) 60, j) 80, k) 100 μmol L⁻¹. Inset: the calibration curve of chromium anions Cr(VI).

Comparison between our proposed method and some methods reported recently for the determination of Cr(VI) is presented in Table 1. The present

method showed lower detection limit and wider linear range.

Table 1 Comparison of the present methods for Cr(VI) determination.

Method	Linearity range	Detect limit
Spectrophotometry [6]	0.1–14.0 $\mu\text{g mL}^{-1}$	0.01 $\mu\text{g mL}^{-1}$
Fluorescence quenching [7]	3–4000 $\mu\text{g L}^{-1}$	1 $\mu\text{g L}^{-1}$
Fluorescence quenching [8]	0.01–1.00 $\mu\text{g mL}^{-1}$	0.008 $\mu\text{g mL}^{-1}$
Fluorescence quenching [9]	5–500 $\mu\text{g L}^{-1}$	0.5 $\mu\text{g L}^{-1}$
Electrochemical method [12]	0.5–50 μM	0.1 μM
SP–RLS [20]	40–320 nM	20 nM
FAAS method [21]	1–40 $\mu\text{g L}^{-1}$	0.8 $\mu\text{g L}^{-1}$
The proposed method	0.1–60 $\mu\text{mol L}^{-1}$	5.2 n mol L^{-1}

3.7 Influence of common coexisting substances

As mentioned above EAB fluorescence probe is very suitable for Cr(VI), as the common metal ions do not produce any noticeable effect on the fluorescence emission of EAB except Cu^{2+} ion. Experiments showed that the interference of Cu^{2+} can be removed using EDTA as a masking reagent. As Cr(VI) in anion forms (CrO_4^{2-} or $\text{Cr}_2\text{O}_7^{2-}$) in water, the influence of anions was also examined. The tolerance limit of some coexistence for the detection of 1.00 $\mu\text{mol L}^{-1}$ Cr(VI) were examined in the presence of EDTA (0.001 mol L^{-1}), as the relative error less than $\pm 5\%$. The results shown that 1 000 times of Ca^{2+} , SO_4^{2-} ; 500 times of Ag^+ , Mg^{2+} , Ba^{2+} , Ni^{2+} , Zn^{2+} , Co^{2+} , Pb^{2+} , Cr^{3+} , Fe^{3+} , Al^{3+} , NO_3^- , Cl^- ; 300 times of Cu^{2+} , Hg^{2+} , Mn^{2+} , CH_3COO^- , BrO_3^- ; 100 times Fe^{2+} ; 50 times of Br^- ; and 20 times of I^- and NO_2^- have no interference on the determination. So this method has relatively high selectivity.

3.8 Mechanism of quenching

Generally, metal ions react with protein to form metal-protein complex, which often cause the change of the secondary structures of the protein. The FT-IR spectra of EAB and Cr(VI)-EAB are shown in Fig. 6. One can see from the infrared absorption spectrum in Fig. 6 b that the absorption peak at 1654 cm^{-1} decreases with a hyperchromic shift (about 7 cm^{-1}) slightly and the relative intensity of the absorption peak at 1530 cm^{-1} increases compared with curve a in Fig. 6. These absorption peaks are characteristic amide I and II bands from amide groups in EAB. So the information of FT-IR indicated that the formation of Cr(VI)-EAB may cause the change of the secondary structures of EAB resulting the fluorescence quenched.

Fig.6 FT-IR spectra for EAB(a) and the complex of Cr(VI)-EAB(b).

3.9 Application of the method

Under optimal experimental conditions, the proposed method was applied to determine Cr(VI) in real samples using EDTA as a masking reagent and the results were recorded in Table 2. On the other hand, Cr(VI) in table-water, well water and the Dongting Lake water was 0.013, 0.010 and 0.046 mg L^{-1} , respectively obtained by DPCI standard method [22]. As seen from Table 2, there was good agreement between the two methods, which indicate that the

present method can be applied to determination of Cr(VI) in real samples.

Table 2 determination of Cr(VI) in samples.

Samples	Found (mg L^{-1})	Added (mg L^{-1})	Total found (mg L^{-1})	Recovery (%)	RSD (%)
Table-water	0.012	0.052	0.067	105.8	2.8
Well water	0.009	0.052	0.065	107.7	3.2
The Dongting Lake water	0.042	0.052	0.096	103.8	2.6

4. CONCLUSION

In summary, we have developed a novel method to detect trace Cr(VI) using egg albumin as fluorescence probes at pH 9.18. The proposed method has wider linear concentration and lower detection limits compared with the other method. The common metal ions and common anions do not produce interference obviously by cooperating with masking of EDTA, and this high sensitive and selective method was successfully used to detect trace Cr(VI) in drinking water. The information of FT-IR showed that the possible physiological mechanism of fluorescence quenching may be the change of the secondary structures of EAB in the presence of Cr(VI). EAB is an environment-friendly, rich in source and cheap bio-chemical. This research will open new application of the extensive prospects bio-chemical.

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