DETERMINATION OF CERIUM (IV) USING RHODAMINE 6G FLUORESCENCE QUENCHING

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The interaction between rhodamine 6G (Rh6G) and cerium sulfate was studied by the fluorescence quenching method. In a sulfuric acid medium, the interaction of Ce(IV) with Rh6G results in Rh6G fluorescence quenching. The maximum excitation wavelength (λ_{ex}) and the maximum emission wavelength (λ_{em}) are 530 nm and 555 nm, respectively. A good linearity between the relative fluorescence intensity (ΔF) and Ce(IV) was observed in the range ~0.12–1.08 μg/mL. The detection limit was 1.4×10^{-3} μg/mL. The optimum reaction conditions, influencing factors, and effect of coexisting substances were investigated in the experiment. We found that the concentration of Rh6G was 3.2×10^{-6} mol/L, and the fluorescence intensity was maximum.

Keywords: *rhodamine 6G, cerium (IV), fluorescence quenching.*

ОПРЕДЕЛЕНИЕ ЦЕРИЯ(IV) МЕТОДОМ ТУШЕНИЯ ФЛУОРЕСЦЕНЦИИ РОДАМИНА 6G **

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УДК 535.371:546.655

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(Поступила 22 сентября 2016)

Методом тушения флуоресценции изучено взаимодействие между родамином 6G (Rh6G) и сульфатом церия. В сернокислой среде Ce(IV) взаимодействует с родамином 6G, что обусловливает тушение флуоресценции родамина 6G. Максимальные длины волн возбуждения и испускания λ_{ex} = = 530 нм и λ_{em} = 555 нм. Линейная зависимость между относительной интенсивностью флуоресценции (ΔF) и количеством Ce(IV) наблюдалась в области концентраций [Ce(IV)] ~ 0.12–1.08 мкг/мл. Предел обнаружения церия 4×10^{-3} мкг/мл. Установлены оптимальные условия реакции, исследованы факторы, оказывающие влияние на результаты эксперимента, в том числе наличие других веществ. Обнаружено, что в исследуемом образце концентрация [Rh6G] = 3.2×10^{-6} моль/л, интенсивность флуоресценции достигает максимума.

Ключевые слова: *родамин 6G, церий (IV), тушение флуоресценции.*

Introduction. In recent years, the rare earth elements have been widely used in industry. Among them, we should single out cerium. This element has two forms Ce(III) and Ce(IV). Ce (IV), possesses strong oxidability [1, 2], and is of exceptional importance to animals and humans, in particular, to their immune and central nerve systems [3]. Therefore, the determination of Ce is paramount. As a rule, in order to determine Ce(IV), the methods of inductively coupled plasma atomic emission spectrometry (ICP-AES) [4], inductively coupled plasma-mass spectrometry (ICP-MS) [5], and flame atomic absorption spectrometry (FAAS) [6] are applied. However, these methods have a number of disadvantages: complexity, expensive equipment,

**Full text is published in JAS V. 84, No. 5 (http://springer.com/10812) and in electronic version of ZhPS V. 84, No. 5 (http://www.elibrary.ru/title_about.asp?id=7318; sales@elibrary.ru).

long duration. Plenty of photometric methods are used for determination of cerium, such as catalytic kinetic spectrophotometry [7], chemiluminescence spectrophotometry [8], the UV method [9], and fluorescence spectrophotometry [10]. It should be noted that the application of these methods generally requires conducting a reaction in which three or more substances take part. This obstructs controlling the reaction conditions. In this paper, we present a simple method for determination of Ce(IV) using fluorescence quenching based on the interaction between rhodmine 6G (Rh6G) and Ce(IV) in a sulfuric acid medium.

Rhodamine 6G is an alkaline xanthene dye based on mixed oxygen which absorbs the energy of incident light and fluoresces due to the aerobic bridge between the benzene ring and a rigid plane structure. Compared with other fluorescent dyes, rhodamine dyes possess a number of advantages: high molar absorption coefficient, good stability, insensitivity to pH levels, wide wavelength range, and high quantum yield [11, 12].

Herein, we proposed a new method of detecting Ce(IV) using the fluorescence quenching based on the interaction of Rh6G and Ce(IV). The relative fluorescence intensity of the system depends linearly on the Ce(IV) concentration within a certain range. Compared with other methods, this simple method is characterized by a low detection limit.

Experimental. For our fluorescence measurements, we used a LS45 spectrofluorophotometer (Perkin-Elmer Corporate of America) and a 970CRT spectrofluorophotometer (Shanghai Sanke Instruments Company). The pH was measured by a pHS-3C meter (Shanghai Leici Instruments Company, China). The absorption spectra were recorded on a UV-8500 spectrometer (Tianmei, Shanghai China)

The stock solution of cerium sulfate hydrate ($Ce(SO_4)_2 \cdot 4H_2O$) (Tianjin Chemical Reagent Company, China) (1 g/L) was prepared as follows. To 0.2491g of $Ce(SO_4)_2 \cdot 4H_2O$ solution in water, we diluted it to 250 mL. The concentration of Rhodamine 6G (Rh6G) (Aladdin Industrial Corporation, Shanghai, China) was 1.0×10^{-4} mol/L, whereas the concentration of H_2SO_4 solution was 1.0 mol/L.

Producer for fluorimetric detection of Ce(IV). We added 0.85 mL of 1.0×10^{-4} mol/L Rh6G, 1.50 mL of 1.0 mol/L H_2SO_4, and a suitable amount of Ce(IV) diluted with doubly diluted water into a 25.0 mL colorimetric tube. The content of each flask was mixed in boiling water for 15 min. After that, we cooled the compounds down to room temperature using water. As is seen from Fig. 1, the fluorescence spectra of the reagent blank and the reaction systems were recorded, and the fluorescence intensities of the reagent blank (F_0) and the reaction system (F) were measured at $\lambda_{em} = 555$ nm. Herein, $\Delta F = F_0 - F$.

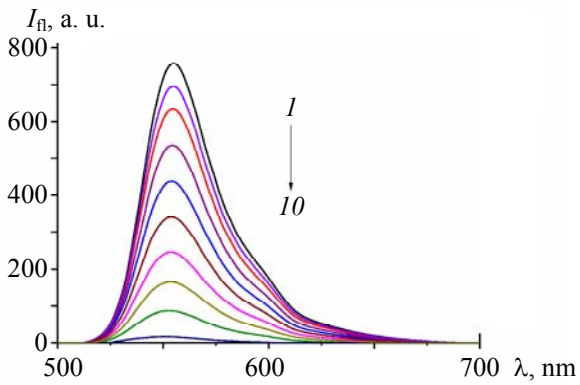

Fig. 1. Fluorescence emission spectrum of Rh6G (3.4×10^{-6} mol/L) in the presence of H_2SO_4 (0.06 mol/L) and Ce(IV) of various concentrations: 0 (1), 0.12 (2), 0.24 (3), 0.36 (4), 0.48 (5), 0.60 (6), 0.72 (7), 0.84 (8), 0.96 (9), and 1.08 μg/mL (10).

Results and discussion. *Influence of the dye concentration on fluorescence intensity.* The fluorescence intensity of the dye is affected not only by its own molecular structure but also by its concentration in the system. Owing to this, we investigated the effect of the concentration of Rh6G in H_2SO_4 aqueous solution on the fluorescence intensity. As is seen from Fig. 2, the fluorescence intensity was observed at different concentrations of Rh6G. Upon addition of H_2SO_4, the fluorescence intensity of Rh6G increased distinctly. We observed the maximum fluorescence intensity at the concentration of Rh6G equal to 3.4×10^{-6} mol/L. In the next stages of the experiment, we kept the concentration of Rh6G at the level of 3.4×10^{-6} mol/L.

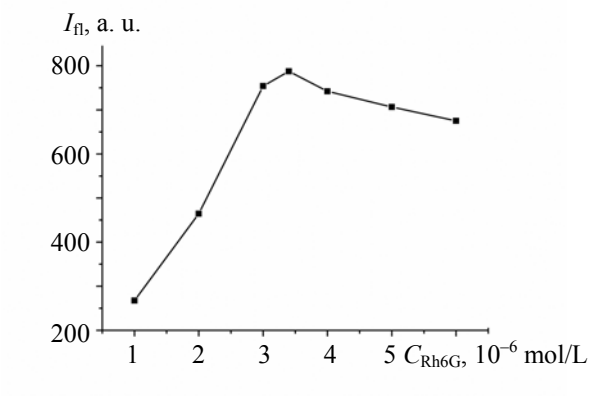

Fig. 2. Influence of the Rh6G concentration on the fluorescence intensity of the system. $C_{Rh6G} = 1.4\times10^{-4}$ mol/L.

Optimum experimental conditions. Ce(IV) can interact with Rh6G distinctly in a strongly acid medium. This results in fluorescence quenching. We tested the influence of different acids (HNO_3, H_2SO_4, HCl) on this process. The results show that the relative fluorescence intensity of the Rh6G system in the sulfuric acid medium was maximal. Therefore, the sulfuric acid medium was selected to control the pH of the solution.

The influence of the reaction time on the fluorescence intensity of the system was studied. In H_2SO_4 the relative fluorescence intensity reaches its maximum at 15 min after the start of the reaction and remains stable during 2 h. This experiment determined the possible time for further measurements.

The different reaction temperatures of the system (in the range ~273–368 K) were explored. As is shown in Fig. 3, ΔF of the reaction system is maximal when the reaction temperature is 298 K. Hence, the reaction temperature should be kept at 298 K.

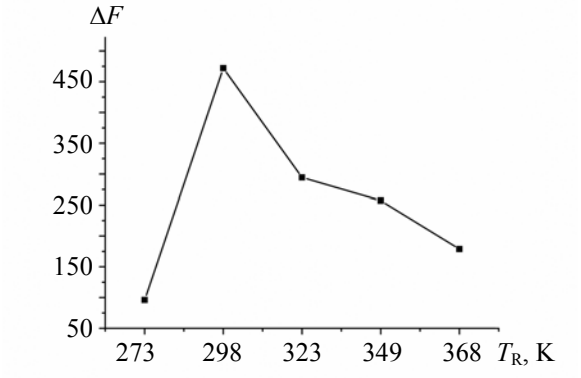

Fig. 3. Temperature dependence of the fluorescence intensity of the system.

Selectivity of the method. Using the established optimum conditions for the experiment, we studied the effect of other substances on the determination of 1.5 μg of Ce(IV). The working wavelength for which we determined Ce(IV) was λ_{em} = 555 nm. As is seen from Table 1, the fluorescence intensity of different ions (Na^+(1000), K^+ (1000), Ca^{2+} (160), Zn^{2+} (30), Ag^+ (250), Cu^{2+} (30), Mg^{2+} (15), Cd^{2+} (15), Mn^{2+} (1.5), Mo(VI) (3), and MnO_4^- (0.03)) was investigated.

TABLE 1. Effects of Coexisting Substances ($C_{Ce(IV)}$ = 1.5 μg/mL)

Species	Concentration, μg /ml	Relative error in determination of Ce(IV), %
Na^+	60	4.99
K^+	60	1.19
Ca^{2+}	10	1.38
Zn^{2+}	2	3.43
Ag^+	16	3.89

Continue Table 1

Species	Concentration, μg /ml	Relative error in determination of Ce(IV), %
Cu^{2+}	2	4.98
Mg^{2+}	1	4.87
Cd^{2+}	1	1.65
Mn^{2+}	0.1	4.06
MoO_4^{2-}	2	4.46
MnO_4^{-}	0.002	3.92

Work curve and detection limit. As is shown in Fig. 4, the calibration curve is calculated after a number of similar experiments for various concentrations of Ce(IV). We prepared 11 tested samples and determined their fluorescence intensities. The results show that the relative fluorescence intensity (ΔF) offers a good relationship with the concentration of Ce(IV). The detection limit is 1.4×10^{-3} μg/m, which is lower than for previous works (see Table 2). The linear regression equation has the form $\Delta F = 220.4C$ (μg/mL) – 34.69 ($R^2 = 0.9969$).

TABLE 2. Determination of Ce(IV) in Different Human Hair Samples

Samples	Ce(IV) present at first, μg/mL	Added, μg/mL	Total found, $n = 6$	Recovery, %	RSD, %
1	0.281	0.20	0.475	97	4.34
2	0.563	0.20	0.770	103	2.67
3	0.745	0.20	0.951	104	1.84

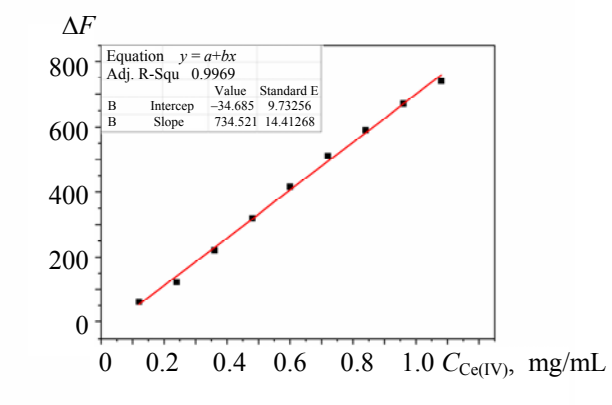

Fig. 4. Standard work curve.

Detection of Ce(IV) in human hair. We used the obtained results to determine Ce(IV) in human hair. For comparison, we applied a standard addition method to measure the concentration of Ce(IV) in human hair. The results are summarized in Table 3.

TABLE 3. Comparison of Sensitivity of This Method with Other Methods for Determination of Ce(IV)

Method	Reagent	Detection limit, μg/mL	Reference
Catalytic spectrophotometric	Malachite Green	8.8×10^{-3}	[9]
Discoloration spectrophotometric	Rhodamine B	2.5×10^{-3}	[10]
Fluorescence quenching method	Rhodamine 6G	1.4×10^{-3}	Present work

Before the measurement, a 3.006 g hair sample was digested with a 60 mL solution of concentrated acids H_2NO_3 and $HClO_4$ (their ratios in the compound were 5:1) at room temperature for 3 h. After that, the sample was kept on a hot plate until a residue was obtained. After cooling, the residues were again digested with 30 mL mixtures of HNO_3 and H_2O_2 (their ratios in compound were 2:1), following the same procedure as described above. The final residue was dissolved in distilled water and transferred to a 50 mL volumetric flask.

Discussion of the quehching mechanism. Generally, fluorescence quenching can occur by two different mechanisms: dynamic and static [13]. Dynamic quenching is due to the collision between the fluorophore and the quencher. Collisional quenching only affects the excited states of fluorophores, and the absorption spectra undergo no changes. Static quenching is caused by the formation of complexes between the quencher and the ground state molecules of the luminescent material. The ground-state complex formation results in changes in the absorption spectra of the fluorophore [14].

As is seen from Fig. 5, the absorption spectrum of the system was investigated. There is strong absorption of Rh6G at 527 nm (curve 1). The broad intensive visible band in the absorption spectrum is caused by the $S_0 \rightarrow S_1$ transitions of dye molecules. These $S_0 \rightarrow S_1$ transitions are spin-allowed electronic transitions with a high transition probability. This can enhance the fluorescence emission. However, the absorption spectrum decreases after adding Ce(IV) into Rh6G. The maximum of the absorption spectrum occurs with the blue shift $\Delta\lambda$ = 10 nm (curve 2). The new peak is located at 517 nm. The absorption coefficient of Rh6G decreases when it interacts with Ce(IV), which can be explained by the strengthening of $T_1 \rightarrow S_1$ transitions in the dye [15].

In aqueous solutions, Rh6G has a strong yellowish green fluorescence because of its large conjugate bond and rigid structure. In an acid environment, the Rh6G dye can interact with Ce(IV) and generate a substance without fluorescence. In a sulfuric acid medium, the Ce(IV) substance reacts with SO_4^{2-} forming the $Ce(SO_4)_3^{2-}$ ion, which can electrostatically interact with Rh6G and generate a new nonfluorescent compound. Thus, it can be concluded that the fluorescence quenching of Rh6G is a static one. As is shown in Fig. 6, it is caused by the reactions in the system.

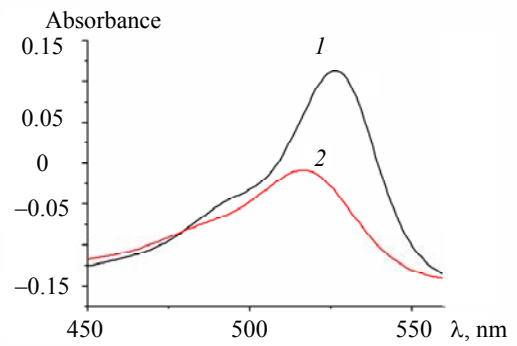

Fig. 5. Absorption spectra of Rh6G in the presence of Ce(IV) in H_2SO_4 medium: 1) 3.4×10^{-6} mol/L Rh6G; 2) 3.4×10^{-6} mol/L Rh6G+0.6 μg/mL Ce(IV).

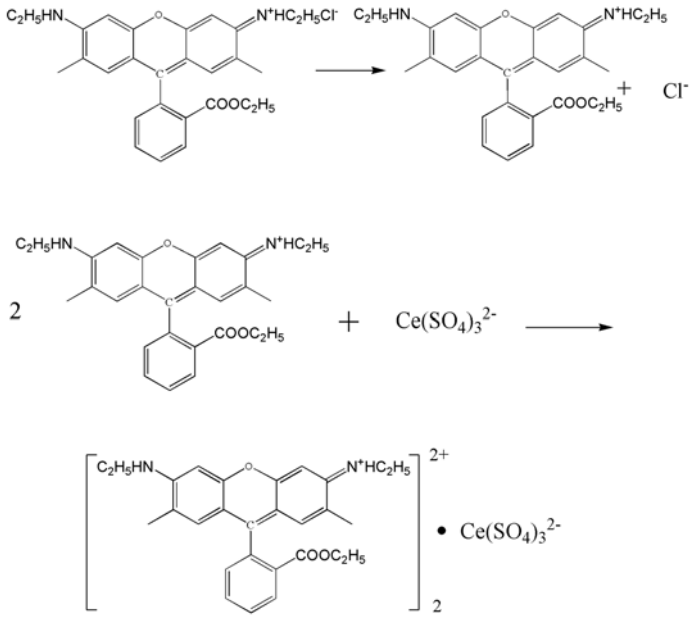

Fig. 6. Mechanism of the reaction.

The fluorescence quenching mechanism can also be distinguished by its dependence on the temperature. It is known that if the quenching constants increase with increasing temperature, we have dynamic quenching, while in the case of static quenching, the reverse effect is obtained. In this paper, the type of the quenching mechanism is established quantitatively using the Stern–Volmer equation [16, 17]:

$$F_0/F = 1 + K_{SV}[Q] = 1 + k_q\tau_0[Q],$$

where F_0 and F are the fluorescence intensities of Rh6G in the absence and presence of Ce(IV), respectively, k_q and K_{SV} are the quenching constant and the Stern–Volmer quenching constant, respectively, τ_0 is the fluorescence lifetime in the absence of the quencher, and [Q] is represented for a definite concentration of the quencher. We investigated the fluorescence intensity at two temperatures (298 and 368 K). As is seen from Fig. 7, the quenching constant decreases with increasing temperature, which indicates that the Rh6G fluorescence quenching is static.

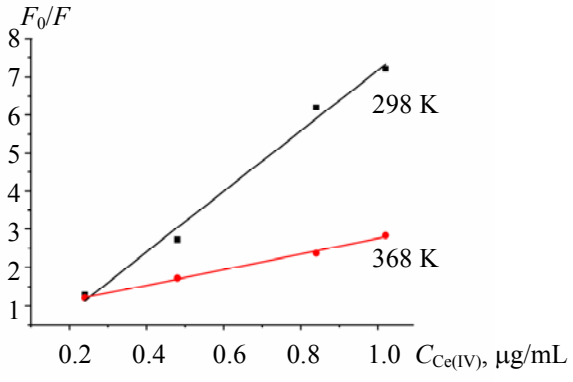

Fig. 7. Stern–Volmer plots for the solution in the H_2SO_4 medium at two different temperatures.

Conclusion. We proposed a simple, inexpensive, and highly selective method for detection of Ce(IV) based on the phenomenon of fluorescence quenching. A linear relationship between the fluorescence intensity and the Ce(IV) concentration was established under optimal experimental conditions.

Acknowledgment. This work was supported by the Experimental Education Reform Program of Lan-Zhou JiaoTong University (No.201711).

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