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Rapid and sensitive spectrofluorimetric determination of trace amount of Cr(III) with *o*-vanillin-8-aminoquinoline

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Abstract

A novel fluorescent reagent *o*-vanillin-8-aminoquinoline(OVAQ) was synthesized, and its infrared spectrum, elemental analysis and acid–base dissociation constants were obtained. The fluorescent reaction of this reagent with Cr(III) was studied. In acetonitrile–water (1:1, (v/v)) medium of pH 6.00, Cr(III) could react with fluorescent reagent OVAQ ($\lambda_{ex/em} = 280/314$ nm) to form a 1:1 non-fluorescent complex. The linear range of the spectrofluorimetric method proposed was from 8.2 to 130 μg l^{-1}, and the detection limit was 2.5 μg l^{-1}. The interferences of 25 foreign ions were also studied. This method could be easily performed and was successfully applied to the determination of Cr(III) and total chromium in domestic and industrial waste water samples.

Keywords: Determination of chromium(III) and total chromium; Spectrofluoremetry; *o*-vanillin-8-aminoquinoline; Quenching

1. Introduction

Cr(III) and Cr(VI) are two common forms of inorganic chromium in environment, and their biochemical properties and toxicity are greatly different for the difference of their valences: trace amount of Cr(III) is essential to human beings, while Cr(VI) is harmful to our health especially for its effect of causing cancer. In environmental analysis, it is always necessary to determine Cr(III), Cr(VI) or total chromium respectively. There are kinds of methods for the determination of chromium, such as spectrophotometry [1–6], spectrofluorimetry [7–13] and atomic absorption spectrophotometry [14–18] etc., spectrophotometry is widely used, while it has a low sensitivity. When chromium with different valence was determined, the procedure of atomic absorption spectrophotometry was always complicated. Spectrofluorimetry is rapid and sensitive, but these methods for the determination of chromium are mainly based on catalysis oxidation [7,8], oxidative action of Cr(VI) [9,10], and fluorescent quenching action [11,12]. In the quantitative determination of domestic and industrial waste water, the atomic absorption method [18,19] and spectrophotometry method [20] for the determination of Cr(VI) were widely used, while there were few reports about the direct determination of chromium(III) with spectrofluorimetry.

In this paper, a novel fluorescent reagent OVAQ was synthesized. The fluorescence of OVAQ quenched at $\lambda_{ex/em} = 280/314$ nm for the formation of complex between Cr(III) and OVAQ, and a fluorescence quenching method for the determination of Cr(III) directly was developed based on this reaction. In proposed method, 40 times of co-exist Cr(VI) ions was tolerated, and the determination of Cr(VI) can be carried out by reducing Cr(VI) to Cr(III) with Na_2SO_3. The method was successfully applied to the determination of Cr(III) and total chromium in domestic and industrial waste water samples.

2. Experimental

2.1. Apparatus

All fluorescent measurements were carried out on a Perkin-Elmer (Norwalk, CT, USA) LS-5 spectrofluorimeter, equipped with a xenon lamp and a 1.0 cm quartz cell.

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An UV-265 recording spectrophotometer (Shimadzu, Kyoto, Japan) equipped with 1.0 cm quartz cells was used for the UV spectrum scanning and determination experiments. All pH measurements were made with a pHS-3C digital pH-meter (Shanghai Leici Device Works, Shanghai, China) and a combined glass–calomel electrode. Perkin-Elmer 983 infrared spectrophotometer (KBr discs, Norwalk, CT, USA) and Perkin-Elmer 2400 elementary analysis apparatus (Norwalk, CT, USA) were also used in experiments.

2.2. Reagents

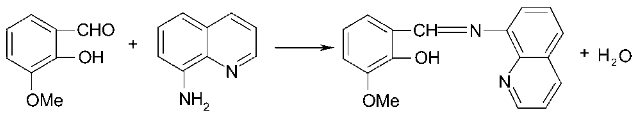
All chemicals were of analytical reagent or higher grades. Doubly distilled deionized water was used throughout. Working standard solution of $1.0 \times 10^3\,\mu g\,l^{-1}$ Cr(III) was prepared by diluting stock solution ($1.0 \times 10^6\,\mu g\,l^{-1}$) with doubly distilled deionized water. Ethanol solution of $2.0 \times 10^{-3}\,mol\,l^{-1}$ OVAQ, acetonitrile, and buffer solutions of HCl (pH = 1, 2), CH3COONa–CH3COOH (pH = 3–6), CH_3COONH_4 (pH = 7) or $NH_4Cl–NH_3{\cdot}H_2O$(pH = 8–11) were also used.

2.3. Synthesis and properties of OVAQ

0.760 g *o*-vanillin (0.50 mol) was dissolved in 10 ml absolute ethanol. Then it was added dropwise into a solution, which was prepared by dissolving 0.720 g (0.50 mol) of 8-aminoquinoline in 10 ml absolute ethanol. Then the mixture was refluxed for 3 h, cooled to room temperature and filtered. After recrystallization from ethanol and dried in vacuum, a 0.890 g amount of red crystal product was obtained (yield 60%). The synthesis reaction was shown in Scheme 1.

The melting point of OVAQ is 103–104 °C. Elemental analysis gave a composition (%) of: C 73.40, H 4.94, N 10.46, which was in a good agreement with the theoretical composition (%) of OVAQ: C 73.38, H 5.04, N 10.07. The infrared spectrum of OVAQ (KBr discs, cm^{-1}) was obtained and the peaks were assigned as: $\upsilon_{O–H}$ (3400), $\upsilon_{C=N}$ (1610), $\upsilon_{Ar–O}$ (1250). The appearance of the characteristic infrared absorption peak $\upsilon_{C=N}$ ($1610\,cm^{-1}$) indicates the formation of OVAQ. Its acid–base dissociation constants were also obtained with spectrophotometry in acetonitrile–water (1:1, (v/v)) medium. Firstly we studied the influence of different pH on the absorption spectra of OVAQ (Fig. 1). Then at 340 nm, the absorbance (A) of OVAQ with variable pH were measured (Fig. 2).

The results showed that OVAQ assumed properties of binary acid. According to the method proposed in a paper [21], we calculated the dissociation constants of OVAQ, $pKa_1 = 2.88 \pm 0.03$ and $pKa_2 = 4.22 \pm 0.02$ ($0.10\,mol\,l^{-1}$ KCl, $20 \pm 1\,°C$).



Scheme 1.

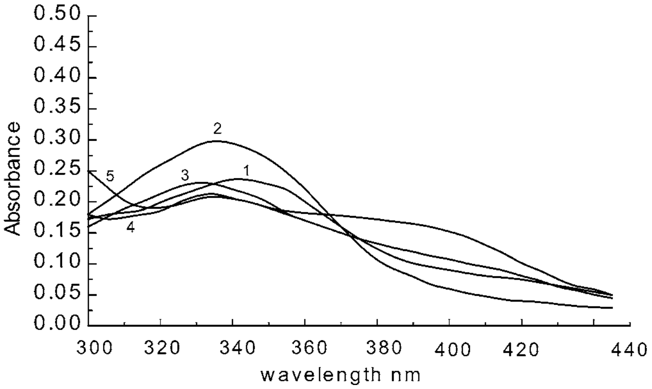


Fig. 1. Absorption spectra of OVAQ at different pH ($1.0 \times 10^{-4}\,mol\,l^{-1}$). (1) pH 2.50, (2) pH 4.30, (3) pH 5.50, (4) pH 6.70, (5) pH 8.90.

2.4. Determination procedure of Cr(III)

Into a 10 ml colorimetric tube, a certain amount of Cr(III), 0.50 ml of $2.0 \times 10^{-3}\,mol\,l^{-1}$ OVAQ solution, 5.00 ml of acetonitrile and 1.00 ml of $CH_3COONa–CH_3COOH$ (pH 6.00) were added in turn. Then the mixture was diluted to volume with doubly distilled water. The solution obtained was mixed thoroughly and equilibrated at room temperature for 10 min, then the fluorescence intensity was measured at $\lambda_{ex/em} = 280/314$ nm.

3. Results and discussion

3.1. Fluorescent excitation and emission spectra

According to the dissociation constant of OVAQ, it can be concluded that OVAQ exists in basic form at pH 6.00, which has intense fluorescence at $\lambda_{ex/em} = 280/314$ nm. Under this conditions, the formation of complex Cr(III)–OVAQ caused static fluorescence quenching at $\lambda_{ex/em} = 280/314$ nm (Fig. 3). It could be explained

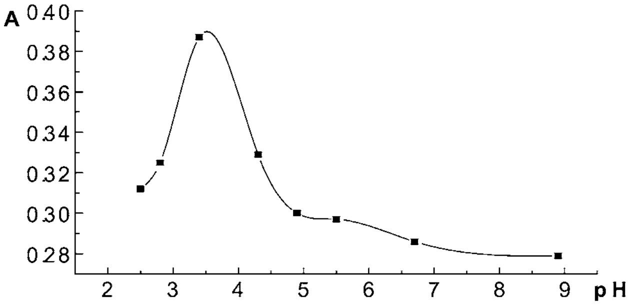


Fig. 2. Absorbance of OVAQ with variable pH at 340 nm ($1.0 \times 10^{-4}\,mol\,l^{-1}$).

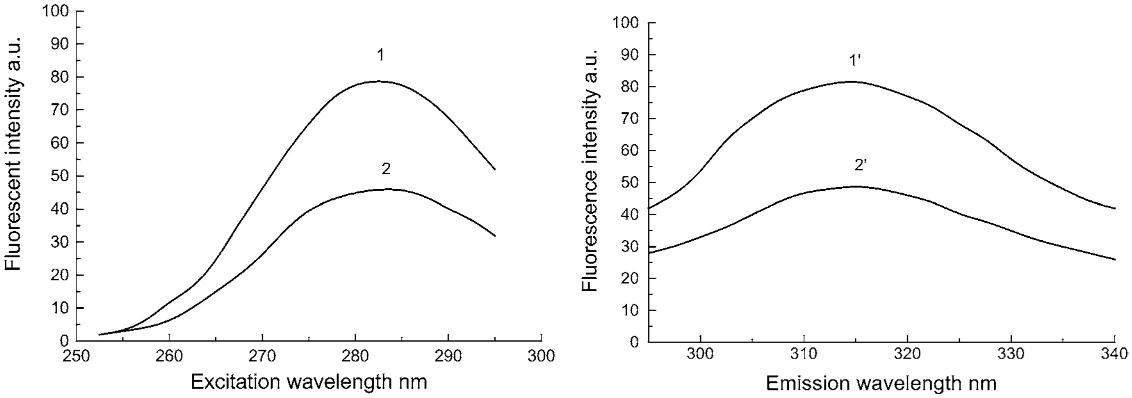


Fig. 3. 1 and 1′: OVAQ, 2 and 2′: OVAQ + Cr(III). OVAQ: 1.0×10^{-4} mol l^{-1}, Cr(III):1.0×10^{2} μg l^{-1}, pH 6.00.

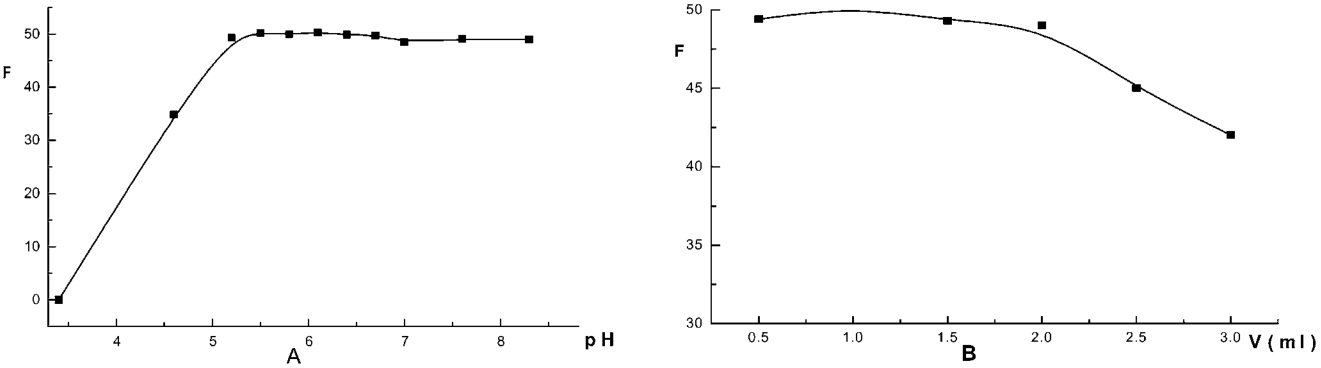


Fig. 4. Effect of (A) pH and (B) amount of buffer solution OVAQ: 1.0×10^{-4} mol l^{-1}, Cr(III):1.0×10^{2} μg l^{-1}.

that, as a paramagnetic substance, chromium had effect of invertible charge transfer or electron spin-orbit coupling, which accelerated the singlet–triplet transition and internal conversion deactivation of excited OVAQ molecules.

3.2. Effects of pH and the amount of buffer solution

The acidity of the medium determines the existence form of OVAQ, the coordination and stability of OVAQ–Cr(III) complex, so it has a great effect on the fluorescence intensity. The experimental results (Fig. 4A) indicated that the difference of fluorescence intensity (*F*) between OVAQ and complex was high and remained constant in the range over pH 5.20–8.30, so the optimum pH 6.00 was selected. Effect of the amount of buffer solution was also studied (Fig. 4B), and 1.00 ml of buffer solution (pH 6.00) was selected in the subsequent experiments.

3.3. Effect of organic solvent

Addition of organic solvent could improve the solubility of OVAQ and the coordition of OVAQ with Cr(III), so the effect of organic solvents on *F* was studied. Table 1 showed that *F* had a higher value in acetonitrile than in any other organic solvent under the same conditions. Moreover, *F* had the highest value and remained constant when the amount of acetonitrile was between 5.00 and 6.00 ml (Fig. 5), so 5.00 ml of acetonitrile was selected.

3.4. Effects of the amount of reagent and the order of addition

The concentration of OVAQ had a great effect on the coordination reaction and the fluorescence intensity, so the effect of the amount of reagent was studied under the conditions established above. The experimental results (Fig. 6)

Table 1
Effect of organic solvent (2.00 ml)

Organic solvent	No addition	Methanol	Ethanol	Acetonitrile	1-Propanol	2-Propanol	DMF
F	7.7	9.7	13.5	32.6	5.1	15.3	15.6

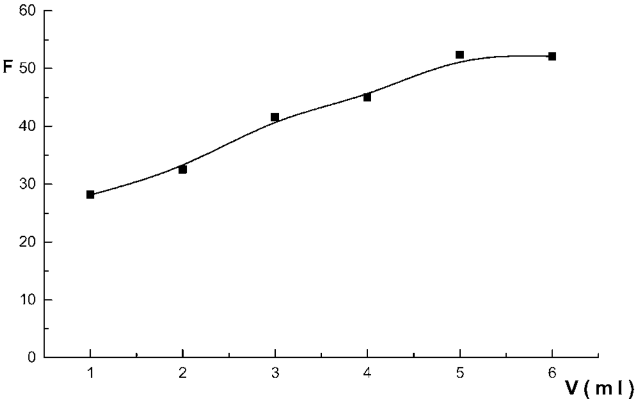


Fig. 5. Effect of the amount of acetonitrile OVAQ: 1.0×10^{-4} mol l^{-1}, Cr(III):1.0×10^{2} μg l^{-1}, pH 6.00.

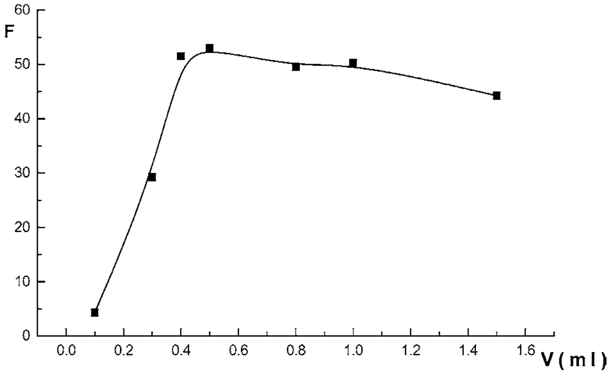


Fig. 6. Effect of the amount of reagent Cr(III):1.0×10^{2} μg l^{-1}, pH 6.00.

indicated that F had the highest value with the amount of 2.0×10^{-3} mol l^{-1} OVAQ in the range of 0.40–1.00 ml, so 0.50 ml of OVAQ solution was used. Furthermore, the optimum order of addition was solution of Cr(III), OVAQ, acetonitrile and buffer solution.

3.5. Effects of reaction temperature and time

Raising temperature could improve the coordination reaction, so the effect of temperature was studied. The experimental results (Fig. 7) showed that F remained comparatively constant with temperature in the range of 15–30 °C, therefore the room temperature was adopted. The effect of reaction time was also studied. Results (Fig. 8) showed that F reached a maximum after 10 min and remained constant for at least 20 min, then decreased slowly. So all coordinations were carried out for 10 min at room temperature, and all measurements were made at room temperature within 30 min.

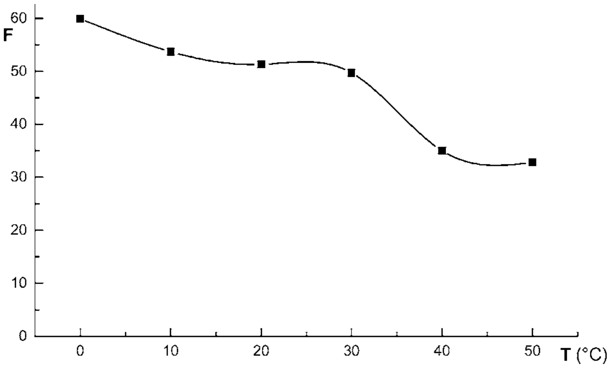


Fig. 7. Effect of reaction temperature (°C).

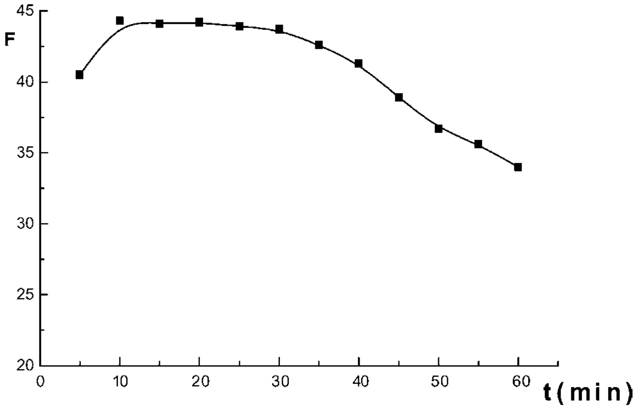


Fig. 8. Effect of reaction time OVAQ: 1.0×10^{-4} mol l^{-1}, Cr(III):1.0×10^{2} μg l^{-1}, pH 6.00.

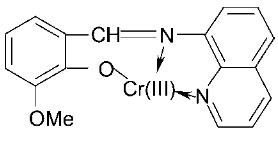
3.6. Determination of fluorescence quenching constant and the stoichiometry of Cr(III)–OVAQ

The change of absorption spectrum ($\lambda_{\max}$ changing from 340 to 385 nm) caused by the addition of Cr(III) indicated that the fluorescence quenching of OVAQ resulted from the formation of non-fluorescent Cr(III)–OVAQ complex. According to the formula of $F_0/F = 1 + K[Q]$ [22], static quenching constant $K = 4.7 \times 10^{5}$ l mol^{-1} was obtained.

The stoichiometry of the complex was studied under the established conditions by the molar ratio method and the continuous variation method with 2×10^{-5} mol l^{-1} solutions of Cr(III) and OVAQ. Both methods showed that the molar ratio of Cr(III) and OVAQ was 1:1, so we concluded that the model structure of the complex was as Scheme 2.

3.7. Interferences of foreign ions

A systematic study of the interferences of foreign ions was carried out under the conditions established above. The concentration of Cr(III) was fixed at 1.0×10^{2} μg l^{-1} and the tolerable error was fixed at a ±5.0% variation of fluorescence intensity. The results were listed in Table 2.



Scheme 2.

Table 2
The interferences of foreign ions

Interference times	Foreign ions
>1000	Na^+, K^+, $NH_4{}^+$, Cl^-, $NO_3{}^-$, CH_3COO^-, $SO_4{}^{2-}$, $C_2O_4{}^{2-}$
500	Ca^{2+}, Sr^{2+}, Ba^{2+}, $PO_4{}^{3-}$
40	Cr(VI)
20	Cd^{2+}
15	Ag^+
8	Mn^{2+}
1	Cu^{2+}, Zn^{2+}, Ni^{2+}, Al^{3+}, As^{3+}
0.5	Co^{2+}, Pb^{2+}, Hg^{2+}, Be^{2+}

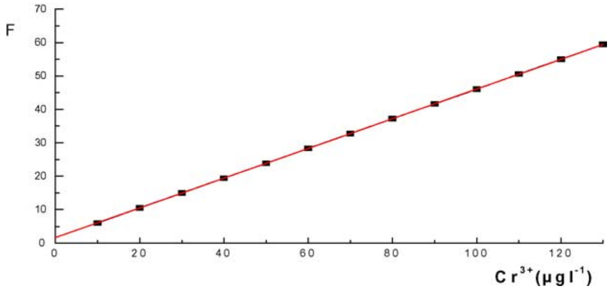


Fig. 9. Calibration graph.

3.8. Analytical characteristics

Under the optimum experimental conditions, there was a linear relationship between F and the concentration of Cr(III) in the range of 8.2–130 $\mu g\,l^{-1}$ with a correlation coefficient of 0.9986. The calibration graph was shown in Fig. 9 and the regression equation was $F = 0.4444C(\mu g\,l^{-1}) + 1.653$. The detection limit, as defined by IUPAC [23], was determined to be 2.5 $\mu g\,l^{-1}$ according to formula of $C = KS_0/S$, where the value of K was taken as 3, the standard deviation was 0.36 obtained from a series of 10 reagent blanks, and S was the slope of the standard curve. The relative standard deviation (R.S.D.) was 0.50% obtained from a series of 11 standards each containing 50.0 $\mu g\,l^{-1}$ of Cr(III). This method was compared with other spectrofluorimetric methods (Table 3). We could see this method had high sensitivity.

3.9. Determination of chromium in domestic water

According to the national criterion of domestic water quality in China (GB5750-85), the artificial sample 1 and sample 2 were prepared as following.

Sample 1 ($mg\,l^{-1}$):	Cr(III) 0.05, Fe 0.30, Mn 0.10, Cu 1.00, Zn 1.00, As 0.05, Hg 0.001, Cd 0.01, Pb 0.05, Ag 0.05
Sample 2 ($mg\,l^{-1}$):	Cr(VI) 0.05, Fe 0.30, Mn 0.10, Cu 1.00, Zn 1.00,As 0.05, Hg 0.001, Cd 0.01, Pb 0.05, Ag 0.05

The actual samples of domestic water in Jinan (a city of China) were also prepared. These samples were all operated respectively as described following. Fifty milliliter of sample water was put into a 100 ml beaker, then 1.00 ml of 6.00 $mol\,l^{-1}$ H_2SO_4 solution and 0.50 ml of 0.50 $mol\,l^{-1}$ Na_2SO_3 solution were added. After reaction, the mixture was adjusted to pH 14.00 with 6.00 $mol\,l^{-1}$ NaOH solution, filtrated to remove precipitation of hydroxides, adjusted to pH 5.00 with 0.20 $mol\,l^{-1}$ $H_2C_2O_4$–$(NH_4)_2C_2O_4$ solution, transferred into a 100 ml of calibrated flask and fixed to volume. According to the experimental method described in Section 2.4, 1.00 ml of solution was used in spectrofluorimetric determination of chromium under the optimum conditions. Determination results (Table 4) showed that the measured value was in good agreement with the standard value, which indicated that the proposed method was reliable to determine trace amount of chromium.

3.10. Determination of chromium in industrial waste water samples

Fifty milliliter of industrial waste water (from Jinan Yuxing Chemical Factory, China), equilibrated for 24 h, was put into a conical flask. 1.00 ml of 6.0 $mol\,l^{-1}$ H_2SO_4 solution and 2.00 ml of $KMnO_4$ saturated solution were added to destroy organic compounds and eliminate the potential interferences from Pb^{2+},Ba^{2+} etc. The mixture obtained was equilibrated at room temperature for at least 1 h, and filtrated

Table 3
Comparision with other fluorimetric methods for the determination of chromium

Method	Linear range ($\mu g\,l^{-1}$)	Detection of limit ($\mu g\,l^{-1}$)	Major interference	Reference
Cr(VI)–H_2O_2–SafranineT	0–120	2.6	Cu^{2+},Bi^{3+},Cr^{3+}, Fe^{3+}	[7]
Cr(VI)–H_2O_2–RhodamineB	10–160	3.32	Cu^{2+}, Cr^{3+}, Fe^{3+}, I^-	[8]
Cr(VI)–PTQA	Cr(VI):100–10^4 Cr(III):100–10^3	50 20	Ag^+,Cu^{2+}, Hg^{2+},Ce^{4+} Mo(VI)	[9]
Cr(VI)–8-hydroxy-quinoline-5-sulfonic acid	291–780		Pb^{2+}, Hg^{2+},Sc^{3+}, Ce^{4+}, V(V), $NO_2{}^-$, $BrO_3{}^-$	[10]
Cr(VI)–L-tyrosine	260–5200		Ag^+, Co^{2+}, Pb^{2+}, Y^{3+}, Fe^{3+},Ce^{4+}, V(V)	[11]
Cr(VI)–Rhodamine6G	8–80	0.8	Cu^{2+}, Ce^{4+}, V(V), $NO_2{}^-$	[12]
Cr(III)–OVAQ	8.2–130	2.5	Pb^{2+}, Hg^{2+}, Be^{2+}, Co^{2+}	this work

Table 4
Determination of chromium in water samples ($P = 95\%$, $n = 5$)

Sample	Concentration ($\mu g\,l^{-1}$)	Cr added ($\mu g\,l^{-1}$)	Measured values ($\mu g\,l^{-1}$)	Average recovery (%)	R.S.D.
Sample 1	0	50.0	51.5 ± 0.5	103	1.6%
Sample 2	0	50.0	51.0 ± 0.8	102	1.7%
Domestic water in Jinan	36.9 ± 0.6	30.0	67.5 ± 0.7	102	1.2%

Table 5
Determination of chromium in industrial waste water samples ($P = 95\%$, $n = 5$)

Method	Concentration ($\mu g\,l^{-1}$)	Cr added ($\mu g\,l^{-1}$)	Measured values ($\mu g\,l^{-1}$)	Average recovery (%)	R.S.D.
Atomic absorption method	24.9	25.0	50.0	100	
This method	25.5 ± 0.7	25.0	51.2 ± 0.8	103	1.6%

to remove precipitation. In order to deoxidized Cr(VI) and MnO_4^-, 0.50 mol l^{-1} Na_2SO_3 solution was added dropwise until the purple of $KMnO_4$ disappeared and 0.50 ml Na_2SO_3 solution was excess. To get rid of Mn^{2+} and some potential ions as Co^{2+}, Ni^{2+}, Cd^{2+}, Cu^{2+} Hg^{2+}, Ag^+ etc., the solution was adjusted to pH 14.00 with 6.0mol l^{-1} NaOH solution, fixed to 100 ml and filtrated with dry filter paper. One milliliter of the filtrate was adjusted to pH 5.00 with 0.10 mol l^{-1} $H_2C_2O_4$–$(NH_4)_2C_2O_4$ solution and diluted to 100 ml, in which Al^{3+} may be concealed. One milliliter of the filtrate was used for the determination of chromium. The results (Table 5) showed that the measured value was in agreement with that obtained by atomic absorption spectrophotometry.

4. Conclusion

Based on Cr(III) can react with fluorescent reagent OVAQ ($\lambda_{ex/em} = 280/314$ nm) to form a non-fluorescent OVAQ–Cr(III) complex, a spectrofluorimetry method for the determination of Cr(III) with high sensitivity was proposed. Cr(III) was determined directly and 40 times Cr(VI) was tolerated; Cr(VI) could also be determined by reducing Cr(VI) to Cr(III) with Na_2SO_3. The method proposed, which has been successfully applied to the determination of chromium(III) and total chromium in domestic and industrial waste water samples, has great value in environmental analysis and monitoring.

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References

[1] P.M.A. Singer, J.H. Aldstadt, Microchem. J. 74 (2003) 47.
[2] J.Y. Sun, X.G. Chen, Z.D. Hu, Bull. Soc. Chim. Belg. 104 (1995) 415.
[3] S. Balasubramanian, V. Pugalenthi, Talanta 50 (1999) 457.
[4] J.E.T. Andersen, Anal. Chim. Acta 361 (1998) 125.
[5] M. Kaneko, M. Kurihara, S. Nakano, T. Kawashima, Anal. Chim. Acta 474 (2002) 167.
[6] G.P. Zhao, B.G. An, S.Q. Cai, Mater. Protect. 33 (2000) 14.
[7] L.H. Chen, D.S. Wang, Chinese J. Anal. Chem. 25 (1997) 120.
[8] S.L. Feng, R.Y. Wang, J. Fan, Chinese J. Anal. Chem. 28 (2000) 61.
[9] E.K. Paleologos, S.I. Lafis, S.M. Tzouwara-Karayanni, M.I. Karayannis, Analyst 123 (1998) 1005.
[10] N.Q. Jie, J. Jiang, Analyst 116 (1991) 395.
[11] N.Q. Jie, J.H. Yang, J. Guo, Anal. Lett. 25 (1992) 1447.
[12] N.Q. Jie, Q. Zhang, J.H. Yang, X.R. Huang, Talanta 46 (1998) 215.
[13] E.K. Paleologos, C.D. Stalikas, S.M. Tzouwara-Karayanni, M.I. Karayannis, Anal. Chim. Acta 436 (2001) 49.
[14] P. Szefer, K. Frelek, K. Szefer, Ch.-B. Lee, B-S. Kim, J. Warzocha, I. Zdrojewska, T. Ciesielski, Environ. Pollut. 120 (2002) 423.
[15] M. Wuilloud Gustavo, G. Wuilloud Rodolfo, C.A. De Wuilloud Jorgelina, A. Olsina Roberto, D. Martinez Luis, J. Pharm. Biomed. 31 (2003) 117.
[16] T.A.T.A. Rehber, Talanta 57 (2002) 1199.
[17] S. Stasinakis Athanasios, S. Thomaidis Nikolaos, D. Lekkas Themistokles, Anal. Chim. Acta 478 (2003) 119.
[18] P. Jozsef, B. Harald, L. Shen-Kay, S. Gerhard, Anal. Chem. 65 (1993) 2590.
[19] M. Sperling, S.K. Xu, B. Welz, Anal. Chem. 64 (1992) 3101.
[20] W.E. Gan, L. Yang, Y.Z. He, R.H. Zeng, M.L. Cervera, M.D.L. Guardia, Talanta 51 (2000) 667.
[21] N.U. Persic-Janjic, A.A. Muk, V.D. Canic, Anal. Chem. 45 (1973) 798.
[22] G.Z. Chen, Z.X. Huang, Z.Z. Zheng, J.G. Xu, Z.B. Wang, Fluorescence Analysis Methods, Science Press, China, 1990.
[23] H.M.N.H. Irving, H. Freiser, T.S. West (Eds.), IUPAC Compendium of Analytical Nomenclature, Definitive Rules, Pergamon Press, Oxford (1981).