Determination of ppb Level of Arsenic(V) Based on Fluorescence Quenching of Thorium-Morin Chelate

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Morin (5,7,2',4'-tetrahydroxyflavonol) forms a stable chelate with thorium(IV). The chelate showed a constant fluorescence intensity (λ_{ex} 415 nm, λ_{em} 505 nm) in acidic solutions, pH 2.2. The stoichiometric composition of the chelate was 1:1 (thorium: morin). In the presence of arsenic(V), the chelate dissociated, owing to the formation of a more stable complex of thorium arsenate. By using the experimental result that the fluorescence intensity of thorium-morin chelate decreased in proportion to the concentration of arsenic(V), 0.01–1 ppm of arsenic(V) could be determined. In the reaction, arsenic(III) did not form a thorium arsenite complex. Accordingly, arsenic(V) in the mixture of arsenic(III) and arsenic(V) was accurately determined by the fluorescence quenching method.

Keywords Arsenic determination, spectrofluorometry, fluorescence quenching, thorium-morin chelate

Morin (5,7,2',4'-tetrahydroxyflavonol) has been used as the reagent for spectrophotometry and for spectrofluorometry of many metal ions.¹ Morin forms a stable fluorescent chelate with thorium(IV) in acidic solution. Sulfate ion forms more stable complex with thorium(IV) than its morin chelate.^{1,2} Since the fluorescence intensity of the thorium-morin chelate was inversely proportional to the concentration of the sulfate ion, the determination of sulfate has been achieved.^{2,3}

We have found that the presence of arsenic(V) also quenched the fluorescence of the thorium-morin chelate by the dissociation of the chelate and then by the formation of a new complex of thorium arsenate at pH 2.2. In this acidic medium, the free morin dissociated was not very fluorescent, compared with the thorium-morin chelate. Accordingly, the fluorescence intensity of the chelate decreased in proportion to the concentration of arsenic(V). By using this exchangeable reaction of thorium(IV), the ppb level of arsenic(V) was determined. Since arsenic(III) could not form thorium-arsenite complex, arsenic(V) in the solution containing arsenic(III) could be selectively determined by this fluorometry.

Experimental

Reagents

Highly pure water, obtained by distilling twice doubly de-ionized water, was used throughout the present study.

A standard 1000 mg/l stock solution of arsenic(III) was prepared by dissolving 0.1320 g of arsenic(III)

trioxide (99.5%), obtained from Nakarai Chemicals Ltd., Kyoto, with 10 ml of 1 M sodium hydroxide, and by making up to 100 ml with water after addition of 6 ml of 2 M hydrochloric acid.

A standard 100 mg/l stock solution of arsenic(V) was prepared as follows: Add 5 ml of concentrated nitric acid and 1 ml of 6 M hydrochloric acid to 10 ml of the standard arsenic(III) stock solution. Dry up the solution in a beaker on a boiling water-bath. Dissolve the evaporated residue with water, and make it up to 100 ml with water.

Thorium(IV) 1000 mg/l stock solution was prepared by dissolving 0.2379 g of thorium(IV) nitrate tetrahydrate (Wako Pure Chemical Industries Ltd., Osaka) with water, and by diluting it to 100 ml with water.

Morin 500 mg/l stock solution was prepared by dissolving 0.05 g of morin (Wako Chemical Industries Ltd.) with ethanol (99.5 v/v%) and by making up to 100 ml with ethanol.

Working solutions of arsenic(III), arsenic(V), thorium(IV), and morin were prepared by diluting each stock solution to be 1 mg/l, 1 mg/l (or 10 mg/l), 10 mg/l, and 50 mg/l, respectively.

All other reagents and solutions were of the highest purity available, and were used without further purification.

Apparatus

A spectrofluorometer (Model 650-10S; 150 W xenon lamp) and a pH meter equipped with a glass electrode (Model M-7), both from Hitachi Ltd., Tokyo, Japan, were used.

Analytical procedure

To a centrifugal tube (Vol. 50 ml) with a cap, add 1 ml of a solution containing 0.01–0.1 μg of arsenic, 0.5 ml of thorium(IV) solution (10 mg/l), 0.1 ml of morin solution (50 mg/l) using a micro-pipet, and 16 ml of ethanol. Make up them to 25 ml with water after adjusting the pH to 2.2 with 0.5 M hydrochloric acid. Shake the tube covered with its cap for about 10 s, and warm the tube in a water-bath controlled thermostically to 30°C for 10 min. Without cooling the tube, immediately measure the fluorescence intensity of the solution with the excitation and emission wavelengths of 415 nm and 505 nm, respectively. Determine the concentrations of arsenic(V) in sample solutions by the calibration curve, in which the fluorescence intensity of a standard subtracted from that of a blank is plotted.

For the determination of the higher concentration of arsenic(V) (0.1–1 ppm), carry out the experiment in the same procedure as described above, after changing the volume to 3 ml for the thorium (10 mg/l) solution and to 0.3 ml of the morin (50 mg/l) solution.

Results and Discussion

Excitation and fluorescence spectra of thorium–morin chelate

Figure 1 shows excitation and fluorescence spectra of thorium–morin chelate. In the presence of arsenic(V), the fluorescence intensity of the chelate decreased. In the absence of both thorium(IV) and arsenic(V), the fluorescence intensity of morin was much lower.

Composition of thorium–morin chelate

The stoichiometric composition of thorium–morin chelate was determined to be 1:1 by Job's continuous

variation method and the molar-ratio method, as shown in Fig. 2. Thorium(IV), thus appears to bind to the carbonyl group and the hydroxyl group of morin, as in the case of flavonol.¹

Effect of various factors

The maximum fluorescence intensity of thorium–morin chelate was observed when the pH of the chelate solution was 2.2, as shown in Fig. 3. In the presence of arsenic(V), the maximum fluorescence intensity was also observed at the same 2.2 pH (Fig. 3).

Figure 4 shows that the maximum and constant fluorescence intensity of the chelate was found when more than 15 ml of ethanol was added for the 25 ml of the total volume, i.e., 60% (v/v) ethanol. The increase in fluorescence intensity of the chelate with ethanol is probably due to the effect of solvent.⁴ As another reason, this increase may be due to the formation of more thorium–arsenate complexes rather than forma-

Fig. 2 Composition of thorium–morin complex by Job's continuous variation method and the molar-ratio method.

Fig. 1 Excitation and emission spectra of thorium–morin complex. (A), (B), 5 μg each of Th(IV) and morin; (C), 5 μg each of Th(IV) and morin in the presence of 0.5 μg of As(V); (D), 5 μg of morin; total volume, 25 ml.

Fig. 3 Effect of pH on the formation of thorium–morin complex. $\square$ blank (5 μg each of Th and morin); Δ blank (30 μg of Th and 15 μg of morin); $\bullet$ sample (5 μg each of Th and morin with 1 μg of As(V)); $\blacktriangle$ sample (30 μg of Th and 15 μg of morin with 10 μg of As(V)); total volume, 25 ml.

tion of the solution of thorium hydroxide.

Figure 5 shows calibration curves. A straight line was obtained, when morin 5 μg and thorium 5 μg , or morin 5 μg and thorium 30 μg were used for the determination of the lower concentration of arsenic(V) (0.01–0.1 ppm) or the higher one (0.1–1 ppm).

In Fig. 6, a linear calibration curve was found when 5 μg of morin, in the presence of 5 μg or 30 μg of thorium, was used for the determination of the lower and higher concentration of arsenic. Accordingly, we decided to use those amounts for this fluorometry.

As can be seen in Fig. 7, the replacement reaction of a thorium–morin complex by a thorium–arsenate one depended on the reaction temperature. Linear calibration curves were obtained only at 30°C, though the higher fluorescence intensity was obtained at 5°C

Fig. 4 Effect of the concentration of ethanol. $\circ$ blank (each 5 μg of Th and morin); Δ blank (30 μg of Th, 15 μg of morin); $\bullet$ sample (5 μg each of Th and morin with 0.5 μg of As(V)); $\blacktriangle$ sample (30 μg of Th and 15 μg of morin with 5 μg of As(V)); total volume, 25 ml.

Fig. 5 Effect of the concentration of thorium. (a) Th: $\circ$ 3 μg ; $\bullet$ 5 μg ; $\square$ 10 μg ; Δ 20 μg ; 5 μg of morin; total volume, 25 ml; (b) Th: $\circ$ 3 μg ; $\square$ 10 μg ; Δ 20 μg ; $\bullet$ 30 μg ; $\blacktriangle$ 300 μg ; 15 μg of morin; total volume, 25 ml.

or 10°C. The time required for the replacement reaction was more than about 5 min, as is depicted in Fig. 8. In this work, the reaction time was settled to be 10 min in the recommended procedure.

Recovery of arsenic(V) in the recommended procedure

Table 1 shows the recovery percentages of arsenic(V) in the recommended procedure. Even in the presence of arsenic(III), arsenic(V) was recovered almost 100%, and its relative standard deviation ($n=3$) was about 1–3%.

Effect of coexisting ions

In order to apply this quenching fluorometry to fresh waters, the dissolved chemical species such as Na^+ , K^+ , Ca^{2+} , Mg^{2+} and SO_4^{2-} , and soluble SiO_2 , and reagents used for general pre-treatment such as NH_4OH , HNO_3 and HClO_4 were tested in the presence of 1 μg of arsenic(V). Table 2 lists the results of this experiment. Since the Ca^{2+} , SO_4^{2-} and SiO_2 ions which are dissolved mainly in fresh waters were considerably interfered

Table 1 Recovery of arsenic(V) in the recommended procedure

As(III) added/ mg	As(V) added/ mg	As(V) found/ mg	As(V) recovery, %
0.5	0.5	0.483	96.6
0.5	0.5	0.514	103
0.5	0.5	0.495	99.0
average			99.5 $\pm$ 3.2
1.0	1.0	1.01	101
1.0	1.0	0.986	98.6
1.0	1.0	0.994	99.4
average			99.6 $\pm$ 1.2

Fig. 6 Effect of the concentration of morin. (a) morin: Δ 2 μg ; $\bullet$ 5 μg ; $\square$ 15 μg ; $\circ$ 45 μg ; 5 μg of Th; total volume, 25 ml; (b) morin: $\bullet$ 5 μg ; $\circ$ 15 μg ; $\square$ 50 μg ; Δ 150 μg ; 30 μg of Th; total volume, 25 ml.

Table 2 Effect of coexisting ions

Na ⁺		K ⁺		Ca ²⁺		Mg ²⁺		SiO ₂		NH ₄ ⁺		NO ₃ ⁻		ClO ₄ ⁻		SO ₄ ²⁻	
ppm	RFI	ppm	RFI	ppm	RFI	ppm	RFI	ppm	RFI	ppm	RFI	ppm	RFI	ppm	RFI	ppm	RFI
0	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100
1	108	0.1	100	1	142	0.2	97	1	46	0.4	109	1.3	99	1.3	47	2	12
2	108	0.2	99	2	134	0.4	106	2	45	4	93	13	99	13	23	4	8
4	110	0.4	98	4	123	1.0	96	5	30	40	92	130	96	130	9	10	9
7	111	0.8	94	10	134	2.0	103	10	19	400	98	1300	76	1300	10	20	7
20	117	2.0	93	20	160	4.0	95	20	9	4000	93	6500	49	13000	10	40	5
30	118	4.0	93	40	156	8.0	102	50	3							100	7
50	113	8.0	91	80	145	20.0	85	100	2							200	6
70	113	12.0	85														

RFI (relative fluorescence intensity) was measured in the presence of 1 µg of As(V), 5 µg of Th(IV) and 5 µg of morin. Total volume was 25 ml.

Fig. 7 Effect of reaction temperature. Temp.: ○ 5°C; △ 10°C; □ 20°C; ● 30°C; ▲ 40°C; total volume, 25 ml; reaction time, 10 min; (a) 5 µg each of Th(IV) and morin; (b) 30 µg of Th(IV) and 5 µg of morin.

Fig. 8 Effect of reaction time. ● 0.5 µg of As(V) (5 µg each of Th and morin); ○ 5 µg of As(V) (30 µg of Th and 15 µg of morin); reaction temp., 30°C; total volume, 25 ml.

with, this fluorometry was not applicable to the fresh water samples. However, the proposed fluorometry may be applied to the mixtures of arsenic(III) and arsenic(V) in the presence of Na⁺, K⁺, Mg²⁺, NH₄⁺ and NO₃⁻, if they are contained within the limited amounts found in Table 2.

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