Fluorescence quenching method for determination of trace tungsten in environmental samples with dibromohydroxyphenylfluorone

Zaijun Li[a]; Yang Yuling[b]; Qin Wei[c]; Zhongyun Liu[a]; Jian Tang[a]

[a] School of Chemical and Materials Engineering, Southern Yangtze University†, Wuxi 214036, People's Republic of China [b] Department of Food Science and Engineering, Nanjing Economy University, Nanjing 210003, People's Republic of China [c] School of Chemistry and Chemical Engineering, Jinan University, Jinan 250022, People's Republic of China

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis
Taylor & Francis Group

FLUORESCENCE QUENCHING METHOD FOR DETERMINATION OF TRACE TUNGSTEN IN ENVIRONMENTAL SAMPLES WITH DIBROMOHYDROXYPHENYLFLUORONE

ZAIJUN LI[a,*], YANG YULING[b], QIN WEI[c],
ZHONGYUN LIU[a] and JIAN TANG[a]

[a]*School of Chemical and Materials Engineering, Southern Yangtze University*[†]*, Wuxi 214036, People's Republic of China;* [b]*Department of Food Science and Engineering, Nanjing Economy University, Nanjing 210003, People's Republic of China;* [c]*School of Chemistry and Chemical Engineering, Jinan University, Jinan 250022, People's Republic of China*

A highly sensitive and selective fluorescence quenching method has been developed for the determination of trace tungsten in environmental samples using dibromohydroxyphenylfluorone (DBHPF) as an emission reagent. In the presence of 0.04 mol/L of sulphuric acid and acetyltrimethylammonium bromide, tungsten(VI) reacts with DBHPF to form a 1 : 3 red complex within 5.0 min. In order for the DBHPF–tungsten(VI) complex to form, the fluorescence intensity of the reagent solution was quenched linearly by adding 0.1 to 1.0 µg of tungsten(VI) in 25 mL of solution. This was measured at 528 nm with excitation at 495 nm. In this work, a standard addition method was investigated and used for sample analysis. The decrease in fluorescence intensity of the reagent solution (ΔF) was linear for $0 \sim 0.9$ µg of tungsten(VI) in 25 mL of solution, and the detection limit (3 s) of the standard addition method was found to be 0.012 ng/mL of tungsten(VI). The effects of various metal and nonmetal ions were studied in detail. The experiments clearly showed that most foreign ions can be tolerated in considerable amounts; in particular, 50-fold Mo(VI), V(V), Zr(VI) and Ti(IV) do not interfere, and the selectivity of the proposed method is better than other previously described methods. Moreover, the method proposed here is very stable and simple, the fluorescence intensity of the solution can remain almost unchanged for 2.0 h at room temperature, and the method has been used successfully to determine tungsten in environmental samples.

Keywords: Fluorescence quenching; Dibromohydroxyphenylfluorone; Tungsten; Environmental samples

INTRODUCTION

Tungsten is a transition metal that occurs naturally as a mineral and has been shown to be an essential element for some micro-organisms. Progress in elucidating the biological importance of tungsten has been helped by the purification and structural analysis of a large number of enzymes containing tungsten [1–3]. Recently, Barbera *et al.* [4,5]

*Corresponding author. Fax: +86-0510-5865424. E-mail: Zaijunli@263.net
[†]Formerly Wuxi University of Light Industry.

showed that sodium tungstate can correct hyperglycaemia in insulin and insulin-dependent models of diabetes and can be useful in the long-term treatment of this disease because of its ability to restore glucose homeostasis and prevent various diabetes-related complications [6]. Further work has revealed that the glycemic reduction brought about by sodium tungstate is due to its direct insulinotropic hormone activity [7]. Chronic sodium tungstate treatment might prime the B-cell and result in an over-response to a glucose stimulus. Sodium tungstate is currently under investigation, and several pre-clinical studies using single and multiple doses in rat and dog have been published [8–10].

However, tungsten may interfere with molybdenum metabolism and depress the biological activity of many important enzymes, including molybdenum, phosphatase and adenosine triphosphate enzymes, and it has been found to be toxic to animals [11]. For example, a mote containing tungsten can cause emphysema and pulmonary fibrosis; a tungsten concentration of $5\,\mu g/kg$ can lead to the death of an animal embryo. The amount of tungsten in the environment is closely linked to the reproduction and development of humans, animals and plants, and so it is important to monitor its concentration.

At present, the main methods to determine tungsten involve spectrophotometry [12–14], flame atomic absorption spectrometry (FAAS) [15], inductively coupled plasma atomic emission spectrometry (ICP-AES) [16], inductively coupled plasma mass spectrometry (ICP-MS) [17] and electrochemistry [18]. Tungsten detection in FAAS is poor, even with the use of a nitrous oxide–acetylene flame. Although ICP-AES, ICP-MS and electrochemistry offer better sensitivity and detection limits for tungsten than FAAS, these methods are not directly applicable to water samples because the average concentration of tungsten in water samples is at the level of ng/mL, below the detection limits of these methods. In addition, the effects of the matrix on the direct determination of tungsten warrant suitable separation and pre-concentration procedures prior to trace tungsten(VI) determination in water.

Spectrophotometry offers the best alternative for the routine analysis of tungsten in the absence of ICP-AES and ICP-MS. Many reagents are available in the literature. Among them, the derivatives of fluorone are preferable for routine analysis of tungsten owing to their rapidity and high sensitivity. However, the detection limit of the methods is only at the level of $\mu g/mL$, clearly not sufficiently sensitive to determine tungsten in water and sediment. Thus, there is a great demand for a simple, valid and relatively inexpensive method by which to determine tungsten. In this work, the fluorescence intensity of dibromohydroxyphenylfluorone (DBHPF; Fig. 1) in the presence of tungsten(VI) is investigated in detail. The change in fluorescence intensity of DBHPF, due to the interaction of tungsten and the reagent in water solution, was found to provide a sensitive and very simple method by which to determine tungsten in environmental samples.

EXPERIMENTAL

Apparatus and Reagents

All fluorescence measurements were made with a RF-850 spectrofluorometer using a 1-cm quartz cell. The bandpasses were at 4 nm for both excitation and emission monochromators. The light source was a 150-W xenon lamp.
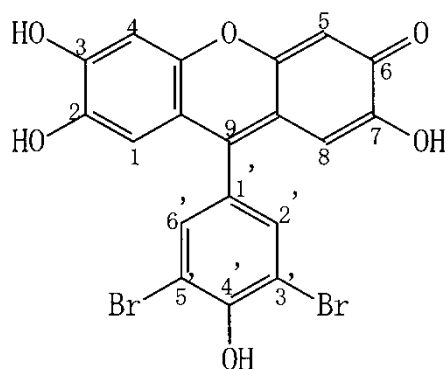
FIGURE 1   Molecular structure of dibromohydroxyphenylfluorone.

Unless stated otherwise, all reagents were of analytical-reagent grade, and all solutions were prepared with distilled water. Tungsten(VI) standard stock solutions (1.00 mg/mL) were prepared by dissolving sodium tungstate (spec-pure, Shanghai Third, Chemicals Factory) in distilled water. Working aqueous solutions (1.0 μg/mL) were prepared by diluting the above stock solution in distilled water. DBHPF solution (2×10^{-5} mol/L) was prepared by dissolving the compound in 95% alcohol containing 1.0 mL of 5.0 mol/L sulphuric acid solution, which was synthesized as described in the literature [19]. Cetyltrimethylammonium bromide (CTMAB) solution (0.2%) was prepared by dissolving CTMAB in distilled water.

General Procedure

Standard solution or sample solutions containing no more than 1.0 μg of tungsten(VI) were transferred into a 25-mL calibrated flask, 1.0 mL of 1.0 mol/L of sulphuric acid, 1.0 mL of 0.20% CTMAB and 1.0 mL of 2×10^{-5} mol/L of DBHPF solution were added successively, and the solution was diluted to 25 mL with distilled water and mixed. After 10 min, the fluorescence intensity of the solution was measured at 528 nm, with excitation at 495 nm. All fluorescence intensity measurements were corrected with blank solution. All experiments were performed at room temperature (20°C).

Preparation of the Samples

Water samples were filtered through a 0.45-μm membrane to exclude insoluble particles. In a 100-mL centrifuge tube, 50 mL of 0.20 mol/L of K_2SO_4 were added to 1.00 g of finely ground sediment. The centrifuge tube was shaken for 16 h at 25°C and then centrifuged for 10 min at 3000 rpm. The upper-layer solution was filtered through a 0.45-μm membrane, and the filtrates were used to determine tungsten directly.

Stream sediment, soil and mote samples collected from different places were dried, ground and sieved to an 80–100 mesh. A small quantity (0.1 ~ 0.5 g) of the sample was weighed and transferred to 100-mL Teflon beakers, and 5 mL of concentrated HNO_3 and 20 mL of concentrated HF were added to each beaker. The sample mixture was evaporated to dryness, heated overnight at 125°C in a sand bath and 2 mL of warm

water and 8.0 mL of concentrated HCl added to each residue and mixed. The mixture was filtered through Whatman no. 42 filter paper, and the resultant solution was transferred to a 100-mL calibrated flask and diluted to the mark with distilled water.

RESULTS AND DISCUSSION

Excitation and Emission Spectra of DBHPF

The excitation and emission spectra of the DBHPF in the presence and absence of tungsten(VI) are listed in Fig. 2. The figure shows that the maximum excitation and emission peaks of DBHPF lie at 495 and 528 nm, respectively. A fluorescence intensity (ΔF) decrease of almost 50% at 528 nm was also observed in the presence of 1.0 µg of tungsten(VI). An excitation wavelength of 495 nm and emission wavelength of 528 nm were selected as the operating wavelengths in the experiments, so as to provide a high sensitivity.

Effect of Acidity

The effect of acidity on ΔF owing to the addition of tungsten(VI) was tested by using the procedure described in the Experimental Section. Although the reaction of tungsten(VI) with the reagent can be completed rapidly in various acidic media, including HCl, H_2SO_4, H_3PO_4 and $HClO_4$, ΔF shows obvious differences. Among these, H_2SO_4 medium gave a maximal ΔF and was selected. Moreover, the effect of H_2SO_4 concentration was tested with reagent solution containing 1.0 µg of tungsten(VI) in
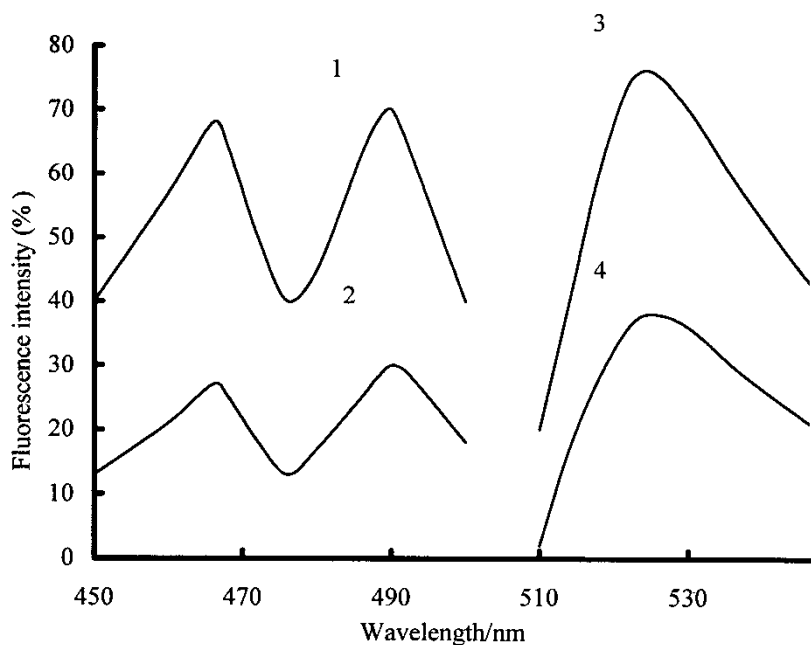

FIGURE 2 Fluorescence excitation (1,2) and emission (3,4) spectra. 1,3: DBHPF 8×10^{-7} mol/L; 2,4: with 2.2×10^{-7} mol/L of tungsten(VI) added to the 8×10^{-7} mol/L of DBHPF.

25 mL. The experiments indicated that ΔF remained at a maximum and almost constant when the sulphuric acid concentration was 0.02–0.1 mol/L. Thus, 0.04 mol/L of H_2SO_4 was recommended in the following studies.

Effect of DBHPF Concentration

Although the fluorescence intensity of the reagent blank and testing solution containing tungsten (VI) shows a remarkable difference with the addition of DBHPF, the ΔF of the corresponding reagent blank and sample solutions remain almost constant in the reagent concentration range of $4 \sim 10.4 \times 10^{-7}$ mol/L. In this work, a concentration of 8×10^{-7} mol/L of DBHPF was chosen.

Selection of Surfactant

In the absence of a surfactant, the reaction of tungsten(VI) and DBHPF was slow due to the reagent and the complex's poor water solubility, and the fluorescence intensity of the testing solution and ΔF was also small. To increase the reaction rate and the sensitivity, the effects of various surfactants including OP, TritonX-100, Tween-80, CPC, CPB and CTMAB on the ΔF were studied in detail. The results indicated that the ΔF of the solution with 1.0 µg of tungsten(VI) in 25 mL of solution was higher in the presence of CTMAB. For this reason, CTMAB was selected as the activator. When $1.0 \sim 10$ mL of 0.2% CTMAB was added, ΔF remained at a maximum and constant. Thus, 2.0 mL of 0.2% CTMAB was used in the following work.

Effect of Foreign Ions

The effect of foreign ions on the fluorescence intensity of the solution was studied in detail. The tolerance limits were calculated as the concentration of a species which resulted in less than 5% deviation in the fluorescence intensity of the solution containing 1.0 µg of tungsten(VI) in 25 mL of solution. The results are summarized in Table I; it can be seen that most foreign ions can be tolerated in relatively high amounts. Only molybdenum in environmental samples may interfere with the trace determination of tungsten. However, the interference caused by molybdenum can be easily eliminated by adding hydroxylamine hydrochloride as a masking agent.

TABLE I   Effect of foreign ions

Foreign ions	Tolerated limit (mg/L)
K(I), Na(I), NH_4(I), Cl^-, Br^-, I^-, SO_4^{2-}, NO_3^-, CO_3^{2-}, PO_4^{3-}	4000
Ca(II), Mg(II), Cu(II)	400
Cd(II), Zn(II), Ni(II), Mn(II)	200
Fe(III)	120
Al(III)	80
Cr(III), Ce(III)	20
Co(II), Bi(III), Sn(IV)	4
Mo(VI), V(V), Zr(VI), Ti(IV)	0.01, 2[a]

[a]2 mL of 1% hydroxylamine hydrochloride added and the mixture heated in boiling bath for 10 min.

Calibration Graph and Standard Addition Method

A calibration graph was constructed by introducing different amounts of tungsten(VI) in 25-mL calibration flasks, adding the reagents and developing the reaction according to the general procedure described above, in the Experimental Section. The calibration graph showed that ΔF was linear for $0.1 \sim 1.0\,\mu g$ of tungsten(VI) in 25 mL of solution.

To help determine low levels of tungsten in real samples, another calibration graph was constructed by using the standard addition method [20], as follows. Tungsten (VI) standard solution $(0.1\,\mu g)$ was added to each of above testing solutions for the reaction development. The fluorescence intensity of the testing solution containing different amounts of tungsten(VI) was determined using the procedure described above. Thus, the fluorescence intensity of the testing solution containing $0.1\,\mu g$ of tungsten(VI) minus the fluorescence intensity of the testing solution containing different amounts of tungsten(VI) yields a series of ΔF. A new calibration curve was obtained, and this is shown in Fig. 3. From the figure, it can be easily seen that ΔF increased linearly in the range of $0 \sim 0.9\,\mu g$ of tungsten(VI). The detection limit (3 s) for $0.53\,\mu g$ of W(VI), defined by IUPAC [21], was found to be $0.012\,ng/mL$, based on the standard addition method.

Effect of Hydroxylamine Hydrochloride

To overcome the interference of Mo(VI), Ti(VI), V(V) and Zr(IV) on the determination of tungsten(VI), the effects of various compounds including citrate, 8-hydroxylquinoline and hydroxylamine hydrochloride were tested. It was found that hydroxylamine
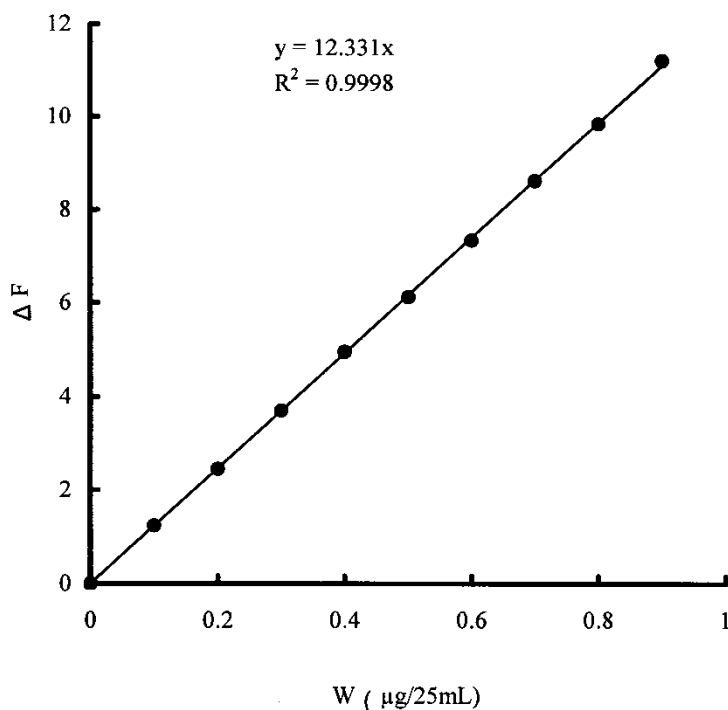

FIGURE 3   Calibration graph of standard addition method.

hydrochloride was the most efficient and was selected as the masking agent. After the reaction was completed, by adding hydroxylamine hydrochloride to the reaction system and heating the solution for 10 min the complexes of Mo(VI), Ti(VI), V(V) and Zr(IV) with DBHPF could be decomposed efficiently and the selectivity of the determination of tungsten improved. Experimental data indicated that the addition of no more than 2.5 mL of 1% hydroxylamine hydrochloride did not effect the sensitivity, so 2.0 mL of 1% hydroxylamine hydrochloride was used. The tolerance limit of Mo(VI), Ti(VI), V(V) and Zr(IV) was calculated in the presence of hydroxylamine hydrochloride, as the concentration of a species producing less than 5% deviation in the fluorescence intensity of a solution containing 1.0 μg of tungsten(IV) in 25 mL of solution. The results are listed in Table I.

Quenching by Tungsten(VI)

The efficiency of quenching a fluorescent species by a quenching species follows the Stern–Volmer relationship [22] if the fluorophore and quencher concentrations are in the appropriate range: $I_o/I = 1 + K_{sv}[Q]$, where I_o and I are the fluorescence intensity in the absence and presence of the quencher, respectively, K_{sv} is the Stern–Volmer quenching constant and $[Q]$ is the concentration of the quencher. If a system follows the Stern–Volmer equation, a plot of I_o/I vs. $[Q]$ will give a straight line with a slope of K_{sv} and a y-axis intercept of 1.

Figure 4 shows the Stern–Volmer plot of the tungsten(VI)–DBHPF system. The I_o/I term increased linearly with increasing concentration of tungsten(VI), $[Q]$. The effect of static quenching is lower at higher temperatures. In the case of dynamic (collision) quenching, quenching efficiency increases with increasing temperature.
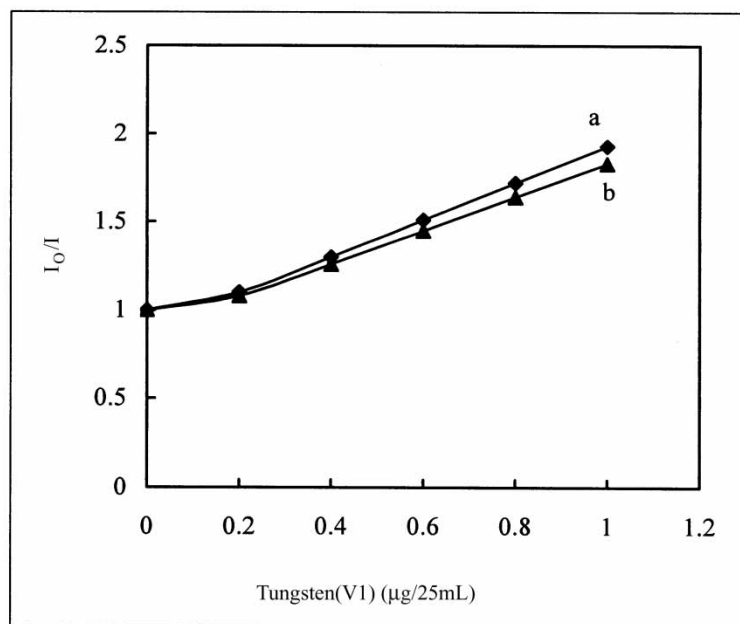

FIGURE 4 Stern–Volmer plots for the quenching of DBHPF fluorescence by tungsten(VI). (a) 25°C; (b) 45°C; DBHPF: 8×10^{-7} mol/L.
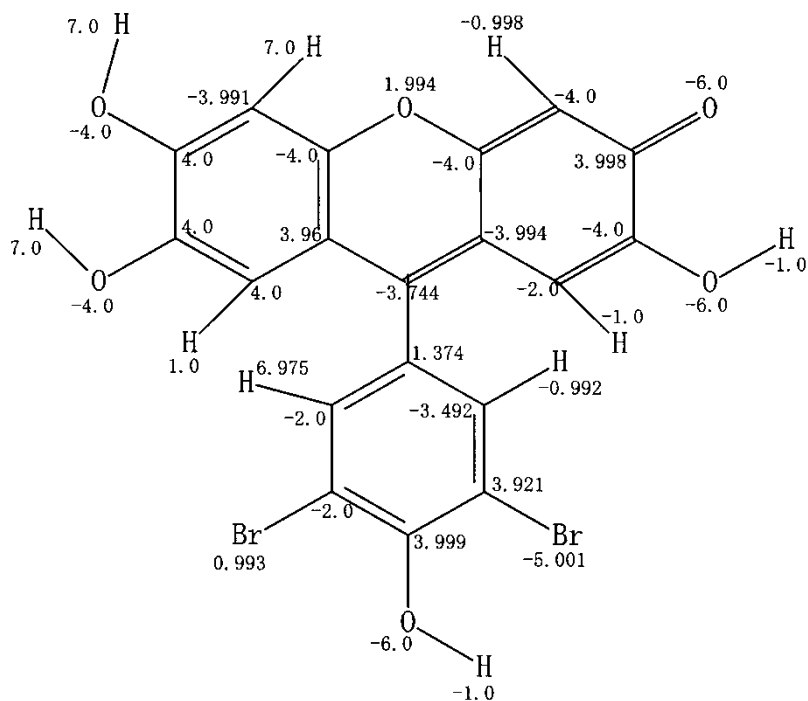
FIGURE 5   Electron distribution of dibromohydroxyphenylfluorone.

The Electron Distribution of DBHPF and Characteristics of the Complex

To research the effect of the structure of reagent on analytical characteristics, the electron distribution of DBHPF was calculated by quantum mechanics. The result is shown in Fig. 5, in which an atom with a higher value has a lower electron density. From Fig. 5, it can be seen that oxygen atoms in the 6- and 7-positions of the DBHPF molecule have a high electron density due to the introduction of two bromide atoms and one hydroxyl into the 9-phenyl ring. Also, the high electron density is beneficial for the reaction of DBHPF with tungsten(VI) and the formation of a stable complex. The above functions result in rapid quenching of the reagent solution's fluorescence. The high degree of electron fluidity in the reagent molecule may produce strong fluorescence and provide a high degree of sensitivity with the fluorescence quenching method.

In H_2SO_4 solution, the reaction of tungsten(VI) and DBHPF can be completed within 5 min and the fluorescence intensity may remain stable for at least 2 h at room temperature. The composition ratio of the tungsten(VI)–DBHPF complex obtained using Job's method of continuous variation and the slope-ratio method was 1 : 3.

Application

Different types of environmental samples were digested, and tungsten was determined by the methods described in detail earlier. The results are listed in Table II

TABLE II Results of determination of tungsten in environmental samples[a]

Samples	Tungsten found by the proposed method	Tungsten found by ICP-AES
Hot spring water[b]	26.12 ± 0.12 $F = 1.36$, $t = 0.165$	26.11 ± 0.14
River water[b]	67.40 ± 0.25 $F = 1.56$, $t = 0.2$	67.42 ± 0.20
Seawater[b]	70.53 ± 0.19 $F = 1.22$, $t = 0.31$	70.50 ± 0.21
Stream sediment[c]	30.31 ± 0.10 $F = 2.56$, $t = 0.40$	30.33 ± 0.16
Mote[c]	0.62 ± 0.05 $F = 6.25$, $t = 0.4$	0.63 ± 0.02
Soil[c]	1.55 ± 0.13 $F = 1.33$, $t = 0.61$	1.51 ± 0.15

[a]Results expressed as: $X \pm s \times t/n^{1/2}$ ($n = 5$) where X is the mean of n observations of X, s is the standard deviation, t is the distribution value chosen for the desired confidence level; the F- and t-values refer to the comparison of the proposed method with the ICP-AES method. Theoretical values at 95% confidence limits: $F = 6.39$, $t = 2.78$.
[b]μg/L.
[c]μg/g.

and show good agreement with those obtained by ICP-AES, carried out for comparison.

CONCLUSIONS

The method proposed here is characterized by a very high sensitivity and selectivity. Its detection limit is slightly better than that of ICP-AES and ICP-MS and much better than that of spectrophotometry. It can be used to determine trace tungsten in various complex samples, including wastewater, alloy, steel, environment and biological samples without prior pre-concentration and separation. Moreover, it is also very simple and economical, and requires only small amounts of reagents. Therefore, it is beneficial in routine analyses, especially in developing countries.

References

[1] A. Kletzin and M.W. Adams, *FEMS Micobiol. Rev*, **18**, 5–63 (1996).
[2] M.K. Johnson, D.C. Rees and M.W. Adams, *Chem. Rev*, **96**, 2817–2839 (1996).
[3] W.R. Hagen and A.F. Arendsen, *Struct. Bond*, **90**, 161–191 (1998).
[4] A. Barbera, J.E. Rodriguez-Gil and J.J. Guinovart, *J. Biol. Chem*, **269**, 20047–20053 (1994).
[5] A. Barbera, J. Fernandez-Alvarez, A. Truc and R. Gomis, *Diabetogia*, **40**, 143–149 (1997).
[6] A. Barbera, R.R. Gomis and N. Prats, *Diabetologia*, **44**, 507–513 (2001).
[7] J. Rodriguez-Gallardo, A.R. Silvestre, E.M. Egodo and J. Marco, *Eur. J. Pharmacol.*, **402**, 199–204 (2000).
[8] S.L. Lamer, P. Poucheret, G. Cros, R.R. Kiesgen, P.A. Bonnet and F. Bressolle, *J. Pharmacol. Exp. Ther*., **294**, 74–80 (2000).
[9] S. Le Lamer, G. Cross, C. Pinol, J. Gernandez-Alcarez and F. Bressolle, *Pharmacol. Toxicol.*, **90**, 100–105 (2002).
[10] S. Le Lamer, G. Cross, J.J. Serrano, C. Pinol, J. Fernandez-Alvarez and F. Bressolle, *Eur. J. Pharmacol. Sci.*, **14**, 323–329 (2001).
[11] Y.D. Gu, G.Y. Xie, Y. Song and S.L. Jin, *Inorganic Chemistry Series*, 8th Edn. Science Press, Peking (1998).

[12] S.X. Li, N.S. Deng, F.Y. Zheng and Y.Z. Huang, *Talanta*, **60**, 1097–1104 (2003).
[13] M. Soylak, L. Elci and M. Dogan, *Talanta*, **42**, 1513–1517 (1995).
[14] S. Lis and S. But, *J. Alloys Compounds*, **303–304**, 132–136 (2000).
[15] R.K. Malhotra, D.S.R. Murthy, G. Srinivasan and K. Satyanarayana, *At. Spectrosc*, **8**, 161–169 (1987).
[16] P. Poucheret, S. Le Lamer and G. Cross, *Anal. Chim. Acta*, **405**, 221–226 (2000).
[17] S. Le Lamer, P. Poucheret, J.L. Perez and F. Bressolle, *Clin. Chim. Acta*, **327**, 39–46 (2003).
[18] P.L. Hagedoorn, P.V. Slot, H.P. Van Leeuwen and H.W.R. Hagen, *Anal. Biochem.*, **297**, 71–78 (2001).
[19] Z.L. Li, J.M. Pan and J. Tang, *The Analyst*, **126**, 1154–1159 (2001).
[20] R. Yang, Y. Lu, G.L. Song, T. Wang and J.M. Pan, *Anal. Chim. Acta*, **324**, 95–99 (1995).
[21] G.L. Long and D. Wineforder, *Anal. Chem*, **55**, 712A–724A (1983).
[22] O.S. Wolfbeis, *Fluorescence Spectroscopy*: *New Methods and Applications*, 1st Edn. Springer, Berlin (1993).