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Determination of Molybdenum(VI) by Optical Sensing Film and Flow Injection Analysis

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Optical flow-through cell detector with incorporated transparent chemosensitive layer of Nile blue has been applied in simple, double-channel flow-injection system for food, environmental and industrial analysis. The reductant analyte converts the Nile blue based sensing layer to colourless form and the attendant colour change is used for sensing. Discoloration of the film is spectrophotometrically at 582 nm wevelength. The flow injection system has been successfully used for selective determination of molybdenum(VI). This detector was fabricated by binding Nile blue to a cellulose acetate film that had previously been subjected to an exhaustive base hydrolysis. The membrane has good durability (> 7 months) and a short response time (< 12 s). Molybdenum(VI) can be determined for the range 0.02–3.40 μg mL^{-1} with 3σ detection limits of 7 ng mL^{-1}. The method is easy to perform and uses acetylcellulose as a carrier. The reagents used for activating the cellulose support are inexpensive, non-toxic and widely available.

Key Words: Optical detector, Molybdenum(VI), Food, Environmental, Flow injection analysis.

INTRODUCTION

The development of optical molybdenum(VI) detectors is of great interest because molybdenum is an essential trace element required by both plants and animals in very small amounts[1–4] and they are used in a variety of manufacturing and environment and analytical procedures[4, 5].

The essential role of this trace element in animal nutrition is due to the fact that flavoprotein enzyme xanthine oxidase contains molybdenum[4]. Molybdenum is also an essential trace element for plants as it is linked to nitrogen fixation by leguminosae and to nitrate reduction processes[5]. On the other hand, at high concentrations molybdenum is toxic[3]. Manifestations of molybdenum toxicity (molybdenosis) vary among different species. Disturbances in phosphorus metabolism in farm animals have been reported in areas of high molybdenum soils[6].

Despite the availability of a number of methods for the routine determination of trace amounts of molybdenum, there is still a need for the development of a method that is superior in accuracy, precision and speed at levels commonly encountered in different natural samples. There are different spectrophotometric methods available for the determination of molybdenum in plants, steel, natural water and food[3–5]. Although the sensitivity can be enhanced by solvent extraction and atomic absorption spectrometry with electrothermal atomization[7], this is achieved at the expense of the speed of the analytical procedure. There are some reports on the catalytic role of molybdenum on some inorganic reactions[8–12]. The new method described in this paper is based on catalytic role of molybdenum on reduction of Nile blue with hydrazine dihydrochloride at triacetyl cellulose, so that sensitive, selective and precise detectors are necessary for the determination of molybdenum(VI) content in environment, industrial and food stuffs. The most important problem with this type of detector is related to the stability of the bond between of the reagents and the carrier. This can be improved by using an efficient procedure for immobilization of the indicator on an appropriate polymer matrix.

The purpose of this work was to modify the above methods for the covalent immobilization of new indicators on an optically transparent acetylcellulose membrane that had previously been hydrolyzed and activated using thiourea and poly (vinyl alcohol). The characteristics of the membrane produced were investigated and the possibilities for its use in the design of optical molybdenum(VI) detectors were evaluated. According to our knowledge, up to now, only dye molecules with amino group on the ring have been used to construct optical molybdenum(VI) detectors based on chemical modification of polymer films. In this paper, we have used thiourea in linking a dye (with amine groups on the ring) to a cellulose acetate film with satisfactory results. The dye used is Nile blue detector, which can be used for direct determination of molybdenum(VI) ion as a catalytic role of molybdenum on reduction of Nile blue with hydrazine dihydrochloride at triacetyl cellulose. It is both very sensitive and precise for determination of low levels of molybdenum and is free from interferences of common interfering ions such as tungsten, iron, etc. The determination of molybdenum(VI) ion is an important factor in the analysis of food, natural waters, industry and environment.

EXPERIMENTAL

All chemicals used in this work were analytical reagent grade (Merck). Distilled water was used throughout.

A standard solution of molybdenum(VI) (1000 ng mL^{-1}) was prepared by dissolving 150 mg of MoO_3 (Merck) in about 50 mL of 0.01 M NaOH solution and diluting to 100 mL with distilled water. Its molybdenum content was established volumetrically by the oxine method[13].

A 2.0 M hydrazine dihydrochloride solution was prepared by dissolving 41.992 g of $N_2H_4{\cdot}2HCl$ (BDH) in water and diluting to 200 mL in a volumetric flask. Nile blue (Merck) solution (2.72×10^{-5}) was prepared by dissolving 0.0020 g of Nile blue A (sulphate salt) in water and diluting to 200 mL. Poly (vinyl alcohol) solution was prepared by dissolving 0.40 g of the reagent in 100 mL of water. Thiourea solution was prepared by dissolving 0.50 g of the reagent in 100 mL of water.

For optical measurements Shimadzu UV-Vis 2100 double-beam spectrophotometer and a thermostated cell at ±0.1°C controlled temperature. Experiments were carried out using conventional system for FIA with a transmission flow-through cell having one of the windows covered with a sensing film. Simple single line manifold consisting of 12 channel peristaltic pump (Desaga, PLG, 70 W) was fitted with three silicon rubber tubes (10 mm i.d.), rotary injection valve and flow-through optical cell.

Preparation of detectors

Triacetyl cellulose was previously hydrolyzed in order to de-esterify the acetyl groups and to increase the porosity of the membrane. Separate pieces of transparent film (34 × 8 × 0.1 mm) were treated in 0.10 mol L^{-1} KOH for 24 h. The films were washed with water and immediately treated with a mixture of 0.50% (w/v) thiourea and 0.40% (w/v) poly (vinyl alcohol) solution for 48 h at 25°C. The cellulose membranes were separately treated with a 2.72×10^{-5} solution of Nile blue at 25°C with magnetic stirring of the solution for 14 h. After washing, the film was dried at 45°C for 20 min. Next the membrane was washed with distilled water until the washings showed no absorbance at the wavelength of the dye during rinsing. Finally the film was dried at 45°C for 20 min.

Effect of reagent concentration and temperature

The effect of HCl + N_2H_4 on the catalyzed reaction was studied with 2.72×10^5 M Nile blue solutions, with various olutions with molybdenum(IV) concentration of 10 ng mL^{-1} and a temperature of 30°C. From the results, 0.35 M was selected as the optimum HCl + N_2H_4 for the study.

The effect of Nile blue concentration in the presence of HCl + N_2H_4 (0.35 M) at a temperature of 30°C was studied. The results show that by increasing Nile blue concentration to greater than 2.55×10^{-5} M, the change in absorbance was diminished. Thus, 2.55×10^{-5} M Nile blue solution was selected for the best sensitivity.

The effect of temperature on the peak height was studied in the temperature range 5–50°C at the optimum condition. From the results, 25°C was selected for the study.

All tests were performed with a 10 ng mL^{-1} molybdenum(IV), Nile blue 2.55×10^{-5} M, N_2H_4, 2HCl concentration of 0.35 M and temperature of 25°C.

The sensitivity of the molybdenum determination largely depends on the flow rate of the reagents. The peak heights increased as the flow rate decreased. Thus, a flow rate of 0.50 mL/min was selected for all reagents.

By increasing the length of the reaction coil from 50–250 cm the peak heights increased, because the longer residence time of the sample zone allowed the reaction to proceed further. However, peak broadening and tailing were observed with longer reaction coils (300 cm) due to dispersion. Thus, 300 cm was selected for the length of the reaction coil.

The effect of sample volume was investigated for the best sensitivity. The sensitivity increased by increasing the sample volume from 50 to 200 μL. However, injection of the larger sample volume into the water stream results in peak broadening and tailing. Therefore a 150 μL sample volume was selected.

Spectrophotometric measurements

The measurements were made on the membrane, which was stretched on a special frame. The size of the aperture was 8.5 × 35 mm (Fig. 1). The control sample against which the measurements were performed consisted of a film treated in the same way but without indicator. The control sample was stretched in the same way inside the cuvette using a frame of the same size. The spectral characteristics of Nile blue were measured by flow injection analysis with molybdenum(VI) in N_2H_4-2HCl 0.35 M, Nile blue 2.55 × 10^{-s} M, temperature 25°C, flow rate 50 mL/min, reaction coil 300 cm and sample volume 150 µL (Fig. 2).

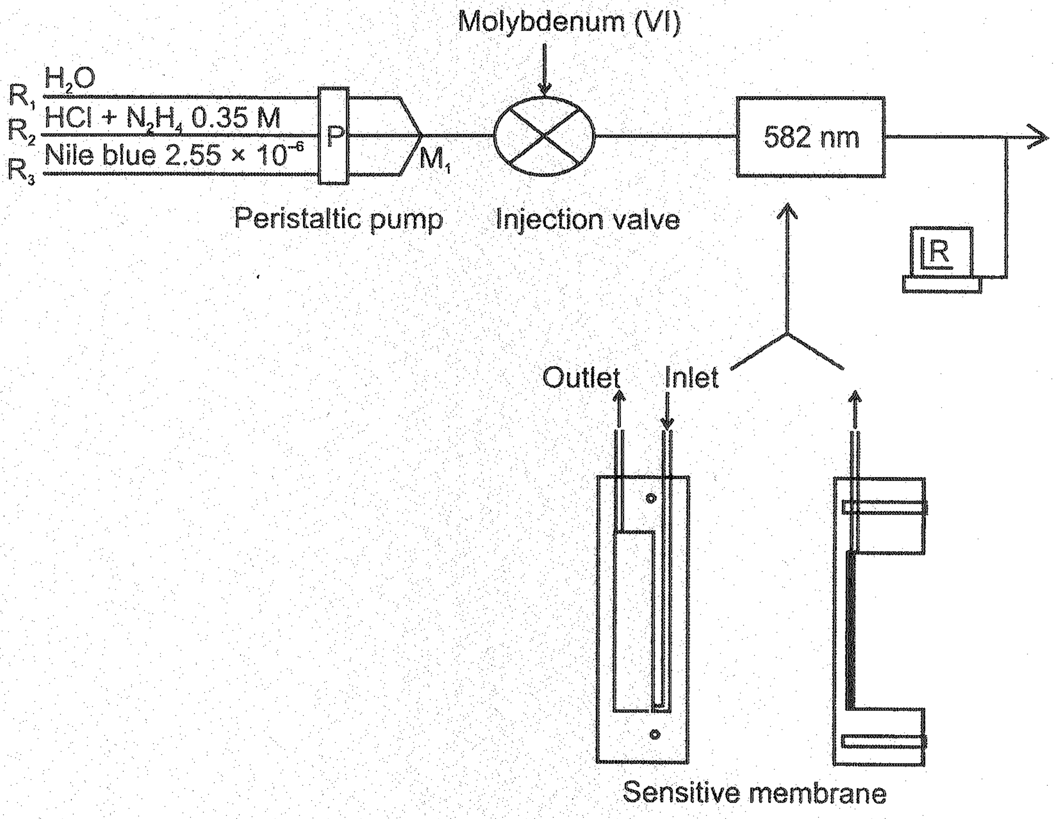

Fig. 1. Schematic diagram of the frame on which the membranes are stretched, inside the cuvette

RESULTS AND DISCUSSION

In the presence of molybdenum(VI) can be catalyzed the reduction of Nile blue by hydrazine. Therefore by this method, total molybdenum concentration can be determined at great speed and high sensitivity. Effect of different variables like reagent concentration, temperature and manifold variables on the determination of molybdenum(VI) was studied.

Nile blue has amino group in the ring. These dye molecules can be linked to the cellulose acetate film by special treatment. Rostov and co-workers[2] showed that only dye with amino groups could be linked with cellulose acetate. We find that using thiourea, dyes with or without amino group can be linked to cellulose acetate film. The optical properties of immobilized Nile blue on a hydrolyzed cellulose membrane as a function of molybdenum(VI) concentration is shown in

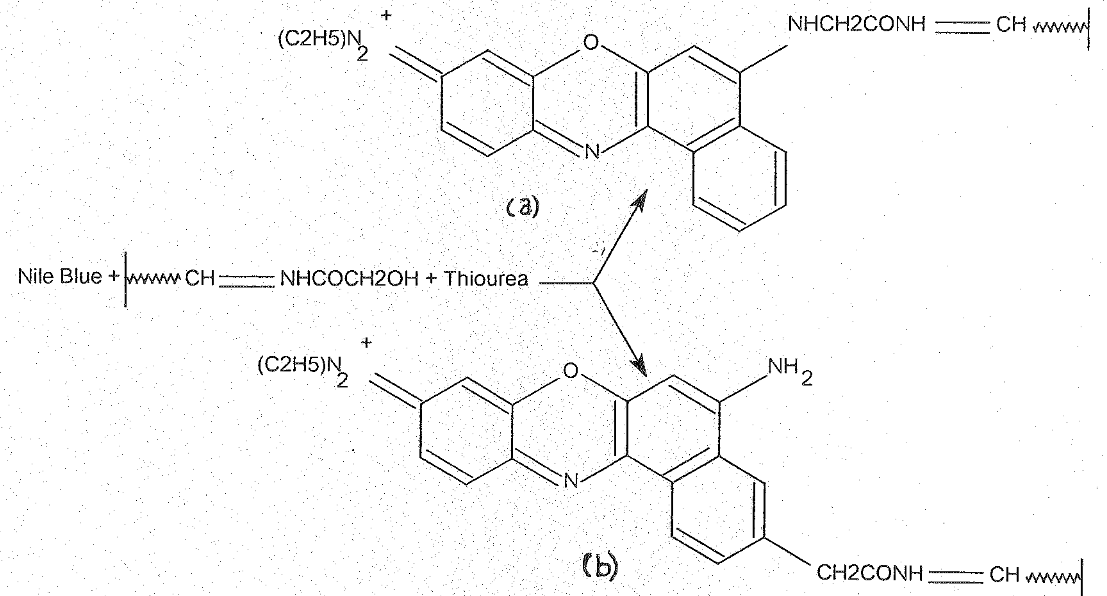

Fig. 2. (a) Absorption spectra for Nile blue in membrane at molybdenum(VI) concentration: (a) 40×10^{-9}, (b) 80×10^{-9}, (c) 120×10^{-9}, (d) 160×10^{-9}, (e) 200×10^{-9}, (f) 240×10^{-9}.

Fig. 2. The absorbance change is linear only for 0.07–3.50 μg mL^{-1} molybdenum(VI). The absorbance maximum of the immobilized Nile blue is located at 582 nm. The above result can be interpreted as the influence of the immobilization procedure on the behaviour of the indicator. Krysteva *et al.*[14] have shown that the condensation between the amino group of the carrier and the protein is accomplished through the transformation of the amino groups in the acidic range. In addition, the reactivity of the activated carrier is so high that it was considered that interaction is possible with low molecular weight compounds that have a free *para*-position in the molecules. For this reason, indicators having hydroxyl group or free *para*-positions in their structure can be used. For this reason, thiourea was used as a bridge to connect the dye molecules to the membrane cellulose acetate film. A possible scheme for the reaction is shown in Fig. 3. This makes it possible to achieve covalent binding to the activated matrix. The fact that the immobilization changes the heights and positions of the absorption maxima to that of free indicators shows that the amine groups are auxochromic elements of the molecule and the loss of the proton after covalent binding influences the charge distribution during the dissociation of the immobilized dye. Nile blue is a dye that can be reduced with hydrazine in an acidic solution in the presence of molybdenum(VI).

Detector stability and response time

It was shown that the changes in the absorbance on making repeated measurements in aqueous solutions for a month were < 5%. The stability of the membrane detectors based on a recycled support is higher than with other methods in which acetylcellulose is also used for producing optical detectors[1]. This is due to the higher mechanical strength of the carrier as well as the covalent binding with the indicator. The change in optical properties of membranes with immobilized Nile blue measured at 582 nm (Fig. 4) shows a typical curve for the transition process. It can be seen that the output signal reaches 98% of the steady-state response of the membrane in 12 s.

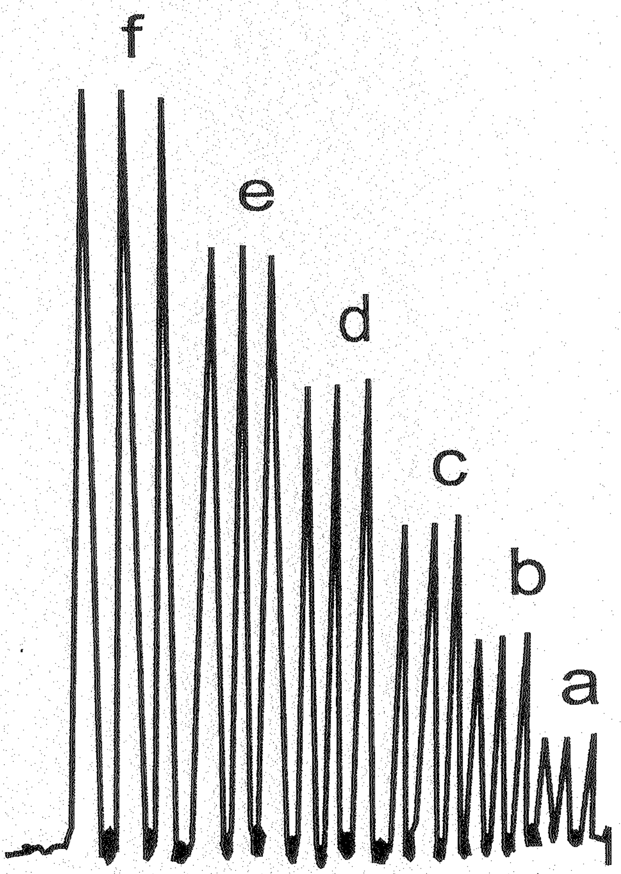

Fig. 3. Possible scheme of reaction between activated membrane and Nile blue

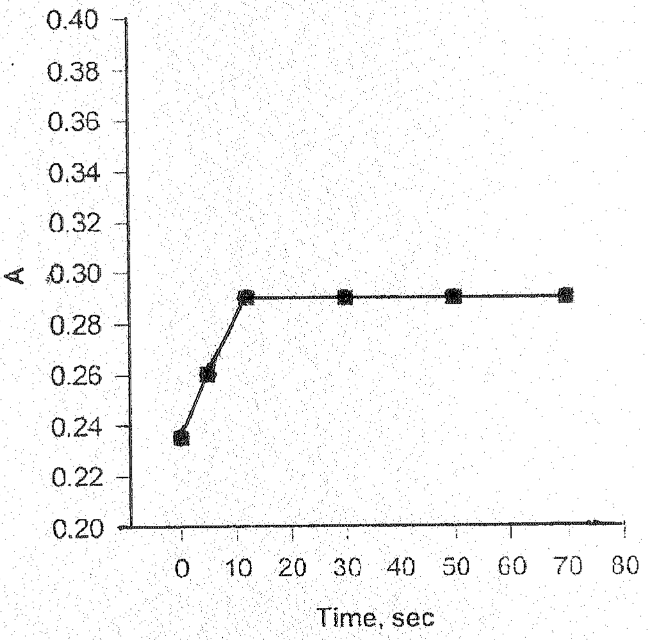

Fig. 4. Transition process of the molybdenum(VI) detector constructed by covalent binding of Nile blue to cellulose in acidic media: (Condition: the initial molybdenum(VI) was 180×10^{-7} μg mL^{-1} at t = 0, the molybdenum(VI) was changed to 60×10^{-7} μg mL^{-1}

Sample Treatment & Digestion

Calibration Curve: Some analytical performance characteristics are given in Table-1.

TABLE-1
CALIBRATION RANGE, DETECTION LIMIT AND REPRODUCIBILITY FOR THE DETERMINATION OF MOLYBDENUM(VI)

Characteristics	Molybdenum(VI)
Linear range (μg mL^{-1})	0.02–3.40
Detection limit (μg mL^{-1})	0.007
Precision (RSD)[a]	
(1) 0.10 (μg mL^{-1}) Mo(VI)	1.75%
(2) 0.50 (μg mL^{-1}) Mo(VI)	1.50%
(3) 1.50 (μg mL^{-1}) Mo(VI)	1.25%

[a] For 10 replicate measurments.

Determination of molybdenum(VI) in real samples: To check the applicability of the method, the determination of molybdenum(VI) was carried out on various steel alloy samples. Synthetic samples were prepared according to ASTM and BCS. A 2–5 g amount of sample was transferred into a 400 mL beaker and 30–50 mL of concentrated hydrochloric acid was added. The beaker was covered and heated gently to dissolve the sample. Concentrated nitric acid (5–10 mL) was added and the heating continued; the cover was then removed and 4 or 5 drops of concentrated hydrofluoric acid were added. The solution was evaporated to dryness in a hot-water bath to remove nitric acid. Concentrated hydrochloric acid (5–10 mL) and approximately 25 mL of water were added and the mixture was heated to dissolve the salts, before dilution to volume in a 100 mL volumetric flask. All aliquot of this solution passes from cation exchanger resin (strongly acidic form) to remove interfering cations and Mo(IV) was determined in procedure. Molybdenum was then determined by the standard addition method. In another experiment molybdenum was determined using the recommended the procedure directly. The results of standard addition method and direct use of calibration graph agreed well. The results are shown in Table-2. The results showed good reproducibility and accuracy in comparison to the standard method.

Preparation of water samples: The proposed method was applied to the analysis of some water samples under optimum conditions. The water sample was initially filtered over Whatmann No. 1 paper. All this solution passes from cation exchanger resin (strongly acidic form) to remove interfering cations and Mo(IV) was determined in the procedure. The results are shown in Table-3. Significant differences between the proposed method and the standard method were found[15].

TABLE-2
DETERMINATION OF MOLYBDENUM(VI) IN STEEL SAMPLES

Samples	Mo(VI) added (μg mL^{-1})	Mo(VI) found (μg mL^{-1})	Concentration of Mo(VI) found* (mg/g^{b})		
			Proposed method	Standard method	
Steel (1)	—	1.18 ± 0.003	2.27 ± 0.002	2.10 ± 0.003	(ASTM, A209, T$_1$)
				2.05 ± 0.002	(ASTM, A 217-65, WC$_1$)
				2.01 ± 0.004	(BAS 33b)
				2.08 ± 0.002	(BCS 261/1)
	1.0	2.12 ± 0.002			
	2.0	3.15 ± 0.002			
Steel (2)	—	1.25 ± 0.002	2.75 ± 0.004	2.62 ± 0.006	(ASTM, A209, T$_1$)
				2.52 ± 0.003	(ASTM, A217-65,WC$_1$)
				2.72 ± 0.002	(BAS 33b)
				2.72 ± 0.004	(BCS 261/1)
	1.0	2.51 ± 0.003			
	2.0	3.58 ± 0.002			

*Mean for five determinations. ^{b}For steel samples.

TABLE-3
DETERMINATION OF MOLYBDENUM(VI) IN ENVIRONMENTAL WATER SAMPLES (MoS$_2$ COMPANY)

Samples	Mo(VI) added (μg mL^{-1})	Mo(VI) found (μg mL^{-1})	Concentration of Mo(VI)* (mg/g^{b}) (sample)	
			Proposed method	Standard method
Sample 1	—	1.04 ± 0.002	1.87 ± 0.003	1.15 ± 0.003
	1.0	2.06 ± 0.002		
	2.0	3.08 ± 0.002		
Sample 2	—	1.20 ± 0.004	1.25 ± 0.006	1.28 ± 0.007
	1.0	2.06 ± 0.002		
	2.0	3.18 ± 0.004		

*Mean for five determinations.

Interference study: In order to assess the application of the proposed method to real samples, the influences of some other substances were studied in the presence of 1 μg mL^{-1} of molybdenum(VI) ion. The tolerance limit of a foreign species was taken as a relative error not greater than 5% (three times standard deviation for 1 ng mL^{-1}). The results are shown in Table-4. In this study, anions had no interfering effect. Cations can be removed by cation exchanger resin (Merck, strongly acidic cation exchanger). Water was used as the eluent.

Conclusion

The detector exhibits a dynamic range of 0.02–3.40 μg mL^{-1} molybdenum(VI). The described method for producing molybdenum(VI)-sensitive optical membranes has the following advantages in comparison with other methods: (a) a waste cellulose material with good optical and mechanical properties is used as a matrix for immobilization; (b) immobilization of the indicators on the membrane's surface reduces the diffusion limitations and allows detectors to be produced with a short response time; (c) its activation is performed by using inexpensive and easily available reagents with suitable time stability for large number of measurements.

TABLE-4
EFFECT OF INTERFERENCE FOR THE DETERMINATION OF 1 μg mL^{-1} OF MOLYBDENUM(VI)

Species	Tolerance limit (μg mL^{-1})
Pb^{2+}, Na^+, K^+, NH_4^+, Ca^{2+}, Zn^{2+}, Mn^{2+}, Cr^{3+}, Co^{2+}, Ni^{2+}, Ag^+, F^-, Cl^-, NO_3^-	10000^a
Cd^{2+}, Al^{3+}, As^{3+}, SCN^-, Tl^-	700
In^{3+}, As^{5+}	500
Sn^{2+}	80
Cu^{2+}, Fe^{3+}, V^{5+}, Cr^{6+}, Ce^{4+}	3

*Maximum concentration tested.

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