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Spectrophotometric determination of low-level concentrations of Se in aqueous solutions

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ABSTRACT

This paper describes the design, testing and validation of a simple and inexpensive spectrophotometric method for total Se analysis in aqueous solution based on the azo coupling reaction of amines. In this reaction, selenite ions react with hydroxylamine hydrochloride ($NH_2OH.HCl$), which in turn diazotizes p-Nitroaniline and is coupled with N-1-naphthyl-ethylenediamine dihydrochloride (NEDA) to form a red coloured azo compound. This azo compound has a maximum absorption peak at 545 nm, an apparent molar absorption of 2.85×10^4 l mol^{-1} cm^{-1} and it follows the Beer's law in the range from 0.01 to 2.50 mg/l with $R^2 = 0.9998$. Interferences between the azo reaction and non-targeted ions often present in environmental samples (i.e., NO_3^-, CO_3^-, Cl^- or metals) were investigated and specific protocols to overcome them were implemented. The advantages of the new method for low levels of Se in aqueous solutions are its high sensitivity and reproducibility, and the fact that measurements are simple, rapid and low-cost.

Introduction

SELENIUM is a common, naturally occurring element in the Earth's crust that has increasingly been released into the aquatic environment via increased human activity (i.e. mining, agriculture, etc.). However, Se can rapidly reach toxic levels in drinking water sources or human diet. In uncontaminated natural waters, Se may occur up to 10 times lower than the WHO or US EPA guideline for drinking water quality (0.01 mg/l; and 0.05 mg/l respectively, WHO, 2003; US EPA, 2003). However, extremely high Se concentrations (up to 0.27 mg/l) have been found in natural ground waters in China, Pakistan, Venezuela, Columbia and the USA, and these can lead to a high risk of Se poisoning (e.g. Holland and Turekian, 2004).

Currently, low concentrations of aqueous Se concentration or speciation are analysed either by Inductively Coupled Plasma-Mass Spectrophotometry (ICP-MS), Hydride-Generation-Graphite Furnace-Atomic Absorption Spectrometry (HF-GFAAS), or Ion Chromatography-Hydride Generation-Atomic Emission Spectrometry (IC-HG-AE; e.g. Muñoz *et al.*, 1994; Adkins *et al.*, 1995). However, these techniques, although they provide high sensitivity, are rather challenging and rely on complex instruments and thus are expensive for regular analysis. This is particularly true for routine water analyses in developing countries where Se toxicity is a well known problem, e.g. China, Colombia (Matamoros, 2002; Zhu *et al.*, 2004).

Spectrophotometric methods for Se analysis have been described previously (Revanasiddappa and Kiran Kumar, 2001 and references therein). However, these methods have been criticized for (1) their lack of sensitivity, (2) their high experimental complexity, (3) the imprecision of the experimental procedure, and (4) their specificity for non-natural aqueous matrices. Thus, these spectrophotometric methods cannot be used routinely for analyses of low levels of Se in natural aqueous waters.

This paper describes the detailed reaction scheme, mechanisms as well as the protocols used for testing and validation of a simple, but reliable and highly sensitive method to analyse Se in aqueous solutions based on a two-step reaction (diazotization and amination) which produces a

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red coloured azo compound that can be analysed spectrophotometrically.

Methods and results

Se determination protocol and mechanism

(a) Protocol: 1–7 ml of either a standard or a sample containing Se (IV) are mixed with 2 ml of a 30% hydroxylamine hydrochloride ($NH_2OH.HCl$), 5.75 ml of concentrated HCl and 3 ml of 1.5% p-nitroaniline dissolved in 2.5 M HCl. The solutions are shaken and heated at 50ºC for 80 min. Next, 4 ml of 1% NEDA (N-1-naphthyl-ethylenediamine dihydrochloride) dissolved in 1% HCl are added and the test tubes heated for an additional 15 min at 50ºC. This leads to the formation of a red azo dye and after dilution with distilled water to 25 ml, the absorbance of the coloured complex is measured with a spectrophotometer at 545 nm (Fig. 1 left hand side).

(b) Reaction mechanism: in the first step, the Se (Se(IV) or selenous acid) oxidizes the $NH_2OH.HCl$ to nitrous acid, followed by the diazotization of p-nitroaniline producing a diazonium salt (right hand side of Fig. 1), followed in the second step, by the coupling between NEDA and the diazotised p-nitroaniline, thus forming a stable red azo compound. The method is based on the oxidizing properties of selenous acid in acid media and these characteristics advocate that the sample matrix or any sample preparation steps (a) do not involve other oxidizing ions (i.e. ClO_4^-, MnO_4^{2-}, NO_3^-) in order to avoid the oxidation of $NH_2OH.HCl$ by non-Se compounds and (b) no reducing agents are present (i.e., Fe^{2+}, SO_3^{2-}) in order to avoid reduction of Se(IV) to Se(0).

Method optimization

The absorption spectrum of the azo dye against the reagent blank is shown in Fig. 2*a*, revealing an optimal absorbance at 545 nm. The procedure as described above was optimized in terms of HCl concentration, temperature, time of reaction and concentration of reagents. Figures 2*b–d* show three of the parameters tested from which it was concluded that the optimal conditions for this coupling reaction would be 3 M HCl, 50ºC and 3 ml of 1.5% p-nitroaniline.

Interferences

Interferences by various ions (e.g. Al^{3+}, Si^{4+}, Fe^{2+}, Mn^{2+}, Mg^{2+}, Ca^{2+}, Na^+, K^+, Cr^{6+}, HPO_4^-, CO_3^-, SO_4^{2-}, Cu^{2+}, Ba^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Pb^{2+},

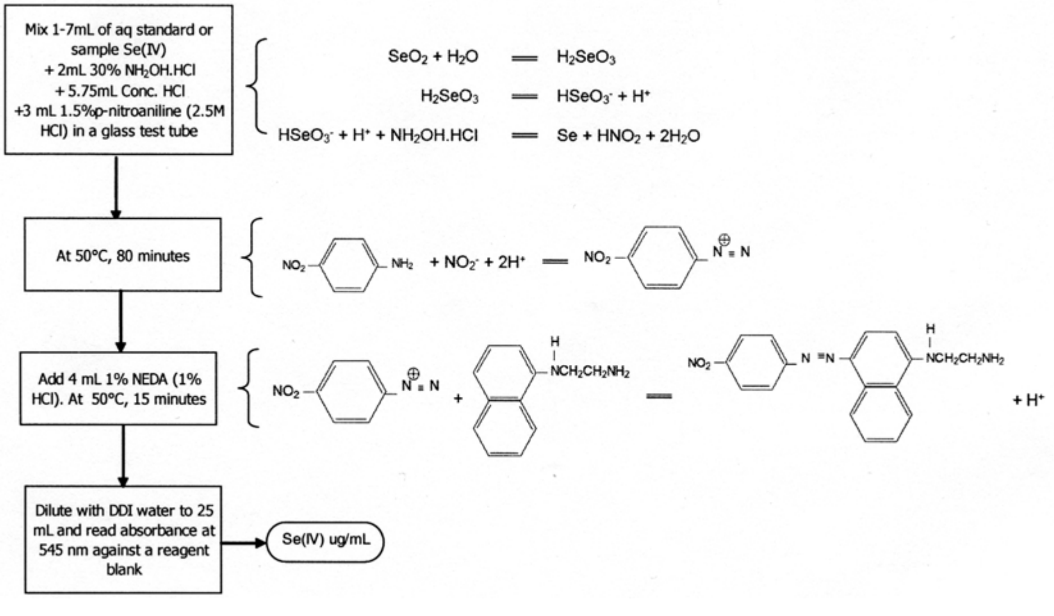

FIG. 1. Schematic flow diagram of reaction progress (left) and the developed mechanism of the reaction for Se determination based on the azo coupling reaction of amines (right).

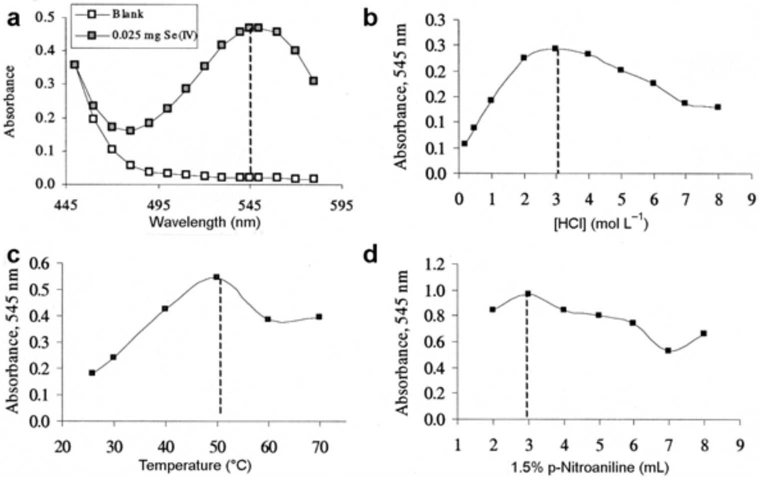

FIG. 2. (*a*) Absorption spectrum of the red azo dye against the reagent blank; (*b*) effect of HCl, (*c*) temperature, and (*d*) p-nitroaniline concentration on absorbance.

SeO_4^{2-}, MoO_4^{2-}, As^{3+}, Cd^{2+}, NO_3^-, SO_3^{2-}, CH_3COO^-, Cl^-, PO_4^{3-}) on the spectrophotometric Se determination method were tested by addition in 100-fold, 1000-fold and 10,000-fold excess. The results show that only the presence of Fe^{2+}, Cu^{2+} and SO_3^{2-} prevented the formation of the azo complex, probably due to their reducing properties or the catalytic decomposition of $NH_2OH.HCl$ by copper. In addition, selenate, molybdenate and nitrate, when present, will oxidize $NH_2OH.HCl$ promoting the formation of the azo dye complex. In natural waters, the prime interfering ion is usually iron and therefore it must be removed or masked prior to measurements. This can be achieved either via cation exchange (Dowex 50W-X8 in acid solution, Martinez *et al.*, 1997) or via masking with 5% EDTA just prior to analysis. In addition, a reduction reaction should be carried out with 6 M HCl to ensure the presence of Se as Se(IV).

Quality parameters

An apparent molar absorptivity of 2.85×10^4 $1\ mol^{-1}\ cm^{-1}$, that obeys Beer's Law (linear relationship between absorbance and concentration of absorbing species) in the range between 0.01 and 2.50 mg/l Se(IV) was derived. The optimal calibration curve is given by the regression equation $y = 0.3545x + 0.0004$ (y = absorbance; x = Se(IV) in mg/l). The sensitivity (S = 0.0124 mg/l) was estimated based on the slope of the calibration curve and concentrations were derived based on S = 0.044/slope, with 0.044 corresponding to the minimum difference measurable with a precision of 1% Transmittance (T).

The accuracy and precision of the method were determined using three standard solutions (made from sodium selenite standard at concentrations of 0.05, 1.00 and 2.50 mg/l). Seven absorbance replicates were used to derive an accuracy of ±1%. Finally, the precision was calculated as % relative standard deviation of these measurements and a highly reproducible method with standard deviations of 2.5, 2.3, 0.3%, respectively for the above concentrations was measured.

It is important to note that the spectrophotometric method developed and tested in this study can be easily combined with standard sample preparation procedures for Se analyses in waters (i.e. 3500-Se, methods B3 and B5, Eaton *et al.*, 1998). The B3 and B5 procedures only require the oxidation of an unfiltered aqueous sample with alkaline H_2O_2 followed by reduction of Se(VI) to Se(IV) with 6 M HCl. These chemical steps are in line with the method described above.

Summary and conclusions

A new simple and inexpensive spectrophotometric method for determining low levels of Se in aqueous solutions was developed. This method relies on a two-step azo coupling reaction of amines and it is applicable for total Se concentrations between 0.01 and 2.50 mg/l. The optimal conditions for sample handling and analyses as well as the ideal protocols for removal or masking of possible interferences were derived and tested. Finally, in an effort to expand this method to non-aqueous samples, tests are in progress to develop a quantitative method applicable to Se determinations in carbonaceous shales and standard reference materials (SRMs). The weathering of shales (Se mostly associated with iron sulphides and organic matter) is the prime source of Se in the environment, yet the pathways and mechanisms of this process are not well understood. Therefore, various methods of shale digestion by alkaline fusion as well as procedures of elimination of interferences (primarily Fe and Cu) by ion-exchange resins, liquid-liquid extraction or masking with chelating agents are being tested. However, the powerful spectrophotometric method described above is easily applicable once a quantitative digestion of the solid samples has been achieved.

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