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Determination of dissolved selenium species in environmental samples

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Abstract—This article reports on the technique for the determination of selenium at ppt level and the procedure for the speciation of dissolved selenium in the environmental samples. By combining the high–performance liquid chromatography (HPLC) and a fluorescent detector (FLD), this technique permits the determination of selenium at 0.001 μg/L for Se(IV) and 0.005 μg/L for Se(VI) and the total concentration of selenium for a sample volume of 20 ml. In the speciation procedure, Se(IV) is firstly determined based on the selectivity of 2, 4 – diaminonaphthalene (DAN), the Se (VI) and the total element concentration are determined after reduced to Se(IV) by boiling in 4 mol/L HC1 and by digesting in HNO_3–$HClO_4$ mixture, respectively. Discussions are given on the relationship between selenium speciation in waters and soil water extract and solution pH, E_H and total organic carbon concentration (TOC).
Keywords: Selenium; speciation; HPLC.

INTRODUCTION

As the biochemical effects of selenium become more and more evident, scientific works on the relationship of the element and human health became prevailed. Deficiency of selenium has been considered as an important factor in causing the Chinese Kaschin–Beck and Kashan diseases (K.D.R.G., 1979; Wang, 1985). The relevant researches include bio–geochemical cycle of the element in chinese low–selenium belt and the pathogenic role through selenium food chain processes. In this aspect, information about the speciation of the element is inadequated. Besides, literature information on the speciation of selenium in environmental waters is very confusing, partially because of different procedure was used (Robberecht, 1982).

DAN fluorometric technique has been used extensively for the selenium determination. The technique is not sensitive enough for the samples from Chinese low–selenium belt, where the total element concentration in drinking water rarely exceeds 0.1 $\mu g.L^{-1}$ with Se(IV) fraction as low as several $ng.L^{-1}$.

In case of DAN fluorometric technique was used as detector, the Se(IV) is selectively determined and other forms should be conversed to this form. When comparing different methods for this conversion, reduction in 0.4 mol/L HCl was found to be the only suitable one (Cutter, 1978). The procedure proposed here includes a direct determination of Se(IV), a reduction of Se(VI) to Se(IV) by boiling the sample in 0.4 mol/L HCl and a digestion step for the total element determination. The combination of these available procedures enables us to determine Se(IV), Se(VI), and organic bounded selenium through differential calculation (with assumption that element selenium is negligible).

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METHODOLOGY

Determination procedure

Waters model 201 HPLC W/510 liquid chromatography equipped with U/6k injector and a Waters u-porosil normal phase column (particle size 8–10 mm, 3.9mm × 20 cm) was used for separation of naphtho-selenodiazol (NSD) from fluorescent interferences derived from extra impurities and from derivation process. The separation was performed at ambient temperature with isocratic elution using cyclohexane–THF (90:10,v/v) as the mobile phase. The flow rate was 1.0 ml/min and fluorescence of eluate was monitored at an excitation wavelength of 376 nm and an emission wavelength of 520 nm.

To 20 ml of sample with pH adjusted to 1.5 in a 100 ml flask was added 1 ml 0.1% DAN solution and 1 ml of 0.1 mol/L EDTA, and the flask was heated at 50 °C for 20 min. 1 ml of cyclohexane was added after the solution was brought to ambient temperature and the organic phase was separated by centralized at 1500 r/min. 20μl of the organic phase was injected into HPLC system and the signal from NSD was recorded by Pandos U-228 model recorder. According to the selectivity of DAN fluorexcent reagent, only Se(IV) can be determined.

Speciation procedure

To 20 ml of sample in 100 ml flask, the solution was adjusted to pH 1.5 and Se(IV) was determined directly as mentioned before. To another 20 ml of sample was added 10 ml concentrated hydrochloric acid (final concentration 4 mol/L). The flask was heated and reflux at 170 °C for 20 min. After heating, the pH of the solution was adjusted to 1.5 and the selenium concentration was determined. This step measures the total of Se(IV) and Se(VI). The total element concentration was determined by digesting 20 ml of sample solution in a mixture of nitric acid and perchloric acid (5/2, v/v). The heating was performed in two sequential steps: heating from room temperature to 170°C, mainly to evaporate water, and heating from 170–200°C for another 30 min to digest the sample. After digestion, the flask was moved to the surface of the sand-bath and 10 ml of 4 mol/L HCl was added after 5 min. The solution was cooled to ambient temperature and adjusted to pH 1.5 followed by the determination procedure. The organic bond selenium fraction of the sample was obtained from differential calculation (elemental Se was ignored).

Samples was collected from different provinces of the country, mostly from village wells of different depth and agricultural surface soils, but also from reservoirs, spring and tap water. Samples of drinking water were under experiment immediately after carrying to the lab. Soil samples were dried in natural condition and ground to 1.0 mm of particle size. 2–3 g of the sample was weighted into a 100 ml flask to which 100 ml of distilled water was added. The soil was extracted for 30 min at room temperature and the supernatant was separated for speciation study.

RESULTS AND DISCUSSIONS

In the conventional selenium determination method by DAN fluoro-reagent, interferences from the reagent (even after purified 5–7 times by cyclohexane) and from those decomposed during derivation process were estimated to be about 5 ng Se correspondence. This interferences are hardly exclusive and therefore limits the detection ability of the technique. It was noticed that interferences from selenium contamination was acceptable if the DAN reagent was purified 4 times before use (Wang, 1988). To increase sensitivity of the technique, separation of NSD from other interferences by liquid chromatography was proposed. As can be seen in Figure 1a, most of interferences have been separated from NSD in the proposed HPLC-FLD system. In Figure 1a, the indicated NSD signal in the chromatogram is contributed by 20 pg Se injection, which corresponds to a initial solution concentration, of 0.05μg/L (in 20 ml). Other peaks in the chromatogram come from the interferences, which would be equally measured in conventional fluorometric technique. It can be seen from the figure that the sensitivity of the proposed

technique was only related to selenium contamination, either from the reagent used or from the distilled water, which, in this lab, is about 1.0 ng/L for Se(IV) and 5.0 ng/L for Se(VI) and the total. Figure 1b shows the calibration curve of Se(IV) standard solution. The determination range can be increased to several dozens μg/L.

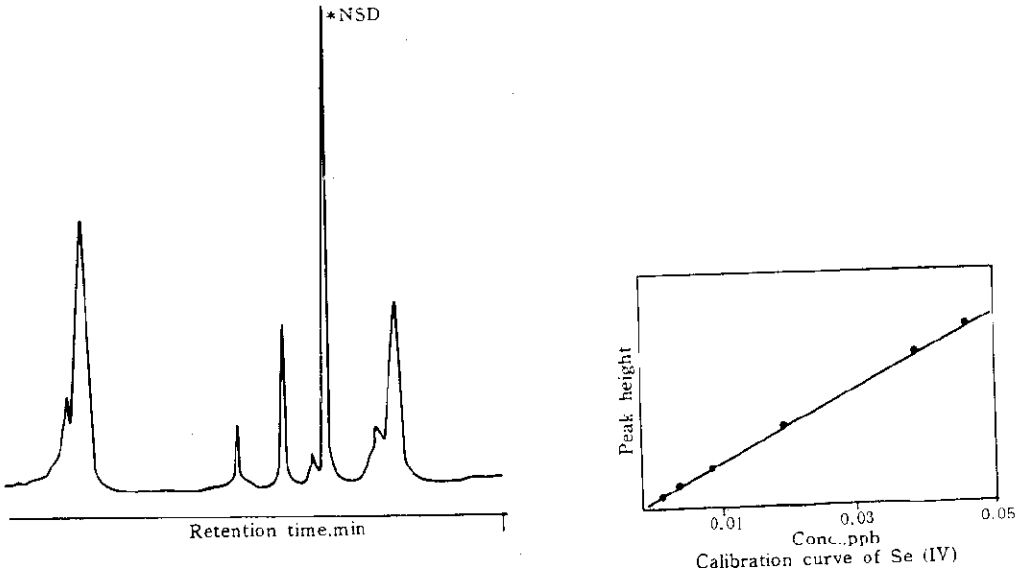

Fig. 1 HPLC–FLD system for the determination of Selenium at ppt level Fig.1a, Chromatogram of NSD (*), 20 pg injection, original concentration 0.05 μg/L (20 ml). Other peaks in the diagram are the interferences. Fig.1b, Calibration of Se(IV), concentration range 0–0.5 μg /L (0–10 ng Se).

Table 1 Calibration of different Se standard solution by different treatment

Treatment step	Se standard	Calibration		Recovery
		Slope mm/ng	C.C.[2]	%[1]
Direct Determ.	Se(IV)	0.8454	0.9999	100
	Se(VI)	0	—	0
	Se–methionine	0	—	0
Reduc. Determ.	Se(IV)	0.8626	0.9990	98
	Se(VI)	0.8813	0.9985	96
	Se–methionine	0.0125	—	1.5
	Se–cysteine	0.0050	—	0.6
Digest. Determ.	Se(IV)	0.8025	0.9998	105
	Se(VI)	0.8214	0.9997	107
	Se–cysteine	0.8034	0.9996	105

Calibration was carried out in 0–400 ng Se range under the condition described in the experimental section

1. Calibration of Se(IV) was taken as 100% recovery;
2. C.C. means correlation coefficient.

Table 1 gives the results on the selectivity of different treatment. In this experiment, the calibration was carried out by using different selenium standard solution (0–400 ng Se) and

by using the proposed speciation procedure individually as described in experimental section. The relative recoveries were calculated from the slope of the calibration curves of different selenium species in the correspondent treatment. A slope of calibration for a species in a specific treatment being near to zero was considered as, therefore, that the species is undeterminable in this treatment, so as those whose slopes are relatively negligible.

It can be seen from the table that only Se(IV) could be determined in the direct determination step, both Se(VI) and seleno-methionine are undetectable. In the reductional determination step, both Se(IV) and Se(VI) are determinable while either seleno-methionine or seleno-cysteine gives a negligible response. The relative recovery of Se(VI) under the described reduction condition is 96%, optimized by testing a standard mixture of Se(VI) and Se(IV). In the digestion step, all selenium standard can be determined with an average relative recovery of 106±1%.

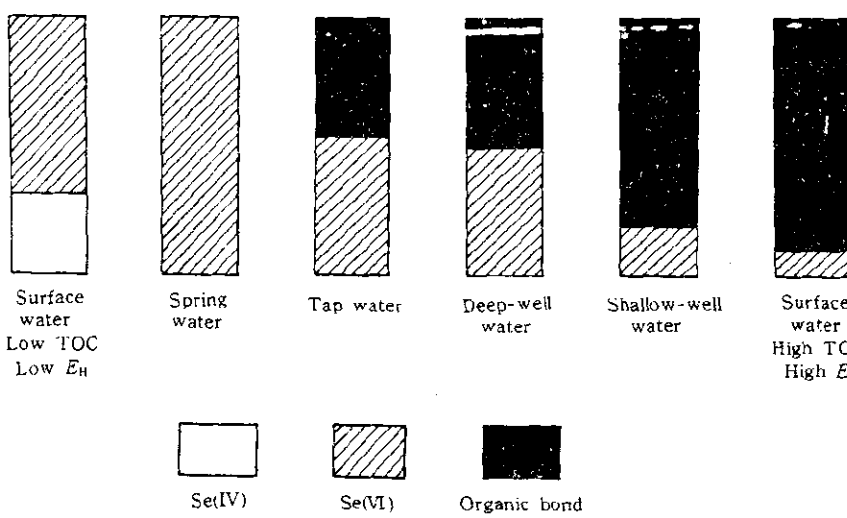

Fig. 2 Selenium speciation in different types of water (samples were selected from chinese low-selenium belt)

Figure 2 shows the analytical results of selenium species distribution in different types of water selected from Chinese low-selenium belt, obtained by applying the proposed HPLC-FLD technique and speciation procedure. As can be seen from the figure, the soluble forms of selenium in these representatives are mainly determined by redox potential and total organic carbon concentration. In the clean water with lower E_H, the dominant forms are inorganic while in the waters with higher E_H and TOC the organic fraction prevails.

It is quite noticeable that the organic fraction means not only those where selenium in minus valency but also those strongly bonded to the organics, such as fulvic acid.

Table 2 shows the selenium speciation in the drinking water samples of different parts of China. The sample from Chinese low-selenium belt (where Kaschin-Beck Disease, KBD, prevails) are remarked by both lower concentration of the total selenium and higher percentage of organic bond fraction. The Se(IV) fraction in these samples is generally less than 6% of the total. Higher inorganic fraction is observed in the sample from acidic and selenoferrous area. No obvious relationship between the ratio of Se(IV)/Se(VI) and solution pH and E_H has been found, probably because the differences of these two parameters among samples are not significant.

Table 2 Selenium speciation in drinking water

Sampling site		Total	Se(IV), g Se/dm^3	Se(VI)	Se(org.)
* HLJ	Fu-yu County	0.083	0.005 (6.0 %)	0.037 (44.6%)	0.041 (49.4%)
* HLJ	Shuiling County	0.026	0.000 (0 %)	0.000 (0%)	0.026 (100%)
* HLJ	Shan-zi County	0.025	0.000 (0 %)	0.007 (28.0%)	0.018 (72.0%)
* NMG	Za-lan County	0.079	0.001 (1.3 %)	0.046 (58.2%)	0.032 (40.5%)
* NMG	Ar-rong County	0.052	0.002 (2.9 %)	0.007 (12.5%)	0.044 (84.6%)
JL	Fu-shon County	0.115	0.000 (0 %)	0.056 (48.7%)	0.058 (51.3%)
* HB	Fengning(1) County	0.084	0.000 (0 %)	0.010 (11.9%)	0.074 (88.1%)
HB	Fengning(2) County	0.122	0.002 (1.2 %)	0.014 (11.6%)	0.106 (87.1%)
BJ	Huairou Reservoir	0.146	0.047 (32.2 %)	0.099 (67.8%)	0.000 (0 %)
BJ	Mi-yun Reservoir	0.146	0.047 (32.2 %)	0.099 (67.8%)	0.000 (0 %)
SD	Juan-lin Spring	0.240	0.000 (0 %)	0.240 (100 %)	0.000 (0 %)
SD	Wen-den County	0.268	0.004 (1.5%)	0.005 (1.9%)	0.259 (96.6%)
HZ	Xi-hu lake District	0.099	0.040 (40.4 %)	0.012 (12.1%)	0.047 (47.5%)
* GS	Tian-shui(1) Taijing Village	0.035	0.001 (4.0 %)	0.006 (16.0%)	0.028 (80.0 %)
* GS	Tian-shui(2) Shuiqing Village	0.154	0.001 (1.0 %)	0.016 (10.0%)	0.137 (89.0 %)
HB	Enshi High Se Area	33.60	15.50 (46.1%)	12.65 (37.6%)	5.45 (16.2%)

* Samples from chinese selenium deficient belt, KBD regions.
Numbers in parenthesis are percentage to total.
HLJ,NMG,JL,HB,SD,HC,GS and SX are the abbreviations of chinese provinces and cities.

Table 3 Selenium speciation in soil water extract

Sampling site		Total	Se(IV), μg Se/kg soil	Se(VI)	Se(org.)
* HLJ	Fu-yu County	3.191	0.750 (23.5%)	0.000 (0.0 %)	2.441 (76.5%)
* NMG	Buteha County	1.235	0.124 (10.0%)	0.580 (47.0%)	0.531 (43.0%)
* NMG	Mianyue County	1.726	0.290 (1.3%)	0.700 (58.2%)	0.732 (40.5%)
* NMG	Ar-rong County	2.219	0.212 (9.6 %)	1.046 (47.1%)	0.961 (43.3%)
* HB	Fengning County	1.459	0.338 (23.2%)	0.440 (30.2%)	0.681 (46.7%)
* SX	Yong-shou(1) Mafang Village	2.505	0.612 (24.4 %)	1.098 (43.8%)	0.795 (31.7%)
* SX	Yong-shou(2) Mujia Village	1.607	0.429 (26.7%)	0.463 (28.8%)	0.715 (44.5%)
* GS	Tian-shui County	2.400	1.600 (66.7%)	0.000 (0.00%)	0.800 (33.3 %)
* SD	Wen-den County	4.770	0.150 (3.2%)	0.020 (0.4 %)	4.600 (96.4%)
HZ	Xi-hu lake District	11.60	0.970 (8.4 %)	0.030 (0.3 %)	10.60 (91.3%)
HB	En-shi High Se District	213.0	108.9 (51.1 %)	17.0 (8.0 %)	87.3 (40.9%)

* Samples from chinese selenium defficient belt, KBD regions.
Abbreviations as Table 2

Selenium speciation in soil water extract varies from sampling sites (Table 3). No obvious relationship between soil water extract and drinking water could be traced because most of the drinking water samples were taken from shallow to deep wells. The results show a transformation process exists in turning the surface water into well water and most of soil organic matter is fixed by soil, so as the bonded selenium. The selenium defficient of agricultural crops in Chinese low selenium belt is therefore mainly due to the insufficient selenium supplement in soil system, i.e. lower total water soluble fraction.

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