Quantitative Analysis of Human Hair for Selenium(IV), Selenium(VI) and Total Selenium by Hydride-Generation Atomic Absorption Spectrometry

Xiaonan DONG, Yuzuru NAKAGUCHI and Keizo HIRAKI[†]

*Department of Chemistry, Faculty of Science and Technology, Kinki University,
Kowakae, Higashi-Osaka 577, Japan*

Optimized procedures were established for the determination of total Se, organic Se, Se(IV), and Se(VI) in human hair by means of hydride-generation atomic absorption spectrometry (HGAAS), respectively. Using this method, the total Se content in a hair sample was determined through pretreating the sample with nitric acid–perchloric acid, and then reducing Se(VI) to Se(IV) with 6.0 mol dm⁻³ hydrochloric acid; however, for individual Se(IV), Se(VI) or soluble Se, after the hair sample was immersed into water and disrupted ultrasonically for 8 h at room temperature, 2,3-diaminonaphthalene (DAN) reagent was added to extract Se(IV). The sum of Se(IV) and Se(VI) was determined by extracting Se(IV) after reducing all of the Se(VI) into Se(IV) with the addition of potassium bromide. Se(VI) was obtained by subtracting Se(IV) from the sum of Se(IV) and Se(VI). The content of organic Se in the hair sample was calculated by subtracting Se(IV)+Se(VI) from the total Se. The detection limit of the method was 0.3 ngSe cm⁻³, and the average precision (relative standard deviation) was less than 5% (*n*=6).

Keywords Hydride generation atomic absorption spectrometry, ultrasonic leaching, human hair sample, selenium(IV), selenium(VI), soluble selenium

Selenium is an interesting trace element in human tissues because of its dual roles as both an essential nutrient at a low concentration level and a toxic substance at a higher concentration level. The toxicity of Se depends greatly on its chemical form.¹⁻³ Thus, much attention has recently been paid to the levels of Se in human tissues. It has been found that its quantity in hair is closely correlated to that inside; the body.^{4,5} Obviously, determining its content in hair could provide a simple way for monitoring the change of the Se status *in vivo*. Since Se is found at trace levels (usually ng g⁻¹ or sub ng g⁻¹), and two forms (inorganic and organic) of the element exist in hair, a sensitive, stable and simple analytical method is required. In previous work we determined the respective concentrations of various Se species in natural water samples by fluorometry. However, this method has several problems during the analytical procedures: 1) the 2,3-diaminonaphthalene (DAN) reagent requires purification; 2) the sample solution requires many repeated extractions; and 3) it takes a long time and a complex operation is required. We thus attempted to use hydride-generation atomic absorption spectrometry (HGAAS)⁶⁻¹³ combined with an ultrasonic leaching method for Se, which has been confirmed to be not only convenient, but also effective for keeping them intact; to determine Se(IV) and Se(VI)

in human hair samples, on the other hand, wet digestion (HNO₃–HClO₄) was employed to measure the total amount of Se. Moreover, optimized instrumental conditions for the HGAAS system in analyzing human hair for Se have been established. The results from a number of determinations on hair samples have shown that these instrumental conditions and sample pretreatment procedures could provide a satisfactory approach for a quantitative analysis for Se species in living systems.

Experimental

Apparatus

A Hitachi (Model 180-70) atomic absorption spectrometer equipped with a commercial hydride-generation system (HFS-2) and a peristaltic pump was used. A Se hollow cathode lamp (Hitachi) served as a light source of 196.0 nm wavelength (with a slit of 1.3 nm, and working current of 12.5 mA). The temperature of the quartz atomizer tube (120 mm in length and 13 mm in inner diameter) was set at 900°C. The absorbance signals in the peak-height mode were recorded on a Rikadenki Model R-01 recorder. An ultrasonic clean bath (Kaijo Denki Co., Ltd. Japan, freq. 38 kHz) was used for leaching the element from hair samples.

[†] To whom correspondence should be addressed.

Reagents

All of the reagents used were of highly pure grade; doubly distilled water was used throughout. A sodium tetrahydroborate (NaBH_4) solution (1.5% w/v) in 0.8% sodium hydroxide (NaOH) was freshly prepared before the experiments. A 1000 mg dm^{-3} stock solution of Se(IV) was prepared by dissolving 0.144 g of Se dioxide in 100 cm^3 of 1.0 mol dm^{-3} hydrochloric acid (HCl). The standard solutions for a calibration were obtained by diluting the stock solution before use.

Sample pretreatment

All human hair used in this study (without permed and dyed) was obtained from a barbershop, and washed with 0.3% polyoxyethylene lauryl alcohol ether for 20 min. After being rinsed with doubly distilled water and dried at 80°C , the sample was powdered under liquid nitrogen, and then homogenized in the presence of argon in a closed acid-washed glass beaker, and finally stored in a desiccator prior to analyses.^{5,14,15}

Determination of total Se

First, 0.2 g of the pretreated hair sample was placed in a 100 cm^3 beaker with 5 cm^3 of concentrated nitric acid (HNO_3) and covered with a glass dish over night; after the beaker was heated at 100°C for 4 h, 2.0 cm^3 of perchloric acid (HClO_4) was added to the sample solution; then, after being subjected to reflux for an additional 3 h, the acid was allowed to evaporate by removing the cover until white fumes of HClO_4 appeared; this procedure was then repeated once again until the solution became colorless. The solution was carefully concentrated to ca. 0.3 cm^3 . At no time was the residue allowed to dry or char, since Se losses could occur. After cooling, 2.0 cm^3 of 6.0 mol dm^{-3} HCl was added to the residue, which was heated at 100°C for 10 min to reduce Se(VI) into Se(IV) .¹⁶⁻¹⁸ Finally, the solution was transferred into a 20 cm^3 volumetric flask for a following determination.

Determination of Se(IV)

About 30 g of a pretreated hair sample was put into a 200 cm^3 glass flask with 100 cm^3 of doubly distilled water; then, ultrasonic leaching was conducted for 8 h in a ultrasonic bath.¹⁰ After being filtered through a $0.45 \mu\text{m}$ membrane filter, the leached solution which was acidified to $\text{pH}=1$ with dilute HCl ; then, after the addition of 5 cm^3 of 0.1 mol dm^{-3} EDTA-NaF and 20 cm^3 of 0.1% DAN reagent, the solution was warmed at 50°C for 30 min in a water bath. After being cooled to room temperature, the solution was transferred to a separatory funnel containing 15 cm^3 of cyclohexane, and shaken for 10 min. The aqueous part was removed and 10 cm^3 of concentrated HNO_3 was added into the organic part. Shaking was performed for 5 min to make the Se extracted back to the aqueous layer, which was then concentrated on a hot plate to ca. 0.3 cm^3 and transferred to a 10 cm^3 volumetric flask¹⁷ for the HGAAS determination.

Table 1 Optimized instrumental conditions for determination of Se(IV)

Wavelength	196.0 nm
Lamp current	12.5 mA
Slit	1.3 nm
Flame temperature	900°C
NaBH_4 concentration	1.5%(w/v)
NaBH_4 flow rate	$6.5 \text{ cm}^3 \text{ min}^{-1}$
HCl concentration	3.0 mol dm^{-3}
HCl flow rate	$5.5 \text{ cm}^3 \text{ min}^{-1}$
Flow rate of argon gas	$500 \text{ cm}^3 \text{ min}^{-1}$
Sampling time	0.2 min
Sample flow rate	$7.5 \text{ cm}^3 \text{ min}^{-1}$

Determination of Se(IV)+Se(VI)

As described, to the sample solution, which was ultrasonically leached and filtered, 2.0 g of KBr was added to reduce Se(VI) to Se(IV) .¹⁶⁻¹⁸, which was then followed by an identical procedure to that used for treating Se(IV) mentioned above; that is, the sum concentration of Se(IV) and Se(VI) was determined. The quantity of Se(VI) was then obtained by subtracting the concentration of Se(IV) from the sum concentration.

Analysis of dissolved organic Se

First, 10 g of a prepared hair sample with 30 cm^3 was ultrasonically leached in a 100 cm^3 for 8 h. After being filtered, 10 cm^3 of concentrated HNO_3 was added and heated to almost dry; then, another 10 cm^3 of HNO_3 and 2 cm^3 of HClO_4 were added. When the solution was nearly dry, 3 cm^3 of 6 mol dm^{-3} HCl was added for reducing Se, after which the total dissolved Se was determined as described above. The level of dissolved organic Se in the sample was calculated from the total dissolved Se, subtracting the sum of Se(IV) and Se(VI) .

Results and Discussion

Optimization of the experimental parameters for the HG-AAS system

It has been reported that, generally, three factors can affect the quantitative analysis for selenium by using the HGAAS system. These include: (1) the concentration of the NaBH_4 and HCl solution, (2) the atomization temperature and (3) the flow rate of the carrier gas.¹⁹ Thus, the optimization of these three factors was carried out. The results are summarized in Table 1. The detection limit (3σ) of this method was 0.3 ngSe cm^{-3} , when the sample volume of 10 cm^3 was applied.

Leaching of Se(IV), Se(VI) and organic Se

According to the method and conditions mentioned above, the dissolved Se species were determined for 2, 4, 6, 8 and 10 h of the leaching duration, respectively. From Fig. 1 it can be seen that the leaching duration would facilitate the dissolution of the inorganic Se from

Fig. 1 Effect of the leaching time on the Se species from hair samples.

Table 2 Effect of diverse ions on the determination of Se(IV) at 5 ng cm⁻³ level

Ion	Concentration/ng cm ⁻³	Recovery of Se(IV), %
Al(III)	500	100
As(III)	100	97
Co(II)	100	100
Cr(III)	200	101
Cu(II)	500	98
Fe(III)	500	97
Fe(II)	300	99
Mn(II)	200	100
Ni(II)	200	100
Pd(II)	300	99
Sb(III)	100	100
Sn(II)	300	98
Zn(II)	300	100
NO ₃ ⁻	40000	99
PO ₄ ³⁻	30000	100
SO ₄ ²⁻	50000	100

the hair sample. However, when the leaching time is over 8 h, such dissolution reaches to a plateau, indicating that 7 to 8 h would be proper for the leaching of Se. On the other hand, the leaching rate of Se(IV) differs from that of Se(VI).

Evaluation of potential interference

It is well known that copper(II), nickel(II), iron(III), and tin(II) are potential interferents for the HGAAS of Se.^{10,20-22} To study the effect of diverse ions on the determination of Se with this procedure, the desired amount of each ion was added into a solution containing 5 ng cm⁻³ Se; the determination was carried out by the recommended method. The results listed in Table 2 show that those ions at the tested concentrations could not affect the determination of Se when a 5 ng cm⁻³ Se standard solution was used.

Fig. 2 Comparison of various Se species in hair. A=Se(IV), B=Se(IV)+(VI), C=Se(VI), D=total dissolved Se and E=dissolved organic Se.

Table 3 Levels of Se species distributed in human hair

Species	Concentration/ng g ⁻¹
Se(IV)	0.57 ± 0.05 (n=6)
Se(IV) + Se(VI)	3.95 ± 0.04 (n=6)
Se(VI) ^a	3.38 ± 0.07
Dissolved Se	8.32 ± 0.03 (n=4)
Dissolved organic Se ^b	4.37 ± 0.08
Total Se	687.14 ± 4.1 (n=7)
Total organic Se ^c	683.19 ± 4.8

a. {Se(IV)+Se(VI)}-Se(IV).

b. (Dissolved Se)-{Se(IV)+Se(VI)}.

c. (Total Se)-{Se(IV)+Se(VI)}.

Soluble Se species in a human hair sample

The levels of different Se species contained in a hair sample were determined by the HGAAS system under the optimized instrumental conditions mentioned above. From Fig. 2, it is noted that the amount of Se(IV) is the lowest among all of the Se species, and the sum of Se(IV) and Se(VI) is nearly equal to the level of dissolved organic Se. All of the Se species in a hair sample are listed in Table 3. The result implies that the organic Se in hair was hardly soluble in water. In addition, it can be determined that over 99% of the total Se in a hair sample is organic Se.

The level of the total Se obtained here is in accordance with that of the previous work¹⁷, in which the content of Se in the prepared hair sample was compared with a certified reference material ("NIES No. 5 Hair"). However, our obtained results concerning inorganic and organic Se were not absolute, because no reference materials act as a standard for estimating the Se species in

a human hair sample at present. Se metabolism is a very complex process *in vivo*, and the organic Se in hair was less soluble in water. Although the main organic Se metabolites are known to be selenomethionine in milk and blood²³, trimethylselenonium, selenoamino acid and selenoniocholine in blood and urine²⁴⁻²⁶ and so on, organic Se species in hair are not yet known.

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