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Simultaneous determination of trace arsenic, antimony, bismuth and selenium in biological samples by hydride generation-four-channel atomic fluorescence spectrometry

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Abstract

A new and sample technique for the simultaneous determination of trace arsenic, antimony, bismuth and selenium in biologic samples by hydride generation-four-channel nondispersive atomic fluorescence spectrometry was development. The conditions of instrumentation and hydride generation of arsenic, antimony, bismuth and selenium were optimized. For reducing hexavalent Se to the tetravalent state was to heat the sample with 6 mol l^{-1} HCl, and then pre-reducing pentavalent As and Sb to the trivalent state was achieved by the addition of 0.05 mol l^{-1} thiourea. The interferences of coexisting ions were evaluated. Under optimal conditions, the detection limits for As, Sb, Bi and Se were determined to be 0.03, 0.04, 0.04 and 0.03 ng ml^{-1}, respectively. The precision for seven replicate determinations at the 5 ng ml^{-1} of As, Sb, Bi and Se were 0.9, 1.2, 1.3 and 1.5% (R.S.D.), respectively. The proposed method was successfully applied to the simultaneous determination of As, Sb, Bi and Se in a series of Chinese certified biological reference materials using simple aqueous standard calibration technique, the results obtained are in good agreement with the certified values.

Keywords: Hydride generation; Four-channel-nondispersive atomic fluorescence spectrometer; Arsenic, antimony, bismuth and selenium; Simultaneous determination

1. Introduction

It is well-known that the arsenic, antimony and certain hydride-forming elements are highly toxic; determination of the mentioned elements in biological samples is an important screening procedure in the studies of environmental pollution and occupational exposure. In recent years, a large amount of different kinds of samples in environment protection were analysed annually in order to control the contents of the elements well below the safety level. Several techniques have been employed for such a purpose, including hydride generation atomic absorption spectrometry (HG-AAS) [1,2], hydride generation inductively coupled plasma-atomic emission spectrometry (HG-ICP-AES) [3], inductively coupled plasma mass spectrometry (HG-ICP-MS) [4] and hydride generation atomic fluorescence spectrometry (HG-AFS) [5,6]. Among the analytical techniques used in the determination of hydride-forming elements, HG-ICP-MS is considered as the most powerful technique in the determination of trace elements, but it is not easy to apply the method for determination of trace As and Se because of the serious interferences poly-atomic ions, e.g., $^{75}ArCl^{+}$ interferes with mono-isotopic $^{75}As^{+}$ and $^{80}ArAr^{+}$ overlap the isotope of $^{80}Se^{+}$; of course, the price of the instrument is not always bearable by most of the common laboratories. HG-AAS is a well-accepted technique, single-element determination; higher detection limits and serious gas phase interference between the hydride-forming elements limit its use in the analysis of real samples. HG-AFS is no doubt the first choice because of its high sensitivity, simplicity, low costs, and less interference; possibility of multi-element

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simultaneous determination is the main advantages of atomic florescence spectrometry, but most of such work has been concentrated on single element analysis [7]. In 1983, Guo et al. [8] had designed a hydride generation double-channel nondispersive atomic fluorescence spectrometer. The instrument has been widely used in China and its method has been successfully applied to the determination of hydride-forming elements (e.g. As, Sb, Bi, Hg, Se, Te, Ge, Sn, Pb, Zn, Cd). To the best of our knowledge, the use of HG-four-channel-nondispersive (ND) AFS for simultaneous determination of trace arsenic, antimony, bismuth and selenium in biological samples has not yet been reported.

In this work, a hydride generation-four-channel nondispersive atomic fluorescence spectrometer has been designed and a sensitive HG-four-channel NDAFS method was developed for simultaneous determination of trace As, Sb, Bi and Se in biological materials. Hexavalent Se is reduced to the tetravalent state by heating with 6 mol l^{-1} of hydrochloric acid and thiourea has been used as a pre-reduction agent for pentavalent As and Sb. At the same time, thiourea has been used as a masking agent also for elimination or minimization the interferences on the determination of hydride-forming elements from coexisting elements such as Fe, Cu and Ni. The developed method was successfully applied to the simultaneous determination of trace arsenic, antimony, bismuth and selenium in biological samples.

2. Experimental

2.1. Apparatus

Fig. 1 shows a schematic diagram of the four-channel-nondispersive atomic fluorescence spectrometer used in this work.

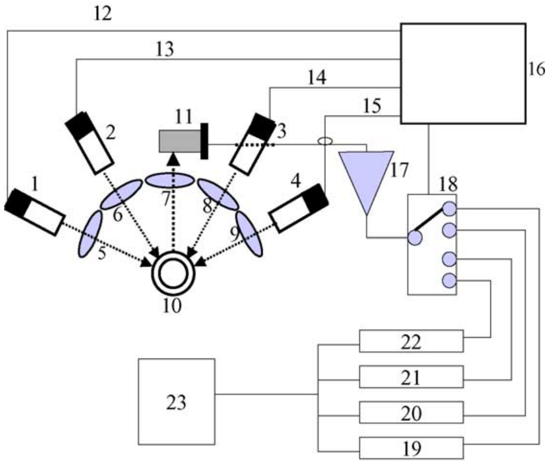

Fig. 1. Schematic diagram of four-channel nondispersive atomic fluorescence spectrometer. (1–4) High-power hollow cathode lamp; (5–9) lens; (10) atomizer; (11) "solar blind" photo-multiplier; (12–15) lamp source controlling; (16) modulated pulsed generator and controller; (17) multiplier; (18) electro-switch; (19–22) channel signal processor; (23) computer.

In Fig. 1, there are four high-power HCls (1, 2, 3, and 4), these lamps are specially designed for AFS, the lamps have a similar construction as those used in atomic absorption spectrometry (AAS), but with a cold auxiliary electrode and a bigger main cathode. The distance between the cathode and the front window of the lamp is much shorter than that of typical HCls used for AAS. This allows an increased in the irradiation area and angle of the light source. Using these lamps, experiments showed that the irradiation intensities were increased 2–5-fold and the detection limits were improved 3–8-fold depending on the tested elements. The lamps are operated in a four-modulated pulsed mode (16), which alternately lightens the lamp so that the light will alternately fall on the quartz furnace atomizer (10), thus producing their respective atomic fluorescence signals for receipt by a signal photomultiplier (11). The signals of all channels are amplified. At the same time, four synchronizing generators produce the demodulating signals (16) to control the electro-switch (18), which is necessary to allow discrimination between the fluorescence signals of the four elements. Thus, the intensities are detected and separated according the electro-switch on or off. The high-powered hollow cathode lamps were operated in high lamp current, which offers generating a high intensity of radiation, improved stability and longer lifetime. A "solar blind" photomultiplier was used as the detector in the nondispersive detection system.

The atomizer used is an electrically heated quartz furnace. In this atomizer, the heating wire (only one loop) is coiled around the top edge of the quartz-tube atomizer. The role of the heated wire is to ignite the argon–hydrogen flame needed for the atomization but not to heat up the body of the atomizer. In this case, the temperature of the quartz tube is maintained at a low temperature that caused no significant decomposition of the hydride, because of its long lifetime and excellent detection limits. The volatile species and hydrogen gas from the reaction are carried by a argon "carrier" gas, the gas mixture is self-ignited at the outlet of the furnace and a hydrogen–argon–air entrained micro-flame is maintained without the addition of any auxiliary hydrogen.

A home-made programmable intermittent flow reactor, as described previously [9], was used throughout the work. As shown in Fig. 2, apart from the addition of storage coil, the configuration of the device is similar to that of a continuous flow reactor. But the operation of the pump can be programmed in several steps for each measurement. At every step, the operator can program the rotation rate and time of the pump. In this work, the operation of the pump during each measurement consisted of three steps. At the first, the sampling tube was placed in the test solution, the sample was propelled by the pump at 6.0 ml min^{-1} and the potassium tetrahydroborate solution at 3.0 for 6 s, which corresponds to an absolution sample volume of 0.6 ml. At the second step, the pump stopped for 3 s thus allowing the sampling tube to be changed over to the carrier solution. At this stage, the sample stayed in the storage coil that was in the front of the mixing joint of the manifold and consequently no reac-

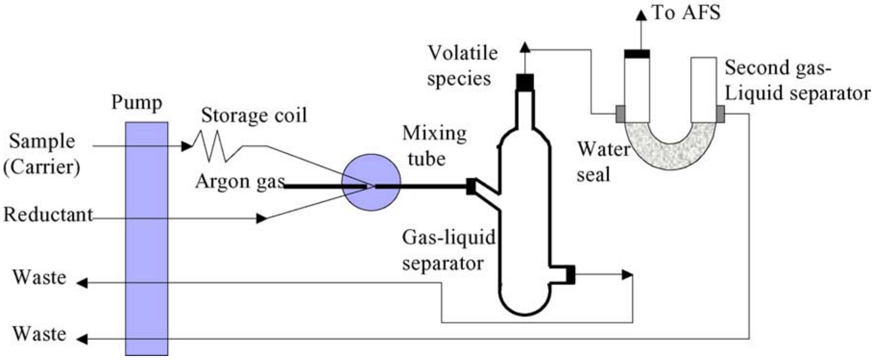

Fig. 2. Schematic diagram of intermittent reactor. Storage coil length 90 cm, i.d. 1.0 mm, PTFE tubing; mixing tube length 70 mm, i.d. 6 mm, silica gel tubing; Perkin-Elmer type 1 gas–liquid separator (made of glass), height 50 mm, i.d. 16 mm; PTFE tubing (from the separator to the second separator) length 25 cm, i.d. 0.7 mm; the second gas–liquid separator constructed from glass with a U-shaped tube, height 30 mm, i.d. 6 mm; PTFE tubing (from the separator to AFS atomizer) length 15 cm, i.d. 0.7 mm.

Table 1
Working program for the intermittent flow reactor

Step	Flow rate (ml min^{-1})	Time (s)	Function
1	Sample: 6; reductant (2% (m/v) KBH_4): 3	6	Insertion of sample tube
2	Sample: 0; reductant (2% (m/v) KBH_4): 0	3	Into sample solution
3	Carrier (3 mol l^{-1} HCl): 12; reductant (2% (m/v) KBH_4): 6	14	Changeover of sampling tube into carrier solution
4	Carrier (3 mol l^{-1} HCl): 0; reductant (2% (m/v) KBH_4): 0	3	Return to step 1

tion occurred between the sample solution and the reductant. At the third step, the pump rate was raised to high value, the carrier solution was propelled at 12 ml min^{-1} for 14 s, rapidly pushing the sample and the reductant into the mixing coil and gas–liquid generator. At this stage, the volatile species were formed and transported to the quartz furnace. In a hydrogen–argon–air entrained micro-flame, the gaseous molecular compounds were atomized and determined by AFS. After the third step, the pump was stopped again and made ready for the next determination. The working program of the intermittent flow reactor used in this work is listed in Table 1. A similar Perkin-Elmer type 1 gas–liquid separator, described in Ref. [10], was used in this work was made of glass. The waste was propelled from the outlet at the bottom of the separator by the same pump.

The operating parameters of the four-channel nondispersive AFS instrument used in this work are given in Table 2.

Table 2
Operating parameters of the four-channel nondispersive AFS instrument

Parameter	As	Sb	Bi	Se
High voltage of PMT (V)	320	320	320	320
Atomizer temperature (°C)	800	800	800	800
Atomizer height (mm)	8	8	8	8
Peak current of lamp (mA)	40	70	80	80
Flow rate of carrier gas (Ar) (ml min^{-1})	400	400	400	400
Flow rate of shield gas (Ar) (ml min^{-1})	800	800	800	800
Read mode	Peak area	Peak area	Peak area	Peak area

2.2. Reagent

All reagents were of analytical-reagent grade and water purified with a Milli-Q system (Millipore) was used throughout. About 20 g l^{-1} of potassium tetrahydroborate was prepared daily by dissolving KBH_4 in 2 g l^{-1} of sodium hydroxide and filtered before used. The single-element stock solutions of 1.0 g l^{-1} of As, Sb, Bi and Se, commercial standard solutions (National standard material center of China) were used. Working standard solutions of As, Sb, Bi and Se were prepared by stepwise dilution of the stock solutions just before use.

The different oxidation state single-element stock solutions of 1.0 g of As(III), As(V), Sb(III), Sb(V), Se(IV) and Se(VI) were prepared from the salts of $NaAsO_2$, $NaAsO_3$, $KSbOC_4H_4O_4{\cdot}0.5H_2O$, $SbCl_5$, $Na_2SeO_3{\cdot}5H_2O$ and Na_2SeO_3, respectively. All solutions were made 2 M HCl and stored at 4 °C. The lower concentration test solutions were prepared fresh daily by dilution.

2.3. Operational procedures

2.3.1. Optimization of AFS instrument parameters and hydride generation conditions

The test solution containing As(III), Sb(III), Bi(III) and Se(IV), was used to optimize the parameters of the AFS instrument and the conditions of hydride generation.

2.3.2. Pre-reduction procedures

When the test solution contained the hydride elements in their highest oxidation state, the following pre-reduction procedure was used. The test solution containing 1000 ng of As(V), 500 ng of Sb(V), Bi(III) and Se(VI), was made 6 mol l^{-1} HCl and heated in water bath at 90 °C for different time to reduce selenium from hexavalent to tetravalent state. After cooling, different amounts of 0.5 M thiourea and HCl were added to reduce arsenic and antimony from the pentavalent to the trivalent state, so that the final acidity was 3 M HCl, and diluted to 50 ml with water. The solution was analysed within the range of 0.5–3 h after the addition of thiourea.

2.4. Sample pretreatment

Approximately 0.2500–0.5000 g of the biological sample, depending on the concentrations of the analytes in the sample, was accurately weighed and placed in a 50 ml beaker, to which 10 ml of concentrated nitric acid was added. Covered with glass and soaked for 6–12 h. After that, 2 ml of perchloric acid was added and gently heated on a hot plate until white fume appearance, then after cooling, washed the wall of beaker and the glass with water and added 0.1 ml of formaldehyde, and gently heated to white fume appearance again. After cooling, 12.5 ml of 6 mol l^{-1} of hydrochloric acid were added and the beaker was gently heated in a water bath at 90 °C for 20 min. After cooling, the solution was transferred in to a 25 ml volumetric flask, to which 2.0 ml of 0.5 M thiourea were added, and diluted to volume with water. After 30 min, the sample was reacted with potassium tetrahydroborate to form the gaseous hydrides, which were carried into the AFS instrument for determination.

3. Results and discussion

3.1. Optimization of AFS instrument parameters

3.1.1. Flow rate of carrier gas

Pure argon was used as the carrier gas and the influence of the carrier gas flow rate on the signal response of the four hydride-forming elements was studied. The relationship between the flow rate of carrier and the signal is shown in Fig. 3. It can be clearly seen that the signal intensity of Sb decreased with increasing carrier gas flow rate. There were the maximum signal intensities as the flow rate of carrier gas of 400–600 ml $\min^{-1}$ for As, 400–500 ml $\min^{-1}$ for Se and 400–500 ml $\min^{-1}$ for Bi. Considering the sensitivities of As, Sb, Bi and Se, 400 ml $\min^{-1}$ carrier gas was used as a compromise.

3.1.2. Flow rate of shield gas

In this work, an argon shield gas flow was employed to prevent extraneous air from entering the flame, in that it decreased the level of the entrained air and as consequence increased the fluorescence efficiency. Studies on the effect of the flow rate of the shield gas revealed that a shield gas flow rate of 800 ml $\min^{-1}$ was optimal for giving higher signal intensities and a better signal-to-noise ratio.

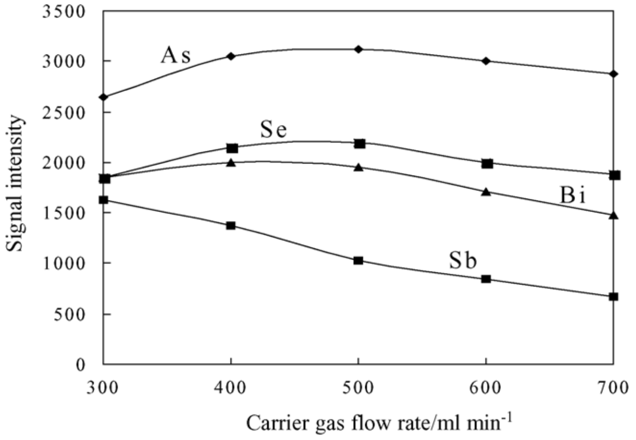

Fig. 3. Effect of the carrier gas flow rate on the atomic fluorescence signals of As (20 ng ml^{-1}), Sb (10 ng ml^{-1}), Bi (10 ng ml^{-1}) and Se (10 ng ml^{-1}); 3 mol l^{-1}; all other conditions as in Tables 1 and 2.

3.1.3. Observation height

Observation height is the distance from the top of the furnace atomizer to the point where the atomic fluorescence signal is measured. The effect of varying the observation height on the signal for As, Sb, Bi and Se was studied and the results are shown that the observation height increased the intensity was found to decrease for all elements, especially, the fluorescence signal intensity of As and Sb became remarkably reduced as the observation height increased. However, the observation height is too low, the background caused by both light scattering and radiation from the furnace will remarkably increased and consequently the noise level. Considering the sensitivity of the method and the noise level, An observation height of 8 mm was found to provide an optimum signal to noise ratio and signal, hence an observation height of 8 mm was used as compromise.

3.2. Optimization of the conditions of hydride generation

3.2.1. Acidic conditions

In this work, we studied the influence of the HCl concentration on the determination of As, Sb, Bi and Se and the results are shown in Fig. 4. It can been seen that optimum acid concentration for As and Sb occurred in the range of 1.8–4.8 M HCl; Se signal was drastically reduced in the range of 0.3–1.8 M HCl and was a plateau in the range of 1.8–4.8 M HCl; the observed Bi signal was virtually unaltered in the acid range studied. Higher acidic concentration was found to be helpful for overcome interference. A compromise concentration of 3 M HCl was chosen as the final working value.

3.2.2. Tetrahydroborate concentration

The relationship between the potassium tetrahydroborate concentration and the peak area signals obtained was tested. The potassium hydroxide concentration (2 mg l^{-1}) in this study was kept constant for different reductant concentra-

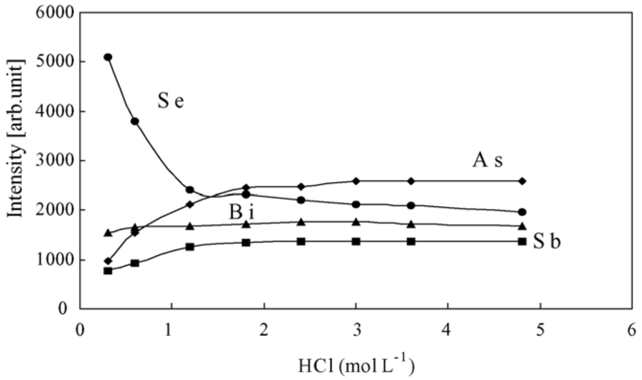

Fig. 4. Effect of hydrochloric acid concentration on the atomic fluorescence signals of As (20 ng ml^{-1}), Sb (10 ng ml^{-1}), Bi (10 ng ml^{-1}) and Se (10 ng ml^{-1}); all other conditions as in Tables 1 and 2.

tions. The influence of potassium tetrahydroborate concentration on the signals of As, Sb, Se and Bi is shown in Fig. 5. It can be clearly seen that at lower concentrations of KBH_4, the signals of As, Sb, Bi and Se increased with the increasing concentrations of KBH_4. However, at higher concentration of KBH_4, the bigger flame was formed and the dilution of evolved volatile species in the atomizer were dominant, leading to gently reduce the signals of Sb and Bi. Considering the sensitivity of the method and the noise of flame, 2% (m/v) KBH_4 was used as reductant.

3.3. *Pre-reduction*

Hydride generation depends strongly on the oxidation states of the elements, the most favourable oxidation states of the elements are As(III), Sb(III), Bi(III) and Se(III). The pentavalent state of As and Sb gives a much lower sensitivity than the trivalent state of the elements, and hexavalent Se is not reduced at all to the hydride by potassium or sodium tetrahydroborate. The predominant oxidation state of an element in a sample solution depends on the procedure that is used for dissolving or pre-treating the sample. In this work, nitric acid will oxidize As and Sb to the pentavalent state and perchloric acid is needed to bring Se to the hexavalent state.

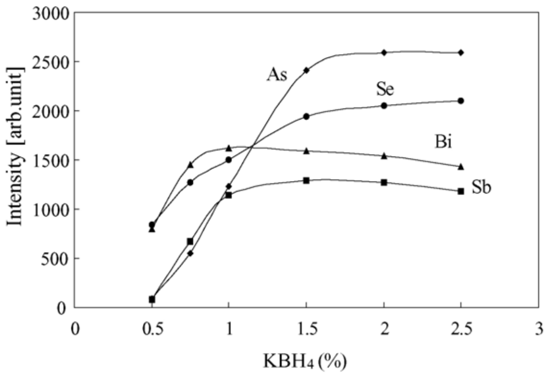

Fig. 5. Effect of potassium tetrahydroborate concentration on the atomic fluorescence signals of As (20 ng ml^{-1}), Sb (10 ng ml^{-1}), Bi (10 ng ml^{-1}) and Se (10 ng ml^{-1}); 3 M HCl; all other conditions as in Tables 1 and 2.

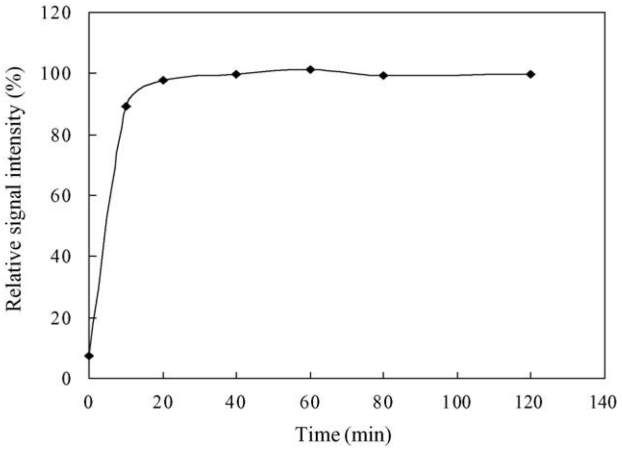

Fig. 6. Effect of the pre-reduction time on the relative signal intensity of Se(VI) (20 ng ml^{-1}); temperature 90 °C; reductant 6 M HCl; the result is given as percentage of the signal obtained with Se(IV).

The most frequently used method for reducing hexavalent Se to the tetravalent state is to heat the sample with HCl [11]. The rate of reduction is function of the acid concentration and temperature. To avoid losing the elements as volatile chlorides, the temperature must be strongly controlled. In this work, we studied the reasonable time of pre-reduction with 6 M HCl at 90 °C and monitored the loss of As, Sb, Bi and Se as volatile chlorides. The relationship between relative signal intensity and reduction time was studied and the results are shown in Fig. 6. It can be clearly seen that the relative signal

Table 3
Relative signal intensities for As(V), Sb(V) and Se(IV) in the addition of thiourea after various pre-reduction time

Element	Thiourea concentration ($mol\,l^{-1}$)	Time (min)				
		30	60	90	150	300
As	0.0075	51	56	68	78	89
	0.0150	61	76	83	96	100
	0.0225	74	91	96	99	101
	0.0300	88	101	99	100	101
	0.0375	93	101	101	100	99
	0.0500	95	101	100	101	100
Sb	0.0075	85	91	96	98	99
	0.0150	96	96	99	100	98
	0.0225	100	99	100	101	100
	0.0300	101	100	101	101	101
	0.0375	100	99	99	100	99
	0.0500	99	101	99	101	100
Se	0.0075	97	97	97	96	96
	0.0150	96	94	94	94	93
	0.0225	93	94	93	93	91
	0.0300	95	93	93	91	90
	0.0375	94	93	92	91	90
	0.0500	92	92	91	90	89

The results are given as a percentage of the signal obtained without thiourea for As(III), Sb(III) and Se(IV). The concentration of each species was 10 ng ml^{-1} and the concentration of HCl was 3 $mol\,l^{-1}$.

intensity, which is given as percent of the signal of Se(IV) is a plateau when the pre-reduction time is more than 20 min. Forty minutes was provide as optimum pre-reduction time for reduction of Se(VI) to Se(IV). It is worth pointing that no loss of As, Sb, Bi and Se as volatile chlorides was found at the above pre-reduction conditions.

Heating with HCl will not reduce pentavalent As and Sb. Pre-reduction of these species has mostly been achieved by the addition of iodide or bromide, L-cysteine and thiourea. The main drawback is that they all reduce tetravalent Se to its elemental state, which is unable to form the hydride. The fact that thiourea can be used to mask interferences in the determination of Se by hydride generation [12] and indicates that this reagent does not interfere seriously with the generation of selenium hydride although thiourea is able to reduce Se(IV) to elemental Se. In this work, the optimization concentration of thiourea and the reasonable reaction time were chosen, whereas pentavalent As, Sb should only be reduced to the trivalent state, but not further to the elemental state of Se. The results are illustrated in Table 3.

From Table 3, it can be seen that the degree of reduction was found to depend on the concentration of thiourea and the time from the addition of thiourea to the actual measurement. An immediate signal increase was observed for As(V) and Sb(V) after addition of thiourea, the signal enhancement became more pronounced with increasing concentration of thiourea and the degree of enhancement was more pronounced for As(V) than Sb(V). However, an signal depression was obtained for Se(IV) and the relative signal intensity did not change much during the next 300 min after the addition of thiourea. Considering the higher thiourea concentration was helpful for overcoming interference of transition element, a concentration of 0.05 mol l^{-1} of thiourea was recommended for the reduction of As(V) and Sb(V) and the samples should be analysed in the range of 30–300 min after addition of thiourea. It is worth pointing that the signal of Bi remained constant when the concentrations of the thiourea were in the range of 0–0.05 mol l^{-1}.

3.4. Interference

The effects of coexisting ions of Fe^{3+}, Cu^{2+}, Cr^{6+}, Co^{2+}, Ni^{2+}, Mo^{6+}, Pb^{2+}, Zn^{2+}, Cd^{2+}, Sn^{2+}, Te^{4+}, Hg^{2+}, Ag^+ on the pre-reduction and hydride generation of As, Sb, Bi and Se were studied, and the results are shown in Table 4. From Table 4 it can be seen that 13 coexisting ions studied do not

Table 4
Relative signal intensity of 10 ng ml^{-1} of the analyte in the presence of coexisting ions, the result are given as a percentage of the signal obtained without coexisting ions in the solution containing 10 ng m^{-1} of As(III), Sb(III), Se(IV), Bi(II) and 0.05 mol l^{-1} thiourea

Ions	Concentration (mg l^{-1})	As				Sb				Se				Bi			
		A	B	C	D	A	B	C	D	E	F	G	H	E	F	G	H
Fe^{3+}	100	101	102	101	101	100	102	101	101	110	110	107	72	99	97	99	98
	50	100	101	100	103	101	103	100	102	108	113	108	85	97	96	101	99
	20	103	102	99	102	100	101	102	100	108	110	107	95	98	96	103	101
Cu^{2+}	50	101	101	99	99	101	102	102	101	40	38	38	83	63	60	60	97
	10	102	102	98	98	99	100	101	100	84	82	84	96	93	95	96	101
Cr^{6+}	50	101	101	90	100	102	101	78	100	110	113	108	102	98	97	96	98
	10	102	103	97	101	100	100	95	102	107	112	109	101	99	97	99	99
Co^{2+}	10	102	101	99	101	102	101	102	102	111	111	106	80	97	97	98	96
	1	100	99	102	100	103	100	101	100	108	109	108	96	101	98	99	98
Ni^{2+}	50	100	99	102	101	102	100	101	100	111	109	107	84	97	97	97	98
	10	101	102	99	100	103	101	102	102	109	111	108	98	98	99	98	99
Mo^{6+}	50	102	101	100	101	102	101	98	101	112	112	106	97	97	97	97	99
	10	100	101	102	100	103	99	99	100	109	111	109	99	99	98	98	98
Pb^{2+}	50	102	101	102	100	101	100	102	101	101	99	97	64	88	87	91	110
	5	100	102	101	102	101	99	100	101	97	98	99	96	96	98	99	103
Zn^{2+}	50	101	102	101	102	101	100	101	100	108	108	106	96	96	97	98	96
	10	100	101	100	100	102	102	100	102	109	110	109	99	100	99	98	99
Cd^{2+}	5	101	102	101	99	101	100	102	103	109	107	108	98	98	99	97	98
Sn^{2+}	5	102	100	102	103	101	100	102	103	111	109	112	99	98	99	97	100
Te^{4+}	2	101	100	100	101	102	101	101	102	112	113	114	103	99	98	99	101
Hg^{2+}	2	100	103	102	102	101	99	100	101	111	109	109	101	98	97	99	98
Ag^+	2	102	102	101	101	103	102	101	102	112	110	109	98	98	101	99	98

A: adding the interferent in the test solution containing As(V) and Sb(V), and then pre-reducing to As(III) and Sb(III) with thiourea; B: pre-reducing As(V), Sb(V) to As(III), Sb(III) with thiourea, and then adding the interferent; C: adding the interferent in the test solution containing As(III) and Sb(III), without thiourea; D: adding the interferent in the test solution containing As(III), Sb(III) and 0.05 mol l^{-1} thiourea; E: adding the interferent in the test solution containing Se(VI) and Bi(II), and then pre-reducing with 6 mol l^{-1} HCl; F: pre-reducing Se(VI) to Se(IV) with 6 mol l^{-1} HCl, and then adding the interferent; G: adding the interferent in the test solution containing Se(IV) and Bi(II), without thiourea; H: adding the interferent in the test solution containing Se(IV), Bi(II) and 0.05 mol l^{-1} thiourea.

Table 5
Detection limits, precision and calibration functions for determination of As, Sb, Bi and Se using the proposed method

Measurement method	Element	Calibration functions[a]	Correlation coefficient	R.S.D.[b] %	DL[c] ($ng\,ml^{-1}$)
Simultaneous determination	As	I_f[d] $= 1.1 + 158.8C_{As}$[e]	0.9998	0.9	0.03
	Sb	$I_f = 0.9 + 130.5C_{Sb}$	0.9996	1.2	0.04
	Bi	$I_f = 1.3 + 183.4C_{Bi}$	0.9997	1.3	0.04
	Se	$I_f = 1.1 + 210.9C_{Se}$	0.9992	1.5	0.03
Single determination	As	$I_f = 0.9 + 158.1C_{As}$	0.9997	0.8	0.02
	Sb	$I_f = 1.4 + 131.1C_{Sb}$	0.9994	1.1	0.03
	Bi	$I_f = 0.9 + 182.8C_{Bi}$	0.9996	1.2	0.03
	Se	$I_f = 1.5 + 209.1C_{Se}$	0.9995	1.0	0.04

[a] Five standard was used for each calibration line.
[b] Relative standard deviation for 5 $ng\,ml^{-1}$ As, Sb, Bi and Se ($n = 7$).
[c] $3\sigma_{n-1}$ of 10 runs of the blank.
[d] Signal intensity.
[e] Concentration ($ng\,ml^{-1}$).

affect the pre-reduction of As(V), Sb(V) and Se(VI), however, the high concentration of Cr^{6+} can oxidize the trivalent state of As and Sb to the pentavalent state in the absence of thiourea. For the determination of As and Sb, no significant interferences from the certainty concentration of coexisting ions studied on the hydride generation are observed. In the absence of thiourea, 100 $mg\,l^{-1}$ Fe^{3+}; 50 $mg\,l^{-1}$ Cr^{6+}, Ni^{2+}, $Zn^{2+}$and Mo^{6+}; 10 $mg\,l^{-1}$ Co^{2+}; 5 $mg\,l^{-1}$ Cd^{2+} and Sn^{2+}; 2 $mg\,l^{-1}$ Te^{4+}, $Hg^{2+}$and Ag^{+} do not significant influence the determination of Se and Bi, the most serious signals depression is caused by 50 $mg\,l^{-1}$ Cu^{2+} on the determination of Se, and to a lesser extent on Bi by 50 $mg\,l^{-1}$ Cu^{2+} and Pb^{2+}. The masking effect of thiourea is also shown in Table 4, in the presence of thiourea, the minimizing interferences from Cu^{2+} and Pb^{2+} on the determination of Se and Bi are observed. On the contrary, the addition of thiourea has no positive effect, in many instances the combined effect of an interfering metalion and thiourea results in lower signal than for the interferent alone, possibly the interferent catalyses the reduction of Se(IV) to the elemental state, the signal depression is caused by 100 $mg\,l^{-1}$ Fe^{3+}; 50 $mg\,l^{-1}$ Cu^{2+}, Ni^{2+} and Pb^{2+}; 10 $mg\,l^{-1}$ Co^{2+} for the combined effect of interfering ions and thiourea.

Experiments were also performed with lower concentrations of interfering ions, the coexisting ions of 20 $mg\,l^{-1}$ Fe^{3+}; 10 $mg\,l^{-1}$ Cu^{2+} and Ni^{2+}; 5 $mg\,l^{-1}$ Pb^{2+}; 1 $mg\,l^{-1}$ Co^{2+} did not depress the signals of Se in the presence of thiourea. The above tolerance levels of coexisting ions permit the use of sample aqueous standard calibration for the determination of As, Sb, Bi and Se in the biological samples of interest.

3.5. *Analytical performance*

The analytical characteristic data of the proposed method are given in Table 5. It should be noted that the calibration functions for simultaneous determination of As, Sb, Bi and Se were in good agreement with those for individual determination of the four elements, indicating that the proposed method for simultaneous determination the four elements is reliable.

Table 6
The result[a] for the determination of As, Sb, Bi, Se in the certified reference materials

CRMs	Element	Certified value ($\mu g\,g^{-1}$)	Found value ($\mu g\,g^{-1}$)
GBW07601 (human hair)	As	0.28 ± 0.04	0.29 ± 0.03
	Sb	0.095 ± 0.012	0.094 ± 0.008
	Bi	0.34 ± 0.02	0.33 ± 0.01
	Se	0.60 ± 0.05	0.61 ± 0.02
GBW07602 (bush twigs and leaves)	As	0.95 ± 0.08	0.92 ± 0.04
	Sb	0.078 ± 0.015	0.081 ± 0.007
	Bi	(0.022)	0.022 ± 0.003
	Se	0.184 ± 0.011	0.186 ± 0.007
GBW07603 (bush twigs and leaves)	As	1.25 ± 0.10	1.24 ± 0.06
	Sb	0.095 ± 0.011	0.098 ± 0.007
	Bi	0.023 ± 0.04	0.024 ± 0.03
	Se	0.12 ± 0.02	0.12 ± 0.01
GBW07604 (poplar leaves)	As	0.37 ± 0.06	0.39 ± 0.04
	Sb	0.045 ± 0.005	0.044 ± 0.003
	Bi	0.027 ± 0.002	0.028 ± 0.001
	Se	0.14 ± 0.01	0.15 ± 0.01
GBW07605 (tea)	As	0.28 ± 0.03	0.29 ± 0.02
	Sb	0.056 ± 0.005	0.058 ± 0.004
	Bi	0.063 ± 0.007	0.061 ± 0.004
	Se	(0.072)	0.070 ± 0.004

[a] Mean ± S.D., $n = 5$.

To demonstrate the accuracy of the present method, we applied it to the simultaneous determination of trace As, Sb, Bi and Se in the following certified biological reference materials: GBW07601 (human hair), GBW07602 (bush twigs and leaves), GBW07603 (bush twigs and leaves), GBW07604 (poplar leaves), and GBW07605 (tea). The results for the simultaneous determination of As, Sb, Bi and Se in these certified reference materials using simple aqueous standard calibration technique are shown in Table 6. It can be seen that the concentrations of As, Sb, Bi and Se in the certified reference materials obtained by the present method were in good agreement with the certified values.

4. Conclusions

This work demonstrated the feasibility for simultaneous determination of trace As, Sb, Bi and Sb in biological samples by HG-four-channel NDAFS. The developed method is simple, fast and sensitive and is promising for routine analysis of trace As, Sb, Bi and Se in biological materials.

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