Sensitive and Simultaneous Determination of As and Hg in Human Hair, Nail, and Saliva by CVG-AFS

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INTRODUCTION

Understanding the effects of trace metals on human health is as complex as it is fascinating (1,2). It can help to facilitate the identification of many abnormalities, thereby being helpful in identifying the possible immune status of the human body. Many metabolic disorders in man are accompanied by changes in the concentration of one or more trace elements. The amount of trace elements is evaluated most often through the determination of their concentrations in the plasma and serum. Non-invasive techniques have been considered a useful tool in determining the trace element status, especially in rural communities (3). More institutions, laboratories, and medical practitioners are now inclined to use hair, nail, and saliva due to the convenience of collection and lack of pain during collection as normally associated in the use of blood (4, 5). Blood contains the component absorbed and temporarily in circulation before excretion and/or storage; mother's milk, urine, saliva, and perspiration contain the component that is absorbed but excreted; hair, nail, and teeth are tissues in which trace minerals are sequestered and/or stored. Concentrations of heavy metals in hair and nail provide an accurate and relatively permanent record of exposure, and there is a strong correlation between concentrations in hair and nail vs. the

ABSTRACT

In this work, an accurate, simple, and sensitive analytical procedure was developed for the simultaneous determination of trace arsenic (As) and mercury (Hg) in human hair, nail, and saliva by chemical vapor generation atomic fluorescence spectrometry (CVG-AFS). The conditions for the generation of volatile As and Hg species, such as pre-reduction conditions, carrier gas and shield gas, and reaction medium (KBH_4 and HCl), were investigated in detail.

The precision for five replicates at the 50 ng mL^{-1} As level and the 5 ng mL^{-1} Hg level was 4.1% and 4.2% (relative standard deviation, RSD), respectively. Under the optimized experimental conditions, the limits of detection (LOD) were 0.11 ng mL^{-1} and 0.06 ng mL^{-1} , with the linear ranges of $2\text{--}200 \text{ ng mL}^{-1}$ ($R^2 = 0.9967$) and $0.2\text{--}20 \text{ ng mL}^{-1}$ ($R^2 = 0.9998$), for As and Hg, respectively. The proposed method was successfully applied to the simultaneous determination of As and Hg in several hair, nail, and saliva samples from volunteers, and the spike recoveries were in the range of 97–112%. The accuracy of method was further validated by analyzing a human hair certified reference material. The obtained values were in good accordance with the certified values.

internal organs. In general, hair and nail analysis is a reliable, simple, and less traumatic method of assessing heavy metal concentrations in the body.

Atomic fluorescence spectrometry (AFS) has been widely used for the determination of chemical vapor generation (CVG) elements (i.e., As, Sb, Bi, Se, Te, Ge, Sn, Pb, Zn, Cd, and Hg) because of its high sensitivity, wide linear dynamic range, speed of analysis, ease of use, and low cost (6–8). The detection ability of AFS is superior to atomic absorption spectrometry (AAS) and similar to inductively coupled plasma mass spectrometry (ICP-MS) (9,10) with regard to sensitivity and linear calibration range, with further advantages (simplicity, lower acquisition and running costs) for chemical vapor-forming elements in routine analysis (11–14). Holak (15) reported the pioneer work of chemical vapor generation (in this case, hydride generation) combined with atomic absorption spectrometry in 1969. Then, Tsujii et al. (16) first applied CVG to atomic fluorescence spectrometry in 1974. The first simultaneous multi-element determination of CVG elements was performed in combination with inductively coupled plasma optical emission spectrometry (ICP-OES) in 1978 (17). Dual-channel CVG-AFS, whereby two elements could be detected simultaneously, was reported by Guo et al. in 1983 (18). However, CVG-AFS is mainly applied for single-element measurements. A literature survey revealed that most of such work concentrated on single-element analysis, whereas, to the best of our knowledge, no CVG-AFS procedure for the simultaneous determination of As and Hg in human hair, nails, and saliva has been published previously (19–27).

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In this work, the simultaneous determination of multi-elements (As and Hg) in human hair, nail, and saliva is presented using CVG-AFS. Arsenic and mercury are treated as severe chemical hazards by the World Health Organization. Epidemiological studies show that arsenic in drinking water causes diseases such as skin cancer and other internal cancers, especially in the lung, bladder, and kidney. Mercury is a common global pollutant due to its potent neurotoxicity as well as its ability to bioaccumulate in food chains. Arsenic and mercury levels in human hair are widely used as biological indicators for estimation of the exposure (28, 29). The experimental conditions of the present study, including pre-reduction conditions, carrier gas and shield gas, reaction medium (KBH_4 and HCl), analytical figures of merit, and method validation, were investigated and are discussed in detail.

EXPERIMENTAL

Instrumentation

A model AFS-2202 non-dispersive atomic fluorescence spectrometer (Beijing Haiguang Instrument Co., Ltd., Beijing, P.R. China) was used in this work. The instrument is equipped with a programmable intermittent reactor and two gas-liquid separators to facilitate

chemical vapor generation and separation. High-intensity hollow cathode lamps (HCL, Research Institute of Non-Ferrous Metals, Beijing, P.R. China) of arsenic and mercury were used as the radiation sources. The working parameters of CVG-AFS are summarized in Table I.

Reagents and Standard Solutions

All reagents were of analytical reagent grade. Deionized water (DIW) was used throughout this study. Standard stock solutions of arsenic and mercury (100 mg L^{-1}) were purchased from the National Center for Reference Material (Beijing, P.R. China). The mixed working standard solutions in the $\mu\text{g L}^{-1}$ and sub- $\mu\text{g L}^{-1}$ range were obtained by stepwise dilution of the stock solution with 7.5% (v/v) HCl solution. A solution of KBH_4 , 25 g L^{-1} , was stabilized with 5 g L^{-1} KOH . The mixed pre-reduction solution was composed of 5% (m/v) thiourea and 5% (m/v) ascorbic acid. High purity argon gas (99.99%) was used as both the carrier and shield gas.

Procedure

The human hair, nail, and saliva samples were obtained from volunteers at the Chengdu University of Technology. Approximately 0.2000–0.5000 g of human hair, nail, and saliva samples were accurately weighed into polytetrafluoro-

ethylene (PTFE) flasks. A 5-mL amount of nitric acid was added to the PTFE flasks. After overnight digestion at room temperature, 0.25 mL H_2O_2 was added to the PTFE flasks, and the solution transferred to an electric hot plate. The vessels were heated until the volume of the solutions was reduced to around 0.5 mL. The resultant solutions were transferred into a 25-mL volumetric flask and finally diluted to volume with deionized water. The solutions for the metal determinations were prepared by diluting 5 mL of sample solutions to a final volume of 25 mL with hydrochloric acid solution (final concentration of 7.5%, v/v) and a pre-reductive solution (final concentration of 5%, m/v, thiourea and 5%, m/v, ascorbic acid). To validate the accuracy of the proposed method, a certified reference material GBW09101b Human Hair was purchased from the National Center for Reference Material (Beijing, P.R. China). Chemical vapor generation was carried out by mixing the sample solutions with 2.5% (m/v) KBH_4 solution using an intermittent flow system.

RESULTS AND DISCUSSION

Pre-reduction Conditions

Arsenic can exist in two different oxidation states: trivalent and pentavalent. It is well known that As(V) has much slower reaction kinetics for hydride formation, which depends on the hydride generation system used and the concentration of the reagents. With a continuous-flow manifold, the hydride generation efficiency is around 40% compared with the trivalent oxidation state. The most common pre-reduction involves the addition of thiourea and ascorbic acid. Preliminary results of this study indicated that a mixture of thiourea and ascorbic acid provides an effective reduction to the trivalent oxidation states after a

TABLE I
AFS Instrumental Parameters

Parameters	
Hollow cathode lamp	As: 193.7 nm, 30 mA Hg: 253.7 nm, 15 mA
Negative voltage of PMT	300 V
Atomizer temperature	200 °C
Observation height	8 mm
Read mode	Peak area
Read time	10 s
Carrier gas flow rate	800 mL min^{-1}
Shield gas flow rate	1100 mL min^{-1}

pre-reduction time of 30 minutes. In addition, thiourea and ascorbic acid used as masking reagents could reduce the interference of coexisting elements.

Carrier Gas and Shield Gas Flow Rate

Pure argon was used as both the carrier gas and the shield gas. Argon transports the chemical vapor from the liquid gas separator to the atomizer, acts as analyte diluent, and stabilizes the flame by forming an appropriate mixture with H_2 . Various flow rates, which influence the sensitivity and stabil-

ity of the instrument, were tested in this study. The effects of carrier and shield gas on the signal of As and Hg are shown in Figures 1 and 2. The carrier gas at a lower flow rate could not quickly sweep the hydride of the analyte into the inner tube of the quartz furnace, but a higher flow rate would dilute the analyte introduced into the furnace tube. The amounts of vapor generated were different for different analytes. Different gas flow rates for As and Hg were required for completely carrying the different amounts of hydride analytes into the atomizer. The shield gas

was used to prevent extraneous air from entering the flame. A carrier gas flow rate of 800–900 mL min^{-1} for As and 700–800 mL min^{-1} for Hg gave the highest signal intensity. A shield gas flow rate of 1100 mL min^{-1} for both As and Hg gave the highest signal intensity. Consequently, a compromised carrier gas flow rate of 800 mL min^{-1} and a shield gas flow rate of 1100 mL min^{-1} was employed for the simultaneous determination of As and Hg.

Reaction Medium (KBH_4 and HCl)

In the CVG-AFS system employed, KBH_4 is used not only as the reductant, but also as the hydrogen supply to sustain the argon-hydrogen flame. The concentration of KBH_4 had a large impact on the vapor generation process and on the stability of the argon-hydrogen flame. The optimization of KBH_4 concentration was carried out between 0.2% to 3% (m/v). Figure 3 illustrates the effect of KBH_4 concentration on the signal intensities of As and Hg. Generation of AsH_3 and Hg vapor could be carried out with KBH_4 concentrations ranging from 0.2% to 3%, but the sensitivities decreased rapidly when the KBH_4 concentrations were below 2.5%. The fluorescence intensity

Fig. 1. Effect of carrier gas flow rate on the fluorescence signal of As and Hg.

Fig. 2. Effect of shield gas flow rate on the fluorescence signal of As and Hg.

Fig. 3. Effect of KBH_4 concentration on the fluorescence signal of As and Hg.

decreased very little when the KBH_4 concentration was increased from 2.5% to 3%. It is also well recognized that some additional drawbacks, such as causing very noisy background, taking more water vapor to the atomizer, and bringing a higher standard deviation, hamper the performance of the CVG-AFS system when the concentrations of KBH_4 are too high. For further experiments, 2.5% KBH_4 was chosen for As and Hg chemical vapor generation.

The generation efficiency of the volatile species greatly depends upon the acidity at which the reaction is performed. Using 2.5% KBH_4 as the reductant and the flow rate of the sample as approximately equal to that of the potassium tetrahydroborate flow rate, the effect of various HCl concentrations on the signal intensities was investigated within the 0.5–15% range. As shown in Figure 4, best results for As and Hg were obtained with an HCl concentration of 7.5–15%. However, the reagent background increased with an increase in HCl concentration. Therefore, a concentration of 7.5% HCl, which was near the lower edge of the optimization plateau, was chosen as the final working value.

Analytical Figures of Merit

Under the optimized conditions, a series of mixed standard solutions of As and Hg were prepared and determined by two-channel CVG-AFS. The fluorescence intensity of the analyte was found to be linearly proportional to the concentration of the analyte. Table II shows the analytical figures of merit of the proposed simultaneous determination method for As and Hg. The calibration curves shown in Figure 5 exhibit good linearity. The limits of detection (LOD, 3σ) were 0.11 ng mL^{-1} and 0.06 ng mL^{-1} for As and Hg, respectively, thus suitable for the sensitive analysis of biological samples.

Fig. 4. Effect of HCl concentration on the fluorescence signal of As and Hg.

TABLE II
Analytical Figures of Merit of the Proposed Method

Analytical Figures	Hg	As
Dynamic range	0.2–20 ng mL^{-1}	2–200 ng mL^{-1}
Equation of linear fit	$y = 149.9x - 0.9751$	$y = 21.01x + 49.81$
Linear correlation coefficient (R^2)	0.9998	0.9967
Relative standard deviation (% $n=5$)	4.2 (5 ng mL^{-1})	4.1 (50 ng mL^{-1})
Limit of detection	0.06 ng mL^{-1}	0.11 ng mL^{-1}

Validation

In order to validate the applicability of the proposed method for trace arsenic and mercury determination, a series of human hair, nail, and saliva samples, collected from volunteers at the Chengdu University of Technology, were analyzed. As shown in Table III, the recoveries of the samples spiked with 4–8 ng mL^{-1} of Hg and 40–80 ng mL^{-1} of As ranged from 97–112%.

In order to further verify the accuracy and precision of the method, the certified reference material GBW09101b Human Hair was analyzed for each element. The sample was prepared in triplicate and analyzed in triplicate. The results of $0.180 \pm 0.02 \mu\text{g/g}$ and $1.06 \pm 0.02 \mu\text{g/g}$ were obtained for arsenic and mercury, respectively,

exhibiting excellent recoveries in comparison to the certified values (i.e., $0.198 \pm 0.023 \mu\text{g/g}$ for As and $1.06 \pm 0.28 \mu\text{g/g}$ for Hg).

CONCLUSION

This work demonstrates the feasibility for the simultaneous determination of trace arsenic and mercury in human hair, nail, and saliva samples by chemical vapor generation atomic fluorescence spectrometry. Using the optimized experimental conditions, the limits of detection (LOD) were 0.11 ng mL^{-1} and 0.06 ng mL^{-1} , with the linear ranges of 2–200 ng mL^{-1} ($R^2 = 0.9967$) and 0.2–20 ng mL^{-1} ($R^2 = 0.9998$), for As and Hg, respectively. The proposed method was applied to the simultaneous determination of As and Hg in several hair, nail,

Fig. 5. Calibration curve for As and Hg.

and saliva samples from volunteers, and the spike recoveries ranged from 97–112%. The accuracy of the method was further validated by using a human hair certified reference material. The proposed method is simple, sensitive, and promising for use in the routine analysis of trace As and Hg in clinical samples and biological materials.

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TABLE III
Determination of As and Hg
in Human Hair, Nail, and Saliva Samples

Samples	Added (ng mL ⁻¹)		Found (ng mL ⁻¹)		Recovery (%)	
	Hg	As	Hg	As	Hg	As
Human nail 1	0	0	0.086±0.020	0.49±0.07	-	-
	4	40	4.30±0.02	43.24±0.24	105	107
	6	60	6.84±0.05	62.26±0.07	112	103
Human nail 2	0	0	^b	^b	-	-
	4	40	3.94±0.11	41.05±0.27	99	102
	8	80	8.35±0.11	79.17±0.96	105	99
Human nail 3	0	0	0.24±0.03	0.065±0.082	-	-
	2	20	2.32±0.09	21.42±0.17	104	107
	4	40	4.72±0.04	38.91±0.55	112	97
Human hair 1	0	0	0.24±0.02	0.54±0.10	-	-
	4	40	4.47±0.07	43.07±0.28	106	106
	6	60	6.70±0.07	62.46±0.15	108	103
Human hair 2	0	0	0.17±0.05	0.075±0.060	-	-
	4	4	4.59±0.07	41.63±0.41	110	103
	8	80	9.06±0.14	79.78±0.32	111	99
Human saliva 1	0	0	0.18±0.08	0.67 ±0.05	-	-
	4	40	4.58±0.03	43.78±0.19	110	108
	6	60	6.78 ±0.08	63.29±0.24	110	104
Human saliva 2	0	0	^b	2.66±0.12	-	-
	4	40	3.80±0.12	43.03±0.55	97	101
	8	80	8.04 ±0.22	80.44 ±2.36	101	97

^a Mean of three determinations.

^b Not detectable.

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