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A low cost and sensitive procedure for lead screening in human whole blood with sequential injection-hydride generation-atomic fluorescence spectrometry

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An automated sequential injection hydride generation (HG) atomic fluorescence spectrometric (AFS) method was developed, with the aim of screening blood lead levels for Chinese citizens, especially for children. Lead hydride was generated from acid solution, with potassium ferricyanide as an oxidizing agent, by reaction with alkaline tetrahydroborate solution. The hydride was separated from the reaction medium in a gas–liquid separator (GLS_1) and swept directly into the atomizer. A thorough scrutiny was made of the various factors, including the modification of the AFS instrument and related parameters, the various wet digestion protocols, the variables of the flow system and the reaction conditions. Under the optimized conditions the blank signal was satisfactorily minimized, giving rise to a limit of detection of 0.014 μg l^{-1}, defined as 3 times the blank standard deviation divided by the slope of calibration graph, and a RSD value of 0.7% (at the 2.0 μg l^{-1} level, $n = 11$), along with a sampling frequency of 120 h^{-1}. The sensitivity of the procedure was found to be significantly superior to those obtained by HG-atomic emission spectrometry (AES), direct sampling electrothermal atomic absorption spectrometry (ETAAS), tungsten filament atomizer ETAAS, and quartz tube atomizer HG-AAS; it was even lower than those by in-atomizer-trapping HG-ETAAS and comparable to ICPMS-based methods. The accuracy and practical applicability of the procedure were validated by analysing certified reference materials of frozen cattle blood GBW 09139 and GBW 09140, and further demonstrated by spiking recoveries of lead in human whole blood samples.

1. Introduction

Lead has been demonstrated to be a non-essential and toxic element that is particularly detrimental to human health.[1] Once absorbed into the blood, lead is rapidly deposited into soft tissues and thence into the bone tissue, where its residence time is of the order of decades.[2] It is confirmed that an increase of 100 μg l^{-1} lead in childhood blood results in a decrease of 0.25 point in intelligence quotient.[3] It is also shown that a blood lead level of *ca.* 65 μg l^{-1} during pregnancy can not only cause harmful effects on the delivery mode but also deteriorate the development of the nervous system of neonates.[4] Extensive studies have shown that childhood lead poisoning in China is becoming a critical issue; it is therefore necessary to launch a public health care program for routine screening of lead poisoning level, related to the child's exposure to lead. In this case, it is not only highly desirable but, in fact, obligatory to keep the sampling volume at a minimum, that is, *ca.* 50 μl, in order to minimize injury to the patients.

Since the mid-1970s, a substantial decrease of the blood lead threshold in childhood blood that is considered detrimental has been enforced,[5] thus posing a challenge in improving the performance of the current analytical procedures. Among the various spectrometric techniques available for lead,[6–20] ETAAS and ICP-MS are the most promising candidates for measuring lead in blood, because of their high sensitivities.[16–20] However, neither the cost of the commercial instruments nor their operations are within the acceptable limit for large scale screening in clinical laboratories in China. In addition, for a direct sampling system, the ETAAS signal is inherently very sensitive to the composition of the matrix and thus creates a serious matrix effect,[21] while the intrinsic low tolerance limit to the total dissolved salts (TDS) restricts the direct applications of ICP-MS in biological samples, *e.g.*, whole blood. AES and quartz tube atomizer AAS cannot be chosen as their sensitivities cannot fulfil the requirements in the case of blood lead.

It is, therefore, highly desirable to develop a low cost, but sensitive and selective procedure for screening the childhood blood lead level in Chinese clinical laboratories.

The atomic fluorescence spectrometer (AFS) has a flow through detector that is compatible with on-line vapor generation and therefore is most suitable for measuring hydride forming elements.[22] Nevertheless, there has been no report concerning its application to the determination of blood lead. The cost of acquiring an AFS instrument in China is only about one fifth to one twentieth of that of an ETAAS or ICP-MS instrument, and the cost of its maintenance is low. At this juncture, it is very promising to explore the feasibility of hydride generation atomic fluorescence spectrometry for assaying blood lead in order to develop a practical procedure for screening childhood blood lead level.

In this work, an automated, low cost sequential injection-hydride generation-atomic fluorescence spectrometric method for blood lead screening was developed. The detection limit of this procedure was significantly superior to those obtained by HG-AES,[6,7,23] quartz tube atomizer HG-AAS,[8–10] direct sampling ETAAS,[16,24] tungsten filament atomizer ETAAS,[25] and even lower than that obtained by in-atomizer-trapping HG-ETAAS.[11–13]

Experimental

Instrumentation

A Titan AFS-920 sequential injection (SI)-atomic fluorescence spectrometric system (Titan Instruments Co. Ltd., Beijing, China), with an electrically ignited concentric quartz tube atomizer, was employed. An argon flow (auxiliary gas) along

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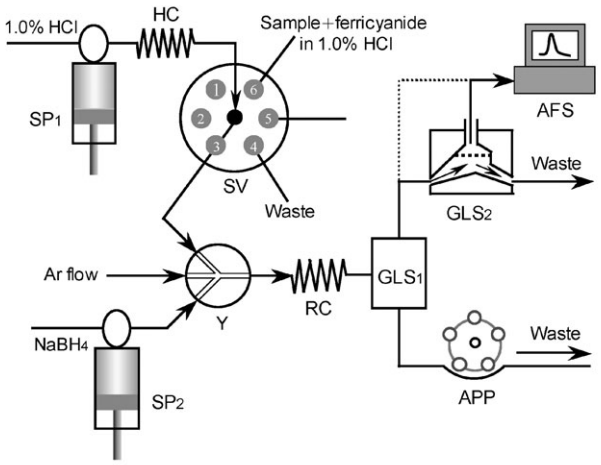

Fig. 1 The flow manifold of the sequential injection-HG-AFS setup. SP_1, SP_2: syringe pumps; GLS_1: W-type glass gas–liquid separator; GLS_2: membrane gas–liquid separator; SV: multi-port selection valve; HC: holding coil; RC: reaction coil; APP: auxiliary peristaltic pump; SV: multi-port selection valve. The broken line denotes that the second stage membrane gas–liquid separator GLS_2 is removed and the hydride out of GLS_1 is directly swept into the atomizer.

the annular space between the walls of the two quartz tubes was applied as a shield gas to avoid the introduction of air into the atomizer. A high-intensity lead hollow cathode lamp (Beijing General Research Institute for Nonferrous Metals, China) was used as the radiation source at 283.3 nm. The lead hydride and the hydrogen generated were separated from the liquid in the first-stage W-type gas–liquid separator (GLS_1), swept by an argon flow (carrier gas) through the second-stage membrane gas–liquid separator (GLS_2) and finally into the atomizer, where the hydride was atomized by an argon–hydrogen flame. In the present study, the GLS_2 was removed and the hydride introduced directly into the atomizer.

The incorporated SIS-100 sequential injection system was furnished with two syringe pumps, a multi-port selection valve and an auxiliary peristaltic pump. The flow manifold of the SI-HG-AFS system is shown in Fig. 1. All the externally used tubes were 0.8-mm id PTFE tubing, except for the reaction coil (RC), which was made from 1.8-mm id PTFE tubing.

All the operations were computer controlled, and the operating parameters of the AFS instrument are summarized in Table 1.

Reagents

All the reagents used were of the highest purity available, but at least of reagent grade; deionized water of 18 MΩ was used throughout.

Working standard solutions of lead were prepared daily by step-wise dilution of a 1000 mg l^{-1} stock solution with 1.0% (m/v) potassium ferricyanide in 1.0% (m/v) HCl. A 0.5% (m/v) $NaBH_4$ solution was prepared by dissolving $NaBH_4$ (Shanghai Chemicals Co., Shanghai, China) in 0.5% (m/v) NaOH (G. R., Beijing Beihua Fine Chemicals Co., Beijing, China). A potassium ferricyanide (A. R., Shenyang Chemicals Co., No. 1) solution was prepared by dissolving an appropriate amount in ion-free water, which was afterwards loaded through an iminodiacetate chelating resin column (Amberlite IRC-718, Sigma, or D401, Shanghai Huazhen Co., Shanghai, China) pretreated to pH 5–11. The solution passed through the column was then diluted to obtain a 1.0% (m/v) ferricyanide solution and its acidity was adjusted to 1.0% (m/v) HCl.

Other chemicals used were: nitric acid (G. R., Dandong Shengli Chemicals Co., Dandong, China), nitric acid (Suprapur, Tianjin Kemiou Chemicals, Tianjin, China), perchloric acid (G. R., Tianjin Dongfang Chemicals Co., Tianjin, China), hydrogen peroxide (G. R., Shanghai Yuanda Peroxides Co., Shanghai, China) and hydrochloric acid (G. R., Tianjin Yaohua Chemicals Co., Tianjin, China), which was redistilled before use.

Human whole blood samples were provided by the hospital of Northeastern University, China.

Table 1 Operating parameters of the AFS instrument

Parameters	Settings
Lead hollow cathode lamp	283.3 nm
Lamp current	60 mA
Atomizer height	9 mm
Atomization temperature	200 °C
Integration time	7 s
Time delay	1 s
Negative high voltage of photomultiplier	300 V
Carrier argon flow	500 ml min^{-1}
Shield argon flow	1000 ml min^{-1}

Sample pretreatment

In the case of measuring trace lead by AFS, sample pretreatment is most critical to ensure accuracy in a complex matrix of blood: thus wet digestion is mandatory in eliminating the matrix and converting lead into a state favorable to hydride formation. In addition, for clinical laboratories, the digestion should be as simple as possible, and preferably the operation will be completed within an hour or less. Thorough investigations have shown the following procedures are most suitable for this purpose.

Nitric acid–perchloric acid scheme 1

50 μl of blood stored by freezing in a low-density polyethylene bottle was taken into a quartz beaker, to which 1.0 ml of 65% nitric acid was added. The mixture was heated gently on a sand bath until fumes appeared (the temperature was controlled at 200 °C), and the solution had nearly dried (*ca.* 20 min). After cooling, 0.4 ml of perchloric acid was then added, and the contents were heated to near dryness again at 260 °C (*ca.* 30 min). 0.2 ml of ion-free water was afterwards added following cooling and the above heating procedure was followed once more (*ca.* 2–5 min), following which a snow-like residue was obtained in the bottom of the beaker. After cooling, the remainder was soaked with an appropriate amount of 1.0% potassium ferricyanide in 1.0% HCl, and finally diluted to 3.0 ml with this solution.

Nitric acid–perchloric acid scheme 2

1.0 ml of 65% nitric acid and 0.4 ml of perchloric acid were added successively into a quartz beaker containing 50 μl of blood. The contents were heated gently on a sand bath at 200 °C for *ca.* 10 min, after which the temperature was increased to 260 °C and the mixture further heated to near dryness (*ca.* 30 min). After cooling, 0.2 ml of ion-free water was added and exactly the same procedure as described in Scheme 1 was then followed.

Nitric acid–hydrogen peroxide protocol

As described in the nitric acid–perchloric acid scheme 1, after treatment with 1.0 ml nitric acid and heating the contents to near dryness, 1.0 ml of hydrogen peroxide was introduced and the mixture gently heated for *ca.* 30 min at 120 °C until virtual dryness. Following cooling, the remainder was soaked with an

appropriate amount of 1.0% potassium ferricyanide in 1.0% HCl, and finally diluted to 3.0 ml with this solution.

The same procedure was also applied to the reference materials, the reagent blanks and the spiked blood samples.

Operating procedures

Using the set-up shown in Fig. 1, before the very first operation syringe pump SP_1 was set to aspirate 4.0 ml of carrier solution (1.0% HCl), which was afterwards dispensed through port 3 to the confluence point, purged with an argon flow through the RC and GLS_1 and finally directed to waste by the peristaltic pump, thereby leaving the tubing between SP_1 and the meeting point, as well as the HC, filled with carrier solution. On operation, 2.0 ml of carrier and 1.7 ml of $NaBH_4$ solution were aspirated into the syringes of SP_1 and SP_2 at flow rates of 80 and 68 ml min^{-1}, respectively. Afterwards, SP_1 was set to aspirate 0.5 ml of sample solution into the HC through port 6 at a flow rate of 20 ml min^{-1}. Subsequently, SP_1 was set to dispense the stored carrier/sample solutions through port 3 to meet the $NaBH_4$ solution directed by SP_2 and the argon carrier gas; at the same time, the peristaltic pump APP was actuated. The confluencing stream flowed through the reaction coil and finally into the first-stage W-type gas–liquid separator (GLS_1) where the lead hydride and the hydrogen generated were separated from the mixture and the liquid was drained by the peristaltic pump at a flow rate of 80 ml min^{-1}. The separated gas was either carried by the argon flow into the atomizer through the second-stage membrane gas–liquid separator (GLS_2) or swept directly into the atomizer according to the experimental design, where the hydride was atomized by an argon–hydrogen flame. During the operation cycle, the previously stored 2.0 ml carrier solution carried out the wash of the reaction system.

Results and discussion

The effect of chemical variables

The level of blank signal is the most critical aspect in lowering the limit of detection. The experiments indicated that blank signal of the present reaction system was attributed mainly to the hydrochloric acid employed and potassium ferricyanide. In this work, re-distillation of hydrochloric acid and pretreatment of ferricyanide solution by loading it through an iminodiacetate chelating resin column eliminated the majority of the residual lead in the reagents, that is, the residual lead was reduced from *ca.* 3 μg l^{-1} to *ca.* 0.5 μg l^{-1}.

The effect of NaOH concentration in $NaBH_4$ solution was investigated and the results suggested that higher net fluorescence intensity could be obtained with a NaOH concentration less than 0.5% (m/v), while the intensity dropped steeply with further increase of its percentage. A 0.5% (m/v) NaOH solution was thus employed to prepare $NaBH_4$ solution.

The experimental results also revealed that the peak area was enhanced significantly with the increase of $NaBH_4$ concentration within the range of 0.3–0.5% (m/v); at higher concentrations, the curve leveled off, as shown in Fig. 2. It also indicated that further increasing the $NaBH_4$ concentration and the corresponding sampling volume enhanced the intensity, while at the same time the increased blank signal increased the limit of detection. As a compromise, a concentration of 0.5% was employed and a 1.7 ml sampling volume was selected.

The fluorescence intensity increased with potassium ferricyanide concentration and a maximum was reached at around 1.0% (m/v); a slight decline was observed by further increasing its concentration, and the blank signal was increased accordingly. A ferricyanide concentration of 1.0% (m/v) was used for further investigations.

The acidity of sample solution has remarkable influence on the formation of lead hydride. No signal was recorded with a HCl concentration less than 0.5% (m/v), while the intensity increased significantly within 0.5–2.0% HCl (m/v), and a decrease was observed when exceeding 2.5% HCl (m/v). In the ensuing investigations, a concentration of 1.0% (m/v) was used.

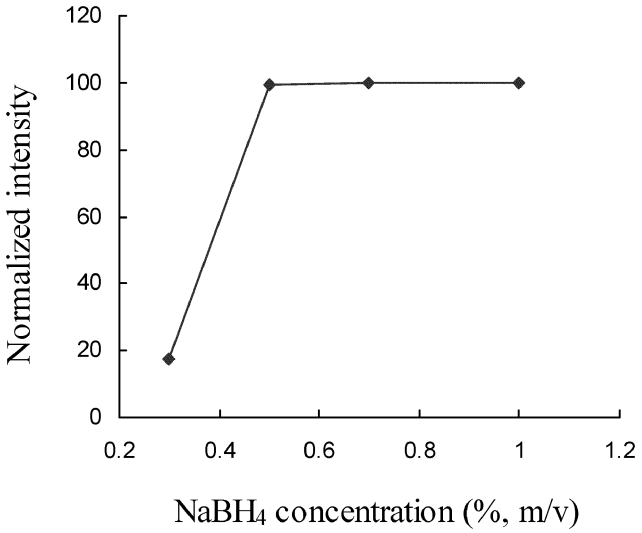

Fig. 2 The effect of $NaBH_4$ concentration. $NaBH_4$ in 0.5% NaOH (m/v); sample volume: 0.5 ml; $NaBH_4$ volume: 1.7 ml; $K_3Fe(CN)_6$ concentration: 1.0%; reaction coil length: 80 cm; sample loading time: 5 s; integration time: 7 s; carrier Ar flow: 500 ml min^{-1}; 2 μg l^{-1} (Pb).

The effect of sequential injection variables

The effect of the sample loading time or loading flow rate for a fixed volume of sample and $NaBH_4$ solution is shown in Fig. 3. The two solutions stored in the holding coil and the syringe SP_2 were propelled simultaneously and mixed after merging to entail the hydride formation process. Obviously, a longer loading time, *i.e.*, 8 s, corresponds to a lower loading flow rate of sample and reductant solutions, *i.e.*, 18.8 and 12.8 ml min^{-1}, respectively, resulting in an irregular peak shape with very low peak (integrated area) intensity. With the decrease in loading time or increase in the loading flow rate, the peak shape was substantially improved and the intensity was enhanced accordingly, *e.g.*, a loading time of 5 s corre-

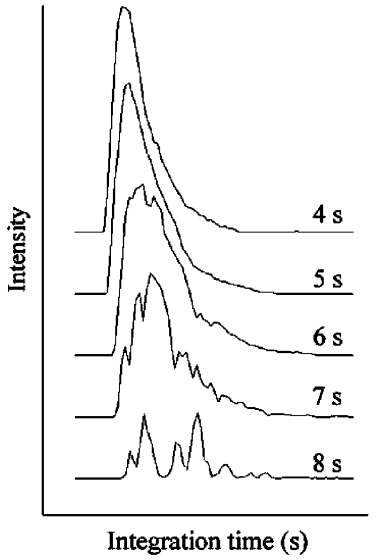

Fig. 3 The effect of loading time or loading flow rate. 0.5% $NaBH_4$ in 0.5% NaOH (m/v); sample volume: 0.5 ml; $NaBH_4$ volume: 1.7 ml; $K_3Fe(CN)_6$ concentration: 1.0%; reaction coil length: 80 cm; integration time: 7 s; carrier Ar flow: 500 ml min^{-1}; 2 μg l^{-1} (Pb).

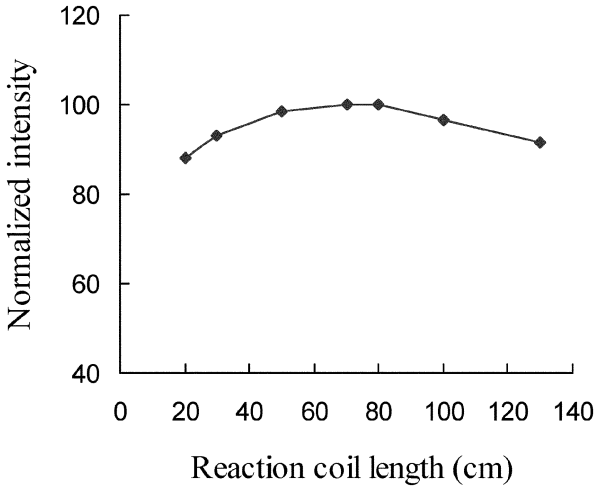

Fig. 4 The effect of reaction coil length. 0.5% $NaBH_4$ in 0.5% NaOH (m/v); sample volume: 0.5 ml; $NaBH_4$ volume: 1.7 ml; $K_3Fe(CN)_6$ concentration: 1.0%; sample loading time: 5 s; integration time: 7 s;. carrier Ar flow: 500 ml min^{-1}; 2 μg l^{-1} (Pb).

sponds to sample and reductant loading flow rates of 30 and 20.4 ml min^{-1}, giving rise to a much improved peak shape and intensity. Virtually no further improvement to the peak shape was observed by further increasing the flow rate, and a slight decrease of the recorded peak area was observed. Thus, a loading time of 5 s was employed for the ensuing study.

Tygon and PTFE tubing of different inner diameters were tested as materials for the reaction coil. It is shown that PTFE tubing of 1.8-mm id was preferential for the formation of lead hydride. Peak areas recorded with various lengths of the reaction coil are illustrated in Fig. 4. The fluorescence intensity increased with the length of the reaction coil, and a maximum was reached at a length of about 70–80 cm: with longer coil lengths the intensity dropped gradually up to 130 cm. For further studies, a coil length of 80 cm was employed.

In order to avoid potential carryover or memory effects, a washing step is essential. In this case, the previously stored 2.0 ml carrier solution in the holding coil was dispensed immediately after the sample loading process, which entailed the rinsing of the reaction system, and no detectable carryover was observed in this circumstance.

The modification of the AFS instrument

One of the most critical steps of the HG-AFS operation is the effective separation of hydride from the reaction medium, as it is mandatory that the water vapor entrained into the quartz atomizer should be kept at a minimum. Thus, the AFS instrument is designed such that the hydride is separated from the liquid phase by means of a standard gas–liquid phase separator (GLS_1), and then carried by an argon flow and swept through a second stage PTFE membrane phase separator (GLS_2) in order to remove the residual water vapor in the gas flow before entering the atomizer.

Table 2 The limit of detection and precision of the present procedure as compared with some of the reported data

Methods/samples analyzed	LOD/ μg l^{-1}	RSD (%) (μg l^{-1})[a]	Refs.
HG-AES			
Calcium carbonate	0.7	1.6 (20)[a]	6
Vegetable and sediments	1.0	1.2 (50)[a]	7
Quartz tube atomizer HG-AAS			
Hair	2.6	4.3	8
Whole blood, hair, *etc.*	1.0	3.1 (20)[a]	9
Urine	5.2		10
HG-ETAAS			
Calcium supplements	0.03	11	
Sediment	0.2–0.5	3 (2)[a]	12
Pine needle, sediment, *etc.*	0.7	2	13
HG-ICPMS			
Limestone and nervous tissue	0.002	<5.7 (1)[a]	14
Water, plant tissue, sediment	0.007	1.4 (4)[a]	15
Direct sampling ETAAS			
Water, plant tissue, sediment	15	6–14 (50)[a]	16
Whole blood	19		24
Filament atomizer ETAAS			
Whole blood	20	4.5 (40)[a]	25
ICP-MS			
Plasma and whole blood	0.015		19
Urine	0.09	3.7 (3.0)[a]	28
HG-AFS			
Whole blood	0.014[b]	0.7(2.0)[a]	This procedure

[a] Concentration level at which the RSD value was obtained. [b] Number of replicates $n = 11$.

The experimental results showed, however, that the lifetime of the PTFE membrane in GLS_2 was very limited in this case, that is, it lasts for only a few hours, which greatly hampers its practical applicability for routine analysis by deteriorating the precision and the peak shape, with progressive decline in the signal intensity. Extensive efforts were thus made in order to solve this problem, including introducing a vapor absorbing medium between GLS_1 and the atomizer, and employing a high draining flow rate from GLS_1. As the organic matrix in the blood had been completely digested, foam caused by the sample solution was not observed during the hydride generation process; thus, the water vapor carried into the argon flow was controlled within a very low level, which made it possible to remove GLS_2 and introduce the hydride out of GLS_1 directly into the atomizer. Further investigations demonstrated that virtually no harmful effects were observed when sweeping the hydride directly into the atomizer by employing a sufficiently high waste outflow rate, *i.e.*, *ca.* 80 ml min^{-1}. As a result, both the peak shape and the precision were significantly improved, *i.e.*, the RSD values obtained with and without the GLS_2 were 2.3% and 0.7%, respectively. In this particular case, GLS_2 proved to be unnecessary for further studies.

It should be noted that too high a waste outflow rate, *i.e.*, *ca.* 120 ml min^{-1}, caused *ca.* a 10% decrease of the integrated

Table 3 The determination of lead content in certified reference materials of frozen cattle blood and human whole blood

		Found/μg l^{-1}				
		HNO_3–$HClO_4$				
Materials	Certified/μg l^{-1}	Scheme 1	Scheme 2	HNO_3–H_2O_2	Spiked (final sol.)/μg l^{-1}	Recovery (%)
GBW 09139	110 ± 20	105 ± 7	117 ± 10	105 ± 6		
GBW 09140	335 ± 30	340 ± 22	347 ± 17	305 ± 5		
Blood 1		45 ± 12			5	100
Blood 2		43 ± 22			5	109

fluorescence intensity, which might be attributed to the partial loss of the hydride along with the waste.

The AFS parameters

The experiments showed that the variations of the diverse parameters associated with the AFS instrument, *i.e.*, the lamp current, atomizer height, atomization temperature, negative high voltage of photomultiplier, carrier argon flow and shielding argon flow, all have a significant effect on the fluorescence intensity and peak shape. Thorough investigations with the aim of optimizing these parameters with univariant optimization, in terms of sensitivity, precision and peak shape, were thus made. The optimal values of these parameters are summarized in Table 1.

The effect of foreign ions

It has been well documented that the nascent transition metals and their borides formed in the reaction medium can severely suppress the formation and release of the hydrides. Fortunately, the reduction rates of these interfering metals are relatively slower than most of the hydride forming metals,[26,27] which made it feasible to eliminate the interfering effects *via* on-line operation, taking advantage of the very short residence time of the hydrides in the reaction medium, and thus minimizing their contact with the nascent transition metals or their borides by immediate separation of the formed hydrides from the reaction medium in a flow injection/sequential injection system. The potential interfering effects of 17 metals at various concentration levels were thus investigated.

The results showed that 10 mg l^{-1} Fe^{3+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, As(III), Cr(VI), Sr^{2+}, Zr(IV), Mn^{2+}, 5 mg l^{-1} Sn^{2+}, Sb(III), Se(IV) and 0.5 mg l^{-1} Cu^{2+}, Bi(III), Ag^{+} did not interfere with the determination of 5 μg l^{-1} of Pb within the error range of ±5%.

The contents of the aforementioned metals in the digests of blood samples are well within the tolerant concentration range by using the present procedure and the sample digestion protocols. Therefore, the blood digests could be analyzed directly and no masking agents or further treatments were necessary.

The performance of the procedure

The limit of detection (LOD) and relative standard deviation (RSD) of the procedure, as well as their comparisons with those of the existing spectrometric procedures for lead, are summarized in Table 2. The LOD of 0.014 μg l^{-1}, defined as 3 times of the blank standard deviation divided by the slope of calibration graph, is not only considerably superior to those obtained by HG-AES, quartz tube atomizer HG-AAS, direct sampling ETAAS and tungsten filament atomizer ETAAS, but also lower than those by in-atomizer-trapping HG-ETAAS and comparable to the ICP-MS-based methods. In addition, a RSD value of 0.7% at the 2.0 μg l^{-1} level was obtained, which was a substantial improvement as compared with those by the protocols in Table 2. A sampling frequency of 120 h^{-1} was derived when running this procedure. As regards the number of samples that could be completely processed in a working day, it depends entirely on the proficiency of the operator as well as the digesting facility. In a worst-case situation, by using a conventional sand bath and small quartz beaker, it is feasible to handle a few hundreds of samples by a skilled worker.

Validation and applications of the procedure

The proposed procedure was applied to the determination of lead in two certified reference materials of frozen cattle blood, *i.e.*, GBW 09139 (low level) and GBW 09140 (high level), and two human whole blood samples. The sample digestion procedures were described in the "Sample pretreatment" section. The results obtained by using nitric acid-perchloric acid schemes (1 and 2) and nitric acid–hydrogen peroxide protocol are summarized in Table 3. The values found for both reference materials by using the three digestion schemes agreed well with the certified values, while the recoveries obtained for spiked whole blood samples were also quite acceptable. The reagent blanks of these schemes were all well controlled within a fluorescence intensity range corresponding to 0.6–1.0 μg l^{-1} Pb, which ensured high sensitivity of the procedure.

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