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Determination of Arsenic and Bismuth in Biological Materials by Total Reflection X-ray Fluorescence After Separation and Collection of Their Hydrides

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A combined procedure was developed for the determination of As and Bi in biological materials by total reflection X-ray fluorescence (TXRF). The materials were first digested by an open wet decomposition method. The separation of both elements from the sample matrix was then achieved by generation of their volatile hydrides and subsequent trapping in a collection solution. The quantitative determination of As and Bi was performed simultaneously by TXRF using Y as internal standard. The procedure was applied to several different biological certified reference materials. The recovery rate determined for mass fractions between 40 ng g^{-1} and 10 µg g^{-1} was 90–100% after correction with aqueous standard solutions. The RSD for the whole procedure ranged from 8 to 14% and the detection limits were about 10 ng g^{-1} for As and 20 ng g^{-1} for Bi.

Keywords: *Arsenic; bismuth; hydride generation; total reflection X-ray fluorescence; biological materials*

Sample solutions to be prepared for analysis by total reflection X-ray fluorescence (TXRF) have to fulfil certain conditions in order to make use of the high sensitivity of this technique. The solutions after sample decomposition should contain few matrix residues and the sample solvent (*e.g.*, water, nitric acid) should have high volatility. About 10 µl of the final solution have to be pipetted onto clean quartz glass carriers and dried by evaporation. A dry residue of only a few micrograms or less deposited as a thin layer several micrometres thick and several millimetres in diameter has to be applied for TXRF measurements. The detection limits of the method can deteriorate by up to three orders of magnitude if the matrix content is too high.[1] However, such interference can be reduced by using suitable techniques of analyte/matrix separation. Apart from extraction or chromatographic separation, which often only reduces the problem, volatilization of the analyte and its collection in a suitable solvent can be a successful approach.

In this paper, a hydride separation/collection technique and its adaptation to TXRF is described. It is well known that a number of elements (*e.g.*, As, Se, Sb, Bi) can be reduced to their corresponding hydrides. These can be transferred *via* a carrier gas stream (nitrogen, argon) into a heated quartz tube, positioned in an atomic absorption spectrometer. Another possibility is the coupling of a hydride generation system to an inductively coupled plasma spectrometer. These techniques have been well known for many years[2–5] and used successfully in routine analysis.[6,7] A combination of hydride generation, collection of the volatile hydrides in a suitable solvent and multi-element determination by TXRF was first proposed by Haffer *et al.*,[8] but the method was only applied to aqueous standard solutions. A similar technique is described in this paper and was applied to biological certified reference materials.

EXPERIMENTAL

Accessories

Equipment

For sample decomposition an open digestion device was used, consisting of an aluminium heating block with drill-holes for the insertion of 10 ml quartz glass tubes. The separation and collection of the element hydrides was carried out in a laboratory-built hydride generation and collection system (Fig. 1), consisting of a peristaltic pump (LKB 2132, Pharmacia, Freiburg, Germany), a 10 ml quartz glass tube, a system for the supply of $NaBH_4$ solution and carrier gas (N_2), glass capillary tubes (Blaubrand intra end 2 µl and intra mark 50 µl; Brand, Wertheim, Germany), an angular tube, a collection vessel (1.5 ml Eppendorf-type vessel) and a heating device (Reacti-Therm 18800, Pierce, Rockford, IL, USA).

An EXTRA II TXRF spectrometer, equipped with Mo and W tubes for X-ray excitation (Rich. Seifert & Co., Ahrensburg, Germany) and a QX 2000 Si(Li) detector and analyzer (Link; Oxford Instruments, High Wycombe, Buckinghamshire, UK) including a software package were used to record and process the spectra.

Reagents

All reagents used were of analytical-reagent or high-purity grade (Merck, Darmstadt, Germany). Water was de-ionized by a Milli-Q water purification system (Millipore, Bedford, MA, USA). High-purity grade nitric acid was prepared by

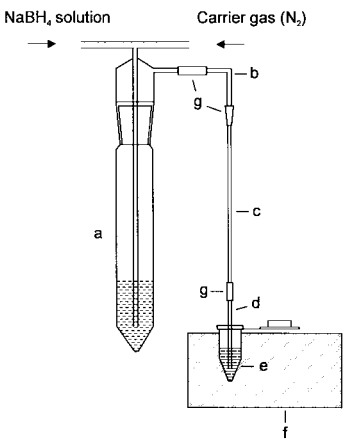

Fig. 1 Hydride generation and collection system: (a) 10 ml quartz glass tube (with digested sample material) and introduction system for $NaBH_4$ solution and carrier gas, (b) angular tube, (c) glass capillary (Blaubrand intra mark, 50 µl), (d) glass capillary (Blaubrand intra end, 2 µl), (e) collection vessel (1.5 ml Eppendorf-type vessel), (f) heating device (105–110 °C) and (g) silicone rubber tubing.

sub-boiling distillation (H. Kürner, Rosenheim, Germany) of analytical-reagent grade acid, and then stored in quartz glass bottles.

For the hydride generation, a $NaBH_4$ solution was prepared by dissolving 3 g of $NaBH_4$ (Merck) in 100 ml of 0.01 M NaOH. This solution was prepared just before use and was stored in a polyethylene bottle.

Stock standard solutions (1000 mg l^{-1}) of As, Se, Sb and Bi were prepared from Merck Titrisol solutions; a stock standard solution of Y (1000 mg l^{-1}) was purchased from Aldrich (Milwaukee, WI, USA). Working standard solutions of each element were prepared daily from the stock solutions.

Samples

First, the procedure (hydride generation, collection and TXRF measurement) was tested using aqueous standard solutions prepared by dilution of stock standard solutions. Subsequently, standard or certified reference materials (SRMs or CRMs) were subjected to the whole procedure including their decomposition by an open digestion method. The following materials were chosen: Bovine Liver (NIST SRM 1577a), Orchard Leaves (NIST SRM 1571), Tomato Leaves (NIST SRM 1573), Plankton (BCR CRM 414) and Tea Leaves (NIES No. 7). All the materials were supplied by Promochem (Wesel, Germany).

Procedure

The whole procedure consists of the following steps: (i) open wet decomposition of the samples, (ii) generation of the hydrides AsH_3, SeH_2, SbH_3 and BiH_3 and their collection in a small volume of a collection solution and (iii) measurement by TXRF.

Open wet decomposition

Portions of 80–100 mg of a biological sample material were weighed in 10 ml quartz glass tubes. To soak the dry samples, 500 µl of de-ionized H_2O were added to each tube. After a few minutes, 500 µl of HNO_3 (65%) and 40 µl $HClO_4$ (70%) were pipetted into the tubes. In a first step, the tubes were heated in the aluminium heating block from room temperature to 140 °C within 60–70 min. The tubes were then allowed to cool outside the block and 500 µl of HNO_3 together with 100 µl of $HClO_4$ were added. In a second step, the tubes were again placed in the heating block and the temperature was increased from 140 to 180 °C within 60–70 min. If the digested sample solutions exhibited a dark colour (undigested material), 400 µl of HNO_3 were added and the temperature was maintained at 180 °C until the solutions became nearly colourless. In a third step, the temperature was increased from 180 to 210–215 °C in 10 min. This temperature was maintained for a further 10 min taking care that the solutions did not evaporate to dryness.

During the described procedure the excess of nitric acid evaporated totally. The colourless residues were dissolved in the small volumes of $HClO_4$ that remained but did not interfere with the following hydride generation process. The temperature and time schedules given above had to be followed exactly to ensure complete mineralization without losses of As and Bi.

Finally, the quartz glass tubes were removed from the aluminium heating block and cooled. The digestion residues were diluted with 400 µl of 5 M HCl after which 1.6 ml of H_2O were added.

Generation and collection of volatile hydrides of As *and* Bi *(* Se *and* Sb*)*

A mixture of 250 µl of HNO_3 (65%), 250 µl of HCl (37%) and 20 µl of Y standard solution (4 ng Y absolute in HNO_3, 65%) was placed in a collection vessel. The vessel was placed in the heating device of the hydride generation/collection apparatus shown in Fig. 1 and pre-heated for 30 s (block temperature 105–110 °C). The quartz glass tube with the digested sample solution was connected to the collection vessel by capillaries. By means of a peristaltic pump, the $NaBH_4$ solution was pumped into the quartz glass tube (60 ml h^{-1}, 45 s). The volatile hydrides were transported to the collection vessel by a stream of nitrogen which acted as a carrier gas (20 ml min^{-1}). The nitrogen was allowed to flow through the apparatus for a further 90 s in order to complete the hydride collection. The collection vessel was then removed from the heating device and closed.

TXRF measurements and quantitative determinations

Aliquots of 30, 50 or 100 µl of the collection solution were dried on quartz glass carriers by means of IR radiation at a temperature of about 80 °C. TXRF spectra were recorded in a preset live time of 300–1000 s depending on the analyte concentration in the sample. Yttrium was used as an internal standard element for quantification. The mass of the analyte element present in the collection solution was determined from the following equation:

$$m_x = \frac{N_x/S_x}{N_Y/S_Y} \cdot m_Y \qquad (1)$$

where m indicates the mass, N the measured net intensity and S the relative sensitivity of either the analyte, x, or the internal standard, Y.[9] The sensitivity values were known from previous investigations on aqueous standard solutions;[10] the mass m_Y was chosen to be 4 ng. The mass fraction c_x of the analyte in the chosen sample material was then determined by the quotient of m_x and the weighed portion of the sample material (80–100 mg). A raw recovery rate was then calculated from the ratio of the value of c_x to the certified value.

The recoveries of As and Bi were high but mostly $<90\%$. To correct for the incompleteness of hydride generation and collection, aqueous standard samples of As and Bi in 1 M HCl were prepared by addition of these elements and measured in parallel with the real samples (the whole procedure except decomposition). The addition of As and Bi was made so as to give concentrations in the aqueous standard samples comparable to the mass fractions of these elements in the real samples. The recoveries of As and Bi in the aqueous standard samples were finally used as scaling factors in order to correct the mass fraction c_x and the raw recovery rate for the real samples by normalization.

RESULTS AND DISCUSSION

Optimization of Hydride Generation and Collection

In view of the simultaneous multi-element capability of TXRF, an attempt was made to separate and collect as many elements as possible *via* their hydrides. The efficiency of an open wet decomposition method had already been tested in earlier experiments with tea leaves.[11] Therefore, this type of digestion was adapted to the above-mentioned biological materials with only small changes. It was necessary to optimize the conditions for hydride generation of the elements and for collection of the hydrides in a collection solution. The usual procedure of drying the solutions on TXRF quartz glass carriers and the standard settings for TXRF measurements could then be applied.

The optimization was first performed with aqueous standard solutions of the elements in question (10–100 ng ml^{-1} As, Se and Sb in 1 M HCl) without any digestion step. Only those reagents that could be dried on TXRF quartz glass carriers without leaving any interfering residues were used as collection

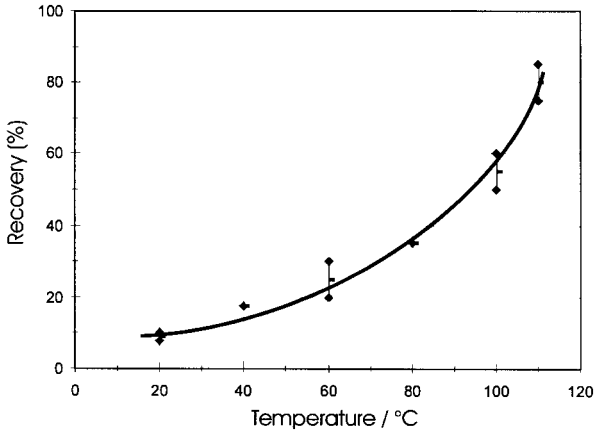

Fig. 2 Influence of the temperature of the collection solution [HNO_3 (65%)–HCl (37%)] on the recovery of As.

solutions. The Se hydride was quantitatively collected in 0.1 M ammonia solution and 0.01 M NaOH. This reaction was controlled by the determination of Se (electrothermal atomic absorption spectrometry). However, drying the collection solution on a quartz glass carrier hampered the quantitative and reproducible recovery of the volatile Se by TXRF measurements.

Pd and Hg salts were added to the collection solutions as chemical modifiers in order to reduce the volatility of Se. Such additions, however, resulted in a new interfering matrix which reduced the sensitivity of TXRF determinations.

An increased recovery rate was achieved by using strong oxidizing agents such as a mixture of HNO_3 and H_2O_2 or HNO_3 and HCl. The latter acidic mixture used as a collection solution provided high recovery rates combined with high precision and accuracy. Fig. 2 demonstrates the influence of the temperature of this solution on the recovery of As. The highest value was found for a temperature of 110 °C in the heating block. The temperature could not be increased further because of the instability of the polypropylene collection vessels used. At 110 °C, the recovery rate for As was about 80%, for Sb about 90%, but for Se only about 10%.

The velocity at which the hydrides are transported to the collection solution is just as important as the temperature. A slow transport to the HNO_3–HCl mixture increased the recovery rates of the elements. Capillaries with a small inner diameter produced small gas bubbles of the hydrides which were obviously collected in a more effective way. Furthermore, the volume of the collection solution had a significant influence on the collection efficiency. A minimum volume of 400–500 µl was necessary and the greatest efficiency was achieved with a mixing ratio of 250 µl of HNO_3 (65%) to 250 µl of HCl (37%).

Yttrium was added to the collection solution as an internal standard before starting the hydride generation procedure so that evaporation of the collection solution or even possible spraying would not affect the accuracy. The optimum efficiency was obtained with the conditions indicated under Experimental. As listed in Table 1, the described procedure led to high recovery rates and low relative standard deviations (RSDs) for As and Sb, but not for Se.

Table 1 Recovery rates and relative standard deviations (RSDs) for the determination of As, Se and Sb in aqueous standard solutions of 1 M HCl

Standard solution	Element	Absolute mass/ ng	n	Recovery (%)	RSD (%)
1 M HCl	As	10	11	75	11
1 M HCl	Se	10	12	6	64
1 M HCl	Sb	10	12	91	9

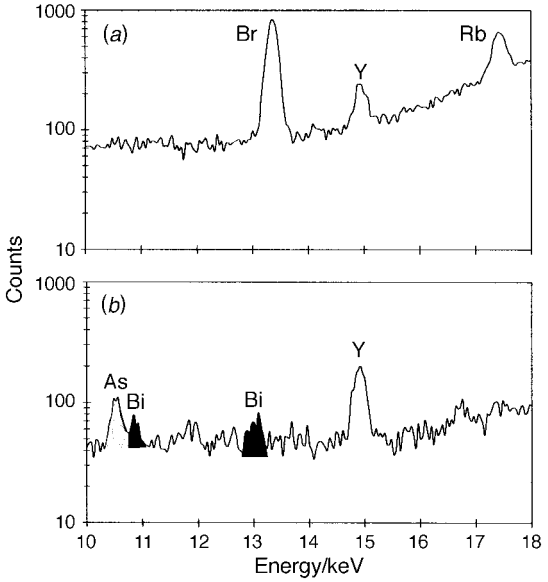

Fig. 3 Determination of As (47 ng g^{-1}) and Bi (50 ng g^{-1} spiked) in Bovine Liver (SRM 1577a). (*a*) TXRF spectrum for direct determination in the digested solution; (*b*) TXRF spectrum for determination after hydride separation and collection. The preset live time for both spectra was 300 s.

Application to Biological Materials

Reference materials (RMs) were used in order to test the separation/collection technique for biological samples. In addition to As, Se and Sb, the element Bi was included in the investigations. First, Bovine Liver (NIST SRM 1577a) was chosen in order to demonstrate the advantages of the proposed sample pre-treatment. As (47 ng g^{-1}) and Bi (50 ng g^{-1} spiked) were determined by TXRF (*a*) directly in the diluted decomposition solution and (*b*) after the described hydride generation/collection procedure. The differences between the correspond-

Table 2 Raw recovery rates for the determination of As, Se, Sb and Bi in different biological RMs

Sample material	Element	Mass fraction/ µg g^{-1}	Raw recovery (%)
NIST SRM 1577a			
Bovine Liver	As	0.04–0.6	60–83
	Se	0.7–2.0	<10
	Sb	0.1–0.6	20–43
	Bi	0.05	70
NIST SRM 1571			
Orchard Leaves	As	10	>95
	Se	0.1–1.0	<10
	Sb	1.0	30–40
NIST SRM 1573			
Tomato Leaves	As	0.27	60
BCR CRM 414			
Plankton	As	6.82	60
	Se	1.75	<10
	Sb	1.0	30–40
NIES No. 7			
Tea Leaves	As	0.04–0.6	80
	Se	0.1–1.0	<5
	Sb	0.1–0.6	30
	Bi	0.05–0.7	70

Table 3 Results of the determination of As and Bi in different RMs

Sample material	Element	Certified mass fraction/ μg g^{-1}	n	Mean found/ μg g^{-1}	s/ μg g^{-1}	RSD (%)	Corrected recovery (%)
NIST SRM 1577a							
Bovine Liver	As	0.047	7	0.046	0.004	9.3	98
	Bi	0.05*	4	0.044	0.005	11	88
	Bi	0.06*	2	0.053			88
NIST SRM 1571							
Orchard Leaves	As	10	8	9.8	0.9	9.4	98
NIST SRM 1573							
Tomato Leaves	As	0.27	9	0.26	0.02	7.7	96
BCR CRM 414							
Plankton	As	6.82	4	6.35	0.91	14	93
NIES No. 7							
Tea Leaves	As	—	6	0.042	0.004	8.8	
	Bi	—	6	0.26	0.024	9.2	

* Element, spiked.

ing two spectra are shown in Fig. 3. No signals of As and Bi can be observed in the spectrum recorded directly [Fig. 3(*a*)], but both peaks are present in the spectrum recorded following the described procedure [Fig. 3(*b*)]. The given amount of about 5 ng of As and Bi in a sample mass of 100 mg may be estimated to be 3–4 times the detection limit (3s) of these elements.

More specific results were obtained for As, Se, Sb and Bi in the different RMs. Increasing amounts of these elements were pipetted onto the samples in the form of aqueous standard solutions. Amounts of 4–1000 ng of each element were added depending on the concentration of the elements in the RMs. The samples were then digested and the elements were measured by TXRF following the described hydride generation/collection procedure. Raw recovery rates were calculated for the range of mass fractions given in Table 2.

High recovery rates were found for As (60–95%) and Bi (70%). In contrast to the recoveries found for aqueous standard solutions in 1 M HCl, unsatisfactory results were obtained for Sb in the RMs (only 20–40%). This was probably caused by the hampered hydride generation in the digested matrix solution and by the incomplete collection of the hydrides in the collection solution. The low recovery of Se (< 10%) also found for aqueous solutions can be explained by the high volatility of this element.

Because of their high recoveries, only As and Bi were included in the following investigations. Individual results found for the different RMs are listed in Table 3. Since these materials are not certified for Bi, samples were spiked with this element. Both elements were determined in a wide range of mass fractions (ng g^{-1} to μg g^{-1}) with detection limits of 10 ng g^{-1} for As and 20 ng g^{-1} for Bi. The RSD for repeated measurements (n = 4–9) is about 10%. The mean values for the mass fractions of As and Bi were found by normalisation to the recoveries of aqueous standard solutions as described above (see under Procedure). By this means, corrected recoveries of 88–98% were found for the different RMs.

CONCLUSION

The developed method allows the simultaneous determination of As and Bi in biological materials with significantly improved detection limits. Following the analyte/matrix separation described in this paper, both elements can be determined by TXRF down to the low ng g^{-1} level, which may be important for biological investigations. Unfortunately other hydride-forming elements such as Se and Sb could not be determined by this method. This was due to the incomplete separation and trapping of the relevant hydrides under the described experimental conditions.

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