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RADIOCHEMICAL SEPARATION METHODS FOR THE DETERMINATION OF SOME TOXIC ELEMENTS IN BIOLOGICAL REFERENCE MATERIALS

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The present paper describes radiochemical separation procedures developed for the determination of the elements As, Cr, Hg, Sb and Se in biological reference materials. The methods chosen utilize ion exchange, retention on inorganic exchangers and solvent extraction techniques for the isolation of the elements of interest. These procedures are more rapid than previously used methods, such as distillation and precipitation. As to the sample dissolution, the following procedures were tested using radioactive tracers and reference materials: treatment of the materials in teflon bombs with a mixture of HNO_3 + H_2SO_4 or HNO_3 only in a normal oven, and with HNO_3 in a microwave oven using specially designed digestion bombs.

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INTRODUCTION

Speed of analysis is becoming more important for neutron activation analysis (NAA) as new and competitive trace analytical methods are being introduced, such as ICP-MS. NAA presents excellent characteristics as regards sensitivity, precision and accuracy, in addition to being an almost contamination-free analytical technique. However, some post-irradiation treatment of the samples, namely radiochemical separations to isolate one element or a group of elements, may convert the analysis into a fairly lengthy process. In the case of determination of elements such as As, Cr, Hg, Sb and Se, present at very low concentrations in biological materials, such as foodstuffs, radiochemical separation procedures have to be included in the analysis to achieve suitable sensitivity, accuracy and precision.

The aim of this work was to develop faster and more reliable radiochemical methods than those previously utilized, such as distillation and precipitation used for the determination of Hg and Se[1]. For sample dissolution, teflon bombs were employed, using conventional and microwave ovens. The main elements studied in this work were As, Cr, Hg, Sb and Se and the separation techniques used were ion-exchange, retention on inorganic exchangers and solvent extraction. Several authors have utilized similar techniques for the analysis of biological materials with good results[2-5]. Table 1 presents some nuclear data of the isotopes used in this study.

The procedures developed were applied to the analysis of the following reference materials: Bowen's Kale, Citrus Leaves (NBS-SRM-1572), Mixed Diet (NBS-RM-8431a), Oyster Tissue (NBS-SRM-1566), Rice Flour

TABLE 1

Nuclear data for the isotopes used in this study

Target isotope	Radioactive isotope	Half-life	Gamma-ray used calculation, keV
^{75}As	^{76}As	26.3 h	559
^{50}Cr	^{51}Cr	27.7 d	320
^{202}Hg	^{203}Hg	46.6 d	279
^{121}Sb	^{122}Sb	2.7 d	564
^{123}Sb	^{124}Sb	60.2 d	603
^{74}Se	^{75}Se	120 d	265

(NBS-SRM-1568), Tomato Leaves (NBS-SRM-1573), Copepod (IAEA-MA-A-1), Fish Flesh (IAEA-MA-A-2), Hay Powder (IAEA-V-10), Mixed Human Diet (IAEA-H-9), Mussel Tissue (IAEA-MA-M-2) and Human Hair (NIES-CRM-5).

EXPERIMENTAL

1. Determination of As, Hg, Sb, and Se using anionic resin (DOWEK 2X8) and inorganic exchanger (TDO, tin dioxide)

Preparation of the resin and inorganic exchanger columns

The inorganic exchanger used in these experiments was tin dioxide (TDO), obtained from Carlo Erba, Italy. The TDO was washed several times with distilled water and the fine particles eliminated by decanting. The TDO was then transferred to Carlo Erba polyethylene columns (5 cm height x 0.7 cm diameter). Each column was conditioned with 15 ml of 3M HCl before using it for the actual separation.

The anionic resin, DOWEX 2X8 (200 - 400 mesh), was also washed several times with distilled water. It was then activated by pouring on it the following solutions successively: NaOH 0.8N, distilled water, 2M HCl and distilled water. About 5 ml of the resin was utilized for each experiment. While on the column and before carrying out the separation, the resin was conditioned with 20 ml of 6M HCl.

Irradiation of reference materials and standards

About 200 to 300 mg of the reference materials Bowen's Kale, Citrus Leaves and Rice Flour were weighed in clean quartz ampoules. A multielemental standard containing the elements As, Hg, Sb, and Se was prepared by mixing appropriate aliquots of solutions of these elements made from spectroscopically pure reagents. Aliquots of the multielemental solution, containing about 2 μg of As and Sb, and 1 μg of Hg and Se, were pipetted into quartz ampoules.

The samples together with the standards were irradiated in the IEA-R1 research reactor, for 8 h, at a thermal neutron flux density of 10^{13} $n.cm^{-2}.s^{-1}$. After irradiation, the reference materials and the standards were processed in the same way.

Radiochemical separation for the determination of As, Hg, Sb, and Se

After two days of decay, the quartz ampoules were washed with diluted HNO_3 and H_2O and cooled in liquid N_2 before opening them. The samples were then transferred to teflon bombs and the following carriers were added: Hg (743 μg), As (532 μg), Se (450 μg) and Sb (110 μg). After addition of 3 ml concentrated HNO_3 and

1 ml concentrated H_2SO_4, the bombs were placed in a conventional oven at 130 °C, from 1 to 4 h, depending on the reference material analyzed.

The dissolved samples were cooled and 3 ml of a 10% urea solution, 5 ml HCl and 0.2 g $NaBrO_3$ were added, as well as some drops of H_2O_2 (120 vol.). The solutions were slowly heated and a clear yellow colour was obtained. After cooling, the solutions were passed through a DOWEX 2x8 anionic resin column, at a flow rate of 1 ml min^{-1} to retain Hg and Sb on the column. The column was washed with 20 ml 6M HCl and transferred to counting vials.

Equal volumes of H_2O were added to the effluent of the resin, and this solution was passed through a TDO column, at a flow rate of 0.5 ml min^{-1}. The column was washed with 15 ml 3M HCl and the resin transferred to a counting vial for measurement with a HP-Ge detector, ORTEC, coupled to a 4096 multichannel analyzer.

Results

The yields obtained for the elements As, Hg, Sb, and Se, for the complete radiochemical procedure, as calculated from the tracer experiments, were 99±5%, 96±5%, 100±13%, 85±11%, respectively.

Table 2 presents the results obtained for the analysis of these elements in the reference materials Bowen's Kale, Rice Flour and Citrus Leaves, as well as the literature values.

2. Determination of Se by retention on the inorganic exchanger TDO (tin dioxide) and of Hg by extraction with Ni(DDC)$_2$

These experiments were carried out at the IAEA's Laboratories at Seibersdorf. The radiochemical proce-

TABLE 2

Results of the determination of As, Hg, Sb and Se in biological reference materials using TDO inorganic exchanger and DOWEX 2X8 anionic resin

Reference material	Concentration, this work, ppm	Relative s.d., %	Relative error, %	Literature values (Ref. 15)
Bowen's Kale	As: 0.128±0.014	10.9	2.3	0.131±0.045
	Hg: 0.136±0.022	16.2	20.5	0.171±0.027
	Sb: 0.068±0.006	8.8	0.7	0.0685±0.014
	Se: 0.123±0.020	16.3	8.1	0.134±0.020
Rice Flour NBS-SRM-1568	As: 0.49±0.07	14.3	19.5	0.41±0.05
	Hg: 0.0079±0.0011	13.9	31.7	0.0060±0.0007
	Sb: 0.0071±0.0030	42.2	6.6	0.0076
	Se: 0.35±0.01	2.9	12.5	0.40±0.10
Citrus Leaves NBS-SRM-1572	As: 3.17±0.54	17.0	2.3	3.10±0.30
	Hg: 0.084±0.004	4.8	5.0	0.08±0.02
	Sb: 0.036±0.006	16.7	10.0	0.04

dure was first tested using tracers of ^{203}Hg and ^{75}Se. These tracers, together with carriers, were added to 200 mg of non-radioactive IAEA mixed human diet H-9 reference material, to have a biological matrix for testing the different dissolution procedures.

Preparation of the column of TDO and of the solution of $Ni(DDC)_2$

The TDO was washed several times with distilled water and the fine particles eliminated by decanting. It was then left overnight in 1M HNO_3 medium. After elimination of the fine particles once more, the exchanger was poured into a Carlo Erba plastic column (5 cm height x 0.7 cm diameter) and 1M HNO_3 was passed through it for conditioning purposes.

The $Ni(DDC)_2$ was prepared according to the procedure described by Gallorini et al.[6].

Irradiation and dissolution of the reference materials

About 200 mg of the biological reference materials of interest were irradiated in quartz ampoules for 6-12 h at a thermal neutron flux density of $8x10^{13}$ $n.cm^{-2}.s^{-1}$, in the Seibersdorf ASTRA nuclear reactor. The samples were allowed to decay for an adequate period of time. The quartz ampoule, containing the irradiated material, was soaked for 15 min in a mixture of 1 part of 50% HF to 1 part of concentrated HNO_3, cooled in liquid N_2 for at least 5 min and opened. The irradiated material was transferred to the dissolution vessel and 100 μg of Hg and Se as carriers were added, as well as 3 ml of concentrated HNO_3.

Two kinds of dissolution procedures were tested: one employing a normal oven and another using a microwave oven. In the first approach, Parr acid digestion bombs containing the reference material, carriers and concentrated HNO_3 were placed in a conventional laboratory oven for 2 h at 140 ^oC and 10 h at 110 ^oC. For the second procedure, Parr microwave digestion bombs were utilized. These bombs, containing the sample and 4 ml of concentrated HNO_3 were placed in a microwave oven for 3 min at 450 W. Both procedures lead, in most cases, to the production of clear solutions of the biological materials.

Radiochemical separation

After cooling of the bombs, about 10 drops of H_2O_2 (120 vol), were added and the solution was made 1M in HNO_3 by adding doubly distilled water. The solution was then passed through the TDO column at a flow rate of about 1 ml min^{-1}. The column was washed twice with 15 ml of 1M HNO_3, allowed to dry, and the TDO transferred to a plastic counting vial for the measurement of ^{75}Se on an ORTEC HP-Ge detector coupled to a CANBERRA multichannel analyzer.

The effluent of the column was adjusted to pH 1 with concentrated NH_4OH and Hg was extracted with 20 ml of nickel diethyldithiocarbamate ($Ni(DDC)_2$) 0.005M in chloroform. A shaking machine was used for the extraction of Hg, the shaking time being 15 min at 225 rpm. The aqueous phase was discarded and the organic phase was measured for ^{203}Hg.

Results

The calculated recoveries of Se and Hg in the TDO column and in the organic phase after extraction with $Ni(DDC)_2$ were $85.2\pm0.8\%$ and $92.0\pm1.1\%$, respectively.

In Table 3 are shown the concentrations obtained for Hg and Se in the biological reference materials analyzed.

3. Determination of Cr by retention on hydrated manganese dioxide (HMD) inorganic exchanger

Preparation of the HMD column

The HMD was washed several times with distilled water and the fine particles eliminated by decanting. It was then left overnight in 1M HNO_3 medium. After elimination of the fine particles once more, the exchanger was poured into a Carlo Erba plastic column (5 cm height x 0.7 cm diameter) and 1M HNO_3 was passed through it for conditioning purposes. The flow rate was adjusted to 0.5 ml min^{-1}.

Irradiation and dissolution of the reference materials

About 200 mg of the biological reference materials were irradiated in quartz ampoules in the ASTRA nuclear reactor at Seibersdorf, in a rotating facility, at a flux density of $8x10^{13}$ $n.cm^{-2}.s^{-1}$ for a 24 h period. After an appropriate decay period, the quartz ampoules containing the irradiated material were soaked for 15 min in a mixture of 1 part of 50% HF to 1 part of concentrated HNO_3, cooled in liquid N_2 for at least 5 min, and opened. The irradiated biological materials was transferred to a Parr acid digestion bomb along with 20 µg of Cr carrier in the form of $CrCl_3.6H_2O$. Two types of dissolution procedures were tested employing either a normal oven or a microwave oven with a suitable digestion bomb in each case.

TABLE 3

Results of the determination of Hg and Se in biological reference materials using TDO inorganic exchanger and $Ni(DDC)_2$ solvent extraction

Reference material	Concentration, this work, ppm	Relative s.d., %	Relative error, %	Literature values (Ref. 15)
Hay Powder IAEA-V-10	Hg: 0.015±0.001	6.7	15.4	0.013±0.004
Human Hair NIES-CRM-5	Hg: 3.81±0.60 Se: 1.78±0.06	15.7 3.4	13.4 27.1	4.4±0.4 1.4±0.2
Mixed Human Diet IAEA-H-9	Hg: 0.0055±0.0004 Se: 0.14±0.02	7.3 14.3	14.6 27.2	0.0048±0014 0.11±0.01
Copepod IAEA-MA-A-1	Hg: 0.24±0.02 Se: 2.85±0.50	8.3 17.5	14.3 5.0	0.28±0.01 3.0±0.2
Oyster Tissue NBS-SRM-1566	Hg: 0.049±0.006 Se: 2.00±0.01	12.2 0.5	14.0 4.8	0.057±0.015 2.1±0.5
Mussel Tissue IAEA-MA-M-2	Hg: 0.87±0.02 Se: 1.86±0.04	2.3 2.2	8.4 18.1	0.95±0.10 2.27±0.43
Fish Flesh IAEA-MA-A-2	Se: 1.47±0.04	2.7	13.7	1.7±0.3

The solution obtained was transferred to a 50 ml beaker and a small amount of water added. Two millilitres of perchloric acid were added and the solution was slowly evaporated to a final volume of about 0.5 ml. This solution was diluted with 15 ml 1M HNO_3 and passed through a preconditioned HMD column at a flow rate of 0.5 ml min^{-1}. The column was washed twice with 15 ml of 1M HNO_3 each time and finally allowed to dry. The HMD resin was transferred to a counting vial and the 320 keV gamma-ray peak of ^{51}Cr was measured with the same counting system mentioned above.

Results

The recovery of Cr when using the HMD column for its separation, was calculated as $99.75 \pm 2.8\%$ as determined from the tracer experiments. Table 4 shows the results obtained for the determination of Cr in several biological reference materials as well as literature concentration values for such materials.

DISCUSSION

From the analytical data obtained in these experiments, shown in Tables 2, 3 and 4, it can be seen that the results obtained using the radiochemical separation schemes presented in this paper, are generally in good agreement with literature and reference concentration values.

When a combination of TDO and DOWEX 2X8 columns is used, the elements As, Hg and Sb can be determined in the reference materials Bowen's Kale, Rice Flour (NBS-SRM-1568) and Citrus Leaves (NBS-SRM-1572) (Table 2).

TABLE 4

Results of the determination of Cr in biological reference materials using HMD inorganic exchanger

Reference material	Concentration, this work, ppm	Relative s.d., %	Relative error, %	Literature values (Ref. 15)
Citrus Leaves NBS-SRM-1572	0.82±0.15	18.3	2.5	0.8±0.2
Tomato Leaves NBS-SRM-1573	4.4±0.2	4.5	2.2	4.5±0.5
Mixed Human Diet NBS-SRM-8431a	0.116±0.018	15.5	13.7	0.102±0.006
Human Hair NIES-CRM-5	1.54±0.04	2.6	10.0	1.4±0.2
Fish Flesh IAEA-MA-A-2	1.110±0.04	3.6	15.4	1.3±0.1
Mussel Tissue IAEA-MA-M-2	1.03±0.03	2.9	20.0	1.25±0.34
Mixed Human Diet IAEA-H-9	0.10±0.04	40.0	33.3	0.15±0.04

Selenium was determined in Bown's Kale and Rice Flour (NBS-SRM-1568) only. Relative standard deviations ranged from about 3-17%, which is acceptable at these concentration levels. The only exception was the case of Sb in Rice Flour (NBS-SRM-1568) for which the relative standard deviation was 42.2%. Relative errors were from about 1 to 32% as in the case of Hg in Rice Flour, but in most cases were less than 15%.

The method using TDO in HNO_3 medium for the retention of Se and $Ni(DDC)_2$ for the extraction of Hg (Table 4) gave similar results. Relative standard deviations were from 0.5-17% and relative errors ranged from 5-27%, i.e., Se in Human Hair and Mixed Human Diet. However, in most cases the relative errors were less than 15%.

The determination of Cr in several biological reference materials, using the HMD inorganic exchanger to retain it and separate it from other interfering elements, was also successful (Table 4), with relative standard deviations and relative errors generally below 15%.

The separation procedures described in this paper, based on ion exchange, element retention on inorganic exchangers and solvent extraction, proved to be much faster and easier than previously utilized methods in our laboratories based on distillation and precipitation[1].

The dissolution procedures employing microwave and conventional ovens were also successful. The dissolution method using a microwave oven is certainly faster than the conventional one. However, it requires specially designed and rather expensive dissolution vessels with a fairly short useful life.

Some of the reference materials analyzed in this work presented some difficulties for dissolving, even when using Parr acid dissolution bombs heated at higher temperatures for longer periods of time than the ones normally used for other materials. That was the case of Human Hair (NIES-CRM-5) and Copepod (IAEA-MA-A-1). The other materials tested did not present any problem when using either dissolution procedure.

CONCLUSIONS

The separation methods outlined in this paper present a good alternative for the isolation and subsequent determination of As, Cr, Hg, Sb and Se in biological matrices. The procedures have proven to be fast and give good results. Although in some cases the concentration values determined by these methods differ from the certified ones, they are within the error indicated by the supplier of the reference material concerned. The two sample dissolution approaches used in this work also demonstrate their applicability for this type of samples. The dissolution process using a microwave oven is the faster of the two but it requires some rather expensive materials. The use of acid digestion bombs with a conventional laboratory oven could be optimized by carrying out the heating step overnight so as to enable starting the separation process early the next day.

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