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RADIOCHEMICAL SEPARATION FOR THE CERTIFICATION OF SOME TRACE ELEMENTS IN BIOLOGICAL REFERENCE MATERIALS BY NEUTRON ACTIVATION ANALYSIS

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A radiochemical separation procedure based on chromatographic separation using Chelex-100 in 0.1M HAc-0.1M NH_4Ac at pH 4.8 and TDO in 6M HCl, has been developed to determine Cd, Co, Cr, Fe, Se, Th, U, W and Zn in three biological materials of botanic origin used as SRM's: 1547 Peach Leaves, 1515 Apple Leaves and the new proposed material Spinach. The aim was to obtain more information for these elements whose values are not yet determined or are given only as "suggested values".

The analyses for trace element determination in biological materials required in studies related to environmental pollution as well as human nutrition and biochemical research, need an accurate data quality control on the obtained results. For example, correct extrapolations to assess the impact onto humans of biologically important trace elements released from pollution sources can be carried out only if reliable concentration data are available in both human tissues and environmental related materials.

This is particulary required when trace element concentrations have to be determined at very low levels (part per bilion) as in the case of biological specimens of unexposed subjects or for the analysis at basal concentrations.[1] A reliable and preliminary quality control testing on the obtained results can be performed analyzing, among the investigated samples and in the same analytical conditions, standard reference materials with identical or similar composition.

For the determination of many trace elements, neutron activation analysis offers the required precision and sensitivity and is normally used as analytical technique for the certification of these materials. However, some specific elements have to be determined with the help of selective radiochemical separations to overcome the interferences deriving from the high background levels of radiation and from the interfering elements.

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In this paper, a radiochemical separation procedure based on the use of ion exchange chromatography is described and used to determine some critical trace elements in biological standard reference materials of botanic origin. Both cation exchange resin Chelex-100 and the inorganic ion exchanger TDO (Tin Dioxide), already adopted in previous works[2–4] have been used in different conditions to determine Cd, Cr, Se, Th, U, W, Fe, Co and Zn.

Experimental

Reagents: The Chelex 100 in Na^+ form (100–200 mesh) was obtained from BioRad Laboratories (USA). It was transformed in the NH_4^+ form as reported below. The inorganic ion exchanger TDO (Tin Dioxide) was obtained from Carlo Erba, Milan (Italy). Primary standard solutions of the elements under investigation were prepared by diluting standard solutions from British Drug House (U.K).

Samples: The samples consisted of three botanic reference materials, all from NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA): the SRM's 1547 Peach Leaves, 1515 Apple Leaves and the Spinach material proposed as new SRM (Standard Reference Material). This latter one was obtained from the IAEA (International Atomic Energy Agency–Vienna) which distributed it for an interlaboratory intercomparison. Sample weights were from 150 to 200 mg.

Columns: Plastic (polyethylene) chromatographic columns were 12 cm height, 1 cm internal diameter and with a reservoir of 50 ml.

Irradiation: All neutron irradiations were performed in the central thimble facility of the TRIGA Mark II Reactor of the University of Pavia at the neutron flux of $9 \cdot 10^{12}$ n $\cdot$ cm$^{-2} \cdot$ s^{-1}. Samples and standard, sealed in quartz vials, were irradiated for seven hours.

Dissolution: According with the last findings by GREENBERG[5] where the dissolution of some biological matrices by microwave system may be not quantitative, in this work conventional high pressure Teflon lined dissolution bombs have been used. The sample dissolutions were carried out in teflon digestion bombs (100 ml internal volume) using HNO_3 and HF (10 : 1) at a temperature of about 150 °C for 10 hours.

Counting equipment: The gamma-ray measurements of the irradiated samples and standards were carried out by a Ge PopTop GMX detector (resolution of 1.93 keV at the 1332 keV γ-ray peak of ^{60}Co) coupled to an ADCAM TM multichannel buffer and associated electronics.

Radiochemical separation: The radiochemical separation procedure was based on ion exchange chromatography using two columns in series filled with Chelex-100 (3 cm height) and TDO (2 cm height), respectively.

The Chelex-100 columns were conditioned with 10 ml of 2M HNO_3, 20 ml of 1M NH_4OH at about 50 °C and then washed with 100 ml of distilled water at room temperature. This procedure allowed to transform the resin from the Na^+ form to the NH_4^+ form. The columns were finally conditioned with 0.1M ammonium acetate buffer at pH of 4.8. The TDO columns were conditioned with 6M HCl and loaded below the Chelex resin columns.

After a cooling time of 24 hours, the irradiated samples were transferred to the teflon vessels of the digestion bombs adding 3 ml of HNO_3, 0.5 ml HF and 0.5 ml of the non-irradiated multielemental standard solution used as carrier. The irradiated primary standard solutions (0.1 ml) were pipetted onto 100–200 mg of non irradiated reference materials (using each time the same SRM under investigation) and dissolved in the same way.

After dissolution, the resulting solutions were brought to dryness by evaporation at 60–70 °C to avoid possible losses by volatilization and the residues were redissolved in 10 ml buffer solution (0.1M ammonium acetate at pH 4.8). The solutions were passed through the Chelex and TDO columns. During the percolation from the Chelex columns the TDO was mantained at a strong acidity by adding dropwise, 6M HCl directly on the TDO columns. The Chelex columns were then washed with 100 ml of buffer solution using the same operating procedure.

In these conditions, elements such as Cd, Cr, Co, Fe, Th, U, W and Zn were retained on the Chelex resin while As and Se were absorbed on the TDO column.

Results and discussion

The radiochemical separation procedure was first tested with non-irradiated samples and radioactive tracers of element analysed to check the recovery of ^{76}As (26.3 h), ^{115}Cd–^{115}In (53.5 h), ^{60}Co (5.24 y), ^{51}Cr (27.8 d), ^{59}Fe (45.1 d), ^{75}Se (121 d), ^{233}Pa (27.0 d), ^{239}Np (2.35 d), ^{187}W (24.0 d) and ^{65}Zn (245 d). The trace experiments showed relatively high and constant recovery for all elements except for As, where the yield was ranging between 40 and 60%. The other yield values were: (95 ± 3)% Cd, (98 ± 2)% Co, (97 ± 3)% Cr, (95 ± 4)% Fe, (96 ± 4)% Se, (96 ± 5)% Th, (96 ± 4)% U, (95 ± 4)% W and (96 ± 4)% Zn. The poor yield obtained in the arsenic determination is still object of evaluation and seems related to the oxidation state of arsenic ions. The retention on TDO from 6M HCl is independent from the As valence while, depending on the As chemical forms, a partial absorption onto the Chelex column may be encountered from the ammonium acetate/acetic acid buffer solutions. Only when present as AsO_4^{3-} form the elution from the Chelex column is quantitative.

Table 1
Results of the determination of trace elements in SRM 1547 Peach Leaves by radiochemical separation (dry weight)

Element	This work	BECKER[8]	SMODIS[9]	Certified value
Cd, ng/g	28 ± 5	27.3 ± 0.3	< 1000	(30)
Co, ng/g	70 ± 14	76 ± 3*	66.1 ± 3.0	(70)
Cr, μg/g	1.13 ± 0.18	1.16 ± 0.04	1.47 ± 0.28	(1)
		1.53 ± 0.10*		
Fe, μg/g	274 ± 15	220 ± 6*	207 ± 15	218 ± 12
Se, ng/g	123 ± 9	120.7 ± 3.6	175 ± 55	120 ± 9
		117 ± 9*		
Th, ng/g	50.3 ± 6.7	N. A.	58 ± 9	(50)
U, ng/g	15.7 ± 1.8	N. A.	18 ± 3	(15)
W, ng/g	23.8 ± 3.3	N. A.	< 100	–
Zn, μg/g	17.1 ± 2.0	17.72 ± 0.40*	17.2 ± 1.3	17.9 ± 0.4

N. A.: not analyzed.
*Determined by INAA.

Table 2
Results of the determination of trace elements in SRM 1515 Apple Leaves by radiochemical separation (dry weight)

Element	This work	BECKER[8]	Certified value
Cd, ng/g	14 ± 2	13.8 ± 0.9	(14)
Co, ng/g	86 ± 4	101 ± 4*	(90)
Cr, ng/g	332 ± 25	312 ± 25	(300)
		391 ± 55*	
Fe, μg/g	80 ± 18	82.9 ± 3.0*	(80)
Se, ng/g	58 ± 4	51.3 ± 1.3	50 ± 9
		55 ± 6*	
Th, ng/g	34.0 ± 4.6	N. A.	(30)
U, ng/g	9.9 ± 0.6	N. A.	(6)
W, ng/g	8.0 ± 2.3	N. A.	–
Zn, μg/g	11.1 ± 2.0	12.65 ± 0.30*	12.5 ± 0.3

N. A.: not analyzed.
*Determined by INAA.

In Tables 1 and 2 are reported the results obtained for Cd, Co, Cr, Fe, Se, Th, U, W and Zn determined in the two NIST SRM's 1547 Peach Leaves and 1555 Apple Leaves. The results were obtained from four independent analysis and are presented as the total mean of the individual data with the standard deviation. In the same tables the certified

Table 3
Analysis of the trace elements in the IAEA 331 Spinach (dry weight)

Element	Mean ± S. D.*	Relative S. D., %
Cd, μg/g	2.60 ± 0.26	10
Co, ng/g	354 ± 22	6.2
Cr, μg/g	1.50 ± 0.13	8.7
Fe, μg/g	256 ± 21	8.2
Se, ng/g	117 ± 5	4.3
Th, ng/g	51.3 ± 3.7	7.2
U, ng/g	143 ± 10	7.0
W, ng/g	15.7 ± 3.2	20
Zn, μg/g	79.1 ± 7.6	9.6

Mean and standard deviation of seven individual determinations.

or suggested values from the NIST Certificates[6–7] and the data found in the literature[8–9] are also reported.

From the analytical data obtained in this work, shown in Tables 1 and 2, it can be seen that the results obtained for Cd, Cr, Co, Fe, Se and Zn using the radiochemical separation, are generally in good agreement with the BECKER[8] and the certified values. For U and Th no certified data are available and the comparison can be done only with the NIST information values.

Table 3 shows the results of mean and standard deviation of seven independent analysis of these elements in the Spinach material proposed as the new SRM.

The possibility of performing a selective separation from ^{24}Na, which is not retained onto the Chelex column, allows the determination of elements such as Cd, U, Th and W at very low level. This may be very interesting for W analysis. Very few data for this element in biological materials are reported in literature. Only recent studies have shown an increasing concern for the role played by heavy metals in human health.[10,11] The use in sequence of the TDO ion exchanger seems very promising for the selenium and arsenic determination if, for this last, better elution conditions from the Chelex resin are found.

Conclusion

The radiochemical separation developed in this work allows the simultaneous determination by neutron activation analysis of 9 elements in biological related materials. Six of them can be determined at very low concentration in the range of parts per billion. If the sample dissolution is carried out during the night following the

irradiation, the overall separation procedure can be performed in few hours allowing the determination of the short lived radioelements ^{187}W and ^{115m}In. Elements such as Th, U and W whose concentration data in biological materials are normally not well established can be correctly determined and their values may be used in the standard reference material certifications.

The application of this radiochemical separation contributed to the trace element certification of the new proposed standard reference material 331 Spinach issued by the International Atomic Energy Agency.

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