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DETERMINATION OF Cd, Mo, Cr AND Co IN BIOLOGICAL MATERIALS BY RNAA

J. R. W. WOITTIEZ, M. DE LA CRUZ TANGONAN*

Interfaculty Reactor Institute, Delft University of Technology, Mekelweg 15, 2629 JB Delft (The Netherlands)

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A method for the determination of trace amounts of Mo, Cd, Co and Cr in biological materials by neutron activation analysis with radiochemical separation is presented. The method is based on the ion-exchange scheme developed by SAMSAHL, where Co and Cr are trapped on BioRad Chelex-100 and Cd and Mo on BioRad AG 2X8. The elements Mo, Cd and Co can be determined without systematic errors. For the element chromium the situation is less clear, partially due to lack of sufficient certified reference materials for Cr. The method has been used in the characterization of candidate reference materials. Detection limits in these materials range from 1.5 μg/kg for Co to 10 μg/kg for Cr. Actual levels as low as 8 μg/kg for Cd and 7 μg/kg for Co were measured.

Introduction

The role of neutron avtivation analysis in the accurate and precise elemental analysis of biological materials is prominent, despite the advent of several competing multielement analytical techniques.[1,2] Neutron activation with radiochemical separation (RNAA) combines characteristics of accuracy and precision with high sensitivity. Routine radiochemical separations for multielement determinations are almost exclusively based on ion-exchange and other forms of selective sorption.[3-5] One of the most widely applied schemes is the SAMSAHL scheme.[5] Some 15 papers have been published on applications and modifications. The important have been referred to in a previous paper.[8] The original scheme leads to one or two elements per column and generates 11 columns per sample. The introduction of high efficiency Ge detectors offers the option to combine different fractions without significant loss of sensitivity and thus time and labor can be saved. To enable a certain minimum sample throughput, we experimented with several different modifications of the scheme to find a compromise

* IAEA fellow, on leave from the Philippine Nuclear Research Institute.

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Akadémiai Kiadó, Budapest

between selectivity and sensitivity. The determination concerns the trace elements Mo, Cd, Co and Cr, which are on the lower μg/kg level in most biological materials and difficult to determine by most trace element analytical techniques.[1] By combining the first two columns from the original scheme, Mo and Cd can be trapped on a BioRad AG 2 × 8 column in a 0.4M HBr environment.[6] Cobalt and Cr can be trapped on a Chelex-100 column in a 0.6M acetate buffer. Simultaneously with the elements mentioned, also W, Au, Th, Zn and Fe will be trapped.

The results obtained with this modified SAMSAHL scheme in the characterization or certification of reference materials have been published elsewhere.[7,8] This paper presents the procedure and analytical aspects for the elements Mo, Cd, Cr and Co and the application of the method to Canadian candidate reference materials.

Experimental

The elements Cd and Mo are determined simultaneously on the anion exchange resin BioRad AG 2 × 8, while Cr and Co are trapped on the chelating resin BioRad Chelex-100. The procedures are given separately, although there is a partial overlap.

Cd and Mo

Up to 300 mg dry biological material is weighed in cleaned Hereaus quartz capsules. The cleaning of the capsules is previously done by consecutive washing with bidistilled water, nitric acid, and bidistilled water again in a closed cleaning set-up. The weighing is performed in a clean laboratory. Samples are irradiated in a thermal neutron flux of $1.4 \cdot 10^{17} m^{-2} \cdot s^{-1}$ for 12 hours. Two days after the end of irradiation, the quartz tubes are unpacked, washed in aqua regia overnight at room temperature, frozen in liquid nitrogen, wrapped in paper tissue and a small plastic bag and finally opened by breaking in a capsule cracker. Sample, quartz pieces and paper tissue are transferred to a destruction vessel, after which 10 μg of carrier Cd and Mo are added. Destruction takes place using 2 ml concentrated H_2SO_4, 1 ml concentrated HBr and as much 30% H_2O_2 as is needed to oxidize the charred digest. The digest is diluted to 30 ml using a solution of 0.3% H_2O_2, 0.05M HBr and 3M NaCl. This solution is passed through a 3 ml (9 × 50 mm) BioRad AG 2 × 8 (200 – 400 mesh) column in the Na^+ form where molybdenum as molybdate and cadmium as the cadmium bromide complex are trapped. The flow rate is 1.5 ml · min^{-1}. The column has previously been equilibrated with a solution containing 0.2M H_2SO_4, 0.3% H_2O_2, 0.05M HBr and 3M NaCl. The column is washed with at least 20 ml of this equilibration solution. The resin is removed from the column and counted after

24 hours for 15 000 seconds on a Ge(Li) detector (2.20 keV FWHM at 1332.5 keV). The 336.2 keV line from the ^{115}Cd-daughter ^{115m}In and the 140.5 keV gamma-ray energy of the ^{99}Mo-daughter ^{99m}Tc are used for analysis.

Always five samples are processed simultaneously in a semi-automated system. The positions 1–3 are used for unknowns, position 4 is for a certified reference material and position 5 for a standard. Standards are dilutions a Titrisol standard solutions (Merck, Darmstadt, BRD) pipetted on half a Whatman 41 filter paper (Whatman, Maidstone, England). The effluent of the column through which the standard has passed is collected and counted, so as to monitor possible break-through of the column.

Cr and Co

Separate samples are processed for the analysis for Cr and Co. The procedures as far as sample weighing, irradiation, number of samples irradiated, digestion and break-through of the standard are concerned, are identical to those for the Cd and Mo determination, except for a decay time of 2 weeks instead of 2 days. The H_2SO_4 digest is diluted to 30 ml, using a solution 0.05M in HBr, 0.3% in H_2O_2, 2.5M in LiCl, 0.5M in NaOH and 0.6M in Na-acetate, the pH of the sample is adjusted to a value of 4.0 using NaOH. This solution is passed through an 8 ml column of BioRad Chelex-100 (200-400 mesh), previously equilibrated with a solution with a composition identical to the sample solution. The flow-rate here is 0.8 ml · min^{-1}. The column is washed with 20 ml of the equilibration solution. The resin is removed from the column and counted during 40000s on a Ge(Li) detector. The 320.1 keV line of ^{51}Cr and the 1173.2 and the 1332.5 keV lines of ^{60}Co are used for analysis.

Results and discussion

Interference

In the analysis of Mo and Co, no sources of interference were discovered. Both elements are relatively insensitive to contamination. Since no detectable amounts of Fe are trapped on the BioRad AG 2 × 8 column the presence of a 142.6 keV gamma-line from ^{59}Fe on the Mo-analysis is not a problem. The contribution of ^{99m}Tc activity from fission of ^{235}U is not important, considering the low levels of U in biological materials.

The levels for Cd are usually on the lower µg/kg level, which makes its analysis more sensitive to interference then in case of the determination of Mo. Firstly, when analyzing on the 1–10 µg/kg level, the number of collected counts is only several hundred. The detector needs to be well shielded to prevent serious interference from the 338.1 keV line

from ^{232}Th in the background radiation. Secondly, depending on the batch of AG 2 × 8 resin, a small fraction of ^{64}Cu may be trapped. Its Compton scattering will negatively influence the peak to noise ratio at 336 keV. Interference from uranium via the 334.4 keV line from the ^{239}Np daughter has not been found.

The analysis of Cr is interfered most by the presence of ^{65}Zn on the Chelex-100 column. Since the content of Zn in biological materials is usually orders of magnitude higher than the Cr-level, the Compton contribution of ^{65}Zn in the 320 keV region outnumbers the ^{51}Cr activity. The same is true for residual amounts of ^{32}P on the resin, where the Bremsstrahlung generates a continuous background.

Sensitivity, limits of detection and blanks

Sensitivity is expressed here as the specific counting result for each of the four radionuclides under the circumstances of actual determination. Limits of detection are defined as 3 times the standard deviation of the background on which the photopeak is situated. Blanks are procedural blanks and consist of half a Whatman 41 filter paper, processed as a sample.

Mo: The counting result for a 12-hour irradiation and a 15000 s counting time after 4 days of decay is about 350 counts/ng. Since the background for this low energy peak (140 keV) may be as high as 7000 counts, a detection limit of 2.5 ng or 7 μg/kg in a 300 mg sample can be obtained. A typical blank value of the Whatman paper ranges from 3 to 5 ng.

Cd: Using the conditions as specified for Mo, the counting result for Cd points to 200 counts/ng. A typical background is about 1500 counts, which means a detection limit of 4 μg/kg in a 300 mg sample. A usual blank value varies between 2 and 4 ng.

Cr: The counting result for a 12-hour irradiation and a 40 000 second counting time after 3 weeks of decay is about 200 counts/ng. Since for a real sample a background of 10 000 counts is realistic, a practical detection limit is 4 ng of Cr or about 10 μg/kg in a 300 mg sample. The blank value was established to be < 2 μg/kg.

Co: Under the circumstances as given for Cr, the counting result is about 450 counts/ng. As the background of the 1332.5 keV gamma ray is only about 500 counts, a detection limit of 1.5 μg/kg can be achieved. The blank was established to be < 0.5 μg/kg.

Accuracy and precision

For all four elements, viz Cd, Mo, Cr and Co, the accuracy of the method is estimated by three different approaches. Firstly, the recovery is established by using radiotracers in digests of non-irradiated samples and by comparing the result for

aliquots which passed the columns to those remaining unprocessed. Secondly, every fifth sample of a bath to be analyzed is a standard (dilutions of Merck Titrisol, pipetted on half a Whatman 41 filter paper), which is processed as a sample. Its column effluent is counted so as to monitor possible losses during separation. Thirdly, the standard reference material NBS SRM1577 Bovine liver, with certified values for Cd and Cr and recommended values for Mo and Co, has been analyzed in 6 runs. This last approach also gives indication on the achievable precision to be obtained using the separation schemes. The results of the measurements on accuracy are summarized in Table 1.

Table 1
Results for the accuracy of the analysis of Mo, Cd, Co and Cr using AG 2 x 8 and Chelex-100

Element	Recovery on column,* (%)	Appearance in effluent**	NBS SRM 1577 Found*** µg/kg	NBS SRM 1577 Certified µg/kg
Mo	99.7 ± 1.1	< 0.22	3336 ± 132	3200 ± 400+
Cd	97.8 ± 1.1	< 0.5	279 ± 25	270 ± 40
Co	98.5 ± 1.5	<0.05	244 ± 13	250 ± 60+
Cr	98.45 ± 0.42	1.74 ± 0.94	66 ± 14	88 ± 12

*Determined by using radiotracers in inactive sample solutions.
**Determined by analizing standards, pipetted on filter paper and processed as samples.
***Determined by RNAA.
+Values marked with this sign are best values from GLADNEY's compilation.[9]

Mo. The absorption efficiency for Mo on BioRad AG 2 x 8 is very high. No measurable activity could be found in the effluent of the standards. The monitoring of the effluent for breakthrough was stopped after 5 runs. The analysis of Mo in the Bovine Liver indicates no major problems for the accuracy. Our value of 3336±132 µg/kg agrees very well with the average literature value as given by GLADNEY (3200±400).[9]

The precision of the Mo-analysis, expressed as relative standard deviation (rsd), points to 4.0%. This value appears to be typical for the technique, since the same value was obtained for different reference materials with levels ranging from 190 up to 4000 µg/kg.[8]

Cd. Also for Cd, the adsorption efficiency on BioRad AG 2 x 8 is high. Monitoring of the effluent of irradiated standards for Cd breakthrough gave a maximum value of 0.5%. The analysis of the Bovine Liver material resulted in a value of 279±25 µg/kg, which agrees well with the NIST certified value of 270±40. The relative standard deviation of the Cd analysis in Bovine Liver is 9%. About 5% is due to statistical

errors originating from irradiation and chemistry, the remaining 7% stems from counting statistics.

Co. The absorption of Co as Co(II) on Chelex-100 is known to be high and constant, provided the pH is higher than 3.5.[10,11] This result was confirmed in the experiments described here, as demonstrated in Table 1. Analysis of the Bovine Liver resulted in a value of 244±13 μg/kg. This value agrees well with the average literature value from GLADNEY's compilation (250 ± 60 μg/kg) and corresponds well with a previous result from our laboratory, 240–250 μg/kg.[16] The RSD of 5.3% for Bovine Liver indicates sufficient precision.

The element Co can be determined in many biological materials with NAA in the instrumental mode, thus presenting an opportunity to compare INAA and RNAA results for the same samples. This was done for a set of reference materials. Detailed results are given in Table 2. The agreement between the two NAA techniques is satisfactory also for the low-level samples.

Table 2
Comparison of determinations of Co by RNAA and INAA.
Values in brackets are numbers of replicates.

Sample	INAA, μg/kg	RNAA, μg/kg
NBS SRM 1577	238	244 ±13[6]
BCR CRM 274	38.3	40.1
IAEA RM A-11	6.1	5.0 ± 1.3[5]
Candidate RM "Bran"	6.8 ± 2.0[4]	5.7; 6.3
Candidate RM "Meat"	8.9 ± 1.3[4]	8.9; 10.6
Candidate RM "Egg"	14.8 ± 1.3[4]	14.6; 15.6
Candidate RM "Gluten"	9.8 ± 0.7[4]	8.3; 8.7

Cr. The element Cr has been the subject of much experimental work. As already reported by SAMSAHL,[12] the retention of Cr on Chelex-100 is not quantitative. Recoveries as low as 70% may be obtained. IYENGAR recognized the problem and achieved considerable improvement.[13] In our original scheme, the recovery of Cr was 91.5±1.8% for a sample volume of 30 ml. Provided that no yield determination is done for every sample, this recovery is too low for accurate analyses. When a loaded column was divided into four slices, it was found that Cr moves through the column during sample application. The upper part contained 66% of total column Cr, the 2nd 20%, the 3rd 8% and the last 5%. Cd, Co and Zn, for comparison, were all exclusively recovered in the upper slice. This result was the reason for performing a systematic

investigation on the absorption behavior using ^{51}Cr as a radiotracer. Various modifications were tested.

(1) The effect of varying column volumes has been investigated first. It was found that for an 8 ml Chelex-100 column an amount of 1.6±0.4% [N = 4] Cr is lost in the effluent, while this number may increase to 22% for a 4 ml column. Doubling the length of the column while retaining the volume at 8 ml, improves retention to 99.1%

(2) The pH of the sample solution was changed from 3.0 to 4.8 with intervals of 0.2 pH units. The average loss in the effluent was 1.4±0.5% [N = 10], and there was no relation between losses of Cr and the pH value.

(3) The same conclusion is true for the amount of carrier Cr added to the sample solution as Cr(III). The average loss was 1.3 ± 0.4% [N = 8], while the amount of carrier added varied from 10–500 μg.

(4) Increment of the flow-rate from around 1 to 3 ml/min decreased the recovery from 98.5 to 90%.

(5) The solution used to equilibrate and wash the column is adapted from the original scheme. It is 0.5M in NaOH, 0.6M in NaOAc and 2.5M in LiCl. LiCl in the original scheme was used to trap Zn and Fe as chloride complexes on AG 1 × 4. Since in the present scheme this step is omitted, the presence of LiCl seems useless. However, keeping the NaOH and NaOAc concentrations and the pH constant, concentrations of 0, 1, 2 and 3M LiCl resulted in Cr losses in the effluent of 8.5, 5.0, 4.3 and 3.0%, respectively. Although this effect is not understood yet, it was the reason for deciding to keep the LiCl concentration at 2.5M.

The value for the recovery under optimized circumstances, 98.5±0.4% [N = 5], obtained by measuring the ^{51}Cr activity on the column, could be confirmed by measuring the effluent of irradiated standards, processed as samples. An average of 1.7±0.9 [N = 10] is found.

Our value for Cr in Bovine Liver, 66±14 μg/kg, is 25% lower then the NBS certified value, 88±12 μg/kg. The reason for this low result is not understood. GLADNEY's average literature mean value, 250±370 μg/kg is too imprecise to be suitable for comparison. Although there is evidence that the method presented here can generate accurate results,[8] there is a shortage of sufficient different reflerence materials, certified for Cr on the lower μg/kg level, to prove this statement.

The precision of the Cr-analysis points to around 20%, which seems to be the state of practice considering the level.[14] The trapping of Cr on Chelex-100 does not allow to determine the yield for each analysis, so it cannot be excluded that the imprecision is partly due to errors in the analysis.

Applications

In a period of time ranging from autumn 1988 until May 1990, the technique described here has been applied to the determination of the four elements in several intercomparisons. Results for specific materials, such as samples from the US Dietary Intake Project, and the appropriate quality control have already been published elsewhere.[7,8] Here, the results of the participitation of Canadian candidate reference materials, as preparated by IHNAT from Land Resource Research Centre Agriculture Canada,[15] are presented. Four materials were analyzed: Gluten, Bran, Meat powder and Egg powder. Results for Cd, Mo, Co and Cr are given in Table 3.

Table 3
Results for candidate reference materials prepared by Land Resource Research Centre Agriculture Canada. All data are in μg/kg. Mean values and standard deviations are based on 4 determinations

Materials	Mo	Cd	Co	Cr
Gluten	797 ± 10	84 ± 10	8.3; 8.7	55 ± 11
Bran	271 ± 14	12.1 ± 1.8	5.7; 6.3	128 ± 13
Meat power	72 ± 8	11.9 ± 1.6	8.9; 10.6	79 ± 23
Egg power	262 ± 6	8.6 ± 1.5	14.6;15.6	402 ± 37

The element Cr has been analyzed twice; once in 1988, which results are included in Table 3 and once in 1990. A comparison of both analyses is given in Table 4. Except for Bran, the agreement is satisfactory. As already mentioned, cobalt has been analyzed by both INAA and RNAA (cf. Table 2).

Table 4
Comparison of Cr results in candidate references materials. All results are in μg/kg

Material	1988 (N = 4)	1990 (N = 2)
Gluten	55 ± 11	59.8; 56.2
Bran	128 ± 13	62.1; 59.6
Meat Powder	79 ± 23	71.1; 80.9
Egg Powder	402 ± 37	398; 358

Conclusion

The results show clearly that the contents for Mo and Co in most biological materials are high enough to be well within the sensitivity range of NAA and to overcome blank problems. According to the results from Tables 1 and 2, accurate results can be generated even on the lower μg/kg level.

The analysis of Cd also appears to be free from systematic errors. It is, however, hard to prove that this is also true on the 10 μg/kg level, where variations due to counting statistics of a single analysis can mask systematic errors of up to 20%.

The analysis of Cr is subject to some uncertainty. Although the procedure has been optimized with regards to the Cr-analysis, it might be that both the low result for NBS SRM 1577, the lack of agreement in the 1988 and 1990 data for Bran and the generally found poor precision (20%, regardless of the level) in fact indicate analytical imperfectness. Many reference materials, on the other hand, including the Canadian candidate materials, are known to be inhomogeneous for Cr.[14,15] Thus, the unsatisfying situation remains to exist. That is why further RNAA work on Cr in our laboratory focuses on yield determination for each individual sample.

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