DETERMINATION OF CHROMIUM IN GELATINE SAMPLES AND IN BIOLOGICAL REFERENCE MATERIALS BY NEUTRON ACTIVATION ANALYSIS

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ABSTRACT - Instrumental and radiochemical neutron activation analysis was applied for Cr determination in gelatine samples and in biological reference materials. The radiochemical separation procedure was based on the solvent extraction of Cr (VI) into tribenzylamine/chloroform solution. Cr concentrations varying from 68 to 610 µg/kg were found in gelatine samples with good precision and the results for reference materials showed a good agreement with the published values.

Key words chromium, gelatine, reference materials, neutron activation analysis

Chromium determination in biological materials has been of great interest for clinical researches and to elucidate the exact role of this element in human nutrition and health. Besides, the attainment of reliable results for Cr in low concentration is a difficult problem in the trace element analysis of biological materials. This element exists in two different oxidation states: Cr (III) and Cr (VI). Cr(III) is considered to be essential to mammals for the maintenance of glucose, lipid and protein metabolism whereas Cr (VI) is known as being toxic to humans. Chromates are better absorbed than inorganic Cr (III). However, when present in diets, Cr (VI) is reduced in the gastrointestinal tract to Cr (III) valence | 1 |. In this paper neutron activation analysis was applied for Cr determination in gelatine samples used by the population for cooking. The reason for analyzing gelatine was a chromium contamination occured in São Paulo, which gave rise to great concern from public health authorities. In order to examine the validity of the Cr results, the following biological reference materials: Fish Flesh Homogenate, Mixed Human Diet (H-9) and Whey Powder/155 from IAEA, SRM 1572 Citrus Leaves from NIST and Pepperbush Nº 1 from NIES were analyzed.

EXPERIMENTAL

Gelatine samples obtained in local supermarkets in the dried powder form were ground to homogeneous fine powder, using an agate mortar. Samples of 200-500 mg were weighed in quartz ampoules previously cleaned with nitric acid solution and water, and irradiated together with Cr standard solution in the IEA-R1 nuclear research reactor for 16h under a thermal neutron flux of 10^{13} $n.cm^{-2}.s^{-1}$. Cr standard solution was prepared by dissolving metallic chromium in the shot form provided by Johnson Matthey Chemical Limited (specpure) with HF and H_2SO_4 and then diluting with distilled water in quartz apparatus. After about 10 days of decay time the ampoules were externally washed with dilute nitric acid solution cooled in liquid nitrogen and then broken to transfer samples into the teflon beakers or into the vials for counting. The ampoule containing Cr standard solution was also broken and an aliquot of 30 µl (2.5 µg of Cr) was transferred using Eppendorf micropipette into a vial or onto a small sheet of filter paper. The radiochemical neutron activation analysis (RNAA) for Cr determination was carried out according to Greenberg and Zeisler | 2 |. Samples transferred into teflon beakers along with 1 mg of Cr (as $K_2Cr_2O_7$) were dissolved with nitric and sulfuric acids and few drops of hydrofluoric acid. Perchloric acid was also added at the second stage of dissolution. Cr (VI) was extracted into a 5% solution of tribenzylamine in chloroform and then it was backextracted into an aqueous solution containing 2M NaOH. The pH was adjusted

to 6.5 ± 0.5 using acetic acid and Cr was precipitated using barium acetate. The precipitate was filtered and washed. To obtain precise results all the steps of dissolution and separation were rigorously accomplished as described in ref | 2 |. The sample (precipitate) and standard pippeted on filter paper were placed between two cellophane sheets for counting in the Ge detector. For instrumental neutron activation analysis (INAA) samples and standard solution were transferred from ampoules to vials using Cr carrier solution and nitric acid solution. The gamma ray energy of 320 keV of ^{51}Cr with half life of 27.7 d was measured using a calibrated ENERTEC hiperpure Ge detector coupled to 4096 channel analyzer.

RESULTS AND DISCUSSION

The overall yield of (87 ± 3)% was obtained in eight determinations by radiochemical separation procedure using a ^{51}Cr tracer solution and different kinds of biological materials. This result indicates the reproducibility of the separation process with a relative standard deviation of 3.5%. Table 1 shows the results obtained for Cr determination in biological reference materials together with literature values for comparison. These results agree with published data and they present a relative standard deviation lower than 23%. Results of Cr determination in gelatine samples (Table 2) indicate the good precision of the results as well as the agreement between the results obtained using INAA and RNAA. The lowest concentrations of Cr were obtained for the flavoured gelatine samples that contain sugar, salt and flavour. Since the sample was transferred using nitric acid solution, blank contribution from irradiated quartz ampoules was examined by irradiating nitric acid solution. ^{51}Cr activity was not detected in this acid nitric solution irradiated in ampoules used in this work. The contribution to the amounts of ^{51}Cr by reaction $^{54}Fe\ (n,\alpha)\ ^{51}Cr$ was also checked. It was found that this contribution is negligible due to the small concentration of Fe (~ 14 µg/g in gelatine samples) and low fast neutron flux. In conclusion, the RNAA and the INAA were successfully applied to Cr determination in gelatine samples, whose concentrations varied from 68 to 610 µg/kg. Cr determination by RNAA or INAA requires a long analysis time however these procedures are free from contaminants and provide reliable results.

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TABLE 1. Chromium Determination in Biological Reference Materials. Results in µg/kg of dried material.

	Fish Flesh Homogenate	Mixed H. Diet (H-9)	Whey Powder 155	Citrus Leaves 1572	Pepperbush № 1
This Work	1087±250 (a) - (*)	- 111±7 (b)	485±30 (a) -	763±59 (a) 813±20 (b)	1604±120 (a) 1012±88 (b)
Published Data	1300±100 | 3 |	150 | 4 |	587 | 5 |	800±200 | 3 |	1300 | 6 |

(a) - Results obtained by INAA ; (b) - Results obtained by RNAA.
(*) - indicates that the analysis was not carried out.

TABLE 2. Chromium Determination in Gelatine Samples.
Results in µg/kg.

Method	Flavourless Natural Gelatine Nº 1	Flavourless Natural Gelatine Nº 2	Strawberry flavoured Gelatine Nº 3	Strawberry flavoured Gelatine Nº 4
INAA	539 ± 39	461 ± 32	68 ± 9	118 ± 13
RNAA	610 ± 79	478 ± 30	85 ± 10	80 ± 12

Samples nº 1, nº 2 and 3 are gelatines from animal collagen origin, and sample nº 4 is from vegetal gel origin.

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