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CHROMIUM IN HUMAN SERUM DETERMINED BY PROTON ACTIVATION

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A method for Cr determination in biological samples based on proton nuclear activation is presented. The activation was induced by a 13.8 MeV proton beam of the AVF Cyclotron of Milan University via a (p, n) reaction on the nuclei of the target. For the quantitative determination Cd has been chosen as reference element. The method has been applied to Cr determination in human serum samples. The experimental procedure is described and results are presented and discussed.

Introduction

Chromium is generally found in human serum at very low concentration; analytical difficulties do not permit to establish normal values and to recognize occupational diseases and toxic effects.

In fact Cr has been measured in biological materials by a variety of methods[1-5] and it has been found in a wide range of concentrations.

As pointed out by other authors[6,7] there are two main factors influencing the determination of Cr: the introduction of contaminations and the losses due to volatilization of the element.

These factors are strictly correlated with the procedure of sample preparation required by the specific method used.

Glassware surfaces, frequently chromium-contaminated, and stainless steel equipments should be avoided.

Many chemical reagents contain sufficient Cr to cause serious errors. Chemical separations and reactions may give low Cr values, due to losses during these operations. Moreover, Cr losses due to ashing and drying treatments are still a subject of discussion.

In this view, the development of an alternative method is welcome. Here we are presenting a method of analysis based on proton nuclear activation (PNA), which has

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sufficient sensitivity, requires a reduced sample handling and allows direct Cr determination in human serum.

Proton nuclear activation has shown good results for the determination of content in human serum of a certain number of trace elements: Cu, Fe, Se, Sr, Zn,[8] Rb,[9] Cd and Ti[10].

Method

The PNA method[8] essentially consists in a bombardment of the sample with a proton beam of appropriate energy to induce predominantly (p, xn) reactions.

The measure of the intensities of the gamma-rays coming from the decay of the radioactive nuclei obtained from the nuclei of interest allows the sample content analysis.

The choice of the particular (p, xn) reaction is determined by the mean life time and the characteristics of the decay of the radioactive nuclei obtainable from the relatively most abundant isotope of the element under study.

For quantitative analysis, the sample is doped with a precisely known amount of a suitable reference element; the ratio between the intensities of the transition relative to the trace element and to the reference element is then measured.

By comparison with the gamma-spectrum of a standard sample containing the same amount of the reference element and also an accurately known quantity of the studied element, one can obtain the trace element content.

PNA allows Cr determination via (p, n) reaction on 52-Cr, which is the most abundant isotope in natural chromium (^{50}Cr 4.31%, ^{52}Cr 83.76%, ^{53}Cr 9.55%, ^{54}Cr 2.38%).

In fact (see Table 1) the radioactive isotope produced, ^{52}Mn, has a mean life time and main gamma-emissions suitable for delayed gamma-spectroscopy.

Table 1
Induced reactions and characteristics of the radioisotopes produced

Reaction	Mean life time, h	Main γ-rays keV	Relative intensity
^{52}Cr(p, n)^{52}Mn	197.4	744.1	0.88
		935.5	0.94
		1434.3	1.00
^{111}Cd(p, n)^{111}In	96.9	150.6	0.10
		171.3	0.90
		245.4	0.94

For the quantitative determination cadmium has been chosen as reference element, because (see Table 1) the mean life time and the gamma-emission of the radioactive ^{111}In produced from ^{111}Cd (12.75% abundance) via (p, n) reaction are suitable for the element under study.

In order to optimize the measurement conditions of Cr, considering the absorption due to tantalum window, the absorption related to the air and to the half-thickness of the sample, we choose a proton beam of 21.8 MeV. In fact, in this way, in the middle of the sample, the energy is that corresponding to the maximum cross section (12.6 MeV) for the reaction considered.[11]

Experimental

As extensively pointed out before, in this kind of measurement attention must be paid to sample preparation.

All the containers for serum and standard solutions were made by polyethylene.

The serum utilized for the samples was a mixture obtained from sera withdrawn from many healthy adults to average individual fluctuations.

The drawing was made by means of particular types of needles surrounded by a teflon sheat. After the introduction in vena, the inner metal needle can be removed and the blood was drawn directly through the teflon capillary tube.

Each sample was prepared by mixing accurately 1 ml of sera with 0.2 ml of a standard aqueous solution of $CdCl_2$, corresponding to 10 μg of Cd that was used as reference element.

The serum, dried at 35 °C and powdered in an agate mortar was then compressed, by means of a bronze device, carefully cleaned, in the form of a self-supporting disc, 12 mm in diameter and ~ 0.7 mm thick. The disc was inserted between two mylar foils, which were completely removed after irradiation and then mounted in a convenient frame.

The standard sample was prepared in the same way, adding to 1 ml of the same sera mixture, 50 μg of Cd and 200 μg of Cr from aqueous standard solutions of these elements.

The pipette used to measure all the previous quantities had an accuracy of 2%.

A proton beam of 300 nA from the AVF Cyclotron of the University of Milan was used for irradiation. Typical irradiation times were 5 h for the samples and 1 hour for the standard samples.

The irradiation was made in air and not in vacuum to reduce the possibility of volatilization of the same elements and moreover the heat produced by energy

losses in the sample was dissipated by cooling the mounting frame at –5 °C with a Freon circulation.

After waiting for a convenient time after irradiation, gamma spectra were collected with a conventional nuclear spectroscopy system.

Interference control

The possible interference reactions on isotopes of intrinsic elements of the matrix, which can produce the same radioisotope of interest, were considered.

The incident energy on the front face of the sample was 13.8 MeV and at this energy the only possible interference reaction is $^{56}Fe(p, \alpha n)^{52}Mn$. In any case, even taking into consideration the large amount of iron compared to Cr and the high relative abundance of the isotope ^{56}Fe (91.66%), this reaction introduces a negligible contribution to the total amount of ^{52}Mn, because the activation energy is just over the threshold energy for the reaction mentioned.

For Cr determination we decided to analyze the 744.1 keV gamma-line instead of the most abundant 935.5 keV and 1434.3 keV gamma-lines, considering the behaviour of the detector efficiency as a function of the photon energy.

Owing to the low concentration of Cr, it was important to verify that the gamma-line chosen had no contributions from spurious effects such as interference with gamma-lines of radioisotopes produced from other intrinsic elements present in the sample.

With this aim, the intensity of the 744.1 keV gamma-line was measured at different times to check the agreement between the experimental intensity decrease and that calculated from the mean life time corresponding to the radioisotope of interest. The mean life time obtained from experimental data was 177.8±19.9 hours, that is in agreement, within 11%, with the known mean life of ^{52}Mn (see Fig. 1).

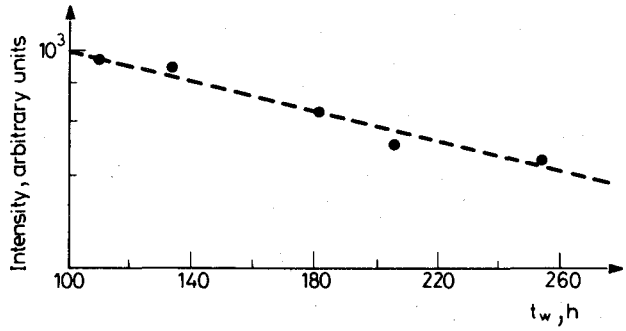

Fig. 1. Intensity of the 744.1 keV gamma-line from ^{52}Mn as a function of waiting time. The dashed line corresponds to the ^{52}Mn mean life time

Contamination control

The Cr contamination of the $CdCl_2$ solution utilized to prepare the solution for the reference element is certified to be much less than the estimated error of the quantitative final determination.

In previous work[9] we reported the results obtained to control the eventual contamination due to the bidistilled and deionized water used for preparing standard solutions.

The analysis was made preparing two serum samples 1 ml each: the first without addition of bidistilled and deionized water, the second with the addition of 10 ml of such water. The two samples were irradiated and the gamma-spectra were collected in the same conditions.

No appreciable difference was found in the intensities of gamma-lines related to Cr from the two samples. It must also be considered that the quantity of water normally involved in sample preparation, as a consequence of the addition of the reference element, was in the order of 0.2 ml per sample.

Temperature control

The protons crossing the sample lose a non-negligible quantity of energy which has to be dissipated in order to avoid volatilization of some elements as a result of increased temperature. This volatilization could influence significantly the determination.

To be sure that the cooling system was adequate to overcome this problem, an indirect measurement of temperature, during irradiation, was carried out by means of a thermistor.

A very small ($\phi \sim 0.7$ mm, L ~ 1 mm) resistance with a nominal value of 60 kΩ at room temperature, was inserted, during the preparation, in a serum sample with the same dimension of the samples under study. The dimension and thermal capacity of the resistor did not affect the temperature level of the sample.

For calibration the change in resistance value, caused by temperature change in an oven, was measured by means of a digital multimeter. Then the sample, with the inserted resistor, was irradiated in the same conditions as all the other samples analyzed. We observed that the temperature rapidly reached the steady value of ~ 40 °C which was then maintained during the whole irradiation.

Also if the accuracy of the determination was not very good, with this measurement we were able to assert that the temperature reached was lower than the one needed for inducing volatilization of Cr, which is at least 110 °C.[12]

Results and discussion

After having optimized the PNA method for Cr determination and controlled eventual sources of errors, we applied the PNA method to Cr determination in human serum samples.

With the procedure described in the experimental part, the serum samples and the standard samples were prepared and irradiated. After a waiting time of the order of 100 hours, gamma-spectra were registered for a measuring time of 72, and 24 hours, respectively, for the analyzed and standard samples. As an example, Fig. 2 shows a gamma-spectrum obtained from a sample.

From three independent samples we determined for Cr concentration a weighted average value of 0.0170 μg/g with a weighted standard deviation of 0.0019 (±11%). One has to remark that our measurements were performed on a mixture of sera

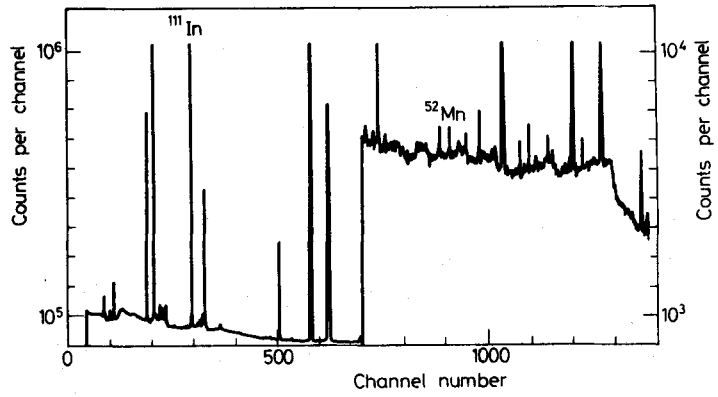

Fig. 2. Typical gamma-spectrum from an irradiated serum sample. The spectrum is presented with two different amplification scales

from 5 individuals, so that the quantitative results have to be considered only an indication of the average contents in human serum and not a mean value determined on a wide statistical population.

In literature we can find results spread over three orders of magnitude and precisely from 0.14 ng/g to 190 ng/g.[13] This range of values seems to be larger than the possible individual fluctuations, taking also into consideration the fact that usually the data reported are already an average referred to many individuals. As pointed out in the introduction, the Cr determination is affected by various problems, which may explain the discrepancies among the results reported in the literature.

We want to emphasize that the proton nuclear activation methodology is a nuclear technique without radiochemical treatment. Furthermore, due to the limited sample handling involved, the result obtained seems to be very useful for intercomparison studies.

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