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Non-invasive methods for express analysis of biological objects based on elemental analysis using synchrotron radiation on hair samples from animals and patients

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Abstract

Non-invasive methods of express analysis for mass monitoring of pathological states of human organism have been developed on the basis of X-ray fluorescence (XRF) elemental analysis of hair, nails, tooth enamel and skin. In addition, methods have been developed for studying sample preparations with the use of a universal X-ray fluorescence spectrometer mounted on a VEPP-3 storage ring. Data have accumulated over several geographical regions (Moscow Region, Chelyabinsk, Middle Asia, Novosibirsk Region, Maritime Region). The data of XRF analysis of hair samples from patients and animals mirror reliably differences in element concentrations. The methods can be useful in clinical medicine.

1. Introduction

Non-invasive methods of express analysis for mass monitoring of pathological states of human organism have been intensively developed using elemental analysis of hair, nails, tooth enamel and skin. The methods can be used both for determining the standard parameters of the so-called 'health status' and for diagnostics when tracing the dynamics of different stages of diseases, for choosing optimal therapeutic strategy, for monitoring the efficiency of treatment and, which is of special significance, for creating a data bank.

It is important to determine the correlation between the element concentration in the hair of patients in areas with different anthropologic 'pressure', especially in those areas where ecological catastrophes occurred (Chelyabinsk [1], Chernobyl). It is also of importance to reveal the influence of environmental conditions, pharmacological and dietary effects on the biological age and on shifts in it, relative to the astronomical age, under the action on the organism of unfavourable physical and chemical cofactors, such as radiation, pesticides, pharmacological and dietary effects, etc., which as a rule decrease the lifetime.

In this paper we attempt to find some integral parameter of cofactors affecting human organism because just a pool of factors is responsible for the shift observed. Researchers from China have undertaken similar studies [2] in which they analyse ratios of quantitative element concentrations for another age group. Joint papers of scientists from Cuba and Germany who studied element contents in hair samples from patients suffering from coronary diseases are available [3,4].

To accentuate the putative integral parameter, we performed model experiments on the hair of mice aging from 1 to 14 months, on the hair of rats of different age groups, on the hair of rats whose diet included nitrates with concentrations of $NaNO_3$ from 0.5 to 50 mg/ml and rats that inhaled highly radioactive plutonium and radioactive iodine, on the hair of dogs whose diet included risky doses of highly radioactive plutonium and that were exposed to external gamma-radiation from 50 to 200 roentgen. Hair samples of patients were collected in Branches No. 1 and No. 4 of the Biophysics Institute (Chelyabinsk), the birth dates of the patients varying from 1926 to 1985. A model sample was that from NIES (The National Institute for Environmental Studies) Certified Reference Material No. 5, Human Hair.

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2. Object of experiments and methods

Hair samples are a convenient material for monitoring the state of biosphere and accumulation of various elements in living organisms. The choice of hair as the main bioptate in multi-element analysis is explained as follows: (1) Hair is a protein tissue with a very low metabolic activity (it grows from 1 to 1.5 cm per month) and contains a 'record' of metabolic processes in the organism for a very long time. (2) The element content in hair mirrors individual peculiarities of a person such as sex, age, dietary and pharmacological effects, environmental conditions, etc. (3) The concentration of elements in hair is several times higher than in other biological preparations. (4) Hair samples can be easily obtained in a non-invasive way. (5) They can be stored for an unlimited time, can be frequently used for repeated analyses without damage, are easy to transport and can be sent by mail.

Synchrotron radiation (SR) used for multi-element analysis of biological preparations allows us to extend remarkably the method application because due to the high SR intensity it is possible for a short time to find a great number of elements with the sensitivity of 10^{-9} g/g in small amounts of a preparation. It appears to be possible to determine the element balance corresponding to a certain biological age as well as permissible deviations from the standard.

3. Sample preparation and spectra of analysed biological preparations

Hair samples and other biological preparations were collected over different geographical regions of Russia. To exclude the influence of external contaminations, the samples were treated preliminarily as recommended by the International Agency on Atomic Energy. The hair samples were washed with acetone for 10–15 min, then they were washed three times with bidistilled water and once again with acetone. After that the samples were dried at 80°C for 24 h, weighed, placed in mailer envelopes and stored in an exsiccator. The dimensions of a mailer envelope were chosen relative to the diameter of Teflon rings of a sample holder forming a cell window.

Survey of the samples was done at the Budker Institute of Nuclear Physics on the SRXRF station on a VEPP-3 storage ring.

XRF spectra of control samples of plants, nails and hair of volunteers from different geographical regions mirror the differences in the intensity of the same element lines in the samples from different regions. To illustrate the method, Fig. 1 shows spectra of hair of a family pair living and working in the same environmental conditions. The spectra of the hair sample of the husband who was treated with bromide preparations revealed enhanced intensity of the bromide line.

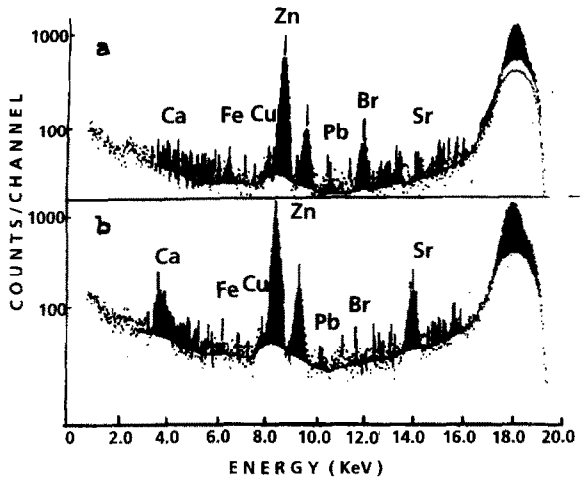

Fig. 1. X-ray fluorescence spectrum of the hair samples from a family pair: (a) husband, (b) wife. Differences in bromide concentrations are seen. The husband was treated with Br-containing preparations.

The spectra of hair samples of male representatives working at atomic industry enterprises (Chelyabinsk 65) revealed increased intensity of the lead line. Much lower lead line intensity was found in hair samples from other groups of patients. The spectra of the hair of a patient with a severe oncological disease showed complete absence of selenium though its traces were found in hair samples of other groups. In addition, spectra of some samples had an intensive bromide line that may be explained by bromide-containing preparations the patients were treated with.

Enhanced intensity of the bromide line was also detected in the spectra of hair samples from mice in a prolonged experiment which is not discussed herein. Such a result was quite unexpected because bromide-containing preparations were not added to the diet of the mice. A series of analyses showed that nutritive briquettes included in the diet of the mice contained high concentrations of bromide.

It should be noted that the dependence of element content in food as well as accumulation of the same elements in hair has no direct linear dependence. The concentration of strontium in the nutritive briquettes was higher than that of bromide, however the hair samples of the mice contained much higher amounts of bromide than those of strontium. Apparently, chemical properties of elements such as the ion charge, ion radius, polarization, etc., affect the process of element interaction with keratins, i.e. proteins that are the main hair components.

4. Processing of XRF spectra

The XRF spectra were analysed in two stages: first, the primary assay and estimation of the areas of the fluores-

Table 1
Dependence of the element concentration in animal hair samples on external conditions and other factors

Age (month)	Sex (1 – male, 2 – female)	External conditions	C (ppm)			Fe/Zn ratio
			Fe	Zn	Ni	
Mouse						
1	1		63.03	243.23	50.37	0.259
	2		57.48	222.76	121.24	0.258
2	1		50.16	226.23	3.03	0.222
	2		139.49	213.74	18.35	0.639
3	1		34.12	200.46	4.10	0.170
	2		52.51	205.85	9.60	0.255
4	1		40.81	175.61	1.16	0.232
	2		56.06	215.75	2.21	0.260
7	1		33.57	209.68	1.55	0.160
	2		100.26	255.95	0.18	0.392
10	1		57.00	211.9	9.43	0.269
	2		68.6	236.89	0.47	0.289
14	1		40.51	195.86	1.14	0.207
	2		71.98	222.71	3.13	0.323
Rat						
3			36.81	234.99		0.157
2		^{90}Sr	103.34	228.08		0.453
1			39.25	212.38		0.185
0.5		γ-rad., $NaNO_3$ – 50 mg/l, J – 50 μR/s	218.70	255.99		0.854
0.5	1	control	33.08	226.67		0.146
1	1	$NaNO_3$ – 0.5 mg/l[a]	46.11	187.7		0.246
1	1	$NaNO_3$ – 50 mg/l[a]	39.64	298.08		0.133
8	1	$NaNO_3$ – 0.5 mg/l	21.25	211.76		0.100
14	2	$NaNO_3$ – 0.5 mg/l	33.98	239.04		0.142
8	1	$NaNO_3$ – 5 mg/l	19.23	192.33		0.1
14	2	$NaNO_3$ – 5 mg/l	17.26	191.84		0.09
8	1	$NaNO_3$ – 50 mg/l	53.75	199.38		0.27
	1	control	26.66	212.53		0.125
	1	$CuSO_4$ – 15 mg/kg per day[b]	18.58	212.54	Cu-14.6	0.087
	1	control	18.16	231.33	Cu-14.9	0.078
	1	Pu-inhalation (^{239}Pu)[c]	16.49	182.15		0.09
	1	Pu-inhalation (^{239}Pu)[c]	20.49	217.36		0.096
Dog[d]						
2–4 years	1	Pu-inhalation (1982)	90.61	141.31		0.641
2–4 years	1	control	206.69	177.46		1.165
2–4 years	1	Pu-inhalation (1985)	121.52	142.49		0.853
2–4 years	2	Pu-inh. (1984) + 100 R γ-rad	80.23	84.09		0.954
2–4 years	2	control	166.8	169.17		0.986
2–4 years	1	50 R γ-rad (1982)	143.15	158.32		0.904
2–4 years	1	50 R γ-rad (1984)	99.00	165.18		0.599
2–4 years	1	100 R-γ-rad (1984)	224.32	147.57		1.52
2–4 years	1	200 R γ-rad (1984)	207.7	159.83		1.299
2–4 years	2	200 R γ-rad (1985)	44.3	220.33		0.201
2–4 years	1	control	198.23	201.18		0.985
Person[e]						
4 y. 11 m	2		173	123		1.41
5 years	2		73.68	57.3		1.286
9 years	2		58.1	130.8		0.444
9 years	2		73.4	258		0.285
10 years	2		63.8	280		0.228
10 years	1		71.6	154		0.465

Table 1 (*Continued*)

Age (month)	Sex (1 – male, 2 – female)	External conditions	C (ppm)			Fe/Zn ratio
			Fe	Zn	Ni	
10 years	1		114.4	190.4		0.601
12 years	1		69.7	296		0.235
13 years	1		105.9	266		0.398
13 years	2		30.65	211		0.145
14 years	1		155	214		0.724
29 years	2		108.8	232		0.469
29 years	2		67.04	188.92		0.355
32 years	2		53.7	236		0.227
46 years	2		30.88	164.05		0.188
51 years	2		83	181		0.459
62 years	2		11.75	123.42		0.095
64 years	1		66.30	157.59		0.421
72 years	2		20.83	187.39		0.111

[a] Exerted on parents during pregnancy.
[b] Duration of external influence is 45 days.
[c] 2 month Pu-inhalation.
[d] The samples were received in December 1992.
[e] Standard NIES CRM No. 5, Human Hair$^{Fe\,-\,225\ ppm}_{Zn\,-\,169\ ppm}$, Fe/Zn = 1.331.

cence peaks, and second, estimation of the element concentrations and monitoring the reproducibility. The peak areas were calculated by a special program using Gaussian non-linear fitting by the least squares method. The estimation of the concentrations was done using the NIES hair standards [5]. Zinc concentrations in the samples studied were calculated followed by calculations of the concentrations of other elements according to the Zn concentration using tables of basic parameters.

When calculating the Zn concentration, normalization to the Compton radiation peak was done since the hair samples were rather non-homogeneous and it was inexpedient to use external monitoring of the SR beam. The choice of Zn as a reference element is explained by its very high concentration both in the samples studied and in the standard samples and, accordingly, by well-grounded statistical analysis.

Correctness and reproducibility were controlled at all the stages of the analysis. On recording the spectra, the standard spectrum was recorded frequently (over 3 samples). The analysis of reproducibility of the element concentration values in the standard sample provides a possibility both to take into consideration smooth changes in the parameters of the synchrotron radiation beam during the lifetime of the electron beam in the storage ring and to make necessary corrections.

5. Results

5.1. General characteristic

The preliminary results reported herein are reproduced and substantiated. However their statistical and correlation analyses are required. Reproducibility by the standard is satisfactory for elements heavier than iron. The main results of the studies are summarized in Table 1. The studies have revealed that the element concentration in the hair of patients and animals, food products and water included in the diet mirrors reliably the age and sex differences, dependence on the diet and water (investigated on animals), and the influence of environmental conditions. It should be stressed that in clinical samples the element concentration varied reflecting the character of pharmacological action and, maybe, illness (oncological disease).

5.2. Age differences

The hair samples collected in Branches Nos. 1 and 4 of the Biophysics Institute were from patients with birth dates from 1926 to 1985. It is clearly seen that the hair samples of children contained much lower amounts of calcium and strontium as compared to those of adult patients. The samples of mouse hair from the age group varying from 1 to 14 months had no tendency of increasing the concentration of calcium, zinc, iron, strontium and copper.

5.3. Sex differences

It was found that the hair samples of female patients contained larger amounts of iron and especially nickel as compared with those of male patients. This is compatible with the available data obtained by other methods.

5.4. Dependence on the element concentration in the diet

When studying the element concentration in the diet, it

was found that the hair of rats under experiments contained high rate amounts of such elements as cobalt, bromide, strontium and copper. This was surprising because the same results were obtained for hair samples from the control animal group. It appeared that high rate amounts of these elements are caused by the diet. The nutritive briquettes for the animals contained excess amounts of the above elements.

5.5. Influence of environmental conditions

The hair samples of patients from Branch No. 1 of the Biophysics Institute contained a greater amount of lead than those from Branch No. 4. It was postulated that this is due to environmental conditions at enterprises where large amounts of lead are used as a protective material. However, researchers from Branch No. 1 believe that high rate amounts of lead in the samples are caused by exploitation of private cars.

5.6. Clinical studies. Influence of pharmacological preparations on the element concentration

High rate amounts of bromide were detected in the hair samples of patients from Branches Nos. 1 and 4 of the Biophysics Institute. The results were checked and reproduced. When decoding them, it was found that the presence of bromide is connected with pharmacological action on the patients. In some cases the presence of bromide indicated its slow release from the organism since its intake had been terminated a few months prior to the studies.

Thus, the XRF analysis of hair samples from patients and animals shows reliable differences in element concentrations, dietary variations, pharmacological and other effects over different geographical regions. The analysis can be used to solve the posed problem.

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