THE POSSIBILITY OF DIRECT ANALYSIS OF BIOLOGICAL TISSUES OF A FEW MILLIGRAMS BY SRXRF METHOD

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The investigations were done in

Nikolaev Institute of Inorganic Chemistry SB RAS

in collaboration with

Budker Institute of Nuclear Physics SB RAS

and

Meshalkin Institute of pathology of circulation of the blood
The main stages of analysis by SRXRF tissues samples
At each stage it is necessary to select the optimum conditions for the analysis of specific samples.

1. Sampling
   - lyophilization
   - Freezing
   - Chemical fixation

2. Sample preparation
   - Pressing of crushed material
   - Drying under load
   - Pressing without rubbing
   - Identity matrix

3. Selection of reference samples
   - Known concentration analysis. elements
   - Accounting corrections to the absorption (\( \mu \))

4. Measurements of the spectra SRXRF
   - Normalization on inside standard
   - Other modes of normalization
   - Normalization on Compton

5. The calculation of the concentration of elements determined
Study changes the elemental composition of the samples of biological tissue by chemical fixation. It is very important to make the correct sampling, especially when samples are very small mass (<20 mg).

**Experimental conditions**: fragments of rat heart. Formalin fixation samples (pH = 7) from 4 hours to 6 days. Determining elements: Cl, K, Ca, Fe, Cu, Zn, Se, Br, Rb, Sr.

Content of element (K, Zn) fall over time. The result of the experiment was shown, what it is advisable to use when sampling is freezing the samples to obtain the most accurate picture of the elemental composition of the tissue.
You see the finished sample 2: the standard and the test sample. We must be sure that the tablet is about 3 mg of the standard sample and dry film (3 mg) are identical.

Experiment on identity: experimental sample weight-560 mg. Drying time - 14 days from the mass of dry samples 15 mg to 1 mg.

Sample preparation thin fragment of tissue (wet tissue), drying **without heating** (within a day), while under pressure by **slow drying**.

Packaging samples Mylar film, after step sample preparation - **dry tissue** - from 0.5 to 15 mg.

This method of sample preparation may be the **only available**, if necessary, quantitative analysis of samples of tissues of small mass, including biopsy material, where the weight of the unit mg.
Specimen: 1- infarct zone, 2-the zone of periphery of infarct, 3-myocardium of left ventricle, 4-myocardium of right ventricle, 5-myocardium of left auricle, 6-myocardium of right auricle

Samples n=20, Standarts n=7.

The value Coh / Incoh varies slightly (Sr = 5%) for the study of muscle tissue, and reference materials.

That confirms in this case, the validity of the use of different standards with a matrix different from the sample matrix.
Mass attenuation coefficients of samples with biological and geological matrices

Curves mass attenuation coefficients of samples with biological and geological matrices were measured and plotted.

For mollusk muscle tissue standards and serum, despite the fact that their biomatrix different in nature, there is no significant difference for both the scattering characteristics, and the relative sensitivity coefficients spectrometer. While the concentrations of many elements differ by an order!
Calculation of concentrations (ppm ± SD) K, Ca, Mn, Fe, Co, Cu, Zn, Br, Rb, Sr, Mo sample in NCS ZC 85005 Beef liver- beef liver (0.0196 g / cm²) of the sample relative to the standard IAEA Soil- 7 - the soil (0.0508 g / cm²) with and without correction for absorption (μ).

When the difference between the mass attenuation coefficients of the analyzed sample and a standard in 6 times vary is possible to obtain correct results of the analysis adjusted for absorption and peak normalization on the Compton scattering.

Experimental Station SRXRF analysis
BINP SB RAS

VEPP-3
$E_{ex} = 2$ GeV, $B = 2$T, $I_e = 100$ mA;

**Station**
Monochromator
Si (111);
Square beam
$2 \times 5$ mm$^2$;
The excitation energy
8 - 42 keV
Determined elements: from S to U
Si(Li) detector (OXFORD, 10 mm$^2$),
energy resolution (at 5.9 keV - 135 eV)
4. Measurements of the spectra SRXRF

The fall of the current storage ring with time
(≈ 1 mA for elemental analysis VEPP-3 during one measuring station).

Needed normalization of the measured spectra to account for changes in the intensity of the exciting radiation

Fe, normalization on Compton

Peak area Kα line Fe with normalization on the peak area of Compton scattering at current values 120-60 mA 43 measurement for standard sample

\[ S_r = 0.7 \% \]
The metrological characteristics of the method were identified: reproducibility, detection limit and accuracy.

The reproducibility of the results of SRXRF analysis and uniform distribution of the chemical elements in samples of myocardium

Reproducibility (Sr1%) SR-XRF analysis of myocardial tissue sample.

The degree of inhomogeneity of distribution of chemical elements in the sample (Sr2%).

The detection limits ($C_{\text{min}}$):

$$C_{\text{min}} = 3.29 \cdot C_{\text{st}} \cdot \sqrt{\frac{N_{\text{bgr}}}{N_p - N_{\text{bgr}}}}$$

$N_p$ - the integrated peak area (Ka-line) defines the elements,
$N_{\text{bgr}}$ - background area,
$C_{\text{st}}$ – concentration determined element in the standard sample.
VALIDATION ACCURACY OF THE RESULTS OF SRXRF ANALYSIS OF TISSUES SAMPLES (m = 8-10 mg) by t-criterion.

<table>
<thead>
<tr>
<th>Element</th>
<th>SRXRF, ppm, liver</th>
<th>AЭС-ДДП, ppm, liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>5400 ± 600</td>
<td>-</td>
</tr>
<tr>
<td>Ca</td>
<td>91 ± 21</td>
<td>86 ± 7</td>
</tr>
<tr>
<td>Mn</td>
<td>5.4 ± 0.9</td>
<td>6.8 ± 0.5</td>
</tr>
<tr>
<td>Fe</td>
<td>1290 ± 230</td>
<td>1050 ± 90</td>
</tr>
<tr>
<td>Cu</td>
<td>14.4 ± 2.7</td>
<td>11.0 ± 0.9</td>
</tr>
<tr>
<td>Zn</td>
<td>98 ± 18</td>
<td>106 ± 11</td>
</tr>
<tr>
<td>Se</td>
<td>5.0 ± 0.9</td>
<td>-</td>
</tr>
<tr>
<td>Br</td>
<td>30 ± 3</td>
<td>-</td>
</tr>
<tr>
<td>Rb</td>
<td>30 ± 5</td>
<td>-</td>
</tr>
<tr>
<td>Sr</td>
<td>0.090 ± 0.018</td>
<td>0.110 ± 0.012</td>
</tr>
</tbody>
</table>

Comparison of test results a liver sample 2 methods: SRXRF, two-jet arc plasma atomic-emission spectrometry (TJAP-AES)

<table>
<thead>
<tr>
<th>элемент</th>
<th>образец 1</th>
<th>образец 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>РФА-СИ</td>
<td>ИСП-АЭС</td>
<td>РФА-СИ</td>
</tr>
<tr>
<td>C, мкг/г (сухой вес)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>1000 - 1600</td>
<td>800 - 920</td>
</tr>
<tr>
<td>Ca</td>
<td>620 - 980</td>
<td>1100 - 1300</td>
</tr>
<tr>
<td>Mn</td>
<td>1.1 - 1.8</td>
<td>1.4 - 1.6</td>
</tr>
<tr>
<td>Fe</td>
<td>120 - 160</td>
<td>140 - 200</td>
</tr>
<tr>
<td>Ni</td>
<td>0.8 - 1.2</td>
<td>-</td>
</tr>
<tr>
<td>Se</td>
<td>0.33 - 0.49</td>
<td>-</td>
</tr>
<tr>
<td>Br</td>
<td>7.9 - 10</td>
<td>-</td>
</tr>
<tr>
<td>Rb</td>
<td>1.4 - 1.7</td>
<td>-</td>
</tr>
<tr>
<td>Sr</td>
<td>1.3 - 2.1</td>
<td>2.5 - 3.5</td>
</tr>
</tbody>
</table>

Comparison of test results 2 samples 2 methods: SRXRF, inductively coupled plasma atomic-emission spectrometry (ICP-AES)
Joint research with Meshalkin Institute of pathology of circulation of the blood System studies lasted for 9 years, was published in print for more than 30 publications in foreign and domestic journals.

**Material:**
pathologic – autopsy, biopsy, operating

**Advantage of synchrotron radiation:**
low-mass analysis of samples, lower detection limits, measurements with variation of energy of the exciting photons.

**Complexity analysis:**

1) the amount of material for analysis are limited (difficulty biopsy);
2) direct analysis of biological objects is often not possible:
   a) effect of the matrix,
   b) low concentrations of trace elements in the samples,
   c) the high volatility of some chemical elements (halogens, S, Se, Hg, As);
3) the complexity of using an internal standard (direct analysis);
4) selection of external standard with a matrix array of such sample
The sampling of myocardium tissue

The parts of myocardium, which were investigated:
1- infarct zone
2- the zone of periphery of infarct
3- myocardium of left ventricle
4- myocardium of right ventricle
5- myocardium of left auricle
6- myocardium of right auricle

**Different pathology:**
- congenital heart defect,
- acquired heart diseases,
- cardiac ischemia,
- vascular disease (aortic aneurysm, aortic dissection)
- cardiomyopathy (**heart transplantation**)
Healthy children (n = 5) and children with congenital heart disease (TMS) (n = 20)

These diagrams show the different parts of the heart the difference between the content elements in a healthy and pathological myocardium, congenital heart disease. Only Cu increasing the concentration is almost an order of magnitude.
Cardiac ischemia, n – 90, Healthy people, n – 7

All elements have increased the content of even one order of magnitude in patients with myocardial infarction. Fe and Zn have maxima in the area of infarction and scarring.

Such distribution pattern of trace element concentrations in different parts of the heart at different pathologies physiologists obtained for the first time.
Human heart transplantation

Studied 60 biopsy samples

Own heart recipient (autopsy)
dilated cardiomyopathy

The donor heart (Biopsy, autopsy)
Heart pathology - heart transplantation

(biopsy)

sample weight of 0.5 mg
The content of trace elements in LV myocardium infarct zone compared with normal LV.

The content of trace elements in the myocardium of the left ventricle in dilated cardiomyopathy compared with LV normal.
Using data on the distribution of chemical elements in the earth's crust, healthy and diseased heart were calculated the Pearson correlation coefficient, and provides a method of evaluating the functional state of the heart.

**Designations**

0-std - Earth's crust  
1-std - the human body  
2-std - normal heart  
3-std - cardiac disease  
X1 - own heart  
X2 - donor heart biopsy  
X3 - donor heart autopsy

K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ge, As, Se, Br

**Method of evaluating the functional state of the heart**

**Patent №2466389**

R01 between 0Std и 1Std, R02 between 0Std и 2Std, R03 between 0Std и 3Std.
Comparison of the Pearson correlation coefficient for the number of elements studied (n=18) by the example of the patient's disease cardiomyopathy (heart transplantation)

- Pearson's correlation coefficient for the 18 elements studied is $R_{03} = 0.144$ in a patient with a transplanted heart, there is **no correlation.**

- The same pattern is observed for the donor heart and to the heart of the recipient in the biopsy specimens and autopsy: $R_{0X1} = 0.022$  $R_{0X2} = 0.106$  $R_{0X3} = 0.020$

- There is no correlation between a healthy body and heart recipient, between the healthy and the donor heart biopsy: $R_{1X1} = 0.114$  $R_{2X2} = 0.030$

- **But there is a correlation between pathological cardiac and remote recipient's heart, the donor heart biopsy and autopsy: $R_{3X1} = 0.753$  $R_{3X2} = 0.746$  $R_{3X3} = 0.935$**

- And the same is a high correlation between the biopsy and autopsy donor heart: $R_{X2X3} = 0.925$
As a result of analysis by SRXRF fragments autopsy and biopsy material physicists to the following conclusions:

• The absence of the Pearson correlation coefficient in the transplanted donor heart (biopsies) and a healthy heart counted for 18 investigated elements makes it possible to assume that in the donor heart does not exist detected lesions. Perhaps it was one of the reasons for the unsuccessful outcome of transplantation of donor heart.

• **Cu** deficiency can provoke Marfan syndrome, aortic aneurysm formation.

• **Zn** deficiency leads to the development of heart disease.

• **Se** deficiency increases the risk of coronary heart disease, myocardial infarction and cardiomyopathy.

• In the myocardium of patients with ischemic heart disease due to lower content to increased content of CE, especially **Ca, Fe, Rb**.

• A high content of **Fe** in the arterial wall may indicate the early development of atherosclerotic processes.

• Dilated cardiomyopathy (**heart transplantation**) - reduced content of basic CE: K, Ca, Sr, Fe, Ni, Se compared with the normal 2 times. The only possible marker of disease cardiomyopathy is increased to 2 times the content of **Rb**.

• This is a direct non-destructive analysis of biological tissues **SRXRF** (weight less than <4 mg), we developed a methodological approach, which allowed for the first time to get the data presented here.
Thank You for attention