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# Method for measuring X-ray reflections of microelements of inorganic compounds in soft tissues

***I.F.Mikhailov[1], V.V.Negoduyko[2], R.M.Mikhaylusov[2], A.I.Mikhailov[1], S.S.Borisova[1]***

[1] National Technical University "Kharkiv Polytechnic Institute", 2 Kyrpychova str., 61002 Kharkiv, Ukraine
[2]Military Medical Clinical Center of the Northern Region, 5 Kultury str., 61000, Kharkiv, Ukraine

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An alternative method is proposed for measuring X-ray diffraction peaks from micro-impurities of inorganic phases in soft tissues. Unlike traditional XRD, each of the diffraction peaks corresponding to the interplanar spacing $d_i$, is measured using radiation with a wavelength of $\lambda_i \to 2d_i$. This makes it possible to register diffraction peaks at large θ angles, thereby increasing the angular resolution ~tanθ. This approach made it possible to increase the beam angular divergence Δθ from 0.05° (for standard XRD) to 3°, gain ~$(\Delta\theta)^2$ in the spectrometer luminosity, and reduce ~$(\Delta\theta)^{-2}$ radiation load per unit area of soft tissue. A criterion for choosing the wavelength and beam divergence based on the micro-impurity structure is proposed. The results of measuring the diffraction peaks of micro-impurities of iron oxides in soft tissues damaged by a fragment wound are presented. The excess iron content in damaged tissues was certified by the XRF method with calibration using standard samples and did not exceed 0.4 mass%. Measurements of the main diffraction peaks of iron oxides $Fe_2O_3$, $d$=2.69Å, as well as $Fe_3O_4$, $d$=1.61Å and FeO(OH), $d$=1.71Å, were carried out in Cl- $K_\alpha$ **($\lambda = 4.728$)** and Sc-$K_\alpha$ **($\lambda = 3.031$)**, respectively. We used the scheme of a portable EDXRF spectrometer with a secondary KCl-Sc target and an angular beam divergence of Δθ≈3°. Without radiation damage to soft tissues, the detection limits were reached: 0.12 mass% for $Fe_2O_3$, 0.059 mass% for $Fe_3O_4$ and 0.034 mass% for FeO(OH).

**Keywords**: X-ray diffraction, micro-impurities, soft tissues, EDXRF spectrometer, secondary target.

**Спосіб вимірювання рентгенівських відбиттів мікроелементів неорганічних сполук у м'яких тканинах**. *I.F.Mikhailov, V.V.Negoduyko, R.M.Mikhaylusov, A.I.Mikhailov, S.S.Borisova*

Запропоновано альтернативний метод вимірювання рентгенівських дифракційних піків від мікроелементів неорганічних фаз у м'яких тканинах. На відміну від традиційного XRD, кожен з дифракційних піків, що відповідає міжплощинному інтервалу $d_i$, вимірюється за допомогою випромінювання з довжиною хвилі $\lambda_i \to 2d_i$. Це дає змогу реєструвати дифракційні піки під великими кутами θ, тим самим збільшуючи кутову роздільну здатність ~tgθ. Такий підхід дозволив збільшити кутову розбіжність пучка Δθ від 0,05° (для стандартного XRD) до 3°, збільшити ~$(\Delta\theta)^2$ у світлосилі спектрометра та зменшити ~$(\Delta\theta)^{-2}$ радіаційне навантаження на одиницю області м'яких тканин. Запропоновано критерій вибору довжини хвилі та розбіжності пучка на основі структури мікроелементу. Наведено результати вимірювання

дифракційних піків мікроелементів оксидів заліза в м'яких тканинах, пошкоджених осколковим пораненням. Перевищення вмісту заліза в пошкоджених тканинах засвідчено методом РФА з калібруванням за стандартними зразками і не перевищувало 0,4 мас.%. Вимірювання основних дифракційних піків оксидів заліза $Fe_2O_3$, $d$=2,69Å, а також $Fe_3O_4$, $d$=1,61Å та FeO(OH), $d$=1,71Å, проводили в Cl- $K_\alpha$ (λ=4,728Å) та Sc-$K_\alpha$ (λ=3,031Å) відповідно. Використано схему портативного спектрометра EDXRF з вторинним випромінювачем KCl-Sc і кутовою розбіжністю променя Δθ≈3°. Без радіаційного ураження м'яких тканин досягнуто межі виявлення: 0,12 мас.% для $Fe_2O_3$, 0,059 мас.% для $Fe_3O_4$ і 0,034 мас.% для FeO(OH).

## 1. Introduction

Soft tissues are amorphous in structure and strongly scatter X-rays, since they contain chemical elements with a low atomic number (H, C, N, O) in the base. As a result of external influence on soft tissues, for example, when a foreign body is introduced, changes occur in their structure. These changes are due to the interaction of the chemical elements of a foreign body with the aggressive environment of the human body. As a result of this interaction, micro-impurities of inorganic phases arise, which can spread in soft tissues, causing harm to the body [1]. Structural analysis of such micro-impurities is a difficult task due to the need to use high-power X-ray beams, while at the object has low radiation resistance. Reducing the radiation load per unit area can be achieved by increasing the beam divergence at a constant beam power.

In the beam line of modern sources of synchrotron radiation for XRD, powerful beams of monochromatic radiation with a wavelength λ in the range from 0.5 to 1.5 Å are formed [2]. These beams have a very small angular divergence $\Delta\theta < 0.05°$ and give a large radiation load per unit area of the object. The most intense reflections from impurity inorganic phases are characterized by interplanar distances $d$ from 4 to 1.5 Å. When using the wavelength from the beam line in accordance with the Bragg law $\lambda = 2\ d\ \sin\theta$, these strongest reflections are at diffraction angles $\theta < 20°$. It follows from the Bragg equation that the angular distance between adjacent peaks with interplanar distances $d_1$ and $d_2$ sharply decreases with decreasing diffraction angle θ, namely,

$$" \theta = \tan\theta \frac{d_1 - d_2}{d}. \qquad (1)$$

Therefore, to separate the diffraction peaks at $\theta < 20°$, it is necessary to use beams with a very small angular divergence $\Delta\theta \le 0.05°$, which leads to a large radiation load per unit area of the object.

In this paper, we consider an alternative method for separating diffraction peaks using radiation with a wavelength of $\lambda \to 2d$. According to the Bragg equation, this makes it possible to significantly increase the diffraction angle θ and increase ~tanθ the angular distance between the peaks. Such an increase in the distance between the peaks allows the use of beams with a large angular divergence Δθ, which leads to an increase in the luminosity of the spectrometer $\sim(\Delta\theta)^2$ and a decrease in the local radiation load on the object.

## 2. Theory

Consider the dependence of the allowable beam divergence on the wavelength λ of the radiation used. In the approximation of the shape of the diffraction peak by the Cauchy function, its angular width $B$ on the diffraction pattern on a scale of 2θ is determined by the expression [3]:

$$B = b + \beta, \qquad (2)$$

where $b$ is the geometric width, $\beta = \frac{\lambda}{L\cos\theta} + 4\varepsilon\tan\theta$ is the physical broadening due to the dispersity of the blocks $L$ and the level of microdeformation $\varepsilon$.

Two adjacent peaks are observed separately in the diffraction pattern if the angular distance between them exceeds the half-width of each of these peaks, i.e.

$$2\frac{|d_1 - d_2|}{d}\tan\theta > b + \frac{\lambda}{L\cos\theta} + 4\varepsilon\tan\theta$$

$$\tan\theta\left\{2\frac{|d_1 - d_2|}{d} - 4\varepsilon - \frac{\lambda}{L}\cdot\frac{1}{\sin\theta}\right\} > b$$

Taking into account $\sin\theta = \frac{\lambda}{2d}$, we get

$$\frac{|d_1 - d_2|}{d} - 2\varepsilon - \frac{d}{L} > \frac{b}{2}\cdot\frac{\sqrt{1 - \frac{\lambda^2}{4d^2}}}{\lambda/2d} \qquad (3)$$

The left side of the inequality is determined by the structure of the sample and sets the measurement conditions – the value of the angular divergence and the wavelength of the probing radiation. In scanning modes θ-2θ and θ-θ, the geometric broadening $b$ is related to the angular divergence Δθ by the approximate ratio $b \approx 2$. It follows from equation (3) that increasing the wavelength λ to $2d$ makes it possible to

increase the divergence Δθ of the primary beam without worsening the resolution of the peaks.

The luminosity of the X-ray optical scheme, and hence the intensity of diffraction reflections, is proportional to $(\Delta\theta)^2$. Therefore, one should choose λ as close as possible to the value of $2d$ for the most intense peaks, i.e. in the range from 3 to 7Å. Evaluation by formula (3) shows that the measurement of the peak with $d$ = 2.0Å in the radiation with $\lambda = \mathbf{3.742}$ Å (K-$K_\alpha$) gives an increase in the luminosity ~$(\Delta\theta)^2$ by 215 times compared with the measurement at $\lambda = \mathbf{0.71}$ Å (Mo- $K_\alpha$). Radiation with this wavelength is not used in traditional XRD. However, in the range from 3 to 7 Å, there are fluorescence lines of the *K*-series of elements with atomic number *Z* from 14(Si) to 21(Sc).

Secondary targets made of appropriate materials can serve as a source of monochromatic radiation for X-ray diffraction studies in the scheme of an EDXRF spectrometer. The compact arrangement of the secondary target, sample, and detector in these schemes makes it possible to reduce the length of the beam path through air to 10–15 mm and not resort to evacuation of the beam path. In a compact measurement scheme on the EDXRF spectrometer, we managed to reduce the angular divergence to the level of Δθ≈3°. This limits the possibilities of analyzing the physical broadening of diffraction peaks. In accordance with Scherrer's formula $\Delta(2\theta) = \dfrac{\lambda}{L\cos\theta}$ [3], such a geometric peak width at $\lambda = \mathbf{4.72}$ Å (Cl- $K_\alpha$) and $\theta$ =70° corresponds to the block size $L$ =130 Å . Therefore, in the compact scheme, it is possible to analyze the peak broadening only from nanocrystalline samples with $L < \mathbf{100}$ Å. On the other hand, such measurements make it possible to increase the luminosity of the spectrometer by two orders of magnitude and achieve an unusually high sensitivity of phase analysis.

## 3. Experimental

The objects of research were samples of soft tissues near a capsule with a foreign body – a fragment of a projectile. According to XRD data, the main structural component of the fragment is the α-phase of iron. The structure of samples that were in the human body for various periods of time, from three months to 23 years, was studied. Samples of healthy tissues were chosen as the object of comparison. The biological material was removed from the paraffin blocks and placed into the working chamber of the spectrometer on ULTRALENE film.

X-ray fluorescence and diffraction spectra were measured on an EDXRF spectrometer SPRUT with a primary radiation source power

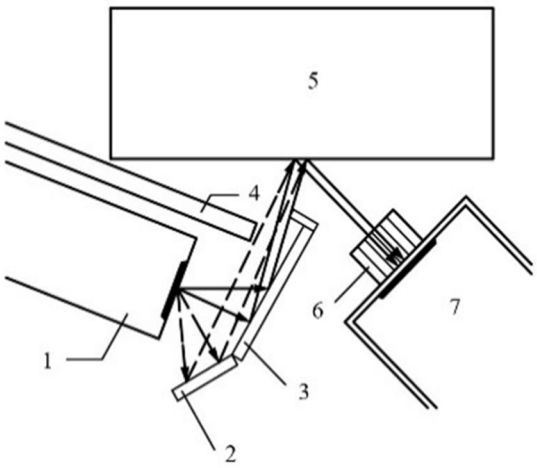


Fig.1 Measurement scheme with secondary emitters: *1* – X-ray tube; *2* – KCl secondary target; *3* – secondary target Sc; *4* – protective screen; *5* – test sample; *6* – collimator; *7* - detector.

of 15 W. A complex secondary target Sc-KCl was used (Fig. 1) [4]. Scanning by the diffraction angle θ was carried out according to the θ-θ scheme with precise displacement of the sample along the normal to the spectrometer axis (Fig. 1). The angular divergence of the collimation system ensured the value $b \le \mathbf{6}°$ of the geometric broadening on the standards. The angular range of scanning in θ is from 54 to 75°. Diffraction patterns were measured in Cl-$K_\alpha$ ($\lambda = \mathbf{4.724}$ Å), K-$K_\alpha$ ($\lambda = \mathbf{3.742}$ Å) and Sc-$K_\alpha$ ($\lambda = \mathbf{3.031}$ Å) radiations. The spectrum accumulation time at each scanning step in θ ranged from 300 to 600 s.

The content of iron $C_{\text{Fe}}$ was controlled by the fluorescence intensity of the Fe-Kα line by the method of calibration using standard samples and was calculated by the formula

$$I = I_b + \frac{\partial I}{\partial C} C_{\text{Fe}}, \qquad (4)$$

where $I_b$ and $\dfrac{\partial I}{\partial C}$ are background intensity and concentration sensitivity, respectively.

We used standard samples of aqueous solutions of RM-24 [5] with iron content from 0.02 to 0.2 wt%. The value of the detection limit was calculated by the formula

$$C_{min} = \frac{\mathbf{3}\sqrt{I_b}}{\dfrac{\partial I}{\partial C}}. \qquad (5)$$

The excess iron content was measured in the damaged soft tissue relative to the healthy one. The content of iron oxide phases was determined using the single-peak method of quantitative phase analysis [6]. The mass fraction $W_\alpha$

Table 1. Results of measuring the fluorescence intensity of the Fe-*Kα* line of standard samples of the PM-24 series in the range of mass fractions from 0.006 to 0.2 mass%.

| *C*, mass% | 0.006 | 0.012 | 0.040 | 0.120 | 0.2 |
|---|---|---|---|---|---|
| *I*, a.u. | 3270 ±60 | 3620 ±80 | 5250 ±75 | 9650 ±110 | 14200 ±130 |

of the α-phase was found from the ratio of the intensity of the diffraction peaks of this phase in soft tissue $I_\alpha$ and the diffraction peak $I_{\text{pure}}$ from a pure standard from this phase

$$\frac{I_\alpha}{I_{pure}} = \frac{W_\alpha \mu_\alpha}{W_\alpha (\mu_\alpha - \mu_m) + \mu_m}, \qquad (6)$$

where $\mu_m(\lambda)$ and $\mu_\alpha(\lambda)$ are the mass absorption coefficients of radiation with wave length λ in soft tissue and pure α-phase, respectively.

## 4. Results and discussion

The basis of the foreign body is iron. According to the XRD data, the structure of the foreign body is the α-phase of iron with a bcc lattice. The content of iron in damaged tissues, at a distance of ~ 1cm from a foreign body, was higher than in healthy tissue. It is this excess of iron, due to interaction with the aggressive environment of the body, that causes the appearance of foreign phases in damaged tissues. To determine the excess iron content in damaged tissues, use the data in Tables 1 and 2.

Calibration by standard samples (Table 1) is described by a linear regularization equation $I$=2952+56160·$C$ (mass%). With background intensity $I_b$=2952 a.u. and sensitivity $\frac{\partial I}{\partial C} = 56160 \frac{\text{au}}{\text{mass\%}}$, we get the detection limit value $C_{min} = 0.0029\text{mass\%}$.

According to formula (4), the excess iron content in samples of damaged tissue No.1, No.2 and No.3 was calculated relative to healthy tissue (Table 2).

Such a low content of excess iron in damaged tissues causes a low content of phases formed as a result of interaction with soft tissues. It is clear that extremely high sensitivity of phase analysis is required to measure the diffraction peaks of such phases.

Let us consider the results of determining phases from structural reflections. According to previous studies of the interaction of iron with soft tissues [7, 8], structural analysis revealed FeO(OH) particles associated with soft tissues, as well as iron nano-oxides. Therefore, we, first of all, carried out measurements of the diffraction patterns in the range of interplanar distances, where the strongest reflections of these

Table 2. Difference in fluorescence intensity of the Fe-*Kα* line for damaged tissues and healthy tissue samples, and the $C_{Fe}$ value of excess iron content in damaged tissues.

| | No.1 | No.2 | No.3 |
|---|---|---|---|
| I, a.u. | 22300±120 | 9100±120 | 6500±60 |
| $C_{Fe}$, mass% | 0.344 | 0.109 | 0.06 |

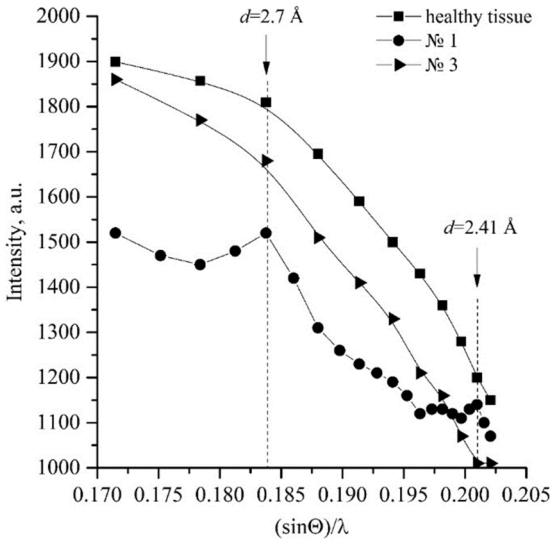


Fig.2. Diffraction patterns of soft tissue samples in Cl-*Kα* radiation (λ=4.724Å) in the range of interplanar distances d from 2.81 to 2.46Å. Initial – healthy tissue; No.1 – iron content 0.344 mass%;No. 3 – iron content 0.06 mass%.

phases are located: $Fe_2O_3$ ($d$=2.69Å, $I$=100), $Fe_3O_4$ ($d$=1.61Å, $I$=64) and ($d$=1.483Å, $I$=80), and γ-FeO(OH) ($d$=1.712Å, $I$=100). To reveal the reflection of $Fe_2O_3$ with $d$=2.69Å, measurements were made in Cl-$K$ radiation ($\lambda = 4.72$ Å) in the range of $d$ from 2.81Å to 2.37Å (Fig. 2). On curve 1 for healthy tissue, this reflection is not detected, but a monotonous decrease in intensity is observed with decreasing $d$. The curve for sample No.1 with the highest iron content revealed two reflections with $d$=2.70±0.01 Å and $d$=2.41±0.02 Å. The first peak has an intensity of about 100 a.u. and corresponds to $Fe_2O_3$. Let us determine the mass fraction of $Fe_2O_3$ by formula (6). The intensity of this reflection from

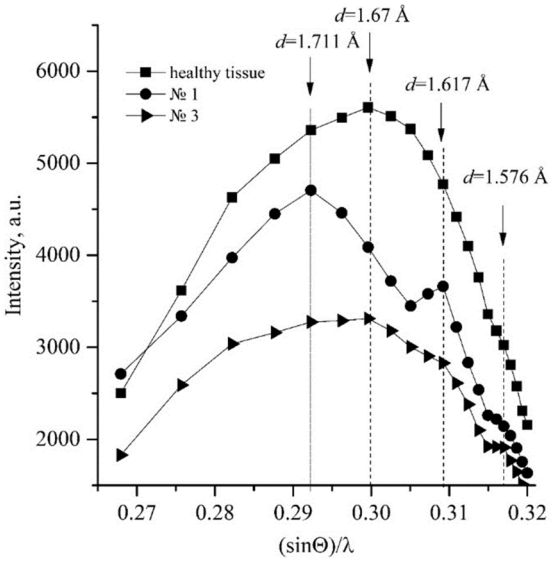


Fig.3. Diffraction patterns of soft tissue samples in Sc-$K_\alpha$ radiation (λ = 3.031Å) in the range of interplanar distances $d$ from 1.87 до 1.56Å. Initial – healthy tissue; No. 1 – iron content 0.344 mass%; No. 3 – iron content 0.06 mass%

the standard $Fe_2O_3$ sample is $I_{\alpha\text{-pure}} = 22000$, and the values of the mass absorption coefficients of Cl-$K\alpha$ radiation in $Fe_2O_3$: in the standard $\mu_\alpha =$ 646.3 and in soft tissue $\mu_m = 222.9$. In accordance with formula (6) $W_\alpha = 0.16$ mass%.

On the curve of sample No.3 with a minimum excess of iron (Table 2), these reflections are not detected. Let us estimate the limit of detection of the $Fe_2O_3$ phase by reflection with $d$=2.70±0.01 Å. In formula (5), we take the background intensity $I_b$=1370, and $\frac{\partial I}{\partial C} = \frac{1520-1370}{0.16} = 938\frac{\text{au}}{\text{mass\%}}$. Then, for the detection limit of the $Fe_2O_3$ phase, we obtain $C_{min} = 0.12$ mass%.

To detect reflections from $Fe_3O_4$ and γ-FeO(OH) with $d$=1.71 Å, measurements were made in Sc-$K_\alpha$ radiation (λ=3.031Å) in the range of $d$ from 1.87Å to 1.56 Å (Fig. 3). In this range, a halo from an amorphous substance is observed on the diffraction pattern of healthy tissue. In the soft tissue diffraction pattern of sample No.1, three diffraction peaks are observed against the halo background: $d$ = 1.711 Å, 1.615 Å, and 1.576 Å. The strongest of them, $d$ = 1.711 Å, corresponds to the most intense reflection of FeO(OH), the second with $d$ = 1.615Å corresponds to the $Fe_3O_4$ phase.

Let us estimate the content of the $Fe_3O_4$ phase. For the standard $Fe_3O_4$ sample, the reflection intensity with $d$ = 1.61 Å was $I$=86200.

By reflection with $d$ = 1.615 Å for sample No.1: $I$=500 a.u. (Fig.3) at $\mu^{Sc-K_\alpha}_{Fe_3O_4} = 196$ $\mu^{Sc-K_\alpha}_{sample} = 60.6$; we get $C_{Fe_3O_4} = 0.18$mass%. for sample No.3: $I$=150 a.u., $C_{Fe_3O_4} = 0.055$mass% . The detection limit estimate for $Fe_3O_4$ at $I_b$ ≈3000 и $\frac{\partial I}{\partial C} = \frac{500}{0.18} = 2780\frac{\text{au}}{\text{mass\%}}$ (sample No.1) gives $C_{min} = 0.059$mass% .

It is not possible to determine the content of FeO(OH) in sample No.1 from the reflection intensity with $d$ = 1.711 Å by direct comparison with the pure phase due to the lack of a standard. To estimate the intensity of the reflection of the FeO(OH) standard, we used the experimental value of the nearest reflection intensity of the $Fe_3O_4$ standard, taking into account the ratio of the structural factors of the $Fe_3O_4$ and FeO(OH) reflections. The estimate gives a value of $I$≈1700000 for the reflection of FeO(OH). With $\mu^{Sc-K_\alpha}_{FeO(OH)} = 178.4$ and reflection intensity $I$≈1000 for sample No.1, we get $W_{FeO(OH)} \approx 0.2$ mass%. For the detection limit of the FeO(OH) phase at $I_b$≈3200 and $\frac{\partial I}{\partial C} \approx \frac{1000}{0.2\text{mass\%}} = 5000\frac{\text{au}}{\text{mass\%}}$ (Fig.3, sample No.1) we get $C_{min} = 0.034$mass% .

The detection limits for iron oxides in soft tissues turned out to be less than 0.1 mass%, which is quite consistent with the best achievements of modern XRD.

## 5. Conclusions

The low radiation resistance of soft tissues makes it difficult to use narrowly collimated monochromatic beams with a wavelength of 0.5 to 1.5 Å from the beam line of modern synchrotron radiation facilities. An alternative to standard XRD is the method of measuring several of the strongest reflections with interplanar distances $d_i$ from impurity phases using radiation with a wavelength of $\lambda_i \rightarrow 2d_i$. Criterion (3) for choosing the wavelength and angular divergence of the probing beam has been developed. The choice of these parameters ensures a high sensitivity of the measurement of each peak at a minimal radiation load per unit area of the object.

In this work, the simplest scheme for measuring diffraction peaks on a portable EDXRF spectrometer is implemented. Even with its help, it was possible to measure the intensities of reflections from impurity phases, the content of which is ≤ 0.1 mass%. The creation of special facilities with adjustable beam divergence in the range from 0.5 to 3°, as well as the possibility of changing the wavelength of the X-ray source in the range from 3 to 8Å, will provide high sensitivity of the phase analysis of impurities in objects of biology and medicine without their radiation destruction.

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## References

1. E.A. Ryan, M.J. Farquherson, *Phys. Med. Biol.*, **52** 6679 (2007)
2. H. Cheng et al., *Synchrotron Radiation X-ray Powder Diffraction Techniques; Progress in Natural Science: Materials International*, **27**, 66 (2017), https://doi.org.//10.1016/ j.pnsc.2016,12,007.
3. M. Ermrich, X-ray powder diffraction –XRD for the analysis, PANalytical GmbH (2011)
4. I.F Mikhailov, A.I. Mikhailov, A.A. Baturin, L.P. Fomina, *Probl. Atom. Sci. Tech.*, **84,** 172 (2013).
5. Multicomponent Samples of the Composition of Metal Ion Solutions for Atomic Absorption Spectrophotometry – 263-00001.
6. F. Gozzo, *Quantitative phase analysis with diffraction methods, 13th Pharmaceutical Powder X-Ray Diffraction Symposium,* (2015).
7. M.H. Schweitzer et al., *Proc. Biol. Sci.,* **281,** 1775 (2014). doi 10.1098/rspb.2013.2741.
8. M. Krispin, *J. of Nanoparticle Research*, 2012. Doi: 10.1007/s11051-011-0664-4.