

Biostandard Surface Density Influence upon the Analysis of Biological Tissues by SR-XFA Method

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Abstract

In the analysis of biological tissue samples (human myocardium and vessels) obtained via biopsy using the SR-XFA method, a necessity arises to study the oscillator density variation effect on determining the content of chemical elements, since the thickness of samples varies depending on the type of tissue under investigation and on the mass of a sample (in the course of preparing samples). In this connection an effect of surface oscillator density on the relative sensitivity coefficients is investigated by the example of the NIST 1577 Bovine liver reference sample. The experiments were carried out at the elemental X-ray fluorescent analysis station on the basis of the Siberian Synchrotron Radiation Centre (Institute of Nuclear Physics, SB RAS, Novosibirsk). It has been established that the variation in density of a reference tablet (the excitation energy being of 17 keV) with the peak areas of elements under determination normalized to the Compton peak area exerts almost no effects on the results of elemental analysis for the elements ranging from Mn to Zn. For K, Ca, Fe and Rb there are considerable differences between the coefficients of relative sensitivity ($R_{i\text{st}}$) observed with the variation of oscillator density from 14.9 up to 45.8 mg/cm².

Key words: synchrotron radiation, biological tissues, X-ray fluorescent analysis

INTRODUCTION

In order to solve the problems connected with the environmental protection and the estimation of the environmental pollution level, new methods are required for the analysis of the content of chemical elements (especially heavy metals) in organic matrices. Research connected with the studies on the state of a human also demand to carry out efficient analysis concerning the content of chemical elements in tissues and biological liquids of humans. Either excess or deficiency of microelements in comparison with a normal physiological level of their content in an organism was revealed in patients with many severe forms of diseases including also the diseases of cardiovascular system [1–5]. Sensitive and selective analytical methods are required in order to determine the content of microelements in biological samples [3, 6].

The X-ray fluorescent analysis (XFA) is widely used for the analysis of various biological objects. Due to fast and simple sample preparing operation XFA has become a popular analytical method in many fields [3, 7, 8, 9]. The most simple method for determining concentrations in XFA consists in the use of a calibration curve plotted basing on data concerning characteristic lines in the spectra of standard samples (certified reference materials, CRM) with the corresponding correction absorption effects. The sensitivity coefficients established in this way for particular elements are used in determining the concentration of elements in the samples of unknown composition, adjusting for self-absorption of a sample.

The matrix of standard samples should be very close (concerning the chemical composition) the matrix of the sample under investigation. However, the analysis of an organic

material (biological tissues and liquids, environmental objects, *etc.*) is complicated due to a discrepancy between the variety of types the objects under investigation and a limited set of the types of standard samples with the matrix corresponding to the sample under analysis [8, 3, 10, 11].

An organic matrix (plants, biological tissues) consists mainly of C, N, H and O. Thus, the absorption of characteristic fluorescent radiation by the matrix relatively low in comparison, for example, with the samples of rocks or soils. It is well known that for a light matrix (with the analysis performed in a thin layer) the effects of absorption could be taken into account in case that the radiation scattered by a sample is used as an internal standard [12].

The authors of [10] were revealed that the thickness of a sample represents a critical factor and thus this value should be determined for each group of elements. In the case that the thickness of a sample is higher than a certain critical size, the intensity of a fluorescence emission line will not be proportional to the concentration due to the effects of radiation absorption by a matrix and the effects of subexcitation. On the contrary, in case that the thickness of a sample is less than a certain critical size, the sensitivity will be insufficient [10].

The need for solving such problems has arisen in the course of investigation using XFA method with synchrotron radiation (SR-XFA) applied to heart muscular tissue samples (myocardium) and the walls of vessels for determining the distribution of chemical elements throughout various parts of heart in normal state and in pathology (coronary heart disease (myocardial infarction), valvular defects, aortic aneurysm). The SR-XFA method allows one to carry out a direct nondestructive multielemental analysis of samples with small mass.

With the use the SR-XFA method one can perform the analysis of various biological objects and environmental objects. Non-identical geometry of samples and standards it is determined by uniqueness and variety of the objects under investigation (tissue fragments, pollen of plants, cereal grains) and represents a general problem in the analysis of any biological objects using the method of SR-XFA.

The present study has been initiated by the Meshalkin Novosibirsk Research Institute

of Circulation Pathology (Novosibirsk). Different types of tissues (myocardium, vascular walls, mitral valves) of both healthy people and at the patients with various cardiovascular diseases were studied.

Sample preparation includes holding under weight the samples under investigation (during 24 h and longer) up to complete drying and shaping the fragments of tissue to a plane-parallel geometry. The mass of samples obtained from biopsy varies over a wide range since the place wherefrom they are taken is strongly determined, whereas the process of sampling is carried out in the course of an operation. Owing to the variety of types (structures) of biological tissues and the difference in the mass of fragments with holding them under a constant weight there are considerable variations in the thickness of samples under investigation observed.

In this connection the present work is devoted to the experiments and calculations by the example of the Bovine liver NIST 1577 reference sample for determining the influence of oscillator tablet surface density (and thus the thickness) upon the coefficients of relative spectrometric sensitivity in order to study the efficiency of the method for taking into account non-identical geometry (thickness) of reference samples (used in calculation of chemical elements concentration in samples), with the peak areas of elements under determination normalized to the incoherent scattering peak area.

EXPERIMENTAL

All the experiments were carried out at the X-ray fluorescent elemental analysis station on the basis of the Siberian Synchrotron Radiation Centre (Institute of Nuclear Physics, SB RAS, Novosibirsk) whose schematic diagram is presented in Fig. 1.

The parameters of the VEPP-3 storage ring are the following: $E_e = 2 \text{ GeV}$, $B = 2 \text{ T}$, $I_e = 100\text{--}200 \text{ mA}$. The characteristics of the station [13]: beryllium film thickness being of 1 mm; the time of continuous measurements amounting up to 6–9 h; the chamber for the analysis is made of elconite; the monochromator represents a Si (111) single crystal; the maximum sample diameter being of 30 mm; the photon

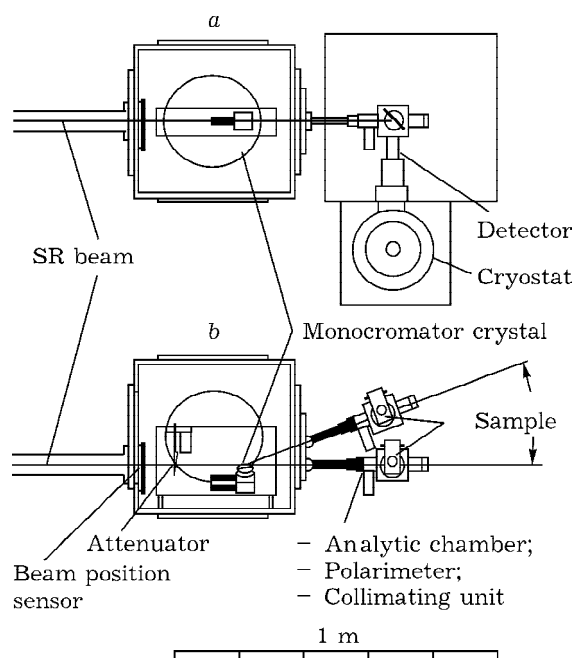


Fig. 1. Schematic diagram of an elemental X-ray fluorescence analysis station: *a* – top view, *b* – side view.

beam area ranging within 1–20 mm²; the exposition time ranging within 20–1000 s; the excitation energy amounting to 12–45 keV; the determination domain for elements ranging from K to Nd. The registration of fluorescent radiation is carried out with the use of an OXFORD Si (Li) detector (Oxford Instruments Inc., the USA; the energy resolution amounting to 130 eV at 5.9 keV line).

The essence of the SR-XFA method consists in the following: the initial synchrotron radiation (SI) beam passing through a monochromator crystal (Si (111)) being tilted at an angle of 45 degrees with respect to the surface of a sample generates the characteristic fluorescent radiation. The latter is registered by a semiconductor Si (Li) detector set at an angle of 90° with respect to the direction of the initial exciting radiation.

The intensity of SR is several orders of magnitude higher than the intensity of the radiation of a traditional X-ray tube. The synchrotron radiation is concentrated within a narrow angular range of the orbital plane of electrons; it is linearly polarized and exhibits a continuous smooth spectrum within a wide energy range. The vector of electric fields strength (*E*-vector) is positioned in the plane of the orbit of electrons. Having a detector located in the

plane of the *E*-vector of the monochromatized SR beam at an angle of 90° with respect to this beam, one could reduce the intensity of elastic scattering and Compton peaks and, correspondingly, the background height [13].

The SR-XFA method provides a unique way to carry out a direct analysis of biological samples (tissues) of small mass (up to 0.5 mg of dry solid matter) without destruction of a sample. In order to estimate the influence of the oscillator (tablet) density we have chosen one of available International standard reference samples such as NIST 1577 Bovine liver. The preparation of samples consisted in pressing a dry material of the reference sample (with the mass of 7.5, 9.2, 11.9, 13.4, 18.8 and 23.0 mg) to obtain tablets of 8 mm in diameter, by means of a hydraulic press ($P = 120 \text{ kg/cm}^2$). Further the pressed tablets were placed between two Mylar films 2.5 μm thick (for performing XFA) and were fixed by Teflon rings.

All the prepared tablets of oscillators satisfy the criterion of thin layer for XFA. The measurements were performed with the use of excitation energy $E_{\text{ex}} = 17 \text{ keV}$. The “live” time of measurement t_{live} amounted to 500 s. Three measurements were carried out for each tablet of the NIST 1577 Bovine liver reference sample with different density. The X-ray fluorescence spectra obtained were processed using a special software AXIL (Canberra Packard, Benelux).

For this reference sample, relative sensitivity coefficients ($R_{i,\text{st}}$) were calculated for each of the elements under determination and corresponding curves of relative spectrometric sensitivity were plotted for reference tablets with different density. The surface density ρ_{st} for the tablets of the reference sample amounted to 14.9, 18.3, 23.7, 26.7, 37.4, 45.8 mg/cm².

The values of detection limit (DL) for each element under determination were calculated according to the formula [14]

$$\text{DL} = 3.29 C_{\text{st}} \sqrt{N_{\text{b/g}}} / (N_{\text{p}} - N_{\text{b/g}})$$

Here N_{p} is the integrated peak area ($K_{\alpha 1,2}$ line) for an element under determination; $N_{\text{b/g}}$ is the background area; C_{st} is the content of an element under determination in the reference sample, μg/g.

The reproducibility of measurements was observed to vary within the range from 2 to 30 % (for different chemical elements).

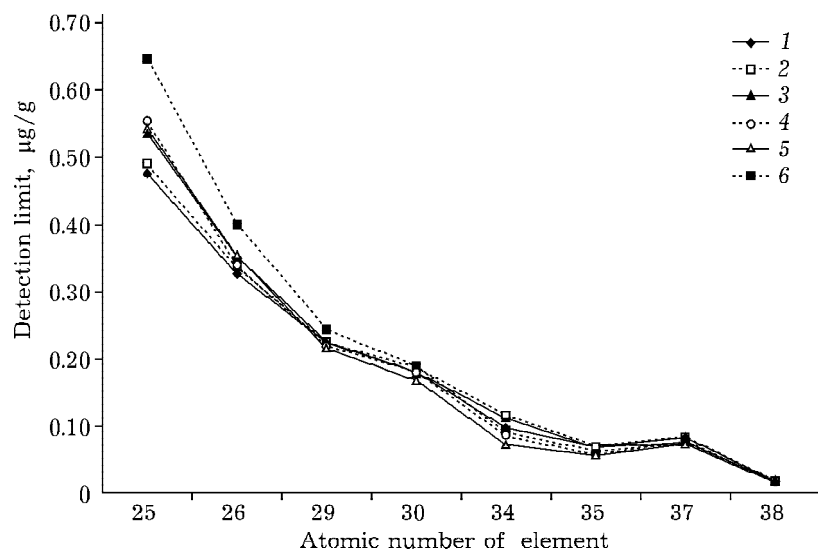


Fig. 2. Detection limits for Bovine liver NIST 1577 reference sample ($E_{ex} = 17$ keV) at different values of the reference sample surface density (ρ_{st}), mg/cm²: 14.9 (1), 18.3 (2), 23.7 (3), 26.7 (4), 37.4 (5), 45.8 (6).

RESULTS AND DISCUSSION

The detection limits for different values of surface density of reference sample tablets are presented in Fig. 2.

The relative sensitivity coefficients (R_{ist}) for each element in each case with different value of density of a reference sample tablet were calculated from the expression [3]

$$R_{ist} = \frac{N_i}{N_{inc}} \frac{1}{C_{ist}} \quad (1)$$

Here N_i is the integrated $K_{\alpha,1,2}$ line peak area of peak for the i -th element; N_{inc} is the peak area for incoherent scattering of radiation (Compton scattering); C_{ist} the concentration of the i -th element in the reference sample (certified value).

The relative spectrometric sensitivity curves obtained were approximated by second order polynoms [15]. The R_{ist} coefficients have been calculated only for those chemical elements whose content in the Bovine liver NIST 1577 reference sample is certified (Table 1).

Figure 3 demonstrates the curves of relative spectrometric sensitivity for all the density values for reference sample tablets with the use of the Compton peak as an internal standard. One can see that the curve corresponding to the minimum density of a tablet (14.9 mg/cm²), is much more flat in comparison with the curve obtained for the maximum density of the Bovine liver NIST 1577 reference sample. The re-

gion of the minimal divergence between the curves (and their crossing) lies within the range of $Z = 25-30$ (i.e. from Mn to Zn). This fact allows one to elucidate what the elements under determination exhibit minimal influence of

TABLE 1

Certification data for the concentration of elements in Bovine liver NIST 1577 reference sample, µg/g

Element	C ± Standard deviation
S	7500*
Cl	2660*
K	9700±600
Ca	121±6
V	0.1*
Cr	0.1±0.12
Mn	10.2±1.3
Fe	263±8
Co	0.2*
Ni	0.2*
Cu	190±10
Zn	131±13
As	0.10±0.005
Se	1.1±0.1
Br	9.1*
Rb	18.2±1.0
Sr	0.2*
Mo	3.2*

* Reference value.

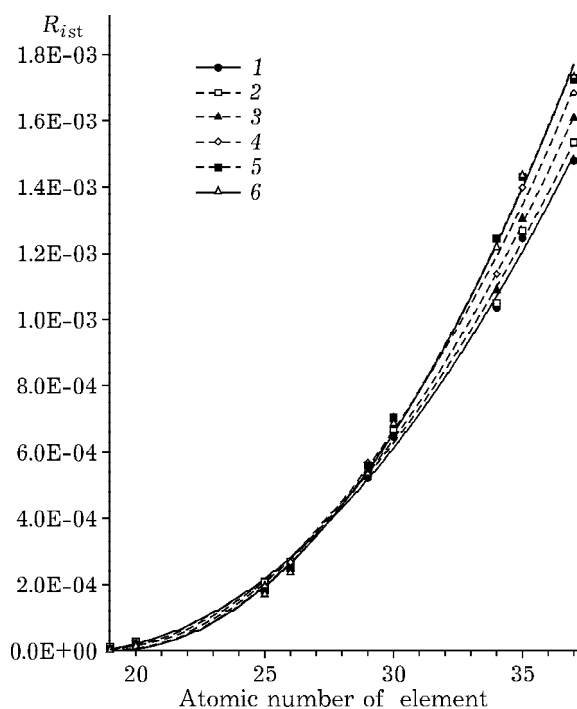


Fig. 3. Relative spectrometric sensitivity curves for Bovine liver NIST 1577 reference sample ($E_{ex} = 17$ keV, Compton peak being used as an internal standard). See Fig. 2 for notations.

reference tablet surface density ($E_{ex} = 17$ keV). For light elements (up to Mn) the determining factor represents the thickness of a tablet (the geometrical factor), whereas the divergence between the curves within the range of heavier elements (from Zn to Rb) can be explained by the reduction of the background contribution (incoherent scattering) to the total fluorescence signal with a simultaneous increase in number of fluorescent radiation quanta due to the increase in the mass (density) of the reference sample tablet.

Figure 4 displays the curves of relative spectrometric sensitivity for all the values of surface density of reference sample tablets obtained with the use of $K_{\alpha 1,2}$ peak of Mn as an internal standard. It is seen that the divergence of curves in this case is greater than with the use of the Compton peak as an internal standard. It is connected with the fact that using Mn as an internal standard the intensity of fluorescent radiation varies with sample thickness variation both for an element under determination, and for Mn. With the increase in the sample thickness, the signal from manganese flattens out faster than the signal from a (heavi-

er) element under determination. Thus, the use Compton peak allows taking into account to a maximal extent the differences in the geometry of samples. The region of minimal divergence between curves (and their crossing) in this case lies within the range of $Z = 22-26$ (from Ti to Fe).

Figure 5 demonstrates data concerning the coefficients of relative spectrometric sensitivity for the two extreme superficial density values for reference sample tablets, minimal value (14.9 mg/cm^2) and maximal one (45.8 mg/cm^2), the Compton scattering peak being used as a standard. The data are presented with taking into account the standard deviation. It is seen that the coefficients R_{ist} are considerably different for K, Ca and Fe, whereas for Mn no significant differences were revealed.

Figure 6 demonstrates similar data concerning relative sensitivity coefficients for chemical elements under determination from Cu and on. Here, any significant differences are observed only for Rb.

The standard deviation for R_{ist} of each chemical element is determined as it follows

$$\begin{aligned}
 (SD_{R_{ist}} / R_{ist})^2 &= (SD_{N_i} / N_i)^2 + (SD_{N_{inc}} / N_{inc})^2 \\
 &+ (SD_{C_{ist}} / C_{ist})^2
 \end{aligned}
 \quad (2)$$

Here N_i is the value of integrated peak area for the i -th element under determination; N_{inc}

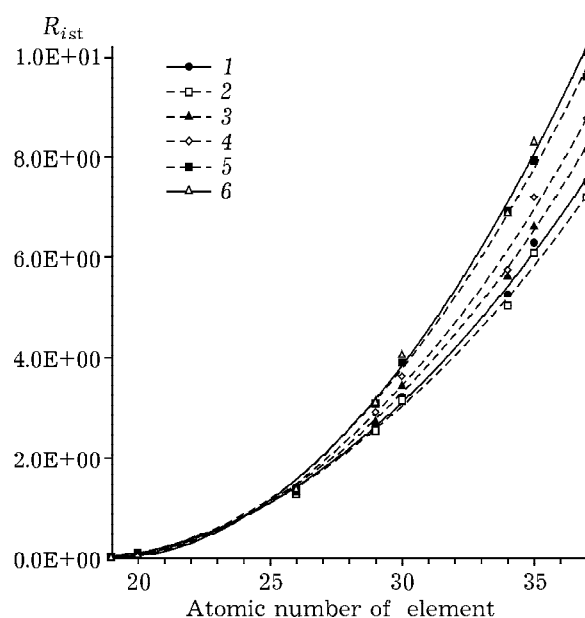


Fig. 4. Relative spectrometric sensitivity curves for Bovine liver NIST 1577 reference sample ($E_{ex} = 17$ keV, K_{α} peak of Mn being used as an internal standard). See Fig. 2 for designations.

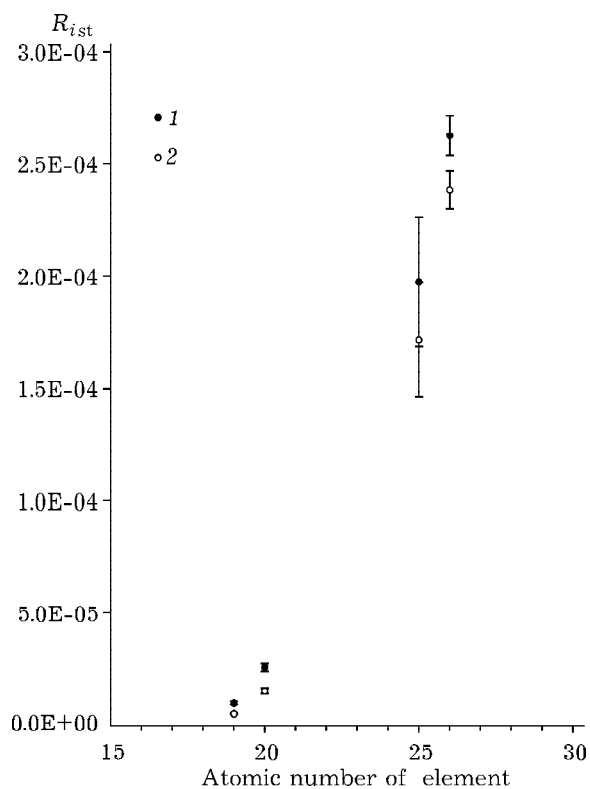


Fig. 5. Relative spectrometric sensitivity coefficients for Bovine liver NIST 1577 reference sample ($E_{\text{ex}} = 17$ keV). ρ_{st} , mg/cm²: 14.9 (1), 45.8 (2).

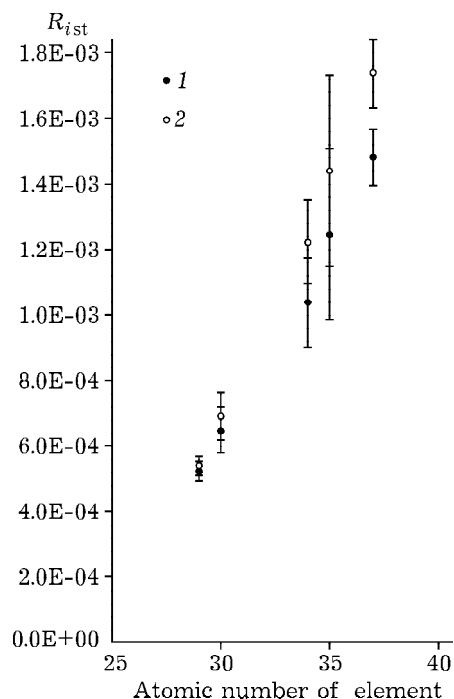


Fig. 6. Relative spectrometric sensitivity coefficients for Bovine liver NIST 1577 reference sample ($E_{\text{ex}} = 17$ keV). See Fig. 5 for designations.

is peak area for incoherent scattering of radiation; $C_{i\text{st}}$ is the concentration of the i -th an element in the reference sample (certified value); SD_{N_i} , $SD_{N_{\text{inc}}}$, $SD_{C_{i\text{st}}}$, $SD_{R_{i\text{st}}}$ are the standard deviation values for corresponding parameters.

Taking into account the reproducibility of results ($n = 3$ for each element and each density value), we obtained final values of the standard deviation for $R_{i\text{st}}$, presented in Figs. 5 and 6. The main contribution to the determination error for $R_{i\text{st}}$ (final) is drawn by the standard deviation of certified values for elemental concentrations and by the reproducibility of measurements (experimental value) (Fig. 7).

After determining the confidence interval for $R_{i\text{st}}$ values in the case of minimal and maximal surface density for tablets of the Bovine liver NIST 1577 reference sample, one can observe significant differences only for K and Ca (Table 2). The operation of the VEPP-3 storage ring is of cyclic nature, thus a decrease in electronic current (I_e) and, correspondingly, in the SR intensity is observed in the course of measurements.

In order to establish the influence of the decrease in the intensity of initial exciting radiation upon the results of measurements and, in particular, upon the type of relative sensitivity curves for different density values of reference sample tablets, the values of $R_{i\text{st}}$ determined according to expression (1) were additionally normalized to the peak area value for coherent radiation scattering. However, no considerable differences were revealed after performing this procedure. Thus, the character of

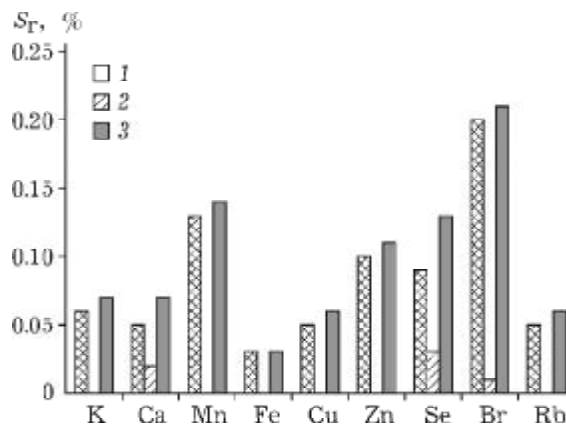


Fig. 7. Relative standard deviation for chemical elements whose content is certified for Bovine liver NIST 1577 reference sample ($\rho_{\text{st}} = 14.9$ mg/cm²; $E_{\text{ex}} = 17$ keV).

TABLE 2

Confidence interval for the values of relative spectrometric sensitivity coefficients $R_{i, \text{st}}$ at minimal and maximal values of sample tablet surface density ρ_{st} ($n = 3$, $P = 0.95$)

Element	ρ_{st} , mg/cm ²	
	14.9	45.8
K	$8.31 \cdot 10^{-6} - 1.2 \cdot 10^{-5}$	$4.3 \cdot 10^{-6} - 5.9 \cdot 10^{-6}$
Ca	$2.1 \cdot 10^{-5} - 3.0 \cdot 10^{-5}$	$1.2 \cdot 10^{-5} - 1.8 \cdot 10^{-5}$
Mn	$1.3 \cdot 10^{-4} - 2.7 \cdot 10^{-4}$	$1.1 \cdot 10^{-4} - 2.4 \cdot 10^{-4}$
Fe	$2.4 \cdot 10^{-4} - 2.8 \cdot 10^{-4}$	$2.2 \cdot 10^{-4} - 2.6 \cdot 10^{-4}$
Cu	$4.5 \cdot 10^{-4} - 5.9 \cdot 10^{-4}$	$4.7 \cdot 10^{-4} - 6.1 \cdot 10^{-4}$
Zn	$4.8 \cdot 10^{-4} - 8.2 \cdot 10^{-4}$	$5.1 \cdot 10^{-4} - 8.7 \cdot 10^{-4}$
Se	$6.99 \cdot 10^{-4} - 1.4 \cdot 10^{-3}$	$9.10 \cdot 10^{-4} - 1.5 \cdot 10^{-3}$
Br	$5.99 \cdot 10^{-4} - 1.9 \cdot 10^{-3}$	$7.12 \cdot 10^{-4} - 2.2 \cdot 10^{-3}$
Rb	$1.3 \cdot 10^{-3} - 1.7 \cdot 10^{-3}$	$1.5 \cdot 10^{-3} - 2.0 \cdot 10^{-3}$

the relative spectrometric sensitivity curves obtained depends mainly on gross composition of the reference sample (matrix), on oscillator layer (reference sample tablet) density and on exciting quanta energy (E_{ex}).

CONCLUSION

In the studies on the influence of oscillator density upon the relative sensitivity coefficients (by the example of the Bovine liver NIST 1577 reference sample) we have established that with the use of the Compton peak as an internal standard the variation in reference tablet den-

sity (at the main operation exciting energy amounting to 17 keV) exerts almost no effect on the results of determining the elements from Mn to Zn. Significant differences in $R_{i, \text{st}}$ (with regard for the standard deviation) in case that oscillator tablets surface density varies from 14.9 to 45.8 mg/cm² are observed for K, Ca, Fe and Rb.

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