

STANDARDLESS X-RAY FLUORESCENCE ANALYSIS OF ENDODONTIC SEALERS USING A PORTABLE SPECTROMETER

IOANA SUCIU¹, ELENA S. PREOTEASA², E. A. PREOTEASA³, CATALINA CHIOJDEANU³,
B. CONSTANTINESCU³, B. DIMITRIU¹, PAULA PERLEA¹,
AL. A. ILIESCU⁴, DANA BODNAR¹

¹Carol Davila Medical University, Faculty of Dentistry, Bucharest, Romania

²Helident Ltd. Dental Surgery, Bucharest, Romania

³Horia Hulubei Institute for Physics and Nuclear Engineering, Bucharest, Romania

⁴Carol Davila Medical University, Faculty of Dentistry, Craiova, Romania

E-mail corresponding author: eugenpreoteasa@gmail.com

Received November 4, 2014

We report a preliminary study on three types of endodontic sealer products carried out by thick target X-ray fluorescence (XRF) using a portable spectrometer. The aim of this study was to assess the potential of this method for the analysis of biomaterials used in the root canal dentistry. The main difficulty of this study was the lack of reference materials. Therefore semiquantitative analysis was performed, and relative concentrations of elements were determined from the XRF spectra with a mean accuracy of about 42% using a dedicated software. To overcome the contributions of matrix effects inherent in thick target analysis, we applied various approximate corrections. All the three formulations contained a major heavy metal like W and Bi for radio-opacity. Two similar products contained both Bi as major element, but they could be differentiated by their minor elements, *i.e.* Ca and Ba in only one of them. Another minor element was Zr, and trace elements were detected in the three materials – Fe and possibly Cu – probably impurities from raw materials of different origin. All three endodontic sealers could be recognized unequivocally by brands based on the XRF analysis, a result of potential interest for forensic applications. Improved quantitative XRF analysis of these and similar materials may bring compositional insight relevant for biocompatibility studies of these materials and for further developments in endodontic research.

Key words: XRF analysis, portable spectrometer, standardless multielemental analysis, thick target, approximate corrections, endodontic sealer, specimen preparation, biocompatibility, dentistry.

1. INTRODUCTION

Endodontic sealers are used in dentistry in the root canal therapy and generally show a clinically acceptable biocompatibility. However certain aspects of their action are not completely understood and further investigation of their composition, characteristics and interactions is needed. Testing of new techniques such as atomic analysis methods may open new perspectives.

Dental research greatly benefits of the atomic and nuclear surface and thin layer analysis methods, although their advent in this field is rather recent. Such techniques analyze elemental and chemical composition, topography, mineral particles morphology and crystallographic structure by monitoring the photon, electron and ion emissions from surfaces of teeth and dental materials when they are excited by ion, electron or photon bombardment [1]. Among the most popular of these analytical methods one can mention *X-ray fluorescence (XRF)*, *particle induced X-ray emission (PIXE)*, *particle induced gamma-ray emission (PIGE)* and *elastic recoil detection analysis (ERDA)*. XRF and related analysis methods are currently used by our group in a wide palette of applications, of whom the study of archaeological artifacts and minerals represents our main concern (e.g., [2–4]). We previously approached the study of teeth and of dental composites and other dental materials by PIXE [1, 5–10], PIGE [1, 8], ERDA [1, 5, 6] and XRF [1, 5]. All these methods make sensitive multielemental analyses, are completely or almost nondestructive and cover a high dynamic range of values in the spectra; therefore they can give at a glance relevant insight from major to trace elements in biomedical applications [11, 12].

Of the above mentioned atomic and nuclear techniques, *energy-dispersive spectrometry (EDS)* XRF is the fastest and most versatile multielemental nondestructive analysis method and it proved already its potential for various types of dental applications. A more cumbersome but more sensitive XRF technique, *wavelength-dispersive spectrometry (WDS)* succeeded to evidence in teeth sex dependent differences in the content of Al, Pb and Fe, age dependent correlations of Mg, Fe, Cu, Sr and Pb concentrations, pollution origin of increased Zn, S and Pb, and high levels of Zn and Pb concentration in smokers [13]. In another kind of application, XRF showed that the levels of Pb in teeth from children residing in Pb-polluted industrial environments were increased, because lead is accumulated for long time in the dental tissue [14]. We previously studied by EDS-XRF excited with an $^{241}\text{Am}/^{237}\text{Np}$ source the diffusion of Ag^+ ions from an AgNO_3 solution in dental enamel, and we found that Ag increased and Ca decreased according to power functions of the incubation time in the presence of Ag (Preoteasa, Preoteasa and Gavrilus, unpublished, presented in [1]). EDS-XRF of dental composites proved to be a useful analysis method for forensic applications [15, 16]. Restorative resins have distinct elemental compositions that allow identification of brand and they survive extreme conditions such as cremation with little change, which may serve for victim's identification in plane crashes or fires. Concerned with the elemental transfer processes at the interface with the dental tissues, we used XRF together with PIXE and ERDA in the analysis of dental composites with different elemental compositions [5]. In addition to composites, we studied by PIXE three calcium hydroxide commercial blends used in endodontics and we found some significant differences both in the concentrations of Ca and at the level of trace elements (Mn, Fe, Cu, Zn, Sr, Ba, Pb) [10].

Endodontic sealers are another class of dental materials which, just like restorative resins, are formed by mineral particles embedded in an organic matrix. They are applied in the root canal of the tooth and must show a good tissue tolerance, which depends on both their organic and inorganic components. They develop at a high rate and the investigation of new products may benefit of the atomic and nuclear analysis methods. To our knowledge they have not been studied before by XRF or any other of these techniques.

As compared to the ion beam analysis methods in standard configurations, which analyze only solid samples in high vacuum, XRF has the advantage that it works in air and may analyze also semi-solid (or liquid) samples. This is important in particular for some endodontic sealers, which do not settle easily in solid form. However, solid or semi-solid samples are thick samples which undergo the so-called 'matrix effects'. The latter diminish the intensity of X-ray fluorescence by self-absorption in the specimen and complicate any attempt of quantitative XRF analysis by non-linear effects, which mean that the intensity of emergent radiation is no longer proportional to the element's concentration. Therefore caution is needed for quantitative analysis, and the most reliable approach requires the use of reference materials or standards with an elemental composition and physical structure close to the analyzed specimen. This procedure is not always possible for the analysis of dental materials, because dedicated and certified reference materials do not yet exist and because the composition information given by the producers is generally very scarce due to the fabrication secret. Thus only more or less approximate standards are available. We applied this technique previously in the PIXE analysis of dental composites [6] and of endodontic calcium hydroxide formulations [10]. However, in the case of endodontic sealers we have not even by far similar standards, as suggested by the qualitative composition given by one producer for such a material (AH Plus) [17]. Accordingly, we have to perform a standardless XRF analysis of endodontic sealers, based on our previous experience with standardless analysis of teeth, bones and dental materials [6].

In the present study we use a portable XRF spectrometer, which is a relatively new instrument and its database has been constructed mostly for metal alloys and environmental soils. Nevertheless, it does not require liquid nitrogen for cooling the semiconductor detector, it is very convenient and simple in use and demonstrated its potential in forensic applications [15, 16].

In brief, here we report a preliminary study on three commercial endodontic sealers carried out by XRF, aiming to evaluate the potential of a portable spectrometer for the standardless analysis of such biomaterials used in dentistry. The ability of the method to evaluate relative concentrations and to assess the sources of errors and inaccuracy in the measurements and in the processing of spectra are analyzed.

2. XRF – A BRIEF OVERVIEW

X-ray fluorescence (XRF) is an atomic thin layer elemental analysis method which uses beams of X- or γ -rays for excitation and which detects the characteristic X-rays emergent from the sample as produced under the primary radiation bombardment. The experimental and technical principles of XRF were established in the second half of the 1920s based on the atom physics revolution spanning the first two decades of the last century, and X-ray spectrometry was developed into a current analysis method after WW2. At various levels and detail, the topic of XRF is widely covered in literature [18–22]. There are many biomedical applications of XRF [12, 23], which include also studies of teeth and dental materials like composites [5, 13–16]. A monographic account of applications of XRF and of other atomic and nuclear surface analysis methods in the study of restorative materials is available [1].

At the core of XRF stands Moseley's law, which shows that the square root of the frequency of the characteristic X-ray lines of the K, L and M series scales like their atomic number Z – and thus makes possible the unambiguous analysis of chemical elements based on their specific spectra. The phenomena underlying the origin of the X-ray spectra are simple in the essence. Consider for example the excitation of a K line. Following the absorption of an X-ray or γ photon, an electron is ejected from the K ($n = 1$) shell and leaves here a vacancy, provided that the energy of the incident X photon exceeds a minimum threshold. Subsequently, an electron from the L or M shell will occupy the vacancy in the K shell, and an X-ray radiation quantum of the $K\alpha$ or $K\beta$ lines will be emitted. The quantum energy $E = h\nu$ will be given by difference between the initial and final energy levels. The X-ray spectra are much more simple and interference of lines from different elements present in samples with complex elemental composition is much more uncommon as compared for instance with the optical emission spectra. This recommends XRF as a method of choice for the analysis of multielemental samples with previously unknown composition.

Any XRF spectrometer is composed of a X- or γ -ray excitation source (X-ray tube, radioisotope or synchrotron), the sample and an X-ray analysis detector. There are two basic types of XRF spectrometers – wavelength-dispersive (WDS) and energy-dispersive (EDX). They differ by the principle of analyzing the secondary X-rays emitted by the sample. Here we are concerned with the EDX spectrometer, which uses an energy-dispersive semiconductor crystal detector. The charge pulse produced in the detector by an X-ray photon is converted to a voltage pulse signal which is further amplified and sent to a multichannel analyzer and then to computer. Because the detector is protected by a thin beryllium window, the detection of elements is usually possible only for elements heavier than F but may include the rest of the periodic table up to U. (In WDS which uses for detection gas proportional counters with a thin Mylar window, one can see also elements lighter than F.)

In EDX the spectrum of multielemental samples is acquired quickly in a simultaneous mode. This is a practical advantage as compared to the slow sequential analysis performed by the WDS spectrometer which analyzes one element after the other.

The energy resolution in EDX depends on the X-ray energy and detector and, with semiconductor detectors at 77 K, is about 150 eV at 5.9 keV (Mn K α); the resolution is poorer than in WDS where the photons are already sorted by diffraction according to their wavelength. The poor resolution produces peak interference in EDX, however less frequently as compared to optical emission spectroscopy.

The minimum detection limit (MDL) in X-ray fluorescence *e.g.* for transition metals, is of the order of 10 mg/kg (or ppm) for thin samples and of about 100 mg/kg (or hundredths of percents) for thick samples; the detection limit depends on the analyzed element and excitation source. At the same time, the XRF spectrum just like in most atomic and nuclear analysis methods covers a high dynamic range of concentrations, from the MDLs to tens of percents.

In the case of thick samples such as dental materials, a major contribution to the loss in sensitivity is caused by the increase of radiation background. This is due among other to bremsstrahlung produced by photo- or Compton electrons ejected from atoms inside the sample material under the action of the primary X-rays, as well as to the so-called *matrix effects*, which diminish the intensity of X-ray fluorescence by self-absorption in the specimen. The matrix effects complicate the analysis because the X-ray intensity is no longer proportional to the concentration, and they are dependent on the composition, structure and heterogeneity of the sample.

Whether WDS or EDS, all XRF spectrometers excited with X-ray tubes (or with synchrotron radiation) have the disadvantage that the *incident radiation is scattered* both coherently (Rayleigh) with the energy unchanged and incoherently (Compton) at lower energy, giving intense background lines at the high energy end of the spectrum. Thus a Rh anode (K α ~20.2 keV) disables practically the 18–21 keV spectrum domain, while an Ag anode (K α ~22.2 keV) interferes with the 20–23 keV region. In addition to the intense reflections of the anode's characteristic lines, Compton peaks from low Z samples like dental materials can give appreciable contributions. The backscattered continuous background from the bremsstrahlung of the X-ray tube will reduce the analytical sensitivity, especially in the low-energy part of the spectrum.

With common semiconductor detectors, when two or three pulses are counted together because they are very close in time, single pulses are recorded at energies equal to the sums of the energies of the two or three quanta. This effect is called *pile-up* and the resulted false lines are a source of errors especially when the latter elements are at trace levels.

Other false peaks which occur in the X-ray spectra recorded with semiconductor (and gas proportional) detectors are *escape peaks*. When the energy of an X-ray photon entering a detector exceeds the absorption edge of the semiconductor (or the gas atoms), it may be absorbed by photoelectric effect by an atom from the detector active medium, and characteristic radiation will be emitted from the ionized atom, carrying away its energy outside the detector. As the two events occur very fast, within the temporal resolution of the detector, the energy of the emitted characteristic X-ray quantum will be subtracted from that of the primary photoelectron ejected by the incident photon.

One important advantage of XRF techniques is that it doesn't require the covering of the electroinsulating samples with a thin conductive layer. The XRF analysis of any type is completely non-destructive and almost totally non-invasive; it does not burn or heat significantly the thin analyzed surface layer of the irradiated specimen and there are no macroscopically visible effects; however at a microscopic level the X-rays produce radiolysis.

In brief, energy-dispersive XRF is a rather sensitive, multielemental, conveniently specific, and nondestructive instrumental technique. Although other methods (like PIXE and SIMS) have a better sensitivity, EDX is faster, does not alter the sample surface, works at atmospheric pressure, is more simple to operate and requires less expensive equipment. At the same time the X-ray spectra are much simpler than optical emission spectra used in LIBS analysis. In qualitative analysis XRF can analyze specimens with previously unknown composition, detecting all elements excepting those with lines in the domain of Rayleigh and Compton scattering of the radiation from the X-ray tube. Additional caution is necessary in eliminating the pile-up and escape peaks and in analyzing peak interferences. In the quantitative analysis of thick samples, important corrections which require complex calculations, as well as reference materials with similar composition and structure, are necessary for accounting the matrix effects. One major difficulty in the analysis of dental materials, both by XRF and by other atomic and nuclear methods is the lack of standard materials and certified information on sample's composition [1, 5, 6].

3. MATERIALS AND METHODS

3.1. MATERIALS AND SAMPLE PREPARATION

Three materials were used: a polymethacrylate resin-based sealer Real Seal (Pentron Clinical Technologies LLC, Wallingford, CT) and two epoxy resin-based sealers, AH Plus and AH26 (Dentsply International Inc, York, PA).

We used a preparation technique similar to that developed previously for the PIXE analysis of dental composites [5]. Disk shaped samples of 7–8 mm diameter with a flat surface were prepared from the pastes on polished glass plates. The

samples were let to solidify, keeping them for several days at room temperature in Petri dishes partially covered to allow vapor to go out but preserved in a closed space to avoid dust deposition. However, the materials required long setting times and were adherent and brittle after solidification, even after use of a photopolymerization light or incubation at 40 °C. Because the samples were not completely solidified and adherent, a special Teflon gondola with a thin Mylar film at the bottom was used for the measurements. The samples were put in the gondola on the Mylar membrane bottom with their flat surface down and the gondola was positioned on top of the portable EDX spectrometers.

As reference materials a pellet of powdered hydroxyapatite and previously analyzed Tetric Ceram and Ariston composites (Ivoclar-Vivadent, Liechtenstein) were used, but the comparison was only semiquantitative because of the different compositions and structures.

3.2. XRF MEASUREMENTS

Two portable EDX-XRF spectrometers outfitted with X-ray tubes having Rh and Ag anodes and with semiconductor detectors cooled with Peltier elements were used. More specifically they were X-MET 3000 TX+ spectrometers (Oxford Instruments) with tubes operated at 40 kV and 0.006 mA anode current for 60 sec, having Si PIN diode detectors. The energy resolution was of 275 eV for the Mn K α line, that is, not so good as that mentioned above for liquid nitrogen-cooled semiconductor detectors, but sufficient for the present preliminary investigation.

These instruments could analyze all elements from a light element like K (or S at high concentration) to U, except those having characteristic radiation in the energy interval of anode's radiation coherently and incoherently scattered by the samples. This interval is defined between the Compton scattered K α line to the Rayleigh scattered K β line of the anode: Rh, Pd, Ag, Cd, In for the Ag anode (K α ~22.2 keV, Compton ~20.6 keV, K β ~24.9 keV) and Ru, Rh, Pd, Ag for the Rh anode (K α ~20.2 keV, Compton ~19.0 keV, K β ~22.7 keV).

To evaluate the concentrations, the spectra were processed with the WinQXAS version 1.30 software (IAEA, Vienna) in the „thick sample” mode in order to make corrections for matrix effects. The estimated concentrations and the count numbers of the characteristic X-ray lines were listed. For Bi only the count numbers were listed.

3.3. THE X-RAY YIELD CURVES PROCEDURE FOR THE ANALYSIS OF XRF SPECTRA

The lack of reference materials inherently resulted in significant limitations of our attempts of quantitative analysis. The samples were thick samples and therefore the WinQXAS software made corrections for matrix effects during the

calculation of elements' concentrations. However, these corrections did not account for the granularity of the materials which further complicate the analysis of spectra. This resulted in obviously wrong concentration values for some elements, especially for Ca, while for the other the results were probably distorted by unknown inaccuracy. This effect is substantial and more important for L lines than for K lines. The matrix effects as measured by the shift from linearity increase steadily with the particle size; in particular, for particles of 12 μm diameter and of mean atomic number 28, they are about 10% for K lines and about 80% for L lines (West 1984). For YbF_3 granules of mean atomic number 24 and of 10–50 μm size as seen by $\mu\text{-PIXE}$ in the dental composite Tetric Ceram, we estimated granularity corrections of about 50% of the evaluated concentrations [1].

In order to minimize as much as possible the largest errors affecting certain elements in particular, we applied a technique similar to that used before for PIXE spectra of dental calcium hydroxide preparations [10]. For this purpose, mean („smoothed”) yield values were calculated by fit of the empirical yields resulting directly from the software output vs. atomic number Z and then the concentrations were recalculated. The yield is defined as the ratio of count number to concentration,

$$Y_i = \frac{N_i}{C_i} \quad (1)$$

where C_i – absolute concentration of element i analyzed in the specimen; N_i – count number of the characteristic line of element i in the specimen's spectrum; and Y_i – yield for element i .

The empirical yield was given by the software output of concentrations and count numbers of the corresponding lines in the spectra. Separate plots were made for K lines and L lines. This procedure allowed also the estimation of concentrations of heavy elements with L lines which were not calculated by the software (Bi). The empirical data were mean values for the three endodontic sealers pooled together with five dental composites (Preoteasa, Preoteasa and Chiojdeanu, unpublished). The composites' (and the hydroxyapatite) data were necessary in order to increase the number of elements in the yield plot of L lines to at least four by including Yb and Hf from the former (and Pb from the later), so as to allow the fit with empirical functions.

The procedure described above provides a simple and convenient approximation aimed to improve the results confronted with the difficult problem of the matrix effects in the XRF analysis of thick targets. It is not a rigorous solution, among other because it does not take into account the absorption edges for the major elements which could introduce discontinuities in the yield curves. Moreover, for the lighter elements like Ca and for the heavier ones like Bi it uses extrapolation and other rough approximations. However, it gives improved Ca values as compared to the direct estimates made by the software processing, and

makes possible the estimate for Bi which otherwise were not possible at all. The evaluation of Bi is particularly important for the analyzed dental sealers, because in two of them (Real Seal and AH 26) Bi was shown to be the major element seen by XRF. "Absolute" concentration values thus obtained are to be considered only indicative and correct only as order of magnitude. Relative concentrations, expressing for example percent values of the total detected elements, while the light XRF-invisible elements are neglected, should be more reliable.

4. RESULTS AND DISCUSSIONS

4.1. THE QUALITATIVE COMPOSITION OF THE BIOMATERIALS REVEALED BY XRF

The XRF spectra (Fig. 1) evidenced regularly the elements from Ca ($Z = 20$) on, with characteristic X-ray energies above 3 keV either in the K and L series. Lighter elements were not visible in the spectra, or produced occasional lines too weak for any quantitative evaluation attempt due to the reduced detector sensitivity and to absorption in the windows and air at low energy. As a rule, with the XRF technique used one can not practically analyze elements having lines with lower energy than ~ 3.3 keV, *e.g.* emitted by elements lighter than K ($Z = 19$), because of detrimental absorption of low energy X-rays; an exception to this rule involving the $M\alpha$ lines of very heavy metals will be mentioned below. The results of the qualitative elemental XRF analysis of the endodontic sealers based on the visual evaluation of the spectra as well as on their software processing are presented in Tab. 1.

All the three endodontic sealers were dominated by the presence of a very heavy metal: W ($Z = 74$) in AH Plus and Bi ($Z = 83$) in AH 26 and in Real Seal (Fig. 1, Tab. 1). Most probably, the presence of these metals was intended to provide a strong radio-opacity, necessary to give sufficient radiological contrast against the hard dental tissues as they were designed to fill the root canal of the teeth. By comparison, usually the dental composites contained only Ba ($Z = 56$) for radio-opacity [5] because these restorative materials are used in more superficial locations in the teeth structure.

In addition to a major heavy metal (W, Bi), AH Plus and Real Seal contained one or two minor elements such as Ca, Zr or Ba, and all contained Fe as a trace element. The software processing of the AH 26 spectra evidenced also Pb as a possible minor element, but the lack of any visible Pb $L\alpha$ and $L\beta$ line in the spectra makes improbable the presence of this element in the material. At the same time low level of Bi was found in AH Plus by the software although it was not seen in the spectrum; although the presence of this element was not clear, we admitted it because the Bi lines were covered by those of W and Zr.

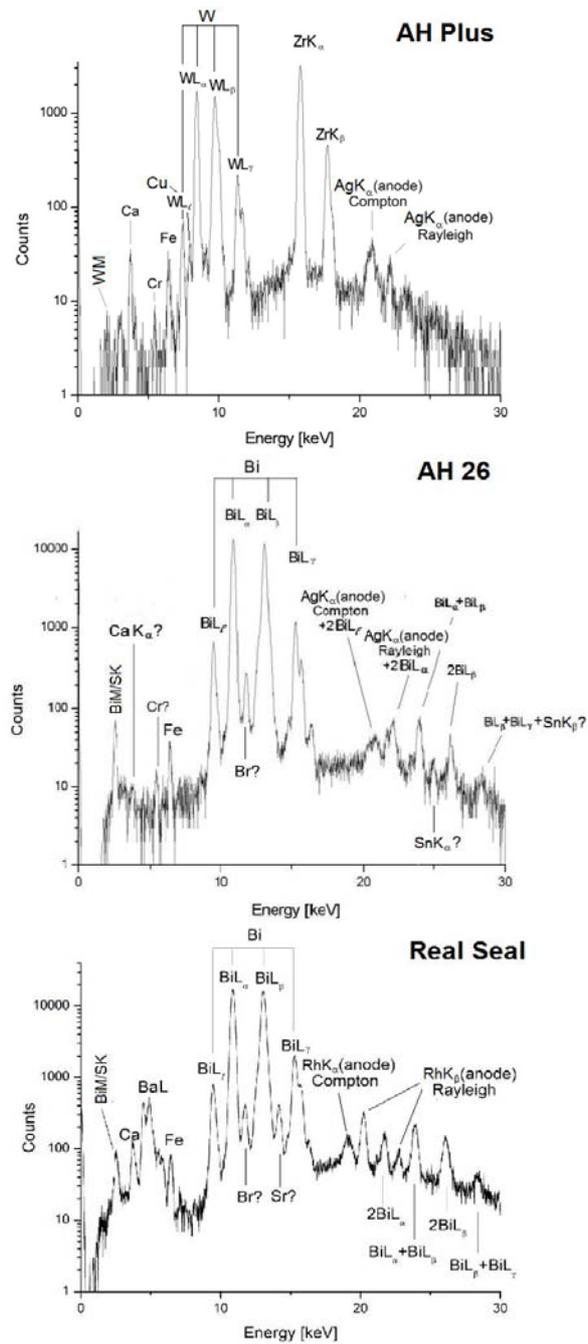


Fig. 1 – XRF spectra of endodontic sealers AH Plus, AH 26 and Real Seal recorded with a portable spectrometer. The X-ray tubes' anodes were made of Ag (AH Plus, AH 26) and of Rh (Real Seal).

In the case of AH Plus, the producer provided qualitative information about the nature of the constituent compounds which were at the origin of the XRF spectrum, namely CaWO_4 , ZrO_2 and FeO . The presence of CaWO_4 , a substance containing both Ca and W as XRF-detectable elements, was important in the assessment of the procedures applied for the relative concentrations' evaluation. The use in one of the investigated sealers of calcium tungstate which is a natural mineral may suggest that Bi detected in AH 26 and Real Seal could be also in the form of natural mineral(s) of bismuth such as Bi_2O_3 , Bi_2S_3 or Bi_2Te_3 [24]. In general, the weak low energy line of S is hardly seen and covered by noise, but a line clearly visible at ~ 2.25 keV in AH 26 and Real Seal could be assigned to S $K\alpha$ to Bi $M\alpha$ lines, or to their superposition, explained by a high concentration of sulfur and bismuth bound stoichiometrically in Bi_2S_3 . Also a very weak low energy signal seen in AH Plus could be assigned to the W $M\alpha$ lines. Thus it appears that the low energy $M\alpha$ lines of the major heavy metal W or Bi could be detected in all three dental sealers.

Table 1

Qualitative elemental composition of endodontic sealers analyzed by XRF*

Endodontic sealer	AH Plus	AH26	Real Seal
Nominal composition	Si, O, Ca, Fe, Zr, W (SiO_2 , CaWO_4 , ZrO_2 , FeO)	-	-
Elements detected by XRF	Major	W	Bi
	Minor	Ca, Zr, Bi?	S?, Ca, Ba
	Trace	Fe, Cu?, Cr?	Fe, Ca, Cr?, Br?, Sn?

*The interrogation mark means that the presence of the respective element in the analyzed sample's composition cannot be clearly assessed.

In Real Seal the simultaneous presence of Ba and Ca (and possible Sr traces) could be explained by their very close chemical properties.

In addition to traces of Fe present in all three sealers, occasionally some trace elements such as Cr and Cu were observed by very low intensity signals, but they (and less probably Fe) could be contaminants (*e.g.* from dust particles). Their presence and that of other possible trace elements like Br and Sn requires further confirmation.

4.2. ON THE TOXICITY AND PHARMACOLOGY OF HEAVY METALS DETECTED IN THE SEALERS

Bismuth. The metallic Bi share some physical properties with Pb and Sn, and many of its chemical properties are similar to those of As and Sb, although Bi compounds are less toxic than derivatives of the later elements. Also Bi and its

compounds are much less toxic compared to Pb, and are not bioaccumulative, have low solubilities in the blood, are easily removed with urine, and showed no carcinogenic, mutagenic or teratogenic effects in long-term tests on animals [24].

Tungsten. Calcium tungstate, CaWO_4 present in AH Plus, is an important tungsten-containing mineral (scheelite); it often contains impurities of rare earths like Pr and Nd [25]. W occurs in biomolecules from some bacteria, but not in eukaryotes; interferes with Mo and Cu metabolism, and is somewhat toxic to animal life [26, 27]. For instance sodium tungstate is much less toxic than lead, but completely inhibited the reproductive ability of earthworms; at the same time it has antidiabetic action [28].

The possible biological influence of the major heavy metals W and Bi has to be studied although they present low toxicological risks.

4.3. INVISIBLE LIGHT ELEMENTS: COMPTON VS. RAYLEIGH SCATTERED RADIATION

Although light elements could not be detected in XRF by their characteristic X-rays of very low energy, one can gather some general information of them by looking at the peaks of coherent (Rayleigh) and incoherent (Compton) scattering of the X-ray tube anode characteristic radiation which is “reflected” by the sample. The relative intensity of Compton vs. Rayleigh scattering of X-rays decreases exponentially with the atomic number Z of the target, so the higher concentration of light elements the higher is this ratio [22].

In AH Plus and AH 26, in contrast with Real Seal, the intensity of the Compton scattering of radiation is greater than that of the Rayleigh scattering of the primary excitation X-rays. This suggests a higher concentration of light elements in the former two biomaterials as compared to the last one. It is known that AH Plus contained silica [17] and it is plausible that the same was true also for AH 26, while we may suppose that SiO_2 was not present in Real Seal in high concentration. Inorganic oxygen was a light element present in AH Plus also in calcium tungstate and in zirconia as specified by the producer. Among the mineral compounds of Bi that could be present in AH 26 and Real Seal one could find Bi_2O_3 and Bi_2S_3 [24] which contain O or S as light elements. Other light inorganic elements like Mg, Na, Al, Si, F, invisible in XRF spectra may be present in the sealers, but probably in low concentration if at all, so that their influence should be negligible. Oxygen was certainly occurring together with H, C and N in the organic polymers in all three materials. Thus the unknown concentrations of invisible light elements such as H, C, N, O, Si, and S could explain the relative intensity of the Compton and Rayleigh scattered X radiation, but on the basis of the available information we can not speculate further (besides the above supposition concerning silica). The presence of light elements should be accounted for in the processing of spectra by making the appropriate options in the software; this could improve the concentrations’ evaluations for the XRF-visible elements in more accurate

analyses. The intensity of Rayleigh (coherent) scattered radiation can be used for normalised calibration of spectra [29] which would be essential for quantitative XRF analysis of dental materials.

Noteworthy, one could say that XRF has some edge on standard configuration PIXE (with outside detector) by this possibility to detect indiscriminately high levels of very light elements such as Si ($Z = 14$) and even O ($Z = 8$). However, high-performance PIXE with the detector inside the reaction chamber can analyze by their characteristic X-rays light elements starting from Mg ($Z = 12$).

4.4. THE X-RAY YIELD CURVES USED FOR THE ANALYSIS OF THE XRF SPECTRA

The yield for the various XRF-detected elements was determined by the ratio of count number to concentration (Eq. 1) which were listed in the output of the WinQXAS version 1.30 software operated in the „thick sample” mode. The plots of yield *vs.* atomic number Z for the K-line and L-line series were fitted with empirical functions (Fig. 2).

The experimental data points were averages obtained partly from the three endodontic sealers and partly from five dental composites (Preoteasa, Chiojdeanu and Preoteasa, unpublished). The later were important especially for the L series curves in order to increase the number of fitted points in the plot so as to extrapolate the curves for Bi (where only the count numbers were listed) and finally to estimate the concentrations of this element. Although differences in the chemical composition and structure of the matrixes of sealers and composites are expected to produce somewhat different matrix effects (self-absorption of X-rays in the sample) and to result in distortion of the yield curves, this was the only procedure at our disposal in the standardless analysis of the three endodontic sealers.

All the empirical functions of the form $Y = f(Z)$ used for the fit were symmetrical or asymmetrical bell-shaped functions characterized by a maximum at a central value Z_c and by a width w , and contained also an additive constant Y_0 and a multiplicative factor A .

The whole set of K-lines data from Ca ($Z = 20$) to Ba ($Z = 56$) could be approximately fitted with a single lognormal function

$$Y = Y_0 + \frac{A}{\sqrt{2\pi w Z}} \exp \left[-\frac{\left(\ln \frac{Z}{Z_c} \right)^2}{2w^2} \right] \quad (2)$$

but a more precise fit could be done using exponential functions on the low-Z domain (from 20 to 30, Ca to Zn) and on the high-Z domain (from 40 to 56, Zr to Ba) as shown in Fig. 2a.

Similarly, the ensemble of L-lines data could be fitted either with the well-known Gaussian function:

$$Y = Y_0 + \frac{A}{w\sqrt{2\pi}} \exp\left[-\frac{(Z - Z_c)^2}{2w^2}\right] \quad (3)$$

or with the more exotic so-called „extreme” function

$$Y = Y_0 + A \exp\left[-\exp\left(-\frac{Z - Z_c}{w}\right) - \frac{Z - Z_c}{w} + 1\right] \quad (4)$$

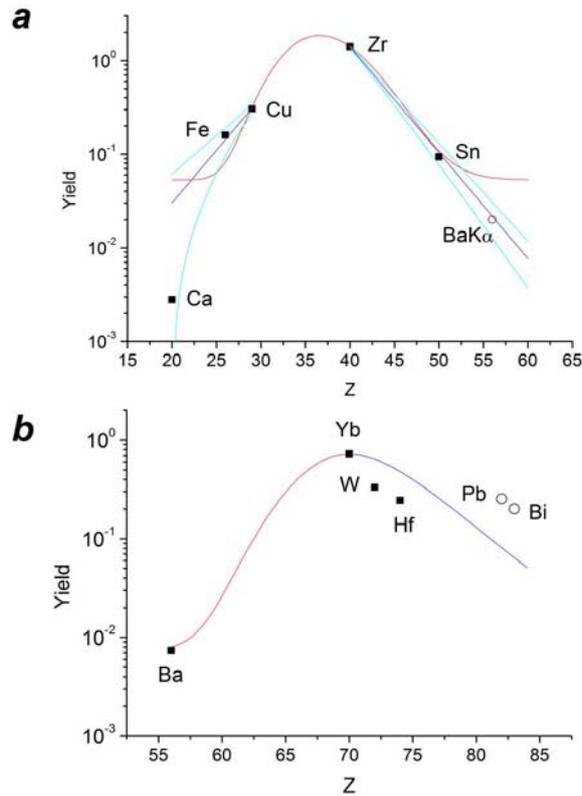


Fig. 2 – Yield vs. atomic number curves for K-lines (a) and L-lines (b) elements detected by XRF. Black squares, experimental data; open squares, extrapolated data. The curves in (a) are lognormal (red), exponential (black), and 95% confidence limits of the exponentials (cyan). In (b) they are Gaussian (red), and extreme function (blue).

We used the Gaussian function from $Z = 56$ (Ba) to $Z = 70$ (Yb), and an „extreme” function was extrapolated from $Z = 70$ to $Z = 83$ so as to include Bi (Fig. 2*b*). Note that Ba is included in the yield plots of both the K and L lines, because both lines of this element were seen in the spectra; however the Ba concentration was estimated from its L lines.

The procedure applied here improves and extends the results of the analysis which, however, remains only preliminary in the absence of adequate standard materials.

4.5. CORRECTIONS FOR Ca CONCENTRATION BASED ON THE ANALYSIS OF Ca AND W IN CaWO_4

The concentrations estimated from the spectra by the software were given in ‘percent’ or ‘ppm’ values, but these values had to be taken only as relative concentrations, because they did not sum up to 100% of XRF-detected elements. Although this shows the limitations of the software, these crude relative concentrations were useful in determining the ratios between elements.

Because the AH Plus sealer contained two XRF-detected elements, Ca and W, only in a single compound (CaWO_4) with a precise stoichiometry [17], we had the remarkable chance to check the correctness of the Ca/W ratio and to make appropriate corrections to the crude Ca and W concentrations, beyond the corrections made with the help of the yield curves. The formular w/w ratio in CaWO_4 is $40.078/183.85 = 0.218$ and it will serve as a reference value. With the experimental values estimated by the software for two distinct samples of AH Plus, the Ca/W ratio was in the range 0.80–1.12. These too large values showed that either Ca was overestimated or W was underestimated from the spectra by the software. On the other hand, the opposite situation is suggested if the concentrations obtained with the help of the yield curves for the K and L series elements from the software-computed values are used, because in this case values of 0.047–0.069 were obtained for the Ca/W ratio, which obviously were too small.

Note that while the effect of the yield curve procedure was less than ~40% for W concentration, the yield curve produced a ~20 times decrease of Ca concentration. This shows that the yield curve procedure may also lead to important inaccuracies, especially for light elements like Ca. Thus the main inaccuracy was in the estimation of Ca and therefore it benefited of appropriate correction while W was assumed to be correct within experimental errors. The diverging results on Ca/W ratio show that the software underestimated the matrix effects while the yield curves procedure overestimated them. Also the magnitude of the matrix effects is emphasized by the results.

In order to escape the contradiction in the two approaches, the geometric means for both experimental and yield curve-determined concentrations and for both samples of AH Plus were computed for Ca and W. With the geometric mean of the two elements' concentrations, a Ca/W ratio of 0.223 was obtained, in excellent agreement with the formular ratio of 0.218. The results allowed the estimation of correction factors for Ca concentration, which were assumed valid for all three biomaterials.

4.6. THE SEMIQUANTITATIVE COMPOSITION OF THE BIOMATERIALS ANALYSED BY XRF

In Table 2 the rough concentrations as given by the software are presented together with the corrected and normalized relative concentrations. In the case of elements for whom only upper limits were given by the software, rough concentrations equal to the half of this value were assumed. The sum of rough concentration values was evaluated; it was between ~1 % for AH 26 and ~21 % for AH Plus, with an intermediate value of ~9.5 % for Real Seal. The low sum value for AH 26 was due to the fact that no rough concentration was given for the major element Bi as it was not computed by the software. The high sum value for AH Plus was due to the fact that the concentration of Ca was overestimated. These aspects evidence the drawbacks of the software with respect to the evaluation of rough concentrations. The later could not be trusted to be absolute concentrations.

Therefore *relative* concentrations were evaluated after making appropriate corrections by use of the yield curves (and by the particular procedure described above in the case of Ca). The relative concentrations have been normalized so as the sum of concentrations of XRF detected elements to be 100 %, and thus to facilitate comparison of biomaterials with each other. The relative concentrations given in "percent" and "ppm" units is an *ad-hoc* convention involving large approximations and are correct only as order of magnitude. Note that the 100 % sum choice of detected elements implies that the XRF-invisible light elements (H, C, N, O, Si, and possibly S) were neglected, although they constituted probably the main part of the materials.

Although the corrected and normalized concentrations were only relative values, several advantages of using this procedure can be listed. 1) The very heavy metal Bi could be evaluated, although by an extrapolation of the L series yield curve. Nevertheless, this was crucial for the AH 26 and Real Seal materials which contained Bi as the major XRF-detected element. 2) The concentration of Ca could be corrected so as to fit the CaWO_4 formular w/w ratio in AH Plus. 3) For many elements the corrected concentrations could be estimated instead of the upper limits of the corresponding rough concentrations.

Table 2

Rough and corrected relative concentrations* of elements in the endodontic sealers analyzed by XRF

Z	Element	X-ray line	Endodontic sealer					
			AH Plus		AH 26		Real Seal	
			Rough data	Corrected	Rough data	Corrected	Rough data	Corrected
20	Ca	K α	9.16 \pm 2.12 %	14.0 \pm 2.8 %	<6589	500 \pm 230	0.0472 \pm 0.0088 %	2.5 \pm 0.6 %
26	Fe	K α	1462 \pm 364	9500 \pm 1970	1184 \pm 286	2575 \pm 170	149,3 \pm 78,9	1545 \pm 820
29	Cu	K α	<589	2500 \pm 870	-	-		-
40	Zr	K α	3.23 \pm 0.72 %	18.0 \pm 3.7 %	-	-		-
56	Ba	L α	<5571	-	-	-	0.63 \pm 0,35	6.6 \pm 3.6 %
74	W	L α	8.17 \pm 0.09 %	62.5 \pm 22.0 %	-	-	-	-
83	Bi	L α	n.c.**	4.4 \pm 0.9 %	n.c.**	99.7 \pm 0.2 %	8.77 \pm 1.62	90.8 \pm 17.0 %
	SUM		21.4 %	100.10 %	1.01 %	100.00 %	9.47 %	100.05 %

*The concentration values (given in ppm (mg/kg) and %) are conventional and represent only *relative* concentrations. The rough relative concentrations are the values as given by the software analysis of spectra. The corrected relative concentrations are normalized to a 100% total for the XRF detected elements.

**n.c. – Not computed by the software. In AH Plus and AH 26, Bi was given by the software only by the counts numbers of the L α lines and was evaluated thereafter.

The average relative errors on the whole body of data are of about 39 % for the rough data and of around 42 % for the corrected data. Thus the correction procedure is responsible of a contribution of about 14 % to the relative errors (one has $(14^2 + 39^2)^{1/2} = 42$, by error propagation formula). This contribution was due to the yield curves and other correction procedures and represents an important source of inaccuracy or systematic errors. According to the above result and after simple operations, the inaccuracy could be esteemed by the approximate formula [1]:

$$\varepsilon_{\text{accuracy}} = 0,2 (\text{concentration})^{1/2} \quad (5)$$

where all values are expressed in percents. The lack of accuracy was specific to a standardless analysis due to partly unaccounted matrix effects. In its turn, this arised because of the use of dental composite data (Preoteasa and Preoteasa, unpublished) as reference data in the yield curves of the sealers. The composites and the sealers have different elemental compositions and physical structures and, therefore, different matrix effects. The yield curves were not the only source of inaccuracy. Systematic errors were due also to the software itself as evidenced by the inaccurate rough concentration estimates for Ca. This demonstrated the limits of the used software for the XRF analysis of the studied dental materials, as it was

dedicated mainly for the analysis of dental alloys. This additional inaccuracy remain unknown within the average ~39 % relative errors of rough concentrations. This figure includes also the statistical errors. Of course, the errors were dependent on the element's nature and concentration, being the highest for the trace elements.

All three sealers contained a major heavy metal (W, Bi). The relative concentrations of the major elements W and Bi were found to be similar (6.2 – 9.8%), which is plausible because they were devoted to the same purpose in all analyzed materials, *i.e.* to provide a high radio-opacity and contrast inside the root canal of the teeth. Divalent ions forming metals Ca and Ba were minor elements in AH Plus and Real Seal with significant relative concentrations (0.2 – 1.4%), which could play a positive role by improving the materials' tissue tolerance. Among the trace elements, Fe was the only present in all materials with reliable relative concentrations values (between 1545 and 9500 ppm), in the order AH Plus > AH 26 > Real Seal.

The semiquantitative XRF analysis, although not very accurate in the absence of reference materials, makes possible the discrimination between Real Seal and AH 26, the two endodontic sealers with high concentrations of Bi, based on the levels of their minor element Ca. In fact they could be recognized by their minor elements. Thus Real Seal is characterized by a Ca concentration about 50 times higher than the level of Ca in AH 26, and the Ca/Bi ratio in the two materials is different by two orders of magnitude (about 0.03 in the former and 0.0005 in the later). Also Ba is present as a minor element only in Real Seal. At the same time AH Plus is easily recognized by its high level of W. In brief, all three endodontic sealers and their brands can be recognized by XRF, which underlines the potential of method for possible forensic applications where the victim may support very severe damage.

The results of the XRF analysis which identified heavy metals (W, Bi) as major elements and minor or trace elements with possible biological activity or influence (Ca, Fe) will serve for correlations with biocompatibility studies now in progress.

5. CONCLUSIONS

Three endodontic sealers (AH Plus, AH 26, Real Seal) were analyzed for the first time by XRF using a portable X-ray spectrometer. The analysis was standardless because reference materials for endodontic sealers were not available and, therefore, only relative concentrations could be estimated by use of a software dedicated mainly for the analysis of metal alloys. Although inexpensive instrumentation designed for field work in archeometry and geology as well as ordinary experimental conditions were used, and in spite of specific difficulties inherent to the analyzed samples – complex and granular structure together with the thick target form of the specimens, resulting in strong matrix effects – XRF demonstrated an important potential for the study of such dental materials. Our

semiquantitative and semiquantitative analysis allowed relevant observations on the endodontic sealers as well as an evaluation of the possibilities and limits of the experimental technique and software, and of the error sources involved in measurement and data processing.

The qualitative analysis evidenced a dozen of elements (Ca, Zr, Ba, W, Bi and possibly S, and Cr, Fe, Cu at trace levels). All three materials contained a major heavy metal – W in AH Plus and Bi in AH 26 and in Real Seal – to provide a strong radio-opacity. However AH 26 and Real Seal could be differentiated by their minor and trace elements, namely by the incidence of Ba and the higher relative concentration of Ca in the later. Therefore all three endodontic sealers could be recognized unequivocally by brands based on the XRF analysis, a result of potential interest for possible forensic applications for the identification of severely damaged victims such as occurring in plane crashes. Among the trace elements, Fe was the only present in all three endodontic sealers. Of the heavy metals detected, the major ones (W and Bi) are not toxic. The different impurities detected in the two Bi-containing endodontic sealers materials suggested also a different origin for the mineral Bi raw materials used in the two formulations.

Although elements lighter than Ca ($Z = 20$), with the possible exception of highly concentrated S ($Z = 16$) at most, could not be detected by their characteristic X-rays, some general information about them was provided in the XRF spectra by the relative intensities of the Rayleigh and Compton scattering of the X-ray tube anode characteristic radiation. Thus in AH Plus and AH 26 the intensity of the Compton scattering was greater than that of the Rayleigh scattering, in contrast with Real Seal. Because AH Plus was known to contain silica as specified by the producer, it was plausible that SiO_2 was also present in AH 26 and probably absent in Real Seal.

The rough results estimated by the software analysis of XRF spectra could not be assumed reliably as absolute concentrations due to the lack of standards and because no corrections were made to account for the granularity of the dental materials. In fact they were valid only as coarse relative concentrations. The software did not estimate the concentrations of Bi. To minimize the errors affecting certain elements, „smoothed” concentrations values were evaluated based on yield *vs.* atomic number curves obtained both for K and L X-ray series of elements by fit of the empirical results provided from the software output. This procedure allowed also the estimation of Bi relative concentrations by the extrapolation of the L series yield curve to higher Z values. The largest uncertainties of the yield curves technique bear on the relative concentrations of the elements at the extremities of the K and L series (Ca and Ba, and Bi, respectively). Further corrections were made for Ca so as to obtain a Ca/W ratio in agreement to the stoichiometry of the CaWO_4 compound which, according to the producer, was present in the AH Plus sealer. The mean inaccuracy of the yield curves and other correction procedures was of about 14 % of the estimated relative concentrations, while the remaining

errors both random and systematic due to the XRF measurement itself and to the software were of about 39 %. All in all, the errors of the relative concentrations were of 42 % in the mean – higher than in PIXE –, but they were dependent on element and its concentration, being highest for the trace elements.

In addition to possible forensic applications already mentioned, the results of the XRF elemental analysis of endodontic sealers could open new prospects for applications in dental research. Thus the acquired compositional insight may be of high value for biocompatibility and tissue tolerance studies of these materials, now in progress. Also the possible biological influence of the major heavy metals W and Bi in the dental use of the sealers raises important questions for further studies. At the same time, while the present semiquantitative analysis provided only preliminary values of relative concentrations, various stratagems may be conceived in order to improve the precision and accuracy of the elemental analysis, such as preparation of standards based on the present XRF analysis as well as intercomparison of XRF with studies by other atomic and nuclear analysis methods (PIXE, PIGE, ERDA). In brief, the XRF analysis of endodontic sealers proved to be a valid and productive approach.

REFERENCES

1. Eugen A. Preoteasa, Elena S. Preoteasa and Ioana Suci, *Atomic and Nuclear Surface Analysis Methods: A Novel Perspective for the Characterization of Dental Composites*, Nova Science Publishers, Inc., NY, 2012.
2. Catalina Chiojdeanu, Daniela Cristea Stan, B. Constantinescu, *Rom. Rep. Phys.* 63, 685-692, 2011.
3. B. Constantinescu, Catalina Pauna, Angela Vasilescu, F. Constantin, Daniela Stan, Gh. Popescu, Antonela Neacsu, *Rom. Rep. Phys.* 62, 47-56, 2010.
4. B. Constantinescu, A. Vasilescu, D. Stan, M. Radtke, G. Buzanich, U. Reinholz and E. Oberlaender-Tarnoveanu, *J. Anal. Atomic Spectrom.* 27, 2076-2081, 2012
5. E.A. Preoteasa, C. Ciortea, B. Constantinescu, Daniela Fluerașu, Sanda-Elena Enescu, D. Pantelica, F. Negoita, Elena S. Preoteasa, *Nucl. Instrum. Methods Phys. Res. B* 189, 426-430, 2002.
6. E.A. Preoteasa, Rodica Georgescu, C. Ciortea, Daniela Fluerașu, Livia Harangus, Andreea Iordan, Feride Severcan, Handan Boyar, Elena S. Preoteasa, I. Piticu, D. Pantelica, Vl. Gheordunescu, *Anal. Bioanal. Chem.* 379, 825-841, 2004.
7. Eugen A. Preoteasa, Elena S. Preoteasa, Andrzej Kuczumow, Dan Gurban, Dieter Grambole, Folker Herrmann, *X-ray Spectrom.* 37, 517-535, 2008.
8. E.A. Preoteasa, Elena S. Preoteasa, C. Ciortea, D.D. Marin, D. Gurban, M. Gugiu, Adela Scafes, *X-Ray Spectrom.* 38, 548-556, 2009.
9. E.A. Preoteasa, Elena Preoteasa, Livia Harangus, A. Moldovan, Maria Dinescu, D. Grambole, F. Herrmann, *X-Ray Spectrom.* 39, 208-215, 2010
10. Ioana Suci, E.A. Preoteasa, D. Gurban, E. Ionescu, D. Bodnar, *Rom. Rep. Phys.* 58, 569-582, 2006.
11. V. Valkovic, *Analysis of Biological Material for Trace Elements Using X-ray Spectroscopy*, CRC Press Inc., Boca Raton, Florida, 1980.
12. M. Thellier, C. Ripoll, C. Quintana, F. Sommer, P. Chevalier, J. Dainty, *Meth. Enzymol.* 227, 535-586, 1993.
13. I. Baranowska, L. Barchański, M. Bąk, B. Smolec, Z. Mzyk, *Polish J. Environm. Stud.* 13, 639-646, 2004.

14. P. Bloch, I.M. Shapiro, L. Soule, A. Close, B. Revich, *Appl. Radiat. Isot.* 49, 703-705, 1998.
15. M.A. Bush, R.G. Miller, A.L. Norrlander, P.J. Bush, *J. Forensic Sci.* 53, 419-425, 2008.
16. M.A. Bush, R.G. Miller, J. Prutsman-Pfeiffer, P.J. Bush, *J. Forensic Sci.* 52, 157-165, 2007.
17. Dentsply International Inc, York, PA, Product information.
18. R. Jenkins, *An introduction to X-ray Fluorescence Spectrometry*, Heyden, London – New York – Rheine, 1976.
19. R.E. Van Grieken, A.A. Markowicz, *Handbook of X-Ray Spectrometry, 2nd edition*; Marcel Dekker Inc., New York, 2002
20. B. Beckhoff, B. Kanngießer, N. Langhoff, R. Wedell, H. Wolff, *Handbook of Practical X-Ray Fluorescence Analysis*, Springer Verlag, Berlin – Heidelberg – New York, 2006.
21. N.G. West, *Trends Anal. Chem.* 3, 199-204, 1984.
22. E.P. Bertin, *Principles and Practice of X-ray Spectrochemical Analysis*, Plenum Press, New York – London, 1978.
23. V. Valkovic, G. Moschini, *Riv. Nuovo Cimento* 16, 1–55, 1993.
24. H. Suzuki, Y. Matano (eds.), *Organobismuth Chemistry*, Elsevier, Amsterdam, 2001.
25. E. Lassner, W.-D. Schubert, *Tungsten: Properties, chemistry, technology of the element, alloys, and chemical compounds*, Springer Verlag, Berlin – Heidelberg – New York, 1999.
26. R. Hille, *Trends Biochem. Sci.* 27, 360–367, 2002.
27. F. Lagarde, M. Leroy, *Metal Ions Biol. Sys.* 39, 741–759, 2002.
28. J.E. Dominguez, M.C. Munoz, D. Zafra, I. Sanchez-Perez, S. Baque, M. Caron, C. Mercurio, A. Barbera, R. Perona, R. Gomis, J.J. Guinovart, *J. Biol. Chem.* 278, 42785–42794, 2003.
29. J.M. O'Meara, J. Börjesson, D.R. Chettle, S. Mattsson, *Appl. Radiat. Isot.* 54, 319-325, 2001.