POTENTIAL OF PIXE FOR THE ELEMENTAL ANALYSIS OF CALCIUM HYDROXIDE USED IN DENTISTRY

IOANA SUCIU¹, EUGEN A. PREOTEASA²*, DAN GURBAN², ECATERINA IONESCU¹, DANA BODNAR¹

¹ Dental Medicine Faculty, Carol Davila Medical University, Calea Plevnei 19, Sector 1, Bucharest, Romania
² “Horia Hulubei” National Institute for Physics and Nuclear Engineering, Atomistilor 407, P.O. Box MG-6, 077125 Bucharest-Măgurele, Romania; Fax: +40-21-432 1701

(Received July 31, 2006)

Abstract. Calcium hydroxide is used in dentistry and generally shows a clinically acceptable biocompatibility. However various commercial assortments show differences in their clinical performances, suggesting that certain aspects of their action are not completely understood and pointing to the necessity of new investigation methods. We report a preliminary study on two types of calcium hydroxide products carried out by thick target PIXE with 3.0 MeV protons, aiming to assess the potential of this method for the analysis of such biomaterials used in endodontic dentistry. (Semi)quantitative analysis was performed by use of reference materials, and relative concentrations of elements with respect to Ca were determined with an accuracy of about 30%; tentative absolute concentrations were also estimated by using Ca as a reference element. In both formulations, a number of trace elements (Mn, Fe, Zn and Sr, and possibly Cu and Pb – probably impurities from the raw materials) were evidenced. These trace elements together with the Ba/Ca ratio are reliable fingerprints for the identification of the materials, and evidenced a different origin for the Ca(OH)₂ used in the two specimens. Possible applications of PIXE in dental research may bring relevant compositional insight, and further studies of such materials are suggested.

Key words: PIXE analysis, multielemental analysis, calcium hydroxide, dentistry, target preparation.

1. INTRODUCTION

Elemental analysis is widely used in biomedicine, from current clinical applications to the investigation of the structure and mechanisms in normal and pathological tissues and to the development of new bio- and medical materials. Instrumental methods, and among them physical (e.g., optical, atomic, nuclear, etc.) methods, distinguish themselves by speed, sensitivity, selectivity, versatility and frequently by a nondestructive and noninvasive character. In particular, ion beam analysis (IBA) methods, which probe the sample composition with accelerated

* Corresponding author: eugen.preoteasa@yahoo.com
protons or heavier ions, are able to characterize in a multielemental manner the composition of the sample, in contrast to the classical chemical techniques but also to the more popular atomic absorption spectroscopy.

Particle induced X-ray emission (PIXE), which identifies the analyzed elements in the sample by their characteristic X-rays generated on the impact of accelerated ions, is a robust method that integrates both spectrochemical and ion beam features. Energy-dispersive detection PIXE classifies as a notably sensitive, multielemental, conveniently specific, and relatively nondestructive technique, which is able to analyze both thin solid samples and thin layers at the specimens’ surface [1–3]. PIXE can cover a high dynamic range of values in the spectra and, therefore – just like other nuclear and atomic analytical methods – it can give at a glance relevant insight on major to trace elements in biomedical applications [4, 5]; but it exceeds most other IBA techniques by its sensitivity in trace element detection. It has been remarked that the real potential of PIXE emerges in the surface analysis of thick specimens, in spite of conceptual and computational difficulties facing the investigation [6]. In this approach, the preparation of the sample is generally simpler, the natural state of the latter is preserved, contaminations are reduced to a minimum, and the information provided is more relevant when the surface differs from the bulk. Moreover, depth profiles of concentrations can be obtained; and, with the use of ion beams focused to micrometer-sized spots (μ-PIXE), the elements at the surface of the specimen can be mapped.

Most applications of PIXE and μ-PIXE in dentistry employed the thick target technique as best fit for dental hard tissues and biomaterials. For instance, in human enamel studies, this method helped correlate the trace elements with the caries score, age and gender of subjects [7, 8], evidence concentration changes of the former in decayed teeth [9], demonstrate distribution changes after CO₂-laser irradiation [10], and identify an ancient tooth inlay [11]. We also applied PIXE to evidence changes in trace elements at the surface of dental enamel following incipient demineralization in vitro [12]. In the analysis of dentistry-used materials and of their interactions with the oral environment, PIXE and its microbeam version have been applied to follow the release into the hard tissues of metals from amalgam fillings [13, 14] and from titanium dental implants [15, 16]. Dental composites were studied by PIXE only recently. Together with ERDA (elastic recoil detection analysis, another IBA technique) and with XRF (X-ray fluorescence), PIXE was used to analyze dental composites, first by our group [17] and subsequently by other investigators [18].

There are several arguments supporting the interest for thick target PIXE studies of dental materials. The fast detection of trace elements at their surface may usefully characterize their composition, and traces may be valuable fingerprints for their identification and for quality control. During their use in the oral environment, dental materials are submitted to chemical and mechanical degradation, and they convey in the organism foreign elements that may cause adverse effects [19, 20].
Transfer processes at the interface with the dental tissues may lead to toxic effects, or at least they may give way to a detrimental influence on the biocompatibility of the material. Therefore, PIXE may lead to significant contributions in the dental research of such issues, particularly also because many dental materials were not yet studied by PIXE.

One such dental material is calcium hydroxide, used in endodontics and known to exhibit a moderate toxicity, but generally showing a clinically acceptable biocompatibility. Accordingly, various calcium hydroxide commercial blends are currently employed in this field of dentistry. However, differences in their clinical performances suggest that certain aspects of their action in the oral cavity are not completely understood. Therefore, new investigation methods may be needed to approach this topics in endodontic research.

Here we report a preliminary study on two commercial types of calcium hydroxide carried out by PIXE with 3.0 MeV protons, aiming to evaluate the potential of this method for the analysis of such biomaterials used in endodontic dentistry. We analyzed in detail the feasibility of sample preparation for PIXE, the experimental problems and sources of errors in the measurements and in the processing of spectra, the ability of the method to evaluate relative concentrations and to estimate absolute ones, as well as its proficiency in detecting impurity trace elements; also, we evaluate the possibilities of PIXE for endodontic dentistry-related research applications.

2. EXPERIMENTAL

2.1. MATERIALS AND TARGET PREPARATION

The two analyzed calcium hydroxide commercial mixtures were Calasept (Nordiska Dental, Angelholm, Sweden), a one-component ready-to-use paste product and Cikal (DOD, DCA – Leskovac, Yugoslavia), consisting of a paste accompanied by a setting fluid. Only the paste of the Cikal product was examined, without mixing it with the fluid.

The composition of the fresh and wet Calasept calcium hydroxide product was given by the producer as consisting of 41.07% Ca(OH)$_2$, 8.33% BaSO$_4$ and 50.60% Ringer solution. To account for the contributions of the Ringer saline, the following composition was considered for this aqueous solution: 8.1 g/L NaCl, 0.38 g/L KCl, 0.45 g/L CaCl$_2$.6H$_2$O, 0.45 g/L NaHCO$_3$, 0.23 g/L MgCl$_2$.6H$_2$O, and 0.45 g/L glucose. The composition of the Cikal assortment was unknown let apart a massive content of Ca(OH)$_2$, and the presence of a fluorine-releasing substance, as specified by the manufacturer.

We used a preparation technique similar to that developed previously for the PIXE analysis of dental composites [17]. Disk shaped samples of 7–8 mm diameter
with a flat surface were prepared from the white pastes on glass plates. The samples were let to solidify by slow evaporation of water, 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, crack formation occurred in the calcium hydroxide samples during drying. The dried specimens were fixed with the flat surface up by means of a cyanacrylic adhesive on Mylar membranes mounted on aluminum diaphragms. Due to the cracks the surface was not perfectly flat. As a reference material a pellet of powdered hydroxyapatite was used. Covering the specimens’ surfaces with a thin film of carbon picked up from an air-water interface, a technique used with the dental composites, was not possible due to the hydro-solubility of calcium hydroxide. Therefore charging of the insulating targets during the PIXE measurements could not be avoided, resulting in relatively high background of the spectra.

2.2. PIXE MEASUREMENTS

Wide beam PIXE measurements were performed with 3 MeV protons at the 8.5 MV NIPNE-HH tandem Van de Graaff accelerator as described before [12, 17]. The proton beam hit the target at 45° with respect to the surface normal. The X-rays emerging from the target were collected with a Canberra hyperpure (HP) Ge detector having a crystal surface of 100 mm² and an energy resolution of 180 eV at 5.9 keV, placed perpendicularly to the beam. The X-rays passed through the Be windows of the scattering chamber (0.25 mm thick) and detector and a 2.4 cm air gap. The spectra were collected without additional absorber.

Beam current integration was used for normalization of spectra. The scattering chamber was insulated from the rest of the system, and the total collected charge was measured using an Ortec model 439 current digitizer. No electron suppression was used. Our technique does not give an accurate measurement of the absolute charge of protons hitting the insulating target and the values provided are largely relative, but they may serve for normalizing the spectra.

The spectroscopic chain consisted of detector preamplifier, a Tennelec amplifier, a Canberra analog-to-digital converter and a Canberra S100 counting system connected to a computer.

2.3. ANALYSIS OF PIXE SPECTRA AND CONCENTRATION ESTIMATES

In order to determine the positions and areas of the lines, the spectra were processed by background subtraction and least square fit of lines with Gaussians; the line areas were used subsequently for the estimation of relative concentrations in the calcium hydroxide samples. For the lighter elements analyzed by their K lines in the X-ray spectra (e.g., up to Sr, Z = 38), a hydroxyapatite pellet and the dried Calasept calcium hydroxide product were used as reference materials.
In hydroxyapatite, the relative concentrations estimated previously for this specimen with the Gupix code at the Forschungszentrum Rossendorf, Germany [21] and corrected for the nominal P/Ca ratio of 0.464 were the following: P, 18.5 ± 8.9%; Ca, 39.9 ± 0.3%; Fe, 432 ± 104 ppm; Cu, 224 ± 72 ppm; Zn, 270 ± 122 ppm; Sr, 1248 ± 799 ppm; Ba, 512 ± 108 ppm; and Pb < 516 ppm (w/w; 1 ppm = 1 mg/kg = 10⁻⁴%). For Calasept, assuming that all water (except crystallization water of CaCl₂⋅6H₂O and MgCl₂⋅6H₂O from Ringer) was removed by drying, the next concentrations were evaluated from the nominal composition for the elements potentially detectable by PIXE: S 2.29%; Cl 0.54%; K 0.02%; Ca 44.52%; and Ba 9.82% (w/w of dry weight).

Using the normalized experimental peak areas from our spectrum of hydroxyapatite together with the above concentrations, the yield curve (area/concentration ratio vs. atomic number Z) for this reference material was plotted for Z < 38. The same route could not be followed in order to use Calasept as a standard for the K-line light elements (S, Cl, Ca), because the concentrations of the latter were too small to be detected in the low-sensitivity, low-energy region of the PIXE spectrum. Then, the concentrations of the elements detected in the spectra of calcium hydroxide samples were evaluated by the formula:

\[ C_i^s = \frac{A_i^s}{Y_i^{Ha}} \]

where \( C_i^s \) – absolute concentration of element \( i \) analyzed in the specimen; \( A_i^s \) – area of the characteristic line of element \( i \) in the specimen’s spectrum; and \( Y_i^{Ha} \) – yield for element \( i \) in the hydroxyapatite reference material.

For the heavier elements yielding intense L lines (Ba and Pb), a similar procedure was applied, however we referred not only to hydroxyapatite but also to a previously analyzed Ca- and Ba-containing dental composite and to a dental enamel sample containing traces of Pb [12]. We used for the yield of Ba the mean value for hydroxyapatite and the dental composite. Using this mean yield the ratio of Ba/Ca concentrations was estimated from the Calasept spectrum and the result was then compared to its nominal value of 0.22, in order to assess the accuracy of our analysis of these two elements. The yield used for Pb evaluation in the calcium hydroxide samples was adapted from a dental enamel spectrum [12].

The procedure described above provides a simple and convenient solution to the difficult problem of the matrix effects in the PIXE analysis of thick targets, but is not a very rigorous one. Although both the analyzed specimens and the standards contain significant amounts of Ca, differences in the chemical composition and structure of the matrixes are expected to produce somewhat different effects and to result in more or less different yield curves. Moreover, systematic errors may occur due to the approximate estimation of collected charge. Therefore absolute concentration values thus obtained are to be considered only indicative. However, they served to evaluate rather reliable values for the relative concentrations, as
given by the ratios of concentrations for a given element and a reference element from the same specimen, e.g., Ca, the best analyzed element due to its very high concentration. Thus the results are reported as the relative concentrations $c_i^r$ in the analyzed specimens:

$$c_i^r = C_i^r / C_{Ca}^r$$

(2)

Although the accuracy of the relative concentrations is expected to be not very good for the trace elements, similar procedures applied in previous work have lead us to medically and biologically relevant results in PIXE studies of thick biomineral samples [12]. This is particularly true when, as the case is in the present work, the analysis is aimed for the comparison of two related materials and variations of relative concentrations represent valuable information.

However, we finally evaluated also the absolute concentrations in the endodontic formulations, using the nominal concentration of Ca in Calasept (44.52% of dry weight) as a reference value. But in doing so we kept in mind that all the results thus obtained should be eventually corrected by a constant factor $f < 1$, in order to account for an unknown fraction of “bound” water which possibly was not removed on drying of the calcium hydroxide paste.

2.4. PIXE DETECTION LIMITS

Rough empirical detection limits were evaluated from the PIXE spectra using the concentration estimates. The detection limits were established imposing that the minimum detectable number of pulses in the peak, $N_p$, must satisfy the relation:

$$N_p \geq 3 \cdot N_B^{1/2}$$

(3)

where $N_B$ is the number of pulses in the background under the peak in an interval having the width equal to the fwhm (full width at half maximum) of the peak corresponding to the considered K$\alpha$ or L$\alpha$ line [1]. For some trace elements showing relative errors close to 100% (e.g., Cu and Pb), upper limits of concentrations equal to 3 times the detection limits were given, however keeping in mind that these values were probably overstimated.

3. RESULTS AND DISCUSSIONS

3.1. THE QUALITATIVE COMPOSITION OF THE BIOMATERIALS

The PIXE spectra of the three calcium-rich biomaterials were obviously dominated by Ca and showed only a limited variability (Fig. 1). In the HA spectrum
the other major element, P, was practically not visible to the naked eye and could be evidenced only by fit with a relative error of at least 50%, due to reduced detector sensitivity and to absorption in the windows at low energy. This reference material evidenced also traces of Fe, Cu, Zn, Sr, Ba and Pb, of which the last two were hardly seen. In the spectrum of Calasept a substantial amount of Ba was verified, and also traces of Mn, Fe, Sr (and possibly of Cu and Pb) were seen. The light minor and trace elements in this biomaterial (S, Cl, K) gave no distinct signals. The Cikal product showed Ba only at trace level in addition to the traces of Fe, Cu, Sr, and Pb, but in addition traces of Zn seemed to be present in its spectrum. Apparently for this specimen, at low energy a very week line of K might be suspected, but the unlikely presence of potassium was not given further attention in the subsequent quantitative analysis because of the high errors affecting this insensitive region of the spectrum. Both Ca(OH)$_2$ materials show a sufficiently high purity, containing only in trace amounts the clearly detected impurities (Mn, Fe, Zn, Sr, Ba). The occurrence of Cu and Pb traces is at first sight uncertain. All these elements are divalent ion-forming metals and their presence
can be explained by chemical similarity to Ca$^{2+}$, supposedly arising from the raw materials used for the preparation of the products. Thus Mn, e.g., is known as a wide-spread impurity in calcite. The trace elements are not specified by (and probably unknown to) the producer. The main difference between the two Ca(OH)$_2$ biomaterials appears to be the high Ba detected only in Calasept, where it was introduced to improve the radiological opacity of the product.

3.2. ANALYSIS OF SPECTRA: X-RAY YIELD CURVE AND DETECTION LIMITS

To evaluate element concentrations from the peak areas of the thick target PIXE spectra, a procedure is needed to establish the X-ray yield function converting the second to the first, e.g., by 'prime principles' calculations which may account well for the matrix effects in the thick targets under study. Alternatively, in the absence of such calculations, we constructed the X-ray yield curve of one of the reference materials in order to use it for (semi)quantitative analysis. The known composition of Calasept for S, Cl; K; Ca; Ba was not helpful to this end, as the K-line elements lighter than Ca could not be detected in our spectra, while for the heavy L-line elements we referred to the spectra of some dental composite and enamel [12]. Thus the only available choice was to use the HA spectrum. Its X-ray yield curve, used throughout to estimate relative concentrations of the analyzed K-line elements, showed a deeply asymmetric bell-shape for Z = 15–40 (Fig. 2A). This curve may be affected by considerable inaccuracy for low Z elements depending on the choice of correction for the P/Ca ratio, and its use may also lead to significant errors when analyzing trace elements, due to the differences among the calcium hydroxide specimens and the HA standard (e.g., elemental composition, density, structure, granularity, surface shape and smoothness, and conductivity). But in spite of the approximations involved in our procedure, it usefully allowed an acceptable accurate analysis of the elements from Ca to Sr in the Ca(OH)$_2$ materials.

Using the yield curve of HA, provisional detection limits were evaluated for each spectrum recorded in the present experimental conditions. These limits reached their best of about 20 ppm for Fe in Calasept (Fig. 2B). This numerical value was higher than detection limits attained previously in our PIXE analysis of other thick target biominerall materials [12, 17]. Apparently, this might suggest a relatively low sensitivity of the analysis, but the above figure should be considered only as an indicative one. At the same time, the shapes of the detection limit curves were similar for the Calasept and Cikal Ca(OH)$_2$ products and different for HA. This should be related to the comparable/different elemental compositions and matrix structures of these samples. However the numerical values of the detection limits were generally rather close in all three Ca-rich materials, which confirms that
Fig. 2 – A) X-ray yield (ratio of peak area vs. concentration) of the hydroxyapatite reference material used in the analysis of the spectra of Ca(OH)$_2$ specimens. B) Detection limits of the analyzed endodontic Ca(OH)$_2$ products.

HA was adequate enough as a reference material for the analysis of Ca(OH)$_2$ mixtures. The rapid raise of the detection limits for elements lighter than Ca is due to causes already mentioned (absorption of X-rays in the Be windows of the
specimen chamber and detector and to the reduced detector sensitivity at low energies), and explains our not detecting S, Cl and K in Calasept in spite of their concentrations rising up to the percent range. The overall detection limits amounted to ~20–300 ppm for the medium- and high-Z elements of primary interest in our study (Ca, Mn, Fe, Cu, Zn, Sr, Ba and Pb). Such values did not show any drawback for the analysis of these elements.

### 3.3. TOWARDS QUANTITATIVE ANALYSIS: RELATIVE CONCENTRATIONS AND TENTATIVE PERCENT CONCENTRATIONS

Based on the X-ray yield curve of HA, we evaluated the relative concentrations of Ca, Mn, Fe, Cu, Zn, Sr, Ba and Pb in the Calasept and Cikal products. For all these elements, the basic results of the PIXE analysis were expressed as relative concentrations, i.e., mass ratios of the analyzed elements with respect to Ca (Table 1).

Although the source results in this unrefined form might seem to some degree not very inviting, they benefit as such of a higher reliability in regular PIXE analysis, since additional assumptions involved in the derivation of data are kept at a minimum. The statistical counting errors were below 1% for the major elements Ca and Ba, but were between 17 and 83% for the ascertained trace elements (42 ± 23% in the mean). Moreover, caution is needed in addition for the relative concentrations of trace elements because of systematic errors; consequently the analyzed values may be several times higher or lower than the real ones. The reason for this uncertainty is the lack of appropriate standards for the trace elements.

#### Table 1

Basic results of the semiquantitative PIXE analysis of two calcium hydroxide formulations for dental use: relative mass concentrations of the analyzed elements with respect to Ca (w/w) and the corresponding relative errors.

<table>
<thead>
<tr>
<th>Ca(OH)₂</th>
<th>Detected element</th>
<th>K-line elements</th>
<th>L-line elements</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ca</td>
<td>Mn</td>
</tr>
<tr>
<td>Calasept</td>
<td></td>
<td>0.0007 ± 0.0003</td>
<td>0.00012 ± 0.00003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.06%)</td>
<td>(43%)</td>
</tr>
<tr>
<td>Cikal</td>
<td></td>
<td>0.00007 ± 0.0001</td>
<td>0.0006 ± 0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.07%)</td>
<td>(17%)</td>
</tr>
</tbody>
</table>

* The relative errors are written in brackets and include only statistical counting fluctuations. Uncertainties due to fit errors of the spectra and to inaccuracy (systematic errors) are not included. Fit errors are of the same order of magnitude as the statistical errors. An accuracy of about 27% was estimated for the Ba/Ca ratio.
elements in Ca(OH)₂ matrix – a problem encountered frequently when a material is studied by PIXE for the first time, as in our case. On the other hand, the assessment of an element vs. Ca mass ratio is usually sufficient to estimate with good reliability the relative variations of this parameter; this advantage pertains equally to the variations of the trace elements’ levels, notwithstanding the above uncertainties. Thus one can get biomedically relevant information – e.g., by following the changes and kinetics of an element from an endodontic Ca(OH)₂ remedy in model systems simulating the conditions from the oral cavity, in view of a clinical evaluation of the product.

However, in order to facilitate comparison to possible analytical studies by other methods on similar medical materials, we converted the results to percent concentrations (Table 2), by selecting Ca as a reference element and making use of its known absolute mass concentration in Calasept. For Cikal the Ca level was estimated similarly with the use of the integrated charge. With a few exceptions for Ca and Ba, where the mass concentration were in the percent range or higher, the concentrations of the other elements rated as tens to hundreds of parts per million (1 ppm = 1 mg/kg = 10⁻⁶%). The values confirm quantitatively the nature of the trace elements; thus Mn, Fe, Cu, Zn, Sr, and Pb in both materials and Ba in Cikal were most probably impurities from raw materials. One can see further from Table 2 that the mass concentration estimated in this way in Calasept for Ba amounted to 7.21 ± 0.04%, as compared to 9.82% according to the producer’s specification. The comparison ascertains an accuracy of about 27% of our PIXE evaluation for the Ba/Ca ratio – a quite reasonable and satisfactory result, given that Ba and Ca are far apart in the periodic table and were analyzed by their L and K lines, respectively. Yet the results from Table 2 should be regarded only as tentative percent concentrations within the limits of an unknown constant factor, at least in the case of the trace elements and especially for Cikal, where we did not have any reference element of known concentration.

### Table 2

<table>
<thead>
<tr>
<th>Bio-material</th>
<th>Detected element</th>
<th>Ca</th>
<th>Mn</th>
<th>Fe</th>
<th>Cu</th>
<th>Zn</th>
<th>Sr</th>
<th>Ba</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calasept</td>
<td></td>
<td>44.52 ± 0.03*</td>
<td>312 ± 134</td>
<td>53 ± 13</td>
<td>&lt; 89</td>
<td>–</td>
<td>1336 ± 445</td>
<td>7.21 ± 0.04*</td>
<td>&lt; 712</td>
</tr>
<tr>
<td>Cikal</td>
<td></td>
<td>50.26 ± 0.04*</td>
<td>–</td>
<td>302 ± 50</td>
<td>&lt; 50</td>
<td>75 ± 45</td>
<td>302 ± 251</td>
<td>101 ± 30</td>
<td>&lt; 402</td>
</tr>
</tbody>
</table>

* Mass concentrations of dry weigh (w/w) in mg/kg (ppm), unless noted by an asterisk for %.

* Concentration of reference element (Ca) in Calasept according to producer’s specification (44.52%).

* Ba concentration in Calasept according to producer’s specification: 9.82% w/w of dry weight.
Note also that, as expected, the evaluated levels of all trace elements in the Ca(OH)\textsubscript{2} products were significantly above the detection limits shown in Fig. 2A. Still, further studies are necessary to ascertain the possible incidence of Cu and Pb traces in both endodontic products; so far, these elements allowed only a rough estimate of upper concentration limits, but their levels are too low for any toxicological risk. Another result that is worth mentioning shows that the Ca concentration of 50.3\% analyzed in the dried Cikal material is higher than the 44.5\% found in Calasept (due to the absence of BaSO\textsubscript{4} in the former), but it is by 3.8\% below the 54.1\% value corresponding to pure Ca(OH)\textsubscript{2}. Obviously, this significant difference, much above the experimental errors, is not on account of the trace elements. Most probably, it is due to another compound from Cikal not detected by PIXE, \textit{e.g.}, a fluorine-containing substance such as NaF, as the presence of fluorine is indicated by the producer. We recently developed a technique combining PIXE with Particle Induced Gamma Emission (PIGE) for the analysis of fluorine in addition to other elements in biomaterials (Preoteasa \textit{et al.}, to be published), which may help analyze this element also in Cikal-type blends.

A number of analytical limitations were evidenced in the above discussions, \textit{e.g.}, crack formation during the drying of the Ca(OH)\textsubscript{2} samples, low sensitivity for the light elements, some uncertainties in the (semi)quantitative analysis, lack of appropriate trace element reference materials. For most of them improvements are possible – by modifying the preparation technique, by mounting thinner Be windows for the detector and scattering chamber, by using advanced computer programs based on developments in thick-target analysis, and by purchasing a wide variety of standards.

As a final point, we observe the completely different concentrations of the Mn, Fe, Zn and Sr traces in the two dental materials. This suggests that Calasept and Cikal were prepared from Ca(OH)\textsubscript{2} row materials of definitely different origin – an aspect that may explain in part the superior endodontic properties of the former product. The higher quality of Calasept probably involves a better biocompatibility as well as a lower toxicity, which in turn may be related to a lower toxicity of its Ca(OH)\textsubscript{2} due to a particular size distribution of the active substance’s crystals. One can expect that these implications, important from the dental point of view, rely to a great extent on the solubility of Ca\textsuperscript{2+} ions and on their release rate from the material in the dental tissue. The present results suggest PIXE as a most suitable method to address such questions in subsequent studies.

4. CONCLUSIONS

Two endodontic Ca(OH)\textsubscript{2} formulations were analyzed for the first time by thick target PIXE, and the method demonstrated an important potential for the
study of such dental materials. Quantitative analysis was performed by the use of a hydroxyapatite standard and also, one of the biomaterials with a known concentration of Ca and Ba was used as a reference material. Relative concentrations of elements with respect to Ca were determined with a satisfactory accuracy (evaluated to 27% for the Ba/Ca ratio). Tentative absolute concentrations were also evaluated by using Ca as a reference element. Most reliably, PIXE should allow the evaluation of relative changes in the concentrations of the detected elements, as expected to occur in the dental use of the materials. In both products, the analysis evidenced a number of trace elements (Mn, Fe, Zn and Sr, and possibly Cu and Pb, all of them divalent ion-forming metals chemically analogous to Ca), probably impurities from the raw materials. The trace elements, as well as the relative concentrations of Ca and Ba, are reliable fingerprints for the identification of the materials. This may serve for quality assessment, including for trade and forensic purposes. The pattern of trace elements evidenced also a different origin for the Ca(OH)$_2$ raw materials used in the two formulations. The present analytical capabilities can be extended both by experimental and computational improvements and by linking PIXE to other IBA methods such as PIGE, which can detect also the ‘invisible’ lighter elements. Possible applications of PIXE in endodontic research may bring compositional insight of high relevance, and further studies of such dental materials are suggested.

Acknowledgement. One of the authors (E.A.P.) warmly thanks his wife Elena S. Preoteasa (Helident Ltd. Dental Surgery, Bucharest, Romania) for valuable suggestions concerning the sample preparation and for fruitful discussions of the results.

REFERENCES