Structural and morphological properties of HA-ZnO powders prepared for biomaterials

Abstract: The purpose of this study was to investigate the structural and morphological properties of hydroxyapatite – zinc oxide (HA-ZnO) powders prepared to be used in an endodontic sealer formulation. The HA-ZnO was synthesized from hydroxyapatite (HA) in the presence of zinc oxide (ZnO) sol. The starting reagents were CaO, H₃PO₄ and a suspension of ZnO (25% in water). Following precipitation, the obtained sol was subjected to heat treatment at 110°C, 400°C, 850°C and 1050°C. The study focused on the influence of the thermal treatment on the crystallinity of precipitated powders. X-ray diffraction was used in order to study the structural properties of the obtained powder. Fourier Transform Infrared Spectroscopy (FT-IR) was used in order to evaluate the interactions between HA and ZnO. The morphology of the samples was studied by Scanning Electron Microscopy (SEM). Transmission Electron Microscopy and High Resolution Transmission Electron Microscopy (TEM, HRTEM) were used for the determination of particle size and fractal analysis. The fractal analysis of images using the standard box-counting method is presented and the results are discussed. It was demonstrated that the fractal dimension analysis is a useful method to quantitatively describe the complex microstructures and it can reveal the relation between fractal parameters.

Keywords: hydroxyapatite, zinc oxide, nanoparticles, biomaterials, fractal analysis

1 Introduction

In recent years, interest in the development of zinc oxide (ZnO) nanoparticles has been greatly increased. In general, the antimicrobial activity of the zinc ion is involved in two major processes, e.g. protein deactivation, microbial membrane interactions and thereby change in membrane structure and permeability. As an advantage, nano-sized particles have gained importance in biomedical fields since the reduction in size. The activation of the particle’s surface often lead to entirely different behavior during dissolution and precipitation. While Zn hydroxyapatite complex has been studied as catalyst [1] or bioactive glass (known as bioglass) [2-6], only few publications discuss hydroxyapatite – zinc oxide compounds (HA–ZnO) [7,8]. Recently, the fabrications of zinc-releasing biocement combining zinc calcium phosphate to calcium phosphate cement [9] and a single-site hydroxyapatite-bound zinc (Zn-HA) catalyst for highly efficient chemical fixation of carbon dioxide with epoxides have been studied [1].

Zn containing hydroxyapatite glasses of phosphate have been studied in order to establish the influence of Zn on the properties of the obtained material [2]. Incorporation of zinc to bioglass composition helps to maintain the pH in simulated body fluid (SBF) within physiological limits. This is done by controlling the hydroxide ion concentration in solution through the formation of insoluble zinc hydroxide. In vitro experiments with osteoblasts have indicated that bioglass incorporating a limited amount of zinc in their composition stimulates bone cell production [2,10].

Aina et al. studied the reactivity towards hydroxo-carbonate apatite (HCA) formation of a bioglass and of two preparations of zinc-doped derived systems (HZ5, HZ20) immersed in Tris(hydroxymethyl)aminomethane (Tris) and Dulbecco’s modified Eagle’s medium (DMEM) buffer solutions [3]. They concluded that Zn caused
a drastic reduction of the overall leaching activity of glasses, but for 20% Zn concentration, the formation of HCA on the glass surface was thoroughly inhibited. Glass containing 5% Zn also decreased the increment of pH after glass immersion in both Tris and DMEM solution assuring endothelial cell adhesion since the cells are very sensitive to pH change. The presence of ZnO in bioglasses at higher concentrations inhibited the amorphous calcium phosphate transformation to HCA [3].

Antibacterial activity of Zn doped hydroxyapatite (Zn-HA) was investigated by Stanić et al. proving the activities of Zn-HA [4]. Other studies related to zinc incorporation into hydroxyapatite revealed that zinc replaces calcium in hydroxypatite structures [5]. Zhou et al. reported the synthesis through a precipitation process, characterization and antimicrobial activities of a nanohydroxyapatite/zinc oxide complex [6]. The obtained material containing HA and ZnO 9:1 (weight ratio) was calcinated at 300°C for 2 hours. They concluded that Zn\(^{2+}\) ions of ZnO can enter the n-HA crystal lattice. It was proven that HA-ZnO complex has good antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* without sacrificing biocompatibility. The materials containing a HA-ZnO system holds potential for application as an antibacterial biomaterial as well as for dental filing and repair [6,11].

During the last few decades, an important research effort has been directed to use microscopy to understand these complex microstructures. Moreover, Mandelbrot’s fractal geometry has brought a new approach and significant progress in understanding and quantifying the effects of magnification and scaling on morphology as well as the methodological approaches necessary to measure self-similar and self-affine structures [12].

The purpose of this study was the investigation of structural and morphological properties of HA-ZnO powders prepared to be used in an endodontic sealer formulation. The development of nano-HA is more significant because of its specific affinity towards many adhesive proteins and its influence on bone cell differentiation and the mineralization process. It is hoped that the incorporation of ZnO nanoparticles to the HA can provide useful outcomes towards the development of antimicrobial properties.

## 2 Materials and Methods

In the present study, hydroxyapatite powders of submicron- and nano-size synthesized in our laboratory were used. Hydroxyapatite (HA) powders were synthesized by precipitation according to previous studies [13]. The reaction was carried out as follows:

\[
10\text{Ca(OH)}_2 + 6\text{H}_3\text{PO}_4 = \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 18\text{H}_2\text{O}
\]

The reaction was performed at room temperature (23°C) and the resulted suspension was maintained for maturation, at room temperature for 120 hours, in the presence of crystallization admixtures. The solution of ZnO (25% in water) was added to the hydroxyapatite gel and reheated to 80–90°C. The obtained suspension was mixed for 1 hour. After 12 hours, the suspension was vacuum filtered using a Buckner funnel through two blue ribbon filter papers. The powder was washed with distilled water 2–3 times, and then transferred into a ceramic vessel and dried in an oven at 120°C for 4 hours to obtain a yield of 95% (η = 95%). The powders were annealed at 400°C, 850°C and 1050°C for 2 hours. The X-ray diffraction (XRD) patterns were obtained with a Bruker D8 Advance powder diffractometer working at 40 kV and 40 mA, using CuKα wavelength, with a germanium monochromator in the incident beam. The measurement was performed in the range angle 2θ = 20–65° and in a step-scanning mode with a step Δ2θ = 0.01°. Pure corundum powder standard sample was used to correct the data for instrumental broadening. XRD patterns of the investigated samples were used for crystal phase analysis. Phase analysis was carried out using the unit cell parameters calculated through structure refinement using PowderCell software [14].

The microstructural information obtained by single X-ray profile Fourier analysis of the ZnO – hexagonal crystalline phase was the effective crystallite mean size, \(D_m\) (nm) and the mean root mean square (rms) of the microstrains, \(\langle \varepsilon^2 \rangle^{1/2}_m\) [15,16]. The Warren-Averbach X-ray Fourier analysis peak profiles were processed with a XRLINE [17] computer program. The FT–IR absorption spectra of the powder in the 380–4000 cm\(^{-1}\) spectral range were obtained with a JASCO 600 FTIR spectrometer using the standard KBr pellet disc technique. The spectra were carried out with a standard resolution of 2 cm\(^{-1}\). Transmission Electronic Microscopy and High Resolution Transmission Electronic Microscopy (TEM, HRTEM) were used for the determination of the particle size; TECNAI F30 G2 with linear resolution of 1 Å and a punctual resolution of 1.4 Å. The Scanning Electron Microscope (SEM) QUANTA 133 from FEI Company was used for the examination of the samples by electron microscopy. The fractal analysis of binary images corresponding to the TEM micrographs of solid microstructures were analyzed using the Image J software (Wayne Rasband, National Institutes of Health,
3 Results and Discussion

The impact of further thermic treatment on the apatite structure is presented in reference [20]. The process used in our study is more effective in retaining the higher energy from nanoparticles for densification because the size of the obtained particles can influence the sedimentation properties and adhesion decrease of the endodontic sealer in time. This led us to produce more thermally stable zinc apatites with a possible different biological response.

The XRD patterns of the obtained HA and HA-ZnO samples are presented comparatively in Fig. 1. The HA lines corresponding to the Powder Diffraction File (PDF) 74-0566 [HA-hexagonal, \(a = b = 0.9424 \text{ nm}, c = 0.6879 \text{ nm}, \) space group hexagonal P63/m (176)] are well developed in all of these samples [21]. The HA lines' intensity increases as the thermal treatment temperature rises from 110°C to 1050°C. XRD diffraction pattern illustrates the fact that the sample obtained in our synthesis conditions contain a major crystalline component ZnO – hexagonal structure phase [22] (Fig.1): ICDD PDF no. 89-7102, \(a = b = 0.32495 \text{ nm}, c = 0.52069 \text{ nm}, \) the space group hexagonal P63mc (186), with Zn and O atoms in special Wyckoff positions (2b).

The microstructural information obtained by single X-ray profile Fourier analysis of the ZnO hexagonal nanoparticles were the effective crystallite mean size \((D)\) and the root mean square (rms) of the microstrains, averaged along the [\(hkl\)] direction, \(<\varepsilon^2>^{1/2}_{[hkl]}\) [10,11]. The Warren-Averbach X-ray profile Fourier analysis of the (100), (102) and (110) ZnO peak profiles were processed by the XRLINE [17] computer program. The unit cell parameters were calculated by Rietveld refinement using PowderCell software [14]. The X-ray diffraction patterns of the HA-ZnO samples were used for crystal structure refinement. The effective crystallite mean size, \(D_{\text{eff}}\) (nm) and the mean root mean square (rms) of the microstrains, \(<\varepsilon^2>^{1/2}_{\text{rms}}\) data for hexagonal ZnO in the HA-ZnO samples are presented in Table 1.

The lattice microstrain value \(<\varepsilon^2>^{1/2}\) for the HA-1050 sample (Table 1) shows an increased value in the intercrystallite zones with a large amount of crystalline lattice defects that suggest a higher HA-ZnO chemical interaction at the surface of HA-ZnO crystallites in this sample. FT-IR absorption spectra of HA, ZnO and of HA-ZnO are presented in Figs. 2, 3 and 4 respectively.

All spectra (Fig. 2) show the bands characteristic to HA. The weak sharp band at 3572 cm\(^{-1}\) can be attributed to free OH valence vibration while the wide intensity band at 3432 cm\(^{-1}\) can be attributed to hydrogen bonded OH groups. Hydrogen bonded OH groups also give a peak at 1625 cm\(^{-1}\). The presence of those two peaks suggests the presence of water traces in the samples. The water was probably adsorbed during the preparation of KBr pellets. The most intensive absorption bands at around 1090 and 1050 cm\(^{-1}\) correspond to asymmetrical valence vibrations of P-O bonds, while the sharp weak band at 964 cm\(^{-1}\) is attributed to the symmetrical valence bond of P-O. The sharp and less intense bands at 632 cm\(^{-1}\), 605 cm\(^{-1}\), and 570 cm\(^{-1}\) can be attributed to the deformation vibration of the P-O-P bond from the \(\text{PO}_4^{3-}\) ions.

It was reported that carbonate ions (\(\text{CO}_3^{2-}\)) can replace hydroxide ions (\(\text{OH}^-\)) from the structure of HA (type A), phosphate ions (type B), or both hydroxide and phosphate ions (type AB) [24]. Almost all wet methods used by our group to obtain the HA led to achievement of the

![Figure 1: (a) X-ray diffraction pattern for ZnO powder and the analyzed samples with HA (80°C) -crystalline structure phase (b) X-ray diffraction patterns for the analyzed samples with HA-ZnO -crystalline structure phase.](image)
carbonated HA (CHA) [23]. FT-IR spectra of HA revealed the presence of carbonate ion absorption bands at about 1400 cm⁻¹ (ν₃) and 870 (ν₂). The intensity of those peaks decreased after thermal treatments at 850°C, and almost disappeared when the temperature of thermal treatment was higher (1050°C). The amount of carbonate embedded into the powders can be evaluated by comparing the peak intensities from the carbonate and the phosphate ions.

The decrease of this ratio with the rise of temperature reveal the reduction of the carbonate concentration in the HA powders since the calcium carbonate decomposes to CaO, which reacts further with H₂O vapours and forms Ca(OH)₂. Similar results were obtained by Ślósarczyk et al. [23].

Metallic oxides with groups M=O give a valence band between 825–1100 cm⁻¹ while zinc oxides give medium and strong absorption bands in the range 500–600 cm⁻¹ and 400–500 cm⁻¹. In the spectra of HA-ZnO obtained at different temperatures (Fig. 3), the absorption bands at 530 cm⁻¹, 480 cm⁻¹, and 440 cm⁻¹ are present which come from zinc oxide. After the thermal treatment at 1050°C, the intensity of those bands increased significantly. The increase of the intensity of the bands can be explained by the interactions that took place between ZnO and phosphate groups from HA. The presence of ZnO seems to influence the vibrations of the HA. This might be caused by the steric hindrances leading to the reduction of the vibrational energy of the HA molecule.

The absorption bands placed in the range of 400-600 cm⁻¹ can be attributed to tetrahedral ZnO₄ and ZnO bonded to phosphate groups. The wide band at 3412-3435 cm⁻¹ belongs to OH bounded by the hydrogen bonds.

### Table 1: Lattice parameters and microstructure parameters of the ZnO hexagonal crystalline phase in HA-ZnO samples

| Sample      | Lattice parameter ± 10⁻⁵ (nm) | Unit cell volume (nm³) ± 10⁻⁵ | Crystallite mean size Dₜₜ (nm) ± 10⁻² | rms microstrains <ε²>₁/₂ 10⁻³ ± 10⁻³ |
|-------------|-------------------------------|-------------------------------|--------------------------------------|--------------------------------------|
| HA-Zn 110   | a 0.32484 b 0.52024           | 0.04754                       | 46.2                                 | 1.329                                |
| HA-Zn 400   | a 0.32514 b 0.52063           | 0.04766                       | 76.4                                 | 0.824                                |
| HA-Zn 850   | a 0.32548 b 0.52095           | 0.04779                       | 156.7                                | 0.214                                |
| HA-Zn 1050  | a 0.32608 b 0.52215           | 0.04808                       | 60.6                                 | 4.447                                |

### Figure 2: FT-IR spectra of HA.

### Figure 3: FT-IR spectra of HA-ZnO.

### Figure 4: FT-IR spectra of ZnO.
The band at 958 cm$^{-1}$ is characteristic to symmetrical ν(P-O) from phosphate anion from HA. The peaks at 1043 cm$^{-1}$ can be attributed to asymmetrical valence P-O and 596-572 cm$^{-1}$ for P-O-P. A small band at 877 cm$^{-1}$ can be attributed to the presence of P-O-H from HPO$_4^{2-}$, and to the vibrations that correspond to CO$_3^{2-}$ ions. Carbonated HA was also clearly present in the HA-ZnO samples, and the absorption peaks of carbonate ions disappeared after thermal treatment at high temperature as in the case of HA alone. The intensities of the vibrations corresponding to OH from HA decreased in the case of HA-ZnO. In the case of the sample treated at 1050°C, OH adsorption band at 360 cm$^{-1}$ disappeared completely and the one at 3572 cm$^{-1}$ was substantially reduced. This can lead to the conclusion that at high temperatures, new bonds are formed and ZnO strongly interact with HA in the vicinity of OH bonds or directly with the OH groups.

The structure model proposed by Zhou et al. [6] explains the interactions between HA and ZnO and how the presence of the ZnO influences the HA vibrations. With an increase of the temperature, the PO$_4$ vibration band shifted from 602 to 605 cm$^{-1}$. Absorption PO$_4$ band at 570 cm$^{-1}$ disappeared in the case of the sample treated at 1050°C, which lead to the conclusion that ZnO strongly interacts with PO$_4^{3-}$ from HA with bi-dentate coordination of Zn [6]. Mori et al. reported that Zn species in the Zn-HA exists in a tetrahedral geometry having a +2 oxidation state [1].

In our study, HA and ZnO have been obtained separately and their interactions were gained through thermal treatment. Major changes in the FT-IR spectra obtained by our group in comparison to previous reports [1,6] can be explained by the increased quantity of ZnO used in our study and by the interactions of the ZnO with the HA caused by the thermal treatment. With increased temperature, the bands shifted from 1094 cm$^{-1}$ towards higher wave numbers (1123 cm$^{-1}$) while the adsorption band from 1044 cm$^{-1}$ remained unchanged.

SEM images for HA treated with temperatures of 120°C and 1050°C are shown in Figs. 5a and 5b respectively. HA treated with 120°C consisted of grains with a very uniform size distribution with no agglomerations looking very aerated. When the thermal treatment applied rose to 1050°C, the HA particles formed aggregates; the powder seems to be much dense, but the particle size appears to be the same. While the HA-ZnO treated with lower temperatures formed particles of elongated shape, at higher temperatures spherical grains were formed (Fig. 5c). The control of processing parameters (such as pH, temperature, time of maturation, and concentration of reactants during
The synthesis) are very important as these parameters may induce changes in crystallinity, morphology and particle size which consequently influence the applications of HA. The results presented here demonstrate the value of texture analysis to quantify the occurrence of mineral deposits by electron microscopy.

The images obtained at higher magnification using TEMBF (Transmission Electron Microscopy Bright Field Image) for analysis of samples of HA and HA-ZnO treated with 1050°C are presented in Fig. 6. The image of HA (Fig. 6a) shows the assembly of nanoparticles in the form of rods with lengths between 15 and 100 nm and thickness around 10 nm. The image of TEMBF (Fig. 6) presents an assembly of particles with submicron sizes. The particles have regular shapes, and their size starts from 0.2 μm. The effect of temperature on the HA crystallite size was also investigated by other authors who observed similar changes [25,26]. Mobasherpour and co-workers showed that the crystallite size of HA produced by a precipitation method increased with the rise in temperature from 100 to 1200°C [25].

As illustrated in the TEM micrograph (Fig. 6b), the n-HA/ZnO complex possesses a uniform and ultra-fine microstructure with no glassy or amorphous interface along the grain boundaries. Also, the ZnO grains combined with n-HA crystals without phase-separation clearly show that the complex forms well defined crystals. The micrograph confirmed that particle sizes ranged from 0.2 to 0.6 μm and EDAX confirmed the presence of Ca, P, O and Zn in the powders. The observed elemental concentration confirmed the Ca₃(PO₄)₂ rich apatite crystalline phase. As expected, the elemental concentration of Zn was found to increase with increased ZnO content in HA.

The fractal dimension was calculated using the box-counting algorithm as the slope of the regression line for the log-log plot of the scanning box size and the count from a box counting scan using Fig. 6a. The slope of the linear region of the plot is (−Df) where Df is the box-counting dimension that corresponds to the fractal dimension. The correlation coefficient (R²) for the regression line shows the relationship between the log of count and size (Fig. 7). An (R²) of 1.0 indicates that the regression line perfectly fits the data.

Lacunarity (Λ) is a counterpart of the fractal dimension that describes the texture of a fractal and reveal how a fractal fills space. It is used to further classify fractals and textures which have the same fractal dimension and a very different visual appearance. If the fractal is dense, the lacunarity is small; the lacunarity increases with coarseness. The fractal analysis of HA-Zn (1050°C) is presented in Table 2.

HRTEM (High Resolution Transmission Electron Microscopy) images of HA and HA-ZnO (Fig. 7a) demonstrate crystallized nanoparticles with diameters of around 1–3 nm. Energy-dispersive X-ray spectroscopy (EDAX) spectrum (Fig. 7b), associated to the micro-area showed in Fig. 7a, prove the presence of Ca, P, Zn, O and C in the sample of HA-ZnO.
The results presented in Figs. 6 and 7 show that samples of HA and HA-ZnO consist of spherical particles with diameters ranging from 50 to 100 nm and the smaller particles form platelet-looking needles. Generally, there was no strong evidence that Zn$^{2+}$ was part of the apatite lattice. No significant changes in the crystallite size or crystallinity were observed in the XRD pattern as well as the FTIR spectra, which did not show any indication that there was a strong effect of Zn$^{2+}$ substitution on the apatite structure.

This study demonstrates a new route for generating a stable zinc enriched apatite that can be used in endodontic sealer with antibacterial effect. The study reporting formation of HA/ZnO powders by precipitation [6], but despite the successful incorporation of metal ions to the HA, they failed to demonstrate antimicrobial effects. We studied a powder (obtained using ZnO sol) with use of a higher amount of ZnO. Due to a higher percentage of ZnO in combination with the HA, the powders obtained in our study have an improved antimicrobial effect and radiopacity [27] in comparison to the results of Zhou et al. [6]. In the present paper, we report a method of preparation of the hydroxyapatite HA/ZnO powder through a simple precipitation process. This has the potential to provide much better dispersion of n-HA particles in the polymer matrix, yielding a composite with uniform microstructure.

Figure 7: (a) HRTEM comparative image of HA and HA-ZnO, (b) EDAX spectra of HA-ZnO sample treated with 1050°C.
4 Conclusion

The paper presents the results obtained upon investigation of the structural and morphological properties of the hydroxyapatite – zinc oxide (HA-ZnO) powders prepared for use as biomaterials at high temperatures: 110, 400, 850 and 1050°C. X-ray diffraction was used in order to study the structural properties of the obtained powder. FT-IR was used in order to evaluate the interactions between HA and ZnO. The increase of the interactions between HA and ZnO with the rise in the temperature of thermal treatment was observed. The FT-IR spectra revealed the formation of carbonated hydroxyapatite. Carbonate ions disappeared after thermal treatment at high temperature. SEM revealed the formation of a powder with uniform size distribution. The treatment with higher temperatures seems to lead to the agglomeration of the particles. TEM and HRTEM were used for the determination of the particles size and shape, the favorable formation of rods in the case of HA and elongated particles in the case of HA-ZnO were observed. We also demonstrated that the complexities of shapes and distributions in HA-Zn powders can be described by fractal geometry.

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