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# Importance of defect nanocrystalline calcium hydroxyapatite characteristics for developing the dental biomimetic composites



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## ABSTRACT

Applying a set of structural-spectroscopic methods of analysis we have studied the relationships between the structure, morphology and sorption characteristics of the samples of nanocrystalline carbonate-substituted hydroxyapatite (C-HAP) synthesized with the use of biogenic calcium source (poultry eggshell) obtained by the chemical deposition from a solution.

With the use of X-ray diffractometry and X-ray microanalysis it was found that the appearance of impurities in the structure of synthesized material was due to their presence in calcium carbonate of the poultry eggshell and this could result in the changes of parameters in the elementary crystal cell of hydroxyapatite without formation of the additional phosphate phases. Optical and ESR spectroscopy allowed us to find that in the presence of the inherited set of impurities inclusion of carbonate ions into the structure of our synthesized hydroxyapatite takes place with the formation of C-HAP of B-type. An increase in the content of phosphate groups PO<sub>4</sub> in the process of the materials synthesis in the atmosphere results in the reduction of that of one of structurally bound CO<sub>3</sub> groups.

A decrease in the pH value in the solution during the synthesis connected with the increase in the  $PO_4^{3-}$  anions content leads to the change of morphology of the C-HAP samples due to the increase in the size of intracrystalline defects – nanopores at the surface of C-HAP nanocrystals. Such a change in the morphology of the materials resulted in the changes of the sorption characteristics of the samples that were measured by means of thermal desorption of nitrogen technique. The specific surface area of C-HAP powders determined by means of the BET technique was ~55.5 ±  $0.9 \text{ m}^2/\text{g}$ , exceeding by several times that of the known analogs. Meanwhile, despite the developed surface the C-HAP samples remained rather stable in the atmosphere of the saturated water vapour and the main losses under polarization are related to Maxwell-Wagner losses.

Analysis of characteristics for the C-HAP samples obtained from a biogenic source of calcium demonstrated their potential significance for obtaining of biomimetic materials imitating structure, morphology and anisotropy of the native hard tissues of the human tooth.

#### Introduction

The use of nanostructured composites when solving the problems of therapeutic dentistry related to the restoration and regeneration of the native hard dental tissue is due to the hierarchical nature and functional characteristics of the dental enamel and dentin [1-3]. Organic-mineral biocomposites with a composition that is unrelated to the hard dental tissue are actively applied when producing of the dental cements and ceramics [3,4]. Note that their morphology, strength properties and wearable characteristics differ from the properties of the native dental tissue. Dental therapy is frequently performed with the use of these materials results in appearance of the cracks, demineralization, secondary caries, erosion and mechanical deformation of teeth [1,5]. In spite of intensive studies in the field of biomaterial research this problem is yet unsolved and as urgent as it has always been.

Recent investigations of the mineral and organic components of

teeth show the availability of the use of biocomposites with the properties that are closest to the dental tissue [6]. Therefore, the elaborated and already developed organic-mineral biomimetic composites applied in dentistry definitely involve the analog of the mineral component of hard dental tissue, i.e., calcium hydroxyapatite (HAP) in their composition [3]. Hydroxyapatite has a unique structure, but it can also be synthesized within a wide range of the required characteristics in various forms due to a number of the ways and techniques of its production. This makes it a prospective modification agent of the dental ceramics and dental cements [7,8]. It is well known that physical and chemical properties of HAP and its morphological organization can be rather well varied at the expense of the way of production and the type of impurities introduced into HAP structure [9–11], as well due to the sources used for its production [12–14].

Therefore, an important problem in the field of contemporary dental materials science and fundamental medicine is a search for the

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techniques for HAP synthesis. This material can become a basis for biomimetic composites that are highly-tropic to the intact tissue. These biocomposites should reproduce structural, physical and chemical, morphological, optical and biomechanical properties imitating the structure (including that at the nano-level), hierarchy and anisotropic properties of the hard dental tissues. They must also be capable of forming a stable functional bind with the native dental tissue [6,15].

One of the possible ways to obtain HAP is the use of biogenic materials as a source of calcium [12,16]. However, the physical and chemical characteristics of such HAP samples require comprehensive studies since the raw biogenic materials possessing their own morphology, structure, as well as various elemental and phase composition have a tremendous effect on the final properties of the synthesized materials [17,18]. A review of scientific literature demonstrated that there is a great number of works concerned with the study of morphology, structural organization and the influence of the type of the introduced impurities on the properties of HAP. Meanwhile sorption properties of synthesized HAP are not sufficiently investigated, even though they play an important role and are of a great importance when developing dental cements of a new class. Actually, sorption characteristics determine cement shrinkage and adhesion to the native tissue under tooth restoration.

As it was shown previously in the papers concerned with hydroxyapatite production with the use of the poultry eggshell [19-24], this material can be obtained in the nano-form. However, HAP samples obtained by means of the chemical deposition technique with the use of the eggshell can involve impurities of the different ions. Particularly, in a number of studies (see, e.g., [21,24]) possible inclusion of carbonateanion into the structure of HAP was noted if the samples were obtained in the atmosphere. It should be emphasized that using calcium carbonate and orthophosphoric acid, it is possible to obtain carbonate-substituted hydroxyapatite with carbonate-anion content of about 8 mass. % [25]. Using the eggshell from the morphological viewpoint, it is possible to obtain different materials with the mean-size of nanocrystals varying from 40 to 100 nm [23,24], including those with the plate-like shape with the thickness of 8-15 nm and 30-50 nm in the diameter [19]. One should note that applying methods of "wet" chemistry, it is possible to obtain materials with a more developed specific surface area than under the high-temperature treatment of the natural animal bones [26], which is certainly an advantage of this technique under the treatment of biogenic sources.

Therefore, the aim of this work was to determine the dependences of the sorption, morphological and structural characteristics of nanocrystalline hydroxyapatite on the parameters of the synthesis and the following treatment as well as the possibility of the application for the final material for the development of the dental biomimetic composites.

#### Materials and methods

# Technology of HAP synthesis

In the current study nanocrystalline HAP was obtained with the use of poultry eggshell according to the technique described in [12]. HAP was prepared by titration of calcium hydroxide  $Ca(OH)_2$  with 0.3 M solution of orthophosphoric acid H<sub>3</sub>PO<sub>4</sub>. Poultry eggshell was employed as a source of calcium; it consists by 95% of calcium carbonate (CaCO<sub>3</sub>). Applying thermal anneal we obtained high-porous calcium oxide (CaO) from the eggshell and it was in turn for preparing of calcium hydroxide  $Ca(OH)_2$  [12]. Since it is known that preparing of hydroxyapatite in the atmosphere accompanied by absorption of  $CO_2$  [27], titration was conducted with the change of amount of the fed H<sub>3</sub>PO<sub>4</sub> solution. The change in concentration of the carbonate-involved groups was controlled by the changes in the pH value of the solution from 9 to 7 with a step of 0.5 (pH-meter/ionomer IPL 111-1).

#### X-ray spectral microanalysis

An elemental analysis of the samples obtained in our laboratory was performed by means of the X-ray spectral microanalysis (XRMA) with the attachment Inca – 250 to the scanning electron microscope JEOL JSM-6380LV.

### X-ray diffraction analysis

Determination of the phase composition as well as the sizes of the coherence scattering areas was performed by X-ray diffraction technique with the use of diffractometer DRON 4-07. An X-ray tube with cobalt anode and characteristic radiation of (K<sub> $\alpha$ </sub>  $\lambda$  = 1.7902 Å) was applied. The accuracy of the XRD experiment was 0.001 Å. The X-ray phase diffraction analysis was performed with the use of database JCPDS –ICDD for 2016.

#### Infrared and Raman spectroscopy

IR-spectra for the samples were obtained with the use of attachment for attenuated total reflection Diamond ATR with the diamond prism for IR-Fourier spectrometer VERTEX 70 BRUKER. Transmission spectra were recorded with the resolution of  $4 \text{ cm}^{-1}$  within the range of 400–4000 cm<sup>-1</sup>. Raman spectra were recorded with the use of spectrometer Raman Microscope RamMics M532 EnSpectr in the range of 350–3650 cm<sup>-1</sup> employing a laser with the wavelength of 532 nm.

#### Electron paramagnetic resonance

In order to determine radicals in the structure of the obtained nanocrystalline HAP samples spectra of the stationary and echo-detected electron paramagnetic resonance were recorded using spectrometer Bruker E580.

#### Transmission electron microscopy (TEM)

TEM technique was employed for a direct estimation of the sizes of nanocrystals for synthesized HAP samples as well as for obtaining microdiffraction from the local areas of nanocrystals. The study was carried out with the electron microscope of Libra 120 Carl Zeiss. Through the course of the preparation of the samples for the TEM study they were subjected to ultrasonic exposure ( $\sim 1$  GHz).

#### Dielectric spectroscopy

In order to perform some of the experiments, the HAP samples were compacted under the pressure of 250 bars with the tin foil thus forming a plane capacitor. Thus the resulting capacitor was placed in a vessel with the controlled pressure of saturated water vapour. The study of dielectric characteristics of the capacitor was performed with the use of LCR-meter GW Insteck LCR-819.

#### Results and their discussion

The influence of pH value in a solution where the synthesis of the samples was employed on their phase composition was studied by means of the X-ray diffraction phase analysis technique (XRA) of the materials obtained at pH values of a solution varying from 9 to 7 with a step of 0.5 and annealed at the temperature of 400 °C. The XRA results demonstrated that for all of the samples displayed the formation of a single phase, i.e. calcium hydroxyapatite. A typical diffractogram of HAP powder obtained from a solution with pH = 8.5 (curve 1) is presented in Fig. 1 as an example.

Since in the process of development of the dental cements as well as biomimetic materials HAP samples are often subjected to compaction, determination of the structural changes caused by the treatment of the



Fig. 1. Diffracrograms of the HAP sample obtained from a solution with pH = 8.5 and annealed at 400 °C in different forms: curve 1 – powder, curve 2 – compacted pellet.

raw powder-like material was performed by means of the data of the Xray diffractometry analysis. The results of the diffraction analysis (Fig. 1, curve 2) show that following the compaction a redistribution of intensities of the main diffraction reflections can be clearly seen in the diffractogram of the sample. The experiments show a decrease in the relative intensity for the diffraction reflections (0 0 2), (0 0 4) and enhancement of intensity for (3 0 0) reflection. This fact is related to the texture formation in the HAP samples following the compaction at the pressure of 250 bars.

The detailed study and comparison of HAP obtained according to our technology and that one of the samples of enamel performed by us in [28], demonstrated that the obtained materials were characterized by the lower size of nanocrystals as compared with the biogenic sample. Similar results were obtained in [29], where the samples of HAP were also obtained by liquid-phase technique. One should note that nanocrystalline powders of calcium hydroxyapatite obtained in this study were in a better correspondence by their structural characteristics with human dental dentine, that is confirmed by the results of [30,31]. Since dentine is characterized by a greater degree of disorganization the size of its nanocrystals is less than in the enamel. In addition, as it was shown in [28], enamel shows an expressed texture that is related with the hierarchical structure and arrangement of the crystals of the biogenic apatite in amelogenin matrix [32]. At the same time it should be noted that in biogenic materials there is a lot of impurities which can affect on the elementary cell parameters. Based on the experimental data of the X-ray diffraction for all of the HAP samples, the parameters of their elementary cell were determined. The obtained results as well as the data from JCPDS -ICDD database are presented in Table 1.

According to the comparison of the elementary cell parameters with the available reference data, one can see that materials synthesized in our study demonstrate lower values of the crystal lattice parameters compared to the similar samples presented in the database of JCPDS-

#### Table 1

Parameters of the lattice cell for the obtained hydroxyapatite samples in comparison with the those of the database JCPDS-ICDD. The accuracy of the XRD experiment is at the level of 0.001A.

НАР	Parameter <b>a,b;</b> Å	Parameter <b>c; Å</b>
ICDD № 09–432 ICDD № 01–074-0565	9418 9424	6884 6879
$\begin{array}{l} \text{HAP pH} = 9 \\ \text{HAP pH} = 7 \end{array}$	9406 9404	6871 6864

ICDD. It is well-known that conditions of HAP synthesis have an influence on the processes of the crystal growth. In its turn, this might result in the changes of the parameters of the crystal structure [33]. Therefore, the lower values of parameters for the elementary cell **a**, **b** and **c** in the HAP samples obtained in our work can be related to the features of our technique for HAP synthesis from the poultry eggshell as well as with the presence of impurities in its composition.

The results of the X-ray spectral microanalysis of the eggshell averaged by 10 samples are presented in Table 2.

As it is seen from the obtained data composition of the raw reagent for synthesis of HAP samples (eggshell) involves atoms of magnesium, sulphur, potassium and chlorine that can be embedded into the structure of the final material and thus form structural defects. Investigations of HAP samples obtained according to our technology using microanalysis technique demonstrated that only magnesium atoms in a trace concentration were found while those of sulphur, potassium and chlorine were not registered by means of XMA technique. This fact means that in the process of synthesis some part of the impurity atoms involved in the raw solution is removed from the final material or they are present in a lower concentration.

Optical investigations of the synthesized and prepared HAP samples made it possible to specify the molecular composition of the materials as well as their structural properties more accurately. Fig. 2 represent IR-spectra of the HAP samples obtained at the pH values of 9 and 7 and annealed at the temperature of 400 °C. As one can see from Fig. 2, the IR-spectra of the samples show an identical set of the vibration bands. No other changes in the frequency of the main vibration bands can be observed and no redistribution of their intensities takes place. This result is in a good agreement with the data of X-ray diffraction phase analysis on the presence of the only phase – calcium hydroxyapatitee – in all of the samples (annealed at the temperature of 400 °C).

It should be noted that for all of the obtained samples a characteristic feature is the presence of  $CO_3$  group in the spectra of vibration bands at  $1415\,\mathrm{cm}^{-1}$  and  $1450\,\mathrm{cm}^{-1}$ . It is well known that the appearance of these vibration modes in the spectra of HAP is related with the substitution of PO<sub>4</sub> group in the crystal lattice of HAP by CO<sub>3</sub> group and formation of carbonate-substituted hydroxyapatite (C-HAP) of Btype. Formation of C-HAP of B-type under the use of selected synthesis technique in our study B is in favor of the fact that when reducing the pH value of a solution used in the process of the materials production the intensity of vibration band of the carbonate-anion involved in the HAP structure decreases as well. It should be noted that comparison of the molecular composition of HAP synthesized in our work with that one of the human dental enamel and dentine was performed basin on FTIR data in [6]. The obtained results coincide with XRD data [28]. Comparative analysis of the properties in the native dental tissues and the obtained HAP samples demonstrated that the synthesized materials were in a better correspondence with a tooth dentine in the inorganic part of dental matrix while the observed spectral differences could be due to its hierarchical [6] revealed the availability of using HAP obtained from the eggshell as a basis for stomatological materials from the viewpoint of their molecular composition and optical characteristics.

In the number of works [34,35] concerned with the study of C-HAP it was shown that in case of appearance of distortions in the crystal lattice of HAP and inclusion of  $CO_3$  anion in the structure of apatite a shift of  $PO_4$  bands was observed in Raman spectra. For the samples obtained in our work at the different values of pH = 9, 8.5, 8, 7.5, 7, where the presence of  $CO_3$  group in the structure of apatite according to the data of IR spectroscopy can vary within a wide range determination of the structural defects in C-HAP was performed by means of Raman spectroscopy technique.

As seen from Fig. 3, in the Raman spectra of C-HAP samples obtained at the different values of pH for solution of the deposition one can see that besides the main modes corresponding to phosphorus-oxygen group of PO<sub>4</sub> and localized at 961, 587 and 431 cm<sup>-1</sup> respectively, an additional Raman mode is observed at ~1070 cm<sup>-1</sup>. Its

#### Table 2

Elemental composition (in at%) of the poultry eggshell samples used for producing of C-HAP.

Concentration of the elements in the material composition for obtaining of HAP, at%								
Са	Р	0	С	Mg	S	К	Cl	
$10.6 \pm 2.3$	$1.4 \pm 1.1$	55.7 ± 4.60	$30.9 \pm 4.70$	$1.0 \pm 0.40$	$0.1 \pm 0.04$	$0.1~\pm~0.00$	$0.1~\pm~0.00$	



Fig. 2. IR-spectra of hydroxyapatite samples obtained at pH=9 and 7 and annealed at 400  $^\circ\text{C}.$ 



Fig. 3. Raman spectra of the samples with pH 9 and 7, annealed at 400 °C,  $\lambda_{ex}=532$  HM.

appearance in the spectra can be attributed as to the carbonate anion  $CO_3^{2-}$ , as to the complex of  $PO_4^{3-}$ . Note that the ratio of intensities of additional mode and the main phosphate mode localized at 961 cm<sup>-1</sup> in all of the spectra remains approximately the same-

Detailed treatment of the shape and intensity of vibrations observed at the range of 1000–1150 cm<sup>-1</sup> and related to the group of PO<sub>4</sub> (1030, 1048 cm<sup>-1</sup>) and the group of CO<sub>3</sub> (1073 cm<sup>-1</sup>) Fig. 3a shows that with an increase in the intensity of PO<sub>4</sub> mode in the spectrum (pH = 7) a decrease in the intensity occurs for the band of carbonate ion. This fact correlates quite well with the data of IR-spectroscopy on the nature of carbonate-substituted samples of C-HAP of B-type. Analysis of the data on Raman scattering as for enamel as for dentine [36–38] showed a complete coincidence of as in position as in the bands intensities within Raman spectra for all of the synthesized materials and this correlated with the results of XRD  $\mu$  FTIR. The vibration modes observed in FTIR and Raman spectra are presented in Table 3.

In order to specify the data on the radicals which were present in the samples, the electron paramagnetic resonance technique was employed. One should note that EPR signals in the obtained C-HAP samples were not observed prior to the irradiation. The samples were dried at 400 °C (for 1 h) obtained at the different values of pH and irradiated with electrons at the dose of 10 kGr 7 days later following the irradiation were studied by means of the stationary and echo-detected electron paramagnetic resonance (EPR). Method of echo-detecting (ED) EPR was applied in order to separate the overlapping components of EPR spectra at g = 2.0000.

The decomposition of the initial signal into components in the spectra of ED EPR made it possible to separate 2 overlapping responses from  $\text{CO}_3^{3-}$  and  $\text{CO}^{2-}$  radicals characterized by a long time of relaxation and thus observed in the spectrum of ED EPR.

For the determination of the nature of radicals in all of the C-HAP samples simulation of the EPR spectra for each of the samples was performed. This allowed us to determine that a total of 5 signals is observed in EPR spectra and can be attributed to the following radicals:  $O^-$ ,  $CO^{2-}$ ,  $CO^{3-}$ ,  $CO_3^{3-}$  [39] and from the organic radical CH<sub>3</sub> [40]. The parameters of the paramagnetic radicals observed in the EPR spectra of the investigated samples are presented in Table 4.

It was found that at the change of content of the phosphorus-oxygen groups in the samples spin concentration of the paramagnetic carbonate radicals  $\rm CO^{2-}$ ,  $\rm CO^{3-}$ ,  $\rm CO_3^{3-}$  (as well as the intensity of their paramagnetic centers in the IR and Raman spectra) increases while that of  $\rm O^-$  and  $\rm \dot{CH}_3$  radicals reduces. It should be emphasized that biogenic apatite of the dental enamel and dentine has the same set of inorganic radicals as the material obtained in our work. The absence of the other paramagnetic centers allows to state that from the viewpoint of deficiency the material obtained in the work also satisfies the requirements concerning biogenic apatite of the human teeth [39].

Intra-lattice substitutions in the obtained samples and formation of C-HAP must be surely represented in the nanostructure of a material. In order to specify the crystalline structure and to determine the phase composition, which is characteristic of an individual nanocrystallite in the synthesized samples, the TEM technique of a high resolution was employed. The C-HAP samples obtained at the different pH values of 9, 8.5, 8, 7.5, 7 and annealed at 400 °C that were additionally subjected to ultrasound dispersing in the aqueous medium have been studied. The experiments showed that the C-HAP nanocrystals formed as a result of the processes had a rachis-like shape with the diameter of  $\sim 20$  nm and the length of  $\sim 50$  nm for all of the investigated samples. TEM images of the C-HAP nanocrystals are presented in Figs. 4 and 5 as well as the microdiffraction data for the materials obtained according to our technology from the solutions with pH = 7 and 9 and annealed at 400 °C.

Detailed investigations of C-HAP samples by TEM with a nanometer resolution demonstrated the presence of a porous structure in the nanocrystals of these materials (Figs. 4 and 5). According to the data from scientific literature, the observed porous structure of C-HAP is characteristic of the samples of calcium hydroxyapatite obtained by means of the chemical deposition methods [43]. The nanopores in C-HAP are 3D defect. From the obtained microphotographs of the samples

Table 3							
Vibration	modes	observed	in	IR	and	Raman	spectra.

Raman band	Raman Shift, $cm^{-1}$	Molecular group attribution	FTIR band	Wavenumbers, $cm^{-1}$
$v_2 PO_4$	431	O-P-O bending, $v_2$	-	-
$v_2 PO_4$	447	O-P-O bending, $v_2$	-	-
$v_4 PO_4$	579	O-P-O bending, $v_4$	$PO_4 \nu^4$	573
$v_4 PO_4$	590	O-P-O bending, $v_4$	$PO_4 \nu^4$	602
υ <sub>4</sub> PO <sub>4</sub>	607	O-P-O bending, v4	-	-
$v_4 PO_4$	614	O-P-O bending, $v_4$	-	-
-	-	OH	OH	627
P-(OH)	880-885	HPO <sub>4</sub>	$CO_3 (\nu^3)$	872
$v_1 PO_4$	962	P-O stretching	$PO_4 \nu^1$	963
HPO <sub>4</sub>	1005	Sym stretching	-	-
$v_3 PO_4$	1028	PO <sub>4</sub>	-	-
$v_3 PO_4$	1040	Shoulder P-O antysym stretching	-	-
$v_3 PO_4$	1047	Most intensive v3 PO4 P-O antysym stretching	-	-
$v_3 PO_4$	1052	Shoulder P-O antysym stretching	-	-
υ <sub>1</sub> CO <sub>3</sub> B-type	1070-1072	B-type CO <sub>3</sub>	-	-
$v_3 PO_4$	1076-1077	P-O antysym stretching	-	-
υ <sub>1</sub> CO <sub>3</sub> A-type	1106	А-тип	-	-
-	-	PO <sub>4</sub>	$PO_4 \nu^3$	1090
-	-	CO <sub>3</sub>	$CO_3 (\nu^3)$	1415
-	-	CO <sub>3</sub>	$CO_3 (\nu^3)$	1451
OH	3570	OH	OH	3570

presented in Figs. 4 and 5 it is seen that as the pH decreases in the solution, the sizes of the nanopores increase. For the samples obtained at the values of pH = 9 in the solution pores sizes are within the interval of 1–4 nm, while for the samples obtained at pH = 7 – within the interval of 3–8 nm.

It should be emphasized that the previously conducted investigations on microdiffraction of the obtained materials from the local areas of C-HAP samples obtained at pH = 9, 8.5, 8, 7.5, 7 confirmed the X-ray diffraction data on their single-phase character [44].

It is well known that the developed surface of porous C-HAP materials is an important characteristic of the samples of such kind that can be used in the dental materials science including the development of biomimetic materials. Therefore we have also determined specific surface areas of the synthesized C-HAP powders using thermal desorption of nitrogen technique. The conclusions made based on the results of measurements for the volume of gas (nitrogen) retained on the studied samples for the four different values of the partial pressure (Fig. 6). These values were calculated with the use of software suite UniSor bi for the instrument SORBI-MS according to the theory of Brunnauer-Emmet-Teller (BET). Estimations of specific areas were performed in the approximation of the same size of the particles. Curves of thermal desorption of nitrogen (TDN) obtained for the materials synthesized at pH values of a solution equal to 9 and 7 and annealed at 400 °C are presented in Fig. 6. Calculations of specific area for all of the C-HAP powders according to the data on thermal desorption demonstrated (Fig. 6) that the samples obtained at the different values of pH in the solution (pH = 9, 8.5, 8, 7.5, 7) have the same specific area surface of  $\sim 55.4 \pm 0.9 \,\mathrm{m}^2/\mathrm{g}.$ 

The developed surface of the C-HAP samples as well as the fact that hydroxyapatite is a wide band gap dielectric ( $E_g = ~7.7 \text{ eV}$ ), should be unambiguously manifested in its electrochemical properties. The most convenient research method of this issue is impedance-spectroscopy. It was previously shown found that employing this technique the

appearance of dependence of the loss-angle tangent tg $\delta$  in the annealed hydroxyapatite materials was determined by the presence of structural defects appearing during hydroxyapatite fabrication [45]. However, when using materials based on HAP in the real conditions rather researchers rather often deal with the wet media. Therefore, in order to study the dependence of tg $\delta$  on the frequency of the electric field, the investigated samples were studied under the conditions of the controlled water vapour sorption.

Investigations of the dielectric parameters for C-HAP samples were performed under the conditions of the controlled sorption of water vapour. The obtained data concerning loss-angle tangent tg $\delta$  in C-HAP showed (Fig. 7) that within the range of frequencies of 10 Hz – 100 kHz and for the relative humidity of the atmosphere (p/p\_s): 0%  $\leq$  p/  $p_s \leq$  100% the conductivity of the carbonate-substituted hydro-xyapatite is determined based on the water sorbate.

A specific feature of the loss-angle tangent in the C-HAP caused by Maxwell-Wagner polarization (Fig. 7) is the appearance of a broad band with the clearly-expressed maximum in the experimental spectra. The position of this maximum is shifted towards the high frequencies under an increase in the relative humidity  $p/p_s$  in the range of  $65\% \le p/p_s \le 100\%$ . A similar effect is observed for the systems that are identical in their structure with HAP [46].

The authors of the similar known works [47–51] ascribe the character of the changes for the dielectric loss-angle tangent in the pure HAP to the reorientation of OH groups. The explanation of the data obtained in our work can be provided due to the following assumptions. We suppose that the charges in C-HAP samples under the exposure in the external alternating electric field are formed at the interfaces of the conducting and isolating layers. In our case the boundary between the hydroxyapatite nanocrystals and retained water can be considered as a set of dipoles. The direction of the dipole moments changes as does the applied alternating field. From the experiments it follows that the dielectric losses in the samples are enhanced when the period of

Table 4

Parameters of paramagnetic radicals observed in EPR spectra for the samples of HAP, T = 300 K.

Para-magnetic radical	Paramagnetic center [4]	Position [1–4]	<b>g</b> 1	g2	$G_3$	$A_1 = A_2 = A_3, \mathrm{mT}$	Reference
$0^{-}$ $CO_{2}^{-}$ $CO_{3}^{-}$ $\dot{C}H_{3}$ $CO_{3}^{3-}$	O <sup>2-</sup> CO <sub>2</sub> */C O <sub>3</sub> <sup>2-</sup> CO <sub>3</sub> <sup>2-</sup> - CO <sub>3</sub> <sup>2-</sup>	In the inter-layers near the surface of nanocrystals In the positions of $\mathrm{PO_4}^{\mathrm{3-}}$ groups	2.0422(5) 2.0032(3) 2.016(1) 2.0029(3) 2.0048(3)	2.0333(5) 2.0020(3) 2.0100(3) 2.0029(3) 2.0032(3)	2.0023(5) 1.9977(3) 2.004(1) 2.0029(3) 2.0017(3)	- - 2.23 -	[31,33] [39,41] [42] [40] [39]



Fig. 4. TEM of the sample obtained at pH = 9, annealed at 400 °C.

oscillations in the strength of the electric field becomes comparable with the relaxation time of surface polarization.

#### Conclusion

Based on the experimental data obtained with the use of a set of structural-spectroscopic methods of analysis we succeeded to show that employing biogenic source of calcium (poultry eggshell) it is possible to obtain nano-size carbonate-substituted calcium hydroxyapatite with high sorption characteristics. Applying optical spectroscopy it was found that the change of pH value of solution used in the process of the materials synthesis resulted in the changes of the surface of C-HAP nanocrystals. Moreover, these changes influence on the size of the nanopores on the surface of nanocrystals. The structural defects in the C-HAP nanocrystals detected by means of the EPR technique are attributed to the impurities that are present in the raw reactant, i.e. eggshell. The presence of these impurities in the structure of the final material results in the change of the parameters for the elementary cell in the crystal lattice of C-HAP. The presence of carbonate-ions in the structure of C-HAP of B-type is confirmed using the IR-spectroscopy and Raman spectroscopy techniques. Their occurrence does also provide a considerable contribution in the observed surface morphology.

The calculations of the specific surface area for C-HAP powders with the use of BET technique according to the data of thermal desorption of nitrogen demonstrated that the obtained C-HAP samples at the different pH values from 9 to 7 with a step of 0.5 are characterized by the specific surface area of about ~55.4  $\pm$  0.9 m<sup>2</sup>/g, thus exceeding by ~3 times the known analogs. It is an important sorption capacity of the synthesized materials. The observed sorption capacity of our C-HAP powders is due to the method of their production and is determined by means of the developed morphology of material.



Fig. 6. Dependences of TDN for the samples of C-HAP obtained at the values of pH = 9 and pH = 7, and annealed at 400 °C.

Investigations of the synthesized nanomaterials of C-HAP that were also subjected to compaction performed by X-ray diffraction and impedance spectroscopy make it possible to imply that such a pre-treatment of hydroxyapatite powders does not change their phase composition, but they are quite sensitive to the content of OH groups in them.

Thus, owing to their characteristics C-HAP samples synthesized according to our technology from the biogenic calcium source are potentially significant for developing the biomimetic materials that are similar in their structure to the native hard dental tissues of a human.



Fig. 5. TEM of the sample obtained at pH = 7, annealed at 400 °C.



**Fig. 7.** Dependence of the dielectric loss tangent  $tg(\delta)$  on the frequency f in the samples of C-HAP at different values of the relative humidity  $p/p_s$ , %.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rinp.2019.102158.

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