Collagen/silicocarnotite composites, cross-linked with chondroitin sulphate: in vitro bioactivity

Lachezar Radev¹*, Vladimir Hristov¹, Irena Michailova¹, Maria H. V. Fernandes², Isabel M. M. Salvado²

¹University of Chemical Technology and Metallurgy, Sofia, Bulgaria
²University of Aveiro and CICECO, Aveiro, Portugal

Received 6 June 2011; received in revised form 12 September 2011; accepted 18 September 2011

Abstract

In this work we present the experimental results on synthesis, structure evolution and in vitro bioactivity of collagen-silicocarnotite-chondroitin sulphate composites. The obtained samples were synthesised by mixing collagen (C) and silicocarnotite (S) powder with C:S ratio of 75:25 and 25:75 wt.% in the presence of chondroitin sulphate (ChS). Collagen was diluted in 5M CH₃COOH before mixing. The obtained materials were characterized by X-ray diffraction (XRD), Fourier-transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM) before and after in vitro test in 1.5 simulated body fluid. XRD of synthesized samples showed that before immersion carbonate containing hydroxyapatite (CO₃HA) were formed in situ in the composites. FTIR depicts the presence of HPO₄²⁻ and the “red shift” of COO⁻ and SO₃⁻ from ChS was also observed. This “red shift” could be attributed that the ChS prefers to chelate Ca²⁺ from partially dissolved S powder. SEM of the prepared samples show the presence of nanosized hydroxyapatite (HA) whiskers and flower-like HA assemblies. After in vitro test, XRD proved the presence of HA with well-defined crystallinity. According to the FTIR results B-type CO₃HA can be observed. SEM of the precipitated layers show the presence of HA spheres. Inductively coupled plasma atomic emission spectroscopy results lead to a conclusion that after in vitro test of the prepared composites silicon containing carbonate substituted hydroxyapatite (Si-CO₃HA) may be formed on the surface of the immersed samples.

Keywords: silicocarnotite, collagen, chondroitin sulphate, hydroxyapatite whiskers, in vitro bioactivity

I. Introduction

Bones and teeth consist of biocomposites with well-organized nanostructured hydroxyapatite (HA) to perform important biological functions. It is well documented that the ordered structures in mineralized tissues appeared to originate from organized assemblies of biomolecules, such as collagen, polysaccharides and proteoglycans [1–3]. Previous articles indicate that some biomacromolecules are added for controlling the nucleation, growth and size of calcium phosphate phases. Chonroitin-4-sulphate (ChS) is just one of these molecules. It consists of a repeating disaccharide unit of D-glucoronic acid linked to N-acetylglalactosamine. The galactosamine residues are sulphated in position 4 [4]. It is obvious that galactosamine belongs to the family of glycosaminoglycans, which can be found on cell surfaces and the extracellular matrix of cartilage and bone [4–6]. The aim of some preliminary results is to contribute to the understanding of the role of ChS in biomineralization process [7–15]. Some studies show that ChS is an inhibitor of HA formation and growth from the calcium phosphate solutions, as well as an inhibitor of transformation of so-called amorphous calcium phosphate to crystalline HA [8,14]. Other studies show that the combination of sulphate and carboxylate groups gives a very high density of negative charge and may allow them to act as nucleation sites for the HA formation [11,12,15]. Despite these papers, all authors summarized that the self-assembly of the HA crystals is critically dependent on

* Corresponding author: tel: +35 92 816 3280 fax: +35 92 868 5488, e-mail: l_radev@abv.bg
the chemical interactions between the HA crystals and the functional groups of ChS. The nucleation models are based on the binding of Ca$^{2+}$ to the periodically arrayed functional groups of ChS [7–15].

In our previous work we have synthesized some bioactive calcium phosphate silicate (CPS) ceramics [16]. The obtained ceramic powders were in vitro bioactive in 1.5SBF for different periods of time. On the bases of the prepared ceramics, we have produced in vitro bioactive composites with gelatin and collagen [17,18] without cross-linkage.

The purpose of this article is to prepare and characterize collagen/silicocarnotite composites, obtained from collagen (C) and silicocarnotite (S) in the presence of ChS and investigate their in vitro bioactivity.

II. Experimental

2.1 Preparation of the composites

The silicocarnotite powder, the inorganic part of the composites, has been synthesized via multi-step sol-gel method. The molar ratio Ca/P+Si of the chemical composition from initially prepared silicocarnotite sol, as described as 58.12 wt.% CaO, 29.4 wt.% P$_2$O$_5$, 12.45 SiO$_2$ wt.%, was equal to 1.67. The procedure for the synthesis and structure evolution of the obtained ceramic powder was described in our previous work [16]. Collagen type I, taken in amounts corresponding to the composite content, was diluted in 20 ml 5M CH$_3$COOH for 24 h at room temperature. The C-S composite materials, for two types of proportions 25:75 and 75:25 wt.% were prepared by adding S to the C solution with constant intensive stirring for 6 h. pH of the obtained mixture was 2. After homogenation time, pH was adjusted to 9 using 25% NH$_4$OH. ChS was added as cross-linking agent. The obtained composite materials were dried at 70°C for 12 h under vacuum.

2.2 In vitro test for bioactivity

Bioactivity of obtained composites was evaluated by examining the apatite formation on their surfaces in 1.5SBF solution. The 1.5SBF solution was prepared from reagents as follows: NaCl = 11.9925 g, NaHCO$_3$ = 0.5295 g, KCl = 0.3360 g, K$_2$HPO$_4$×3H$_2$O = 0.3420 g, MgCl$_2$×6H$_2$O = 0.4575 g, CaCl$_2$×2H$_2$O = 0.5295 g, Na$_2$SO$_4$ = 0.1065 g, and buffering at pH 7.4 at 36.5°C for 3 days. A few drops of 0.5% sodium amide (NaN$_3$) were added to the 1.5SBF solution to inhibit the growth of bacteria [19]. After soaking, the specimens were removed from the fluid, gently rinsed with distilled water, and then dried at 36.6°C for 12 h.

2.3. Methods for analysis

The structure and in vitro bioactivity of composite materials were monitored by X-ray diffraction (XRD) analysis, Fourier-transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM). Powder X-ray diffraction spectra were collected within the range from 10° to 80° 2θ with a constant step 0.04° 2θ and counting time 1 s/step on a Bruker D8 Advance diffractometer with CuKα radiation and SolX detector. The spectra were evaluated with the Diffracplus/EVA package. FTIR transmission spectra for the obtained samples were recorded by using a Bruker Tensor 27 spectrometer with scanner velocity 10 kHz. KBr pellets were prepared by mixing ~1 mg of the samples with 300 mg KBr. Transmission spectra were recorded using MCT detector, with 64 scans and 1 cm$^{-1}$ resolution. SEM (JEOL, JSM-35 CF, Japan) was used to ascertain the morphology and chemical constituents of the prepared composites before and after immersion in 1.5SBF for 3 days at accelerating voltage of 15 kV. After 3 days of soaking, the samples were taken out from 1.5SBF and the ion concentrations of Ca, P and Si in the solutions were measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES, IRIS 1000, Thermo Elemental, USA).

III. Results and discussion

3.1 Characterization of the obtained composites before in vitro test

Figure 1 presents X-ray diffraction data of the prepared composites with different component weight ratios. From the depicted XRD data, we can observe the presence of carbonate hydroxyapatite (CO$_3$HA) - PDF 21-0145 and Ca$_4$(PO$_4$)$_3$SiO$_4$ (silicocarnotite) - PDF 40-0309. In addition, an unexpected result is the presence of a large crystalline peak, positioned at 32.59°. The presence of this peak could be ascribed to the CO$_3$HA and carbonate phase and then dried at 36.6°C for 12 h in polyethylene bottles in static conditions for 3 days.

The obtained ceramic powders were in vitro bioactive and characterized by observing the presence of ν$_3$(PO$_4$)$^{3–}$ and ν$_4$(PO$_4$)$^{3–}$ groups [22,24–27]. The band with small intensity at around 628 cm$^{-1}$ corresponds to the silicon substitution mechanism, proposed as SiO$_4^{4–}$→PO$_4^{3–}$. This replacement leads to...
to decreasing of quantity of some OH– groups, i.e. silicocarnotite is partially dehydroxylated calcium phosphate silicate [16].

The very important vibrations of the PO4 3– in the FTIR spectra, depicted in Fig. 2, could be distinguished in the three main regions: (i) the peak with small intensity posited at 1059 cm–1 (curve a) is associated with asymmetric stretch vibrations [28]; (ii) a weak band at 954 (962) cm–1 for two samples corresponding to the symmetric stretching [27–30] and (iii) two well resolved peaks at 562 cm–1 assigned to the asymmetric modes [30,31]. Furthermore, the presence of a νCO3 2– mode, posited at 857 (860) cm–1 and 892 cm–1 indicates that the synthesised composites were similar to natural bone [32]. Moreover, in accordance with Garnjanagoonchorn et al. [6], the peak at approximately 857 (860) cm–1 was used to identify ChS in different sources of cartilage. In our samples the presence of this band verified the experimental procedure.

On the other hand, Tanaka et al. [33], assumed that the peak at ~962 cm–1 and these between 1020–1100 cm–1 could be ascribed to the presence of HPO4 2– in the apatite structure. In the FTIR spectrum of the sample with 75 wt.% C and 25 wt.% S, HPO4 2– bands were posited at 960, 1080 and 1208 cm–1 [34,35]. In this case, when silicocarnotite particles are added into acidic collagen solution, some of the silicocarnotite particles will dissolve in the acidic medium and produce Ca2+, PO4 3– and SiO4 4– ions. Furthermore, PO4 3– can produce HPO4 2– according to the reaction: H + + PO4 3– → HPO4 2–. Because of this the dissolution reaction of silicocarnotite is accelerated by the decreasing of pH value, i.e. the acidic collagen solution was supersaturated with Ca2+ and PO4 3– ions during the preparation of the composites, and consequently, the precipitation of other calcium phosphates occurs.

From a fundamental point of view, the amide groups of polypeptides and proteins possess a number of characteristic vibration modes of group’s frequencies. Especially, the amide I, II and III are directly related to the polypeptide conformation. The amide I band, with a characteristic frequency in the range from 1500 to 1700 cm–1, is associated with the stretching vibration of the C=O (carbonyl group) along the polypeptide backbone [36]. This group is a sensitive marker of a polypeptide secondary structure. In accordance with our preliminary investigations, the amide I band is posited at 1656 cm–1. The amide II is centred at 1550 cm–1 and amide III bands have been detected at 1240 and 1281 cm–1. Amide B is centred at 3081 cm–1. The two bands at 1400 and 1340 cm–1 can be assigned to the presence of COOH and COO– in the spectrum of pure gelatin and collagen [17,18].

As shown in the Fig. 2, the amide I bands have changed and shifted to lower wavenumbers from 1656 cm–1 for pure collagen [17,18] to 1613 (1612) cm–1 for the synthesised composites. This change could be assigned to the presence and the crucial role of ChS. It can be explained as following. In a series of papers Rhree and Tanaka [7–9] found that the asymmetrical stretching modes of COO– and SO4 2– ions in ChS were detected at 1632 and 1234 cm–1. They wrote that after formation of hydroxyapatite crystals via biomimetic route, with the use of ChS as a template, these modes were detected at 1613 and 1228 cm–1, respectively. They also noted that the “red shift” caused a chemical interaction between hydroxyapatite crystals and the functional groups of the ChS template during the precipitation [7–9]. In our case, in accordance with the experimental procedure, we can assume that the observed “red shift” (from 1632 cm–1 to 1613 (1612) cm–1 and from 1234 cm–1 to 1227 (1230) cm–1) may be due to the chemical interaction between Ca2+ from partially dissolved silicocarnotite and COO– and/or SO4 2– from ChS.

It is possible and another interpretation, concerning the presence of bands at 1613 (1612) cm–1 in the pre-

![Figure 1. XRD for the prepared C-S samples: 75:25 wt.% (a) and 25:75 wt.% (b)](image1)

![Figure 2. FTIR spectra of C-S samples: 25:75 (a) and 75:25 (b) wt.% before immersion in 1.5SBF](image2)
pared composites. Alili [37] shows that amide I bands for denatured and aggregated proteins are posited between 1610 and 1628 cm⁻¹. Liard et al. [38] and Petibois et al. [39] found that the band at 1615 cm⁻¹ can be assigned to the β-sheet conformation in collagen. Most likely, the presence of the bands at 1613 (1612) cm⁻¹ is due to the two processes: (i) chemical interaction between Ca²⁺ and COO⁻ and/or SO₄²⁻ from ChS and (ii) partial denaturation of collagen in the process of preparation of the composites which leads to the formation of β-sheet structures.

More detailed information about the type of changes of C as an organic part of the synthesized composites was analyzed by the curve fitting in the region 1700–1500 cm⁻¹ (Fig. 3). From the presented results, it can be seen that in the 75C:25S wt.% sample (Fig. 3b), new band centred at 1647 cm⁻¹ is quite visible. The presence of this band could be ascribed to the chemical interaction between Ca²⁺ from partially dissolved S and C=O amide I from C. In brief, the C=O amide I undergoes “red shift” from 1656 [17,18] to 1647 cm⁻¹ for the prepared 75C:25S wt.% sample. From the described data it can be assumed that the collagen goes through two types of changes; (i) “red shift” for C=O amide I and (ii) partial transformation of C molecule to β-sheets conformation under experimental conditions and quantity of C and S of the samples.

The amide II bands in the composites are centred at 1569 (1563) cm⁻¹ and at 1535 (1532) cm⁻¹ for the composite with weight ratio of the C-S components 25:75 wt.% (Fig. 2, curve a). It was difficult to analyze amide II band in the fields of conformation feature, as it has a complex nature with is vibration source, which arises primarily from the combination of the N-H banding coupled to the C-N stretching vibrations of the peptide linkages [40]. The observed bands can be attributed to the conformation change in collagen, as a result of the chosen method for the preparation of the composites. The amide III does not undergo any changes.

Based on other preliminary results, the absorption bands centred at 1451, 1404, 1337 and 1227 (1230) cm⁻¹ may be attributed to the δ(CH₂), δ(CH₃) and

Figure 3. Curve fitting spectra of the C-S samples: 25:75 (a) and 75:25 (b) wt.% before in vitro test in 1.5SBF in the range 1700-1500 cm⁻¹

Figure 4. Curve fitting spectra of the C-S samples: 25:75 (a) and 75:25 (b) wt. % before in vitro test in 1.5SBF in the range 1500-1350 cm⁻¹

164
ν(C-H) absorptions of collagens [39,41]. The band at 1337 cm⁻¹ in the C-S composite with 75:25 wt.% (curve b) could be ascribed to the covalent bond formation with Ca²⁺ from partially dissolute silicocarnotite and COO⁻ from the collagen before soaking in 1.5SBF [7–9]. The band posited at 1227 (1230) cm⁻¹ probably corresponds to dehydrate and monohydrate phosphate groups, respectively [42]. In the Fig. 2, the presence of the absorption bands at 1420 (1418) cm⁻¹, 1461 (1457) cm⁻¹ and 1560 cm⁻¹ indicated the formation of carbonate containing calcium phosphates with two types of substitution A-type and B-type before in vitro test [43,44].

Fig. 4 presents more detailed information about curve fitting in the region of 1500-1350 cm⁻¹ of CO₃²⁻ in the synthesised C-S samples, before in vitro test in 1.5SBF solution. From the results shown in Fig. 4 results it can be seen that the presence of absorption band at 1417 cm⁻¹ (Fig. 4b) indicated the formation of A-type CO₃HA in which CO₃²⁻→OH⁻ [45]. The formation of CO₃HA with B-type substitution in which CO₃²⁻→PO₄³⁻ [46,47] is posited firstly at 1401, 1452 and 1469 cm⁻¹ (Fig. 4a) and secondly at 1400, 1452 and 1469 cm⁻¹ (Fig. 4b). Therefore, B-CO₃HA preferentially observed into the C-S samples without reference to the weight ratio of the C and S components of the composites.
On the FTIR analysis for the prepared composites before in vitro test we can conclude that:
- In the studied samples Ca-deficient HA was formed \textit{in situ} before in vitro test in 1.5SBF: HPO\textsubscript{4}\textsuperscript{2–} ions are present in the composite samples.
- Ca\textsuperscript{2+} from the partially dissolved
- silicocarnotite powder may bind with amid I and/or COO\textsuperscript{–} and form (C=O):Ca and/or (COO)\textsubscript{2}Ca bonds.
- The collagen undergoes conformation changes due to formation of β-sheet structures.
- The FTIR data confirmed XRD results.

SEM micrographs of the synthesized samples before in vitro test in 1.5SBF are given in Figs. 5 and 6. Figure 5 shows that the C-S composite sample (75:25 wt.%) has a porous microstructure, similar to the bone structure. At high magnification values (Figs. 5c and 6a) the SEM depicts the presence of the monodisperse thin HA whiskers. In coincidence with FTIR data these whiskers consist of Ca-deficient HA. In addition, at very high magnification value (Fig. 5b) it can be seen that Ca-deficient HA is in a nanometer scale.

Figure 7 presents SEM images for C-S (25:75 wt.%) sample before in vitro test. From the SEM images presented in Fig. 7 we can see that the prepared samples have a porous structure and that the Ca-deficient HA has a flower like assembly.

3.2 Characterization of the obtained composites after in vitro test

X-ray diffraction data for the C-S samples after in vitro test in 1.5SBF in static conditions is given in Fig. 8. From the presented XRD data it can be seen that the well-defined HA (PDF 9-0432) is formed on the composites after immersion in 1.5SBF for 3 days.

The FTIR spectra of prepared C-S composites with weight ratio of the components 75:25 wt.% and 25:75 wt.%, after immersion in 1.5SBF for 3 days are given in Fig. 9.
Figure 9 shows the IR absorption spectra of carbonate containing hydroxyapatite (CO₃HA), formed on the surface of the immersed samples for 3 days in 1.5SBF. The ν₁PO₄³⁻ mode is represented in the apatite structure by a band at 958 (962) cm⁻¹, while ν₂PO₄³⁻ produces an absorption peak, posited at 465 and 421 cm⁻¹ [7–9]. As other authors have noted, the main absorbance signal of PO₄³⁻ appears in the triply degenerate ν₃ domain [48]. The broad peak at around 1029 cm⁻¹ (1031 and 1027 cm⁻¹ for two samples) is due to both the ν₁PO₄³⁻ and ν₃PO₄³⁻ and the stronger SiO₄⁴⁻ band [48]. In addition, the ν₂PO₄³⁻ is also masked by the Si-O-Si peak, but the well-defined ν₁PO₄³⁻ at 561 (558) cm⁻¹ and 603 (600) cm⁻¹ are visible [7–9]. Furthermore, among the four internal vibrational modes of CO₃²⁻, only two are important for the FTIR analysis: ν₁ and ν₃ [49]. The bands posited at 726 and 795 cm⁻¹ (Fig. 9, curve b) could be assigned to the presence of small amount of calcite [50,51]. In addition, Feki et al. [52] postulated that the bands at ~720 cm⁻¹ and 870 cm⁻¹ could be assigned to the presence of B-type CO₃HA in the immersed samples. The absorption bands at 1357, 1420 and 1461 cm⁻¹ (Fig. 9, curve a) and at 1373, 1418, 1457 and 1505 cm⁻¹ indicated the formation of CO₃HA with B-substitution in which CO₃²⁻ → PO₄³⁻ [53].

The 1340 cm⁻¹ band in collagen, as has been written in [17,18] represents not only the carboxyl group, but a number of bands in the range 1400–1290 cm⁻¹, which are attributed to the presence of type I collagen in biological tissue. As shown in Fig. 9 we confirm the “red shift” of this band for the synthesized composites after immersion in 1.5SBF for 3 days. The band centred at 1335 (1336) cm⁻¹ can be described as a wagging vibration through the covalent bond formation with Ca²⁺ of partial dissolution silicocarnotite. The amount of observed “red shift” is determined by the conditions of the in vitro test, pH, temperature and concentration of the reagents in 1.5SBF solution. The bands at 1641 (1646) cm⁻¹ and 1550 (1556) cm⁻¹ can be assigned to the amide I and amide II from collagen [17,18,54].

Figure 10 concisely presents the evaluation of ionic concentration of Ca, P and Si in the liquid after in vitro test of the synthesized C-S composites after 3 days of soaking in 1.5SBF by ICP-AES analysis. From the data of Fig. 10, we can see that the Ca concentration for the two samples slightly increased from 89 mg/l (which is the concentration of Ca in 1.5SBF before soaking), to 179 mg/l (sample 1) and 164 mg/l (sample 2). Meanwhile, the concentration of P also increased from 1 mg/l in 1.5SBF before the test, to 22 mg/l (sample 1) and 4 mg/l (sample 2). From the depicted results we can conclude that (i) ion exchange of Ca and P increases their concentration in 1.5SBF and (ii) the formation of HA phase on the surface of the immersed samples, which would consume Ca and P, leads to a decrease in Ca and P concentration in 1.5SBF solution. From sample 2, the concentration of P is lower than the one in sample 1, in accordance to the weight ratio of the C and S as the parts of the composite. The lower P concentration leads to a conclusion that P may play an active role in the transformation of the sample surface during in vitro test. For instance, Agathopou-
los et al. [55] observed that in the case of CaO-MgO-SiO2 glass, modified with B2O3, Na2O, CaF2 and P2O5, after very long immersion in SBF phosphorus concentration was rapidly decreased. Because the quantity of S for samples 1 and 2 is 75 and 25 wt.%, respectively, we can conclude from Fig. 10 that the concentration of Si for the sample 2 is higher than the one in sample 1. In accordance with X-ray diffraction data (Fig. 1) we could conclude that these changes (caused by dissolution and precipitation process, respectively) are more pronounced for carbonate hydroxyapatite (Ca10(PO4)6(OH)2·H2O) and the C-S-P-H phase. This conclusion is in good agreement with literature data [56]. From the obtained ICP-AES results, we can conclude that after in vitro test of the prepared composites silicon containing carbonate substituted hydroxyapatite (Si-CO3HA), may be formed on the surface of the immersed samples. This conclusion will be the subject of some future article.

Figures 11 and 12 show SEM images of the samples after in vitro test in 1.5SBF for 3 days in static conditions. The morphology of the samples after in vitro test in 1.5SBF for 3 days shows a relatively dense microstructure. This result indicated that the composites with ChS were endowed with excellent bioactivity. A similar morphology has been observed by other authors [57]. Figure 11b shows detailed microstructure of the fibre-side. From Fig. 11a,b it is noted that the nucleation of hydroxyapatite crystals were initiated from the surface of the collagen fibres and silicocarnotite, leading to the conjecture that there are some types of interactions between the HA crystals and collagen fibres. The presented SEM image at a high magnification value (Fig. 11c) clearly shows the presence of the concentrated spherical particles. They have a bright colour and have some pores denoting the formation and nucleation of an apatite layer. The presented SEM images also show that hydroxyapatite particles are connected to each other in the sample. For the C-S sample with 25:75 wt.% of the components, SEM image (Fig. 12c) shows a mixture of plate-like and needle-like HA crystals.

**IV. Conclusions**

The purpose of this work was to prepare, characterize and evaluate in vitro bioactivity of composites between collagen and silicocarnotite in the presence of chondroitin sulphate. Collagen type I, diluted in acetic acid, was mixed with silicocarnotite powder in a 25:75 and 75:25 weight ratio, in the presence of chondroitin sulphate. XRD of the prepared composites depicts the presence of carbonate containing hydroxyapatite and CaO-P2O5-SiO2-H2O crystalline phases, from the partially dissolved silicocarnotite glass-ceramics. FTIR observed that the chemical interaction between Ca2+ and COO− and/or SO4 2− from the chondroitin sulphate and β-sheets structures might occur. In the sample with 75:25 wt.% collagen:silicocarnotite FTIR observed the “red shift” of C=O amide I, which related to the chemical interaction between Ca2+ and C=O: amide I. On the other hand, the B-type of carbonate containing hydroxyapatite in which CO3 2−→PO4 3− is formed in situ into the composites. SEM of the composite with 75:25 wt.% collagen:silicocarnotite depicts the presence of nanosized hydroxyapatite whiskers. This observation confirmed the XRD and FTIR results.

After in vitro test in 1.5 SBF, XRD observed well defined hydroxyapatite peaks. Based on these results we can assume that the prepared composites are in vitro bioactive. FTIR depicted that the band centred at ~1335 cm−1 could be ascribed to the covalent bond between Ca2+, from the partially dissolved phases and deprotonated carboxylate groups from the collagen. SEM proved that the sample prepared with chondroitin sulphate composites endowed excellent in vitro bioactivity. ICP-AES data lead to a conclusion that silicon containing carbonate substituted hydroxyapatite may be formed on the samples immersed in 1.5SBF solution.

**Acknowledgements:** The authors kindly acknowledge Dr. Tsvetelina Gerganova from AQUACHIM JSC-Bulgaria for her support for fitting of the FTIR spectra.

**References**


