

# Study of Phosphocalcic Glasses

## SiO<sub>2</sub> - CaO - P<sub>2</sub>O<sub>5</sub> System with and without Silver

### I. Synthesis of glasses and characterization by WD- XRF and XRD

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*Synthesis glasses biocompatible made a great step forward in the last few decades, moving from traditional methods of supercooling melts and casting them to the modern, using the action of neutrons, mechanical and deposition in thin layers by different techniques, glass transition or sol-gel technique. In this paper it is presented sol-gel synthesis of two glasses silicofosfocalcic composition of SiO<sub>2</sub>-CaO-P<sub>2</sub>O<sub>5</sub> system with and without silver ions. The investigation of chemical elemental composition of researched glasses, as well as the de novo synthesis of hydroxyapatite, post dip, was done by using modern methods used for this purpose.*

*Keywords: bioactive glass, sol-gel synthesis, in vitro bioactivity, doping silver, WD-XRF, XRD*

Phosphocalcic glasses were widely used in clinical applications of prosthesis and bone reconstruction, orthopedics and maxillofacial reconstructive surgery. Thus, they reached the top of the most promising biocompatible materials due to their excellent biological and mechanical systems that tend to become increasingly better.

The use of sol-gel synthesis method allowed us to obtain a great versatility and the introduction of transition metals (Ag, Cu, Zn), which confer additional biological properties such as the antibacterial agents. Lastly, sol-gel technique was imposed because of the advantages of economic, technological and due to improved biological properties, which allowed the widening of the scope of medicine [1, 2].

The basic principle used in the development of biomaterials was initially thought they would have to be inert chemically point of view [3, 4], although it has been shown that they produce side effects in the host [4] because, in time they lead to the formation a fibrous capsule, hindering the formation of a biochemical link between tissue and implant. Due to mechanical wear and chemical degradation device must be surgically removed within a few years [2, 3].

In this respect, biomaterials research was directed towards the development of potentially bioactive materials that can interact with the host. The main feature of bioactive materials is their property to develop strong bonds at the interface with the bone tissue that comes in contact [5]. The tissue implant link requires the formation of a layer of hydroxyapatite and/or carbonated hydroxyapatite to the implant surface by mechanisms that involve physico-chemical and biochemical complex processes [5, 6].

Hench and all have found that certain compositions of glass, through which the phosphocalcic glass from the systems in this study, are capable of forming a stable bond with the bone once implanted. When they come in contact with biological fluids the deposit on their surface a layer of

hydroxyapatite similar to the mineral phase of bone takes place. Collagen molecules are incorporated into this layer and thus the biological tissue implant bond occurs.

Wilson and Nolletti subsequently showed that the interfacial bond can be formed even with a soft tissue, provided that the rate of formation of hydroxyapatite be large enough [8].

Incorporating silver into glasses silicate network is a relatively new method used in the synthesis of bioglasses, all easily accomplished due sol-gel technique. With the incorporation of silver in the glass structure, besides the property of bioactivity, these gains antimicrobial properties [9] with the undeniable role in preventing and combating hospital-acquired infections occurring after surgical prosthesis [10, 11]. The antimicrobial action of silver ions is based on the diffusion of these ions in the surrounding areas from the implant over a long period of time, bacteriostatic even bactericidal strengths, without being toxic to the human body [12, 13].

#### Experimental part

##### *Synthesis of sol-gel glasses*

For this study were synthesized two calcium silicate glasses system SiO<sub>2</sub> - CaO - P<sub>2</sub>O<sub>5</sub>, of which one of the two compositions is doped with silver by partial substitution of CaO with Ag<sub>2</sub>O.

The chemical composition of these glasses oxide was accomplished by stoichiometry calculation using a computer program, taking into account that most of the materials used are organic substances undergo hydrolysis and condensation reactions.

Table 1 shows the chemical composition of both calcium silicate glass produced by sol-gel technique.

The raw materials used as precursors for oxides are found in glasses composition are summarized tetraethylorthosilicate (Si(OC<sub>2</sub>H<sub>5</sub>)<sub>4</sub> -TEOS), triethylphosphate (TEP - (C<sub>2</sub>H<sub>5</sub>O)<sub>3</sub>PO<sub>4</sub>), calcium nitrate

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Sample	Oxide [% wt]			
	SiO <sub>2</sub>	CaO	P <sub>2</sub> O <sub>5</sub>	Ag <sub>2</sub> O
S <sub>1</sub>	50	41	9	-
S <sub>2</sub>	50	34	9	7

**Table 1**  
COMPOSITION OF BIOACTIVE PHOSPHOCALCIC GLASSES OBTAINED BY SOL-GEL METHOD

tetrahydrate (Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O) and silver nitrate (AgNO<sub>3</sub>) for glasses doped with silver. The synthesis is carried out under acid catalysis using as a catalyst 2N nitric acid (HNO<sub>3</sub>).

In terms of chemical synthesis glasses supposes four major steps:

- acid hydrolysis and condensation of the precursors that lead to the formation of ground bioglass by magnetic stirring at moderate speeds (400-500 rpm) for 2-3 h [1, 14, 15], until the clarifying the solution;
- gelation and aging the gel (fig. 1a, 1b) [1, 14, 15] by drying and curing in a desiccator at the room temperature (24-48 h) in an oven over a period of 5-6 h at approx. 60°C;
- drying the gel in an oven at a temperature below 180°C for 72 h to obtain xerogel structure (fig. 1c) [1, 14, 15];
- stabilizing xerogels by calcination in the oven at temperatures well controlled (5°C/min) under 600-650°C for up to 8 h while maintaining at 600°C maximum for 3 h in order to maintain the desired porous structure (fig. 1d) [1, 14, 15].

The final phase to obtain the silver -doped glass of the hydrolysis and condensation nitrate it adds silver nitrate to the silver oxide as a precursor of the composition calculated. Stirring was continued until the solution is clear and complete dissolution, and it follows the same protocol as for the undoped glass [1].

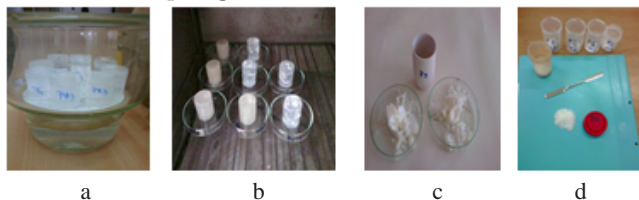


Fig. 1. Obtaining of sol-gel glasses: a - sol gelification, b - aged gels, c - dried gels (xerogels), d - bioglass powder

The amounts of reagents used in the synthesis of the two glass compositions and their order of addition are shown in table 2.

In the first stage it is important to observe that for the hydrolysis a volume ratio H<sub>2</sub>O:HNO<sub>3</sub> 2N 6:1, and for gelation - aging step to proceed in the range of 48-54 h is essential that the molar ratio H<sub>2</sub>O: (TEOS + TEP) to be within 4 - 12 [1, 14, 15]. For this synthesis was chosen molar ratio 8.

In vitro bioactivity of the glass powders was carried out by soaking them in the liquid in the simulated human body

thermostatic conditions for 3 to 21 days at 37°C and pH = 7.20.

#### Characterization of the samples

The elemental chemical composition of the glass powder synthesized was determined by X-ray fluorescence spectroscopy, with the variation in the length of the wave (WD - XRF). Analyses were performed in the laboratory of spectroscopy of SC Mechel Targoviste, according to the methodology standardized operation of the spectrometer Advant X ARL - Thermo Scientific with X - ray tube of 2400 W, 60 kV and 80 mA, using the crystal diffraction LIF 200 and 220 scintillation detector [1].

The structural changes that appear on the surface of the glasses synthesized and subsequently soaked in simulated human fluid were studied by X-ray diffraction [5, 16]. In this study it opted for the static soaked of the samples in order to stimulate reactivity of the material. Thus, the glass powders are soaked for 3 to 21 days without refreshing the SBF solution [17-20].

The XRD analysis was carried out with a diffractometer Rigaku Ultima IV, with the following features: 1αCuk = 1.5405 Å, the source of radiation (X-ray tube with copper anode) has a power of 3 kW, voltage of 40 kV and a current of 40 me. The data was collected in scan range 20-60° (2θ) with a scanning speed of 0.04°/s (2θ).

#### Preparation of SBF

To reproduce as faithfully real biological conditions post-intervention and kinetic reactions that occur on the surface of the material, the choice of solution used for testing is defining. For this purpose, the use of simulated body fluid (LUS = SBF) concentration 1.5 N and the static soaking method.

Simulated body fluid is a solution that mimics the composition, concentration and pH of human plasma and body fluid, being more acellular and non-protein. However the most used medium bioactivity in vitro testing of various biomaterials after 1990, when it was first synthesized by Kokubo et al [6, 21-23]. Both plasma and SBF have in their composition salts that provide sufficient amounts of Ca<sup>2+</sup> and HPO<sub>4</sub><sup>2-</sup>, which support the formation of hydroxyapatite on the surface of biomaterials [21, 24].

The chemical composition and concentration of the ionic concentration per liter of 1.5 N SBF is presented in table 3 [17, 24].

Sample	TEOS [mL]	H <sub>2</sub> O [mL]	HNO <sub>3</sub> 2N [mL]	TEP [mL]	Ca(NO <sub>3</sub> ) <sub>2</sub> · 4H <sub>2</sub> O [g]	AgNO <sub>3</sub> [g]
S <sub>1</sub>	59.4135	44.4267	7.4044	7.2307	57.5070	-
S <sub>2</sub>	54.5693	40.8144	6.8025	6.6412	33.5782	5

**Table 2**  
THE REAGENTS USED  
IN SOL-GEL GLASSES  
SYNTHESIS

mol H<sub>2</sub>O : mol (TEOS + TEP) = 8:1; V<sub>H<sub>2</sub>O</sub> : V<sub>HNO<sub>3</sub></sub> = 6:1

Reagent	Amount [g]	Ion concentration [mmol/L]	
		Cation	Anion
NaCl	11.994	Na <sup>+</sup>	-
NaHCO <sub>3</sub>	0.525	213.0	HCO <sub>3</sub> <sup>-</sup> 6.3
KCl	0.336	K <sup>+</sup>	-
K <sub>2</sub> HPO <sub>4</sub>	0.342	7.5	HPO <sub>4</sub> <sup>2-</sup> 1.5
MgCl <sub>2</sub>	0.458	Mg <sup>2+</sup> 2.3	-
HCl 1M	60 cm <sup>3</sup>	-	Cl <sup>-</sup> 221.7
CaCl <sub>2</sub>	0.417	Ca <sup>2+</sup>	-
Na <sub>2</sub> SO <sub>4</sub>	0.107	-	SO <sub>4</sub> <sup>2-</sup> 0.8
(CH <sub>2</sub> OH) <sub>3</sub> -C-NH <sub>2</sub>	9.086	-	-
HCl 1M	Until pH= 7.2-7.4	pH= 7.25	

**Table 3**  
THE COMPOSITION AND IONIC STRENGTH  
SBF 1.5 N to 1L

## Results and discussions

### Investigations on bioglasses with and without silver ions

The results of analysis oxide, for both samples of glass synthesized by the WD - XRF method are shown in table 4, together with practical yield of the synthesis process.

These results, compared with the theoretical values in table 1 confirm the efficiency of the method of synthesis chosen and accuracy of the calculation of chemical composition, as well as how to conduct the sol-gel synthesis process. The yield of obtaining reactions demonstrates that the working and technological route followed were correct, especially in the conditions in which the specialty literature states that the optimum yields for hydrolysis and condensation reactions of values starting at 85% [25].

The formation of hydroxyapatite crystal structures on the surface of the two glasses after their soaking in SBF

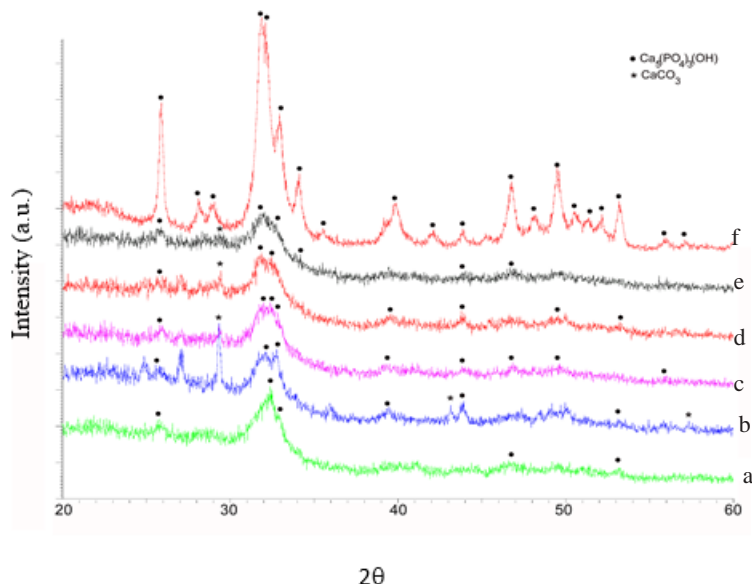
was highlighted by XRD analysis, figure 2 and figure 3, which confirms their bioactive property [26, 27].

In figure 2 (a-f) corresponding diffractograms shows the ternary glass S1 before and after the soaking in SBF, for 3, 7, 14 and 21 days, together with the diffraction pattern of pure hydroxyapatite which considered by comparison according to the ICDD- PD2: 00-009-0432 database.

The diffractogram **a** (fig. 2) shows that unsoaked in SBF sample shows a characteristic spectrum of amorphous materials, with a few peaks at 25.84 2θ (3.44Å), 32.2 2θ (2.77Å), 32.88 2θ (2.72Å), 46.7 2θ (1.94Å), 53.12 2θ (1.72Å). After 3 days of soaking (diffractogram **b**) shows crystallites of hydroxyapatite on the surface of the glass, as evidenced by peaks 39.8 2θ (2.26 Å) and 43.75 2θ (2.06 Å). Also CaCO<sub>3</sub> now appears on the surface of the glass, paintings from 29.33 2θ (3.04Å), 43.1 2θ (2.09Å), 57.38 2θ (1.60Å), which can be placed as it was present in the composition SBF NaHCO<sub>3</sub>.

Sample	Oxides [% wt]				Yield [%]
	SiO <sub>2</sub>	CaO	P <sub>2</sub> O <sub>5</sub>	Ag <sub>2</sub> O	
S <sub>1</sub>	49.95	41.02	8.6	-	97.12
S <sub>2</sub>	52.7	29.94	9.02	6.46	95.4

**Table 4**  
COMPOSITION OXIDE AND YIELD OF  
BIOGLASSES



**Fig. 2.** XRD spectra for S1 sol-gel samples before and after soaking in SBF  
a) unsoaked; after soaked in simulated body fluid for:  
b) 3 days, c) 7 days, d) 14 days, e) 21 days and  
f) hydroxyapatite

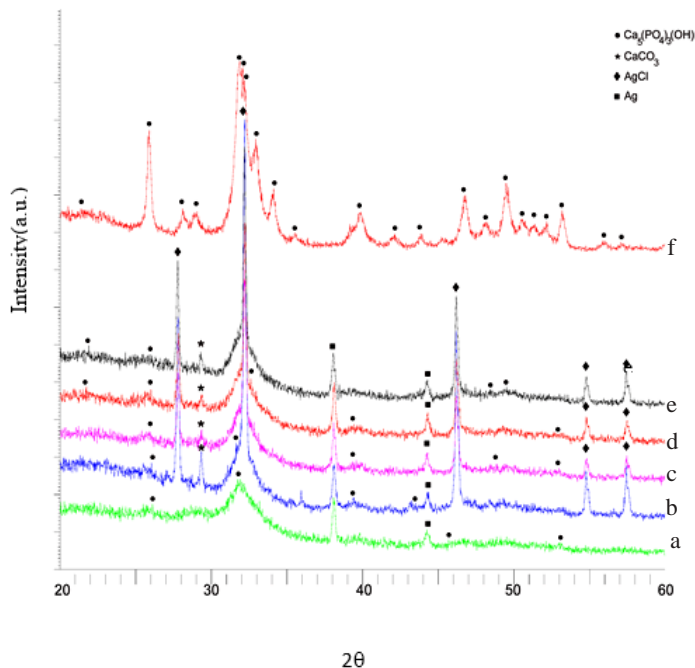


Fig. 3. XRD spectra for S2 sol-gel samples before and after soaking in SBF

a) unsoaked; after soaked in simulated body fluid for:  
b) 3 days, c) 7 days, d) 14 days, e) 21 days and f) hydroxy-apatite

After 7 days (diffraction **c**) it increase the formation of hydroxyapatite, as evidenced by the emergence of 2 new peaks at  $49.47\ 2\theta$  ( $1.84\ \text{\AA}$ ) and  $55.88\ 2\theta$  ( $1.64\ \text{\AA}$ ) and significantly decreases the formation of  $\text{CaCO}_3$ , when there is the presence of a single peak at  $29.31\ 2\theta$  ( $3.04\ \text{\AA}$ ). This can be explained by solubilising  $\text{CaCO}_3$ , especially if it was present as the majority of vaterit ( $\mu\text{-CaCO}_3$ ), which is more soluble than calcite ( $\beta\text{-CaCO}_3$ ) in water or in SBF. Calcium carbonate anions  $\text{CO}_3^{2-}$  solubility issue that may come into carbonated hydroxyapatite composition which forms at the interface with the stoichiometric bioglass - SBF.

After 14 days and 21 days (graphs **d** and **e**), there is a reduction in the number of peaks of hydroxyapatite and constant stoichiometric quantity of calcite. This can be explained by the fact that due to static soaking process,  $\text{Ca}^{2+}$  and  $\text{HPO}_4^{2-}$  ran out of SBF or pure hydroxyapatite that gradually turns into carbonated hydroxyapatite. SBF exhaustion drawback does not occur in real life in vivo than in cases of severe hypocalcaemia or major demineralization due to osteoporosis. Carbonated hydroxyapatite formation can be an advantage in terms of training interfacial bone - implant because the non-stoichiometric hydroxyapatite mineral phase composition is the closest composition of the bone tissue.

In figure 3 (a-f) corresponding diffractograms are presented quaternary  $\text{S}_2$  ( $\text{SiO}_2\text{-CaO-P}_2\text{O}_5\text{-Ag}_2\text{O}$ ) before (diffractogram **a**) and after soaking in SBF, under the same conditions ( $37^\circ\text{C}$  and  $\text{pH} = 7.2$ ) as sample  $\text{S}_1$ , the same duration: 3, 7, 14 and 21 days as figure 3 (diffractograms **b-e**) and together with the pure hydroxyapatite spectrum of figure 3(f).

From figure 3 it notes that if quaternary  $\text{S}_2$  glass doped with silver by substituting  $\text{CaO}$ , the rate of formation of hydroxyapatite on the surface of glass powder is smaller by comparison with ternary composition  $\text{S}_1$ .

This is reflected in the smaller number of peaks of hydroxyapatite identified as follows: four peaks before soaking, figure 3a ( $25.83\ 2\theta$  ( $3.44\ \text{\AA}$ ),  $31.72\ 2\theta$  ( $2.81\ \text{\AA}$ ),  $46.65\ 2\theta$  ( $1.94\ \text{\AA}$ ),  $53.17\ 2\theta$  ( $1.72\ \text{\AA}$ )), after 3 days and 7 days of soaked the same number of peaks, figure 3b ( $25.89\ 2\theta$  ( $3.43\ \text{\AA}$ ),  $31.72\ 2\theta$  ( $2.81\ \text{\AA}$ ),  $39.78\ 2\theta$  ( $2.26\ \text{\AA}$ ),  $44.30\ 2\theta$  ( $2.04\ \text{\AA}$ )), and figure 3c ( $25.77\ 2\theta$  ( $3.45\ \text{\AA}$ ),  $39.80\ 2\theta$  ( $2.26\ \text{\AA}$ ),  $49.45\ 2\theta$  ( $1.84\ \text{\AA}$ ),  $53.15\ 2\theta$  ( $1.72\ \text{\AA}$ )), versus the 7 and 9 peaks identified by the same periods of soaked of the glass  $\text{S}_1$ , a maximum of 6 peaks is identified synthesis

after 14 days of soaking, figure 3d ( $21.83\ 2\theta$  ( $4.06\ \text{\AA}$ ),  $25.83\ 2\theta$  ( $3.44\ \text{\AA}$ ),  $32.80\ 2\theta$  ( $2.72\ \text{\AA}$ ),  $39.90\ 2\theta$  ( $2.26\ \text{\AA}$ ),  $49.50\ 2\theta$  ( $1.84\ \text{\AA}$ ),  $53.15\ 2\theta$  ( $1.72\ \text{\AA}$ )), compared to the 7 peaks of the sample  $\text{S}_1$ .

After 14 days and 21 days there was a reduction in the number of peaks of hydroxyapatite stoichiometric figure 3.e ( $21.77\ 2\theta$  ( $4.07\ \text{\AA}$ ),  $48.42\ 2\theta$  ( $1.87\ \text{\AA}$ ),  $49.53\ 2\theta$  ( $1.83\ \text{\AA}$ )), as compared to day 7 of the dip.

Throughout the period of soaking of the glass  $\text{S}_2$  the amount of calcium carbonate formed in the first three days of the peak corresponding to  $29.37\ 2\theta$  ( $3.04\ \text{\AA}$ ) are kept constant.

It is relevant that both compositions have a maximum synthesis of pure hydroxyapatite (stoichiometric) in the first days (7 days -  $\text{S}_1$  and 14 days -  $\text{S}_2$ ) and then the quantitative training is reduced.

In comparison with  $\text{S}_1$  composition, in the case of silver-doped glass the amount of  $\text{CaCO}_3$  is lower.  $\text{CaCO}_3$  appears from the first 3 days (peak 2  $29.37\ 2\theta$  ( $3.04\ \text{\AA}$ )) and then this amount remains constant throughout soaking.

Specific for glass doped with silver  $\text{S}_2$  is the emergence of AgCl crystallites, corresponding peaks  $27.71\ 2\theta$  ( $3.12\ \text{\AA}$ ),  $32.22\ 2\theta$  ( $2.77\ \text{\AA}$ ),  $46.17\ 2\theta$  ( $1.96\ \text{\AA}$ ),  $54.91\ 2\theta$  ( $1.67\ \text{\AA}$ ),  $57.42\ 2\theta$  ( $1.60\ \text{\AA}$ ) after 3 days of soaking. Are highlighted, two diffraction lines for metallic silver at  $38.17\ 2\theta$  ( $2.35\ \text{\AA}$ ),  $44.22\ 2\theta$  ( $2.04\ \text{\AA}$ ) since the beginning of soaking in SBF, which may influence negatively, the capacity for the synthesis of hydroxyapatite.

This phenomenon is explained by the fact that the content of  $\text{CaO}$  composition glass  $\text{S}_2$  was low, so fewer  $\text{Ca}^{2+}$  were available in this case, to form  $\text{CaCO}_3$  and hydroxyapatite, however the presence of  $\text{Ag}^+$  ions leads to the formation of AgCl as their reaction with anions  $\text{Cl}^-$  is highly sensitive, it underpinning the silvermetry as chemical method of dosing anion  $\text{Cl}^-$  in any system. In addition, this is due to excess of ions and  $\text{Cl}^-$  from SBF to human plasma ( $147.8\ \text{mmol/L}$  to  $103.0\ \text{mmol/L}$  in plasma).

The specialty literature mentions that the crystalline precipitate of AgCl and thus prevents clogged pores of the bioglass, hydroxyapatite layer forming part of silica on its surface [2].

## Conclusions

This study confirms the versatility of the sol - gel synthesis of phosphocalcic glasses of the ternary system of  $\text{SiO}_2\text{-CaO-P}_2\text{O}_5$  and quaternary  $\text{SiO}_2\text{-CaO-P}_2\text{O}_5\text{-Ag}_2\text{O}$ , from organic precursors for  $\text{SiO}_2$  and  $\text{P}_2\text{O}_5$ , respectively TEOS, and TEP and inorganic  $\text{CaO}$  and  $\text{Ag}_2\text{O}$ , respectively  $\text{CaNO}_3\cdot 4\text{H}_2\text{O}$  and  $\text{AgNO}_3$ , in acid catalysis.

The elemental chemical composition of the two glasses, determined by WD- XRF analyses confirms the correctness of the technological route adopted and the method of calculating the theoretical composition. This is supported and yield synthesis process, ranges from 95.4 to 97.12 %, much higher than known from the specialty literature ( $\approx$  85%) for hydrolysis and condensation reactions.

The bioactivity of the glasses was confirmed by XRD analysis after the in vitro study, by soaking the samples 3-21 days SBF at 37°C and  $pH = 7.2$ . It showed that both glasses are bioactive because they generate the formation of hydroxyapatite de novo after the first 3 days of soaking.

The glass composition  $S_1$  is more bioactive than the composition  $S_2$ , but both reach a maximum bioactivity after 14 days, according to the conditions of static soaking method. The bioactivity glass  $S_2$  is diminished by the presence of silver in its composition, but not cancelled.

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