Obtaining simple and doped phosphocalcic glasses by using sol-gel technique

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Phosphocalcic glasses, based on ternary system SiO_2 - CaO- P_2O_5 and those doped with silver (SiO_2 – CaO - P_2O_5 - Ag_2O) can be obtained by the traditional method of sub-cooling melts or modern methods such as process that uses mechanical energy, neutrons action, deposition in thin layers or by sol-gel technique. In this study is presented sol-gel method for obtaining phosphocalcic glasses. Two glass compositions obtained were doped with silver, which has resulted in a new SiO_2 - CaO - P_2O_5 - Ag_2O bioactive glass system. These were tested in terms of bacteriostatic / antibacterial activity in vitro.

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1. Introduction

Sol-gel technique for obtaining phosphocalcic glasses was chosen at the expense of the classical method of foundry casting, because it was found that the glasses obtained have a high level of bioactivity compared with traditional ones [1,2].

Thus, phosphocalcic glasses in ternary system SiO2 – CaO - P2O5 fall into the category of bioactive materials that generate a reaction interface between implant and bone, generates the synthesis of biologically active hydroxyapatite layer to the tissue-implant interface [3, 4].

The traditional method to obtain them by sub-cooling and pouring glass melt shows a number of drawbacks related to both technology and cost.

First, it is difficult to obtain high purity glasses because the raw materials used are inorganic materials to the liquid phase, homogeneous, high temperature of 1000 - 1500°C, a process that causes solid or gaseous inclusions in the structure of vitreous cooled material.

Lack of higher purity negatively affects the bioactivity of the material.

This is diminished by high SiO2 content of glasses obtained, knowing that bioactivity varies inversely with silica content; not to lose bioactivity property, it should not exceed 60wt%, considering that maximum bioactivity is obtained at an optimum silica content of about 45wt% [5].

Also, getting by melting involves the use of alkaline materials or alkaline-earth materials that can significantly reduce bioactivity as they print a high reactivity to melted material that can attack the crucible, and thus bring a number of impurities in the glass composition [6].

Last but not least, the high cost deriving from melting technology, due to high temperatures and multiple stages of handling, is another argument in favor of giving up the traditional methods of obtaining bioactive glasses.

Compared with the traditional method, getting through sol-gel process has the advantage of using low temperature reaction and obtaining homogeneous glass compositions with high purity, mainly due to the porous nature of the organic raw materials, used as precursors.

This synthesis technique has been used since the 1990s, by LL Hench and glasses obtained proved to have a high level of bioactivity and biocompatibility [4].

Vitreous bioglass powders obtained by sol-gel have a nanoporous microstructure and a composition well determined.

Through rigorous control of heat treatments faced along the technological process, the glasses may have a structure with pore sizes ranging within 100-600 μm, fully interconnected, which greatly enhance the degree of bioactivity, and tissue-implant interface processes, as they allow the development of osteocytes and osteoblasts in the porous structure of prosthesis implanted [7, 8].

Glasses obtained by sol-gel technique can be included in the binary system, SiO_2 -CaO [9], tertiary - phosphocalcic glasses, SiO_2 -CaO-P_2O_5 [7], or quaternary SiO_2-CaO-P_2O_5-Na_2O [10], all of which have a certain degree of bioactivity, proved by immersion in simulated body fluid [11].

In order to minimize the risk of microbial contamination, due to potential bacteriostatic activity resulting from diffusion of Ag ions in the synthesized porous structure, Ag2O was added in SiO_2-CaO-P_2O_5 sol-gel glass compositions [12-17].

Adding silver in glass structure is a recent method, used in surgical bone reconstruction, by giving certain antimicrobial properties of the implanted material, without being toxic, and reduce the incidence of postoperative infections.

Bacteriostatic and bactericidal properties have been studied by using Escherichia coli and Staphylococcus

aureus, as representative strains for the microorganisms potentially pathogenic / pathogens.

2. Experimental procedure

2.1. Glasses synthesis

The aim of this study was to obtain phosphocalcic glasses in ternary system SiO_2 -CaO-P_2O_5 and those doped with silver by sol-gel technique.

Stoechiometrically equivalent for chemical compositions of oxide contents of glasses, given that the raw materials used are organic substances undergo hydrolysis and condensation reactions for obtaining sol and then gel was made by computer program.

The chemical compositions of the glasses synthesized and presented in this study are presented in Table 1.

Table 1. Composition of phosphocalcic glasses obtained by sol-gel method

Sample				
Oxide [Weight %]	P_1	P_2	PA_1	PA_2
SiO_2	50	55	47	50
CaO	45	40	45	40
P_2O_5	5	5	5	5
Ag_2O	-	-	3	5

Precursor materials used in the synthesis of phosphocalcic glasses by sol-gel are:

- Tetraetilortosilicate (Si $(OC_2 H_5)_4$ = TEOS);
- Triethylphosphate ($(C2H5)_3 PO_4$ = TEP);
- Calcium nitrate hydrated four water molecules (Ca (NO3)2 · 4H2O);
- Silver nitrate ($AgNO_3$) - for glasses doped with silver.

From the chemical point of view, the glass synthesis involves hydrolysis and condensation reactions of the precursors leading to the formation of sol and then the physico-chemical processes of gelation-aging.

The gel aged and stabilized by well-controlled heat treatments, is calcined at temperature up to 600°C, which allows maintaining the desired porous structure.

The flow chart for sol-gel glass synthesis is presented in figure 1.

To describe explicitly the obtaining process, I will show the way of obtaining glass P1, with the composition shown in Table 1: 50SiO_2-45CaO-5P_2O_5.

In the first stage of hydrolysis and condensation of synthesis glass P1, were used 45.75wt% TEOS, which were mixed with 43,86 ml ultrapure water and 7,31 ml nitric acid 2N in a glass container, with a magnetic stirrer at moderate speed (n = 400-450 rot / min) to completely rinse of the solution.

In this phase TEOS is partially hydrolyzed in acid catalysis. Mixing up the rinsing is required, considering that TEOS is insoluble in water.

The volume of acid used as catalyst is calculated based on the amount of water added in the report V_{H2O}: V_{HNO3} = 6: 1. The amount of water added at this stage is defining for the next processes such as condensation (coalescence), maturation, aging and drying of the gel.

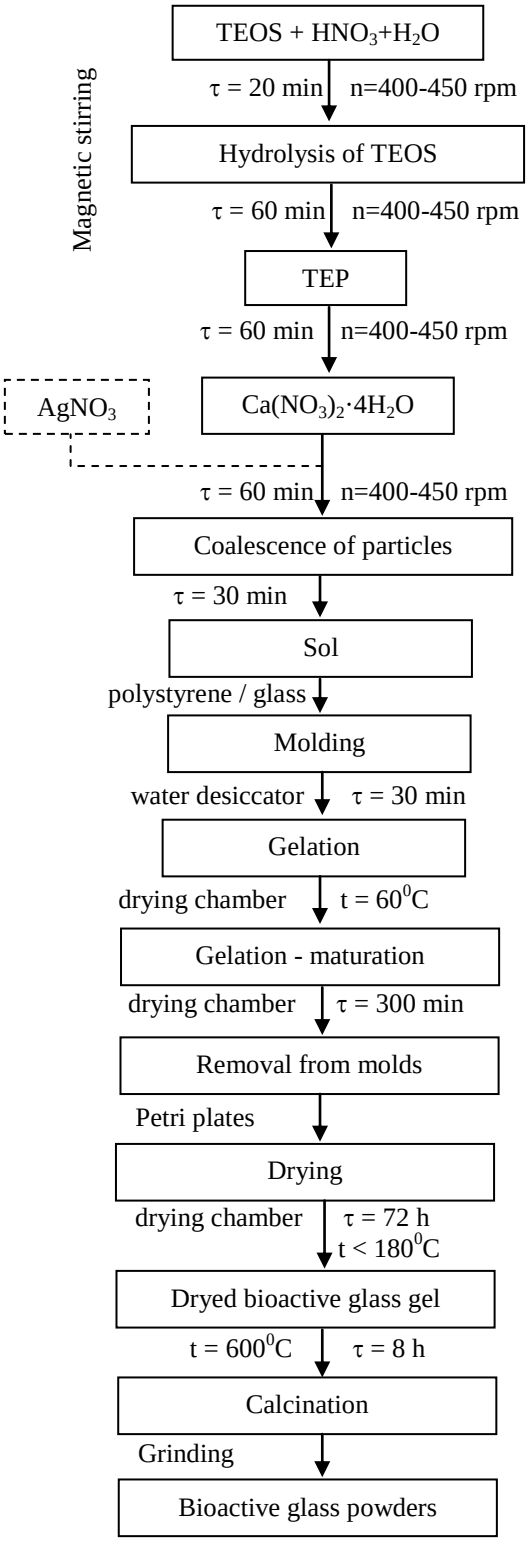


Fig. 1. Flow chart for synthesis of sol – gel bioactive glass powders

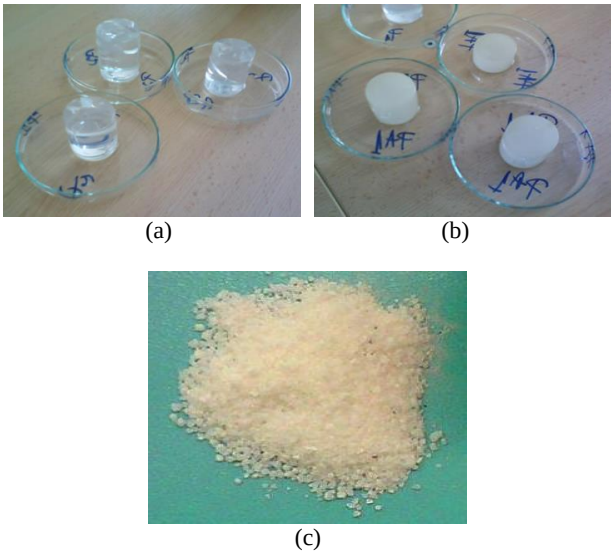
In the second stage, for the complete hydrolysis of the TEOS and homogenizing the sol was added 4.57wt% TEP. The amount of TEP added influence the time of coalescence of particles, thus forming the gel, as well as the time of aging and drying it. For optimal duration of 24 - 48 hours for coalescence, or 72 hours for aging and drying can be respected, it requires compliance with a molar ratio between the amount of water and the amount of TEOS and TEP, of 4 and 12. The molar ratio H_2O: (TEOS+TEP) chosen was 8. The mixture was stirred at the same speed for 20 to 30 min., then it was added 49.67wt% $Ca(NO_3)_2 \cdot 4H_2O$ and stirring continued for at least one hour, to obtain bioglass sol.

In the case of obtaining glasses doped with silver, the content of SiO_2 is reduced and the glass is replaced with the equivalent amount of $AgNO_3$, as it has been shown in Table 1.

The sol resulted, with extremely low viscosity is moulded and maintained 24 hours in an airtight container in a saturated atmosphere in moisture, in order to gelation. Gelation process consists of polycondensation reactions leading to interconnection colloidal particles (sol) in a three-dimensional network [18]. Aging and drying the gel formed is accomplished by boiling it at 60°C in an electric furnace (stationary atmosphere) for 54-60 hours.

During aging of the gel, it take place the completion of polycondensation processes, followed by contraction of the gel and liquid removal from its pores (syneresis) [18].

Figure 2 presents gels aged, after keeping them in the furnace at 60°C (a) and glass powder after calcination (b).



(a) (b)

(c)

Fig. 2. Synthesis of sol-gel glasses: gel samples (a) without and (b) with silver, respectively glass powders after heat treatment (c)

The last step of the process involves drying the gel to maintain the increasing temperature from 60°C to 180°C, for 12-18 h, resulting in partial stabilization thereof.

Final stage of the technological process consists of stabilized gel calcination at 600°C, with a minimum holding time of 3 h, according to a well-defined program [19].

Drying and calcination program choice is decisive for obtaining mesoporous structure and interconnecting pore size desired.

2.2 Antimicrobial activity of sol-gel glasses doped with silver

For this study have been selected two species of bacteria commonly found in different works that have studied the bacteriostatic and bactericidal activity of silver-doped glasses: Escherichia coli - bacteria resistant gram negative, with extremely high intrinsic resistance to antibiotics and Staphylococcus aureus – gram positive bacteria with high risk of postoperative infections in prosthetic and reconstructive surgery.

The antimicrobial activity was determined at various dilutions for pure cultures of microorganisms, incubated at 37° C and 48 hours to Staphylococcus aureus, respectively 44^o C and 24 hours for the Escherichia coli at various concentrations of Ag + (0.005 - 0.03 mg glass / ml) in the same volume of culture medium.

Bacterial strains used were: Staphylococcus aureus ATCC reg. 25923 and Escherichia coli MG 1655. Both were incubated aerobically, each on selective culture medium.

2.3. Characterization techniques

Chemical composition of the glass powders obtained was determined by X-ray fluorescence spectroscopy, wavelength dispersive (WD-XRF), according to the instructions of the instrument Advant'X ARL - Thermo Scientific with X-ray tube of 2400 W, 60 kV and 80 mA, using a crystal diffraction LiF 200 and 220 scintillation detector. Powders has been grinding in Herzog oscillating mill until a particle size less than 100 μm, followed by sieving. Hydraulic press Herzog HTP 60 was used in order to obtain discs with a diameter of 40 mm. Quantitative data processing was made by using OXSAS software. Morphological analysis of glass powders has been made by using Field Emission - Scanning Electron Microscope Hitachi SU- 70 with resolution of 1.0 nm at 15kV.

Relative density of glasses was measured by pycnometer method. For this purpose have been used pycnometers glass, analytical balance, glass pipettes, funnels and beakers.

In order to identify bacteriostatic properties of silver-doped glass were used electric incubators, microbiological niche, Petri plates, glass pipettes, Pasteur pipettes, test tubes.

3. Results and discussion

3.1. Study of glass composition by WD – XRF analysis

The results of WD-XRF analysis are presented in the following table, for each of the four samples synthesized (P_1, P_2, PA_1, PA_2).

The results of chemical analysis achieved by X-ray fluorescence spectroscopy revealed obtain results close to those initially set, in case of phosphocalcic sol-gel gasses and those doped with silver ions.

Table 2. Chemial composition of synthesized glasses

Sample	Oxidic composition			
	SiO_2	CaO	P_2O5	Ag_2O
P1	47.83	45.73	5.6	-
P2	52.97	40.69	5.76	-
PA1	48.14	41.6	5.95	3.7
PA2	49.5	37.41	6.2	6.02

3.2. Results of relative density for glasses synthesized

A qualitative assessment of porosity of glasses obtained was achieved by correlation with relative density of powders obtained. Density determination was performed by standardized pycnometric method.

Relative densities of glasses samples are presented in table 3.

Density values determined confirm data from the literature, showing that the density of the glasses varies inversely with temperature synthesis and directly proportional to their porosity. The density of glasses obtained by melting varies from 1833 Kg/m^3 up to 3300 kg/m^3 and even 8000 kg/m^3, for those with heavy metal oxide in composition, being compact glass, vitreous mass. Glasses obtained by sol-gel technique have densities of 650-816 kg/m^3 and have a degree of porosity of 80-87% [20].

Table 3. Relative density of the samples synthesized

Sample	Density [Kg/m^3]
P_1	651,4
P_2	773,3
PA_1	760,9
PA_2	816,0

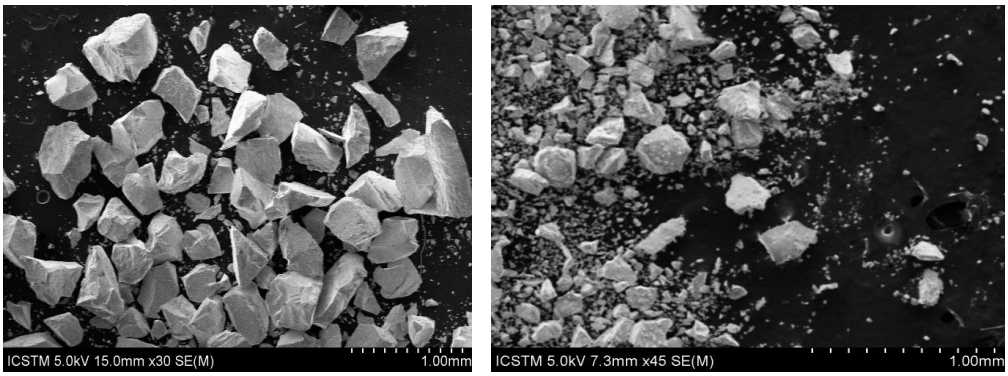
3.3. Surface morphology of glass powders

For morphological analysis of glass surfaces preliminary study was done on compositions 50SiO2 - 45CaO -5 P_2O_5, denoted P1 and 55SiO_2 - 40CaO - 5P_2O_5, denote P_2.

In case of the glass composition P_1 at a magnification of 30X, shown in figure 3a, can be observe an agglomeration of particles with well-defined shapes and sizes. It can be noticed particles with sharp edges, the result of the obtaining process, in particular heat treatment applied but also of insufficient processing in stages that followed synthesis.

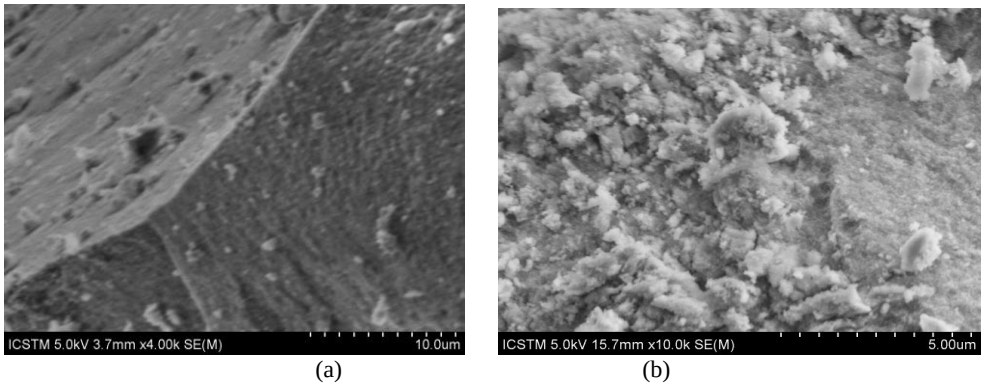
It can be highlight particle size particle uniformity, ranged around of 500 mμ.

By composition P_2, SEM analysis highlighted at low magnification (45x) and resolution of 1mm (fig. 3.b), reveals glass powders characterized by unevenness dimensional size. On this subject, can be may seen particles with dimensions of less than 100 mμ, but also a series of particles whose dimensions reach 300 mμ.



(a) (b)

Fig. 3. SEM micrographs for sol-gel glass powders at low magnification: 30X and 45X



(a) (b)

Fig. 4. SEM micrographs for sol-gel glass powders at high magnification 4000X and 1000X

SEM micrograph of glass powder P_1, achieved at magnification of 4000x and resolution of 10mμ, shown in figure 4a, highlight an slightly rough area, by comparison with the sample P2 (fig. 4.b), which is observed a surface with a pronounced roughness. Both compositions analyzed shows the synthesis of glasses characterized by a high specifics area, ideal for further development of a carbonated hydroxyapatite layer on their surfaces, after immersion in simulated body fluids human or growth of bioactive interface between the implant and tissue, when tests are performed in vivo.

3.4. Study of antimicrobial activity in case of sol-gel glasses doped with silver

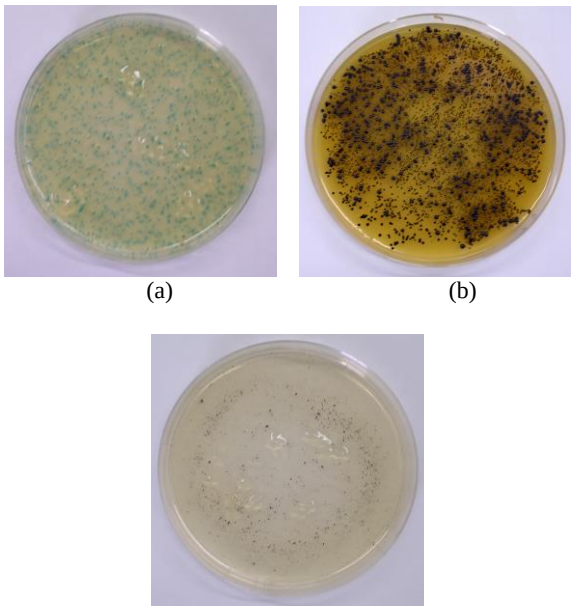
Bacterial strains used were aerobically incubated on selective culture media; Baird - Parker medium (bovine fibrinogen and rabbit plasma) for coagulase-positive staphylococci and TBX medium (trypsin, bile and glucuronide) for Escherichia coli [21-25].

As regards antimicrobial activity has been found that silver doped glasses exhibited bacteriostatic and even bactericidal activity, depending on the dilution used, as follows:

- in the plates were particulate glass embedded undiluted included, in the fluidified culture medium, at a concentration of 0.1g/10ml culture medium, and 0.1ml pure culture have not been developed specific colonies for any of the bacteria (Fig. 5.c), than the control plates (without silver) - bactericidal effect, as can be seen in figures 5.a and 5.b;

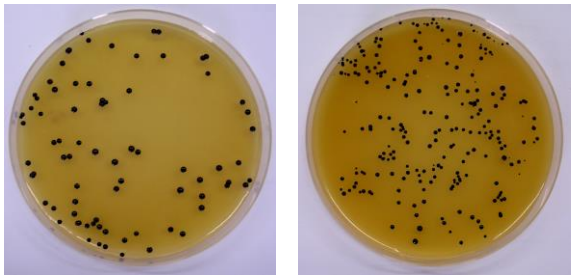
- Staphylococcus aureus has grown from third decimal dilution (10^{-3}) of 0.1g glass powder suspended in 9.9ml peptone saline (physiological serum) and allowed to diffuse 6h under continuously stirring. Inoculum suspension and 0.1ml of pure culture have been incorporated by striation on the surface of the solidified medium. Fig. 6 shows the bacteriostatic effect of the glasses according to the dilution.

- Escherichia coli has begun to grow up at concentrations of the glass powders from two decimal dilution (10^{-2}), but starting from initial concentrations of powder by 0.03g/9.9ml in peptone saline. Incorporation and quantity of pure culture inoculum have been identical as in the case of coagulase positive staphylococci. The control plates, bacterial colonies have been developed normally (Fig. 7.a), in large numbers. Figures 7b and 7.c are relevant to the bacteriostatic effect of glass dilution with silver.



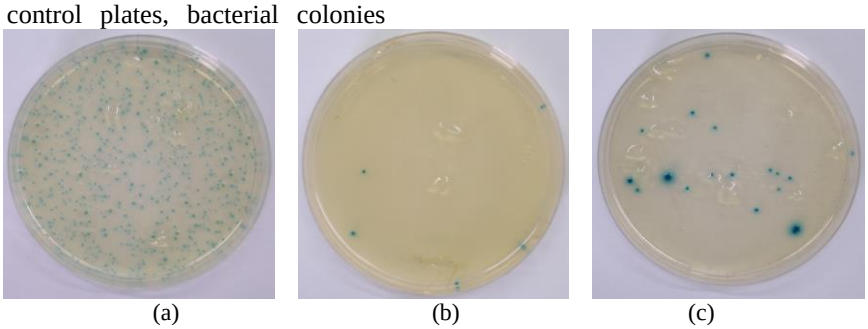
(a) (b) (c)

Fig. 5. Control plates without silver for (a) Escherichia coli, (b) Staphylococcus aureus and (c) glass powders with silver



(a) (b)

Fig. 6. Glass powders with silver soaked on peptone saline (physiological serum) at different dilutions: (a) $0.01 \cdot 10^{-3}$ g/ ml, (b) $0.01 \cdot 10^{-5}$ g/ml



(a) (b) (c)

Fig. 7. Control plates without silver (a), glass powders with silver soaked on peptone saline (physiological serum) at different dilutions: (b) $0.03 \cdot 10^{-2}$ g/ ml, (c) $0.03 \cdot 10^{-4}$ g/ml

4. Conclusions

In this study, bioglasses from the ternary system of SiO_2-CaO-P_2O_5, and those doped with silver, have been successfully synthesized by the sol-gel process using as precursors tetraetilortosilicate, triethylphosphate, calcium nitrate tetrahydrate, and silver nitrate.

Chemical composition determined quantitatively by X-ray fluorescence spectroscopy demonstrated that the technology chosen and the method of calculating theoretical compositions of glasses are appropriate, the results demonstrating the efficiency hydrolysis and polycondensation of over 95-98%.

The density value is dependent on the proportion of silica from glasses composition and substitution degree of SiO_2 with $AgNO_3$.

In glass samples when some percents of SiO_2 has been substituted with $AgNO_3$ was observed a slight increase of glass density. Also, there is a higher density in glass samples with a higher content of SiO_2, as seen in Tables 2 and 3.

Glasses particles morphology has been influenced by the synthesis conditions. Also, SEM analysis for the two glass compositions analyzed (without silver ions) highlights obtain of rough surfaces, able to develop an apatite layer to these or to generate biologically active interfaces, in vivo.

Sol-gel glasses doped with silver have a certain bacteriostatic activity even at extremely low concentrations: 0.1g/10ml ($0.01x10^{-3}$g/mL) for Staphylococcus aureus and 0.03g / 10ml ($0.003x10^{-2}$ g/ ml) for Escherichia coli.

At concentrations of 0.1g glass /10ml medium, powders have a bactericidal effect; therefore, the initial concentrations of glasses silver obtained could be considerably reduced, even to 0.5-1% in glass composition.

Glasses without silver had no effect on bacterial growth.

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