SYNTHESIS, CHARACTERIZATION AND IN VITRO BIOACTIVITY OF $SiO_2 – CaO – P_2O_5$ SOL – GEL GLASSES HIGHLIGHTED BY XRD TECHNIQUE

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Abstract. Synthesis of bioactive glasses can be achieved by two major methods: traditional fabrication by melt – casting and sol – gel process. Bioactive glasses obtained by sol–gel process generally reveal a high bioactivity than melt derived glasses of similar compositions. A common feature of this materials is the modification of their surface reactivity immediately after soaking in simulated body fluids. On the glass surface a layer of carbonated hydroxyapatite, biologically active, is formed. In this paper, bioactive glasses based on SiO_2-CaO-P_2O_5 system have been synthesized by sol – gel process. The powder glass obtained has been characterized by X-ray diffraction, X-ray fluorescence spectroscopy (XRF)*. In vitro study reveals formation of apatite layer at surface of powder glass, after 3 days of soaking in simulated body fluid.*

Keywords: Bioactive glass, Sol – gel synthesis, Apatite, In vitro bioactivity

1. INTRODUCTION

Bioactive glasses and glass ceramics are among the most promising systems, due to the excellent biological and remarkable mechanical properties. A common feature of oxide materials with bioactive properties is the modification of their surface reactivity immediately after implantation. On the glass surface a layer of carbonated hydroxyapatite, biologically active, is formed. This layer provide an interface connection with the bone. The new phase formed (carbonated hydroxyapatite) is chemically and structurally equivalent whit the bone mineral phase [1].

Melt derived bioactive glasses are obtained from a mixture of inorganic materials. This mixture is transformed into a completely homogeneous melted liquid, without solid or gaseous inclusions, by heating at temperatures between 1000^0C and 1500^0C, according to its composition.

Liquid glass phase is transformed into an amorphous substance that solidifies while the viscosity increases to ambient temperature, without allowing crystallization [2].

Traditional fabrication methods by melt-casting and grinding of bioactive glasses have a number of disadvantages: first, it is difficult to obtain a melt derived glass characterized by a high purity. This parameter leads to obtaining optimal values in terms of bioactivity. The reasons behind this shortcoming is related to the high temperatures associated with melting and mixing processes, but at the same time, because of low content of silica (SiO_2) or high percentage of alkali or alkaline earth metals, which are found in compositions of bioactive glasses obtained by traditional methods. These compositions are very reactive and therefore tend to dissolve the crucible made by refractory material, bringing a series of impurities in the melt. This phenomenon will lead to a final product with high degree of contamination [3].

Stages of technology process in which glass powders are crushed, sieved or sintered powder has a negative effect on the glasses and glass ceramics bioactivity.

Not in the last, high production costs resulting from technological process that occur at high temperatures and the many stages in which glass is handled. Therefore, it is aimed at decreasing of melting temperature which will lead eventually to a significant reduction of all costs of production

Bioactive glasses synthesized by sol – gel process allowed to obtain an improved bioactivity compared to melt derived glasses, which is due to porous nature of the materials used as precursors.

Sinthesis of bioactive glasses by sol – gel method has been applied since the 1990s. This new class of bioactive glasses has a wide range of biocompatibility [4].

Bioactive glasses obtained by sol – gel process can be placed in the binary system CaO - SiO_2 [5], tertiary SiO_2 - CaO - P_2O_5 [6] or quaternary SiO_2 - CaO - P_2O_5 – Na_2O [7]. All these glass compositions have a certain degree of bioactivity, after immersion in simulated body fluids. Sol – gel derived glasses from the system SiO_2 - CaO - P_2O_5 can form a hydroxyapatite layer on their surface, while the proportion of silica does not exceed 90% [8].

Most of the inconveniences arising from the synthesis performed at high temperature can be eliminated through a rigorous control of purity. The sol - gel process is also offers potential benefits for the obtaining of powdered materials with a wider range of bioactivity and a better control of it by controlling the composition or microstructure, by a rigorous control of heat treatment parameters. The enhanced bioactivity is due to the nanoporosity and enhanced surface area. In fact, sol–gel glasses can be considered as mostly surface as the nanopores are thought to be completely interconnected [9].

The aim of this paper was to synthesize SiO_2-CaO-P_2O_5 based glasses with chemical compositions shown in Table 1, prepared by using sol - gel technique. Sol-gel glass based on SiO_2-CaO-P_2O_5 system has been characterized by X-ray diffraction in order to identify phase transformations before and after immersion in

simulated body fluids (SBF), chemical composition of sol-gel derived glass has been determined by using X-ray fluorescence spectroscopy (XRF).

2. EXPERIMENTAL

The composition of bioactive glasses synthesized in this study is presented in table 1.

Table 1. Composition of bioactive sol-gel glasses

Composition		SiO_2	CaO	P_2O_5
S1	Mole, %	50	46	4
	Weight, %	49	42	9
S2	Mole, %	60	36	4
	Weight, %	58	33	9

The sol-gel precursors used in this study were tetraethyl orthosilicate ($Si(OC_2H_5)_4$, TEOS), triethylphosphate ($(C_2H_5)_3PO_4$, TEP) and calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$).

Sol-gel derived glasses synthesis involves a series of processes that include hydrolysis and condensation reactions by mixing the glass precursors into a sol, gelation, aging of the gel, drying of the gel, stabilization or calcination of dry gel.

2.1. Synthesis of sol – gel glasses

Two types of gel-derived glasses are presented in this papter: S1 and S2. The glass compositions are listed in Table 1.

Synthesis process for the first compositon of bioactive glass, S1, is presented below. In the first stage of synthesis, reactions of hydrolysis and condensation are involved. Thus, 54 ml of tetraethylorthosilicate (98%, Acros Organics, Be) is mixed with distilled water and hydrochloric acid in a glass container.
As tetraethylorthosilicate is insoluble in water, the hydrolysis reaction requires a catalyst, usually acidic. In order to accelerate hydrolysis reaction of tetraethylorthosilicate, 4.71 ml hydrochloric acid (2N) was added to 28.3 ml ultra pure water. The amount of water added is very important (the degree of gelling, homogeneity and, respectively, reasonable aging and drying time are function of wather amount). The aims of the second phase were to complete hydrolysis of tetraethylorthosilicate and, consequently, a homogenization of the sol. In order to obtain an optimal time for gelling between 24 and 48 hours, respectively, up to 72 hours for maturation and drying of gel, H_2O / (TEOS + TEP) ratio was fixed at 6. The mixture was allowed to react for 30 min for hydrolysis of precursor during stirring at room temperature. After that, 6 ml of triethylphosphate (99%, Acros Organics, Be) have been added to the stiring solution and 51.6 g calcium nitrate tetrahydrate (99%, Acros Organics, Be) after another 50 min. Solution stiring will continue for another hour, in order to obtaining a sol.

In case of second sol – gel derived bioactive glass composition, S2, the technological process is similar to that described above.
The synthesis of glass was carried out by hydrolysis and polycondensation of 63 ml tetraethylorthosilicate (TEOS), 4 ml triethylphosphate (TEP), and 38.5 g calcium nitrate tetrahydrate ($Ca(NO_3)_2\_4H_2O$). Hydrolysis reaction of tetraethylorthosilicate was achived by mixing 44.5 ml of deionized water with 7.42 ml hydrochloric acid (2N). In order to obtain optimal times for the tree processes of gelling, maturation and drying, in this case less than 96 hours, H_2O / (TEOS + TEP) ratio was fixed at 8. At the same time, this value this value allows the obtaining of sol – gel glasses in powder form [10].
After complet hydrolysis of tetraethylorthosilicate, in condition of intense stirring (600 rpm), triethylphosphate is added into solution and after another 60 minutes calcium nitrate tetrahydrate.
The solution obtained after this stage of technological process is stirred for another 60 minutes. In order to obtain a sol, solution is stirred for another 60 minutes.
The next stages involved in sol – gel derived glasses are identical for the two compositions studied [10].

Gelation process involves the interconnection of colloidal particles (sol) in a three-dimensional network. Since the sol is a low-viscosity liquid, it can be cast into a mold. The mold must be selected to avoid adhesion of the gel or nucleation of the bubbles at the mold-gel interface [11]. In this case, the sol was stored in polystyrene recipients placed on a sealed glass container for one day at ambient temperature to form the gel.

Aging and drying: First the samples are placed in a drying oven at temperature of 60^0C, for about 60 hours.
Aged gel samples after drying process are presented in Fig. 1.

Fig. 1. Gel samples in different shapes formed after keeping sol for about 60 hours in drying oven

To avoid water evaporation a sealed glass container with a proper amount of water has been used. In this case the conditions for gel hydrolysis are maintained. Three processes can occur during aging of the silica gels:

polycondensation, shrinkage of the gel and expulsion of liquid from the pores and as result a decrease of surface area due dissolution and re-precipitation processes [11].

The aged gel was remove from the polystyrene recipients and dried by heating at 175^{0}C for three days, on a glass plate, into a drying oven.

Dried gel is placed, then in alumina crucible for calcination. The dried sample was stabilized at 600^{0}C for 4 hours in order to remove undesired compounds. The flow chart for preparing sol-gel glass powder, in agreement with procedure above mentioned, is illustrated in Figure 2.

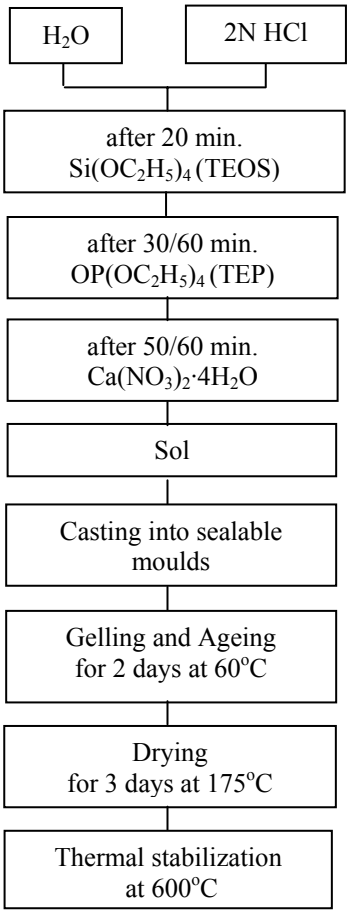

Fig.2. Flow-chart for production of sol-gel glass powder

After thermal stabilization, the powders was lightly ground in an agate mortar and pestle and sieved to 75–150 μm and 45–75 μm, by using a planetary ball mill with tungsten carbide balls, for 2 hour.

Sol-gel derived glass obtained after heat treatment at 600^{o}C are illustrated in figure 3.

Fig. 3. Sol-gel powder glass obtained after calcinations process

2.2. Characterization of the samples

The chemical composition with respect to SiO_2, CaO and P_2O_5 contents of glass powders has been measured by X-ray fluorescence (XRF) on Panalytical Axios spectrometer. In order to produce high-quality samples, for a highly accurate and reproductible analysis an automated sample preparation system Pearl X`3 was used.

The structural characterization of powder glass was carried out by X-ray diffraction using a Bruker AXS D8 ADVANCE diffractometer with $Cu_{k\alpha}$ = 1.5405 Å radiation generated at a voltage of 30 kV and a curent of 20 mA. Data were collected in the 2θ range of 20–60^o, with a step size of 0.04^o 2θ. X-ray analysis was used to assess the present of amorphous glass structure and formation of apatite phase after immersion in simulated body fluid.

2.3. Preparation of simulated body fluid

The importance of in vitro bioactivity tests compared with those achieved in vivo is evident. In vivo studies encounter many difficulties, they involve ethical problems, difficulty in reproducibility of results, high costs etc. For these reasons, before making the testing of biomaterials in vivo, the products obtained in the laboratory must be tested in vitro.

Simulated body fluid soaking was used to evaluate in vitro behaviour of bioactive glass powder.
The composition of this solution, described by Kokubo et al [12], has similar composition to human blood plasma.
SBF is able to produce the same type of hydroxyapatite layers in vitro, such as that would form on the bioactive glass surface in the human body [13].
Bioactive glass powders has been immersed in simulated body fluid for various times up to 14 days. In order to achieve good resuts, glass surface / SBF volume ratio was setup at 0.005 cm^{-1}. SBF solution was changed after three days to have fresh reactive ions always present reacting with powder. The temperature of the reaction was maintained in the range from 36.5^{0}C to 37^{0}C.

The ion concentrations and proper amounts of reagents used in preparation of simulated body fluid are given on Table 2.

Table 2. Reagents for preparation of SBF

Reagents	Amount	Concentration SBF	
		Ion	
NaCl	7.9950 g	Na^+	142.0
$NaHCO_3$	0.3528 g	K^+	5.0
KCl	0.2239 g	Mg^{2+}	1.5
K_2HPO_4	0.1742 g	Ca^{2+}	2.5
$MgCl_2$	0.1428 g	Cl^-	147.8
1 kmol/m^3 HCl	40 cm^3	HCO_3^-	4.2
$CaCl_2$	0.2775 g	HPO_4^-	1.0
Na_2SO_4	0.0710 g	SO_4^{2-}	0.5
$(CH_2OH)_3CNH_2$	6.0568 g		
1 kmol/m^3 HCl	To adjust the pH to 7.4		

3. RESULTS AND DISCUSSION

3.1. X-ray diffraction analysis

In figure 5 are presented X – Ray diffraction patterns of SiO_2 – CaO – P_2O_5 sol-gel glass, S1, before and after soacking in simulated body fluid at 37°C and pH = 7.4

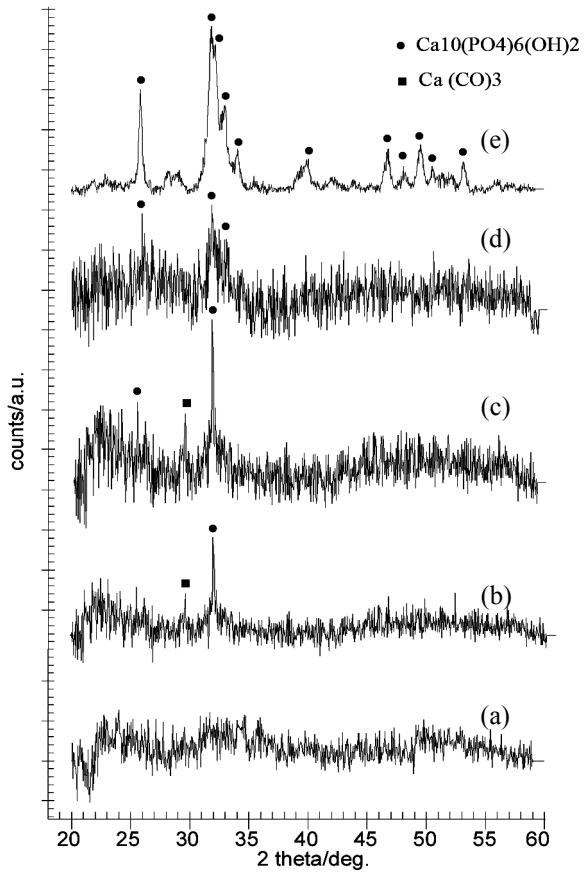

Fig. 4. X – Ray diffraction patterns of S1 gel derived glass immersed in SBF from 0 to 14 days.

The X- Ray diffraction pattern presented in figure 4 a are characteristic of amorphous silica–lime–phosphate glasses synthesized by sol – gel process.

In this study, the substance formed on glasses surface became detectable after 3 day of immersion in simulated body fluid, thus a new peak situated at 31.77 2θ and 29.42 2θ, were assigned to be (211) apatite and (104) calcite in according with ICDD – PDF2 cards 00-09-0432 and 00-005-0586 as shown in figure 4 b.

In case of the sample immersed for 7 days (Fig. 4 c) in simulated body fluid apatite formation on the glass surface is revealed by peaks situated at 3,44 Å (2θ = 25.81°) and 2.81 Å (2θ = 31.77°). As well the calcite phase was identified at 2θ = 29.42.

In case of sample immersed for 14 days in simulated body fluid, XRD pattern presented in figure 4d reveals new peak characteristic of hydroxyapatite at 32.9 2θ. Also, in figure 4 e are revealed peaks characteristics of apatite new phase formed on the glass surface.

All these patterns that reveal formation of apatite layer on glass surface are related to hydroxyapatite sample obtained by precipitation process, as are shown in Fig. 4 e.

X-Ray diffraction patterns of S2 gel derived glass before and after immersion in simulated body fluid up to 14 days, in the same condition with sample S1, are presented in figure 6.

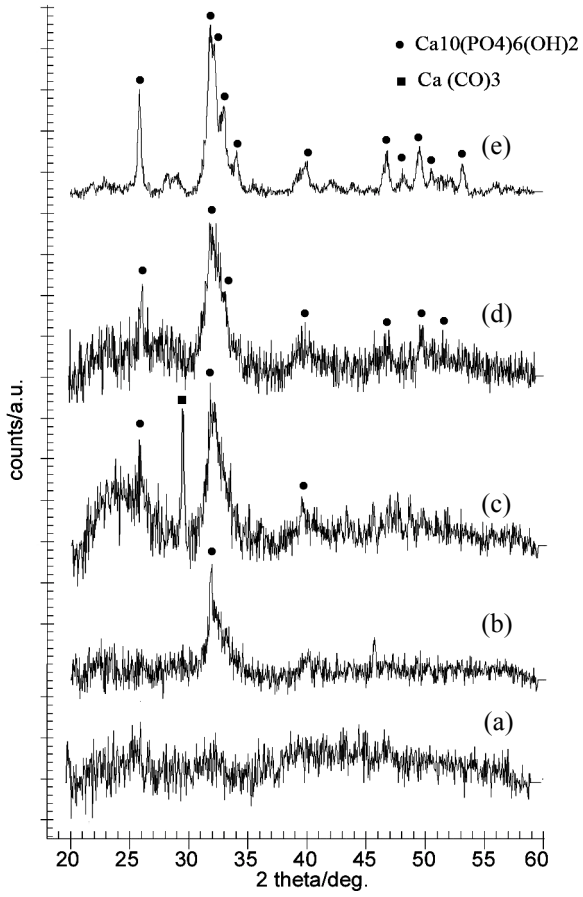

Fig. 5. X – Ray diffraction patterns of S2 gel derived glass immersed in SBF from 0 to 14 days.

XRD spectra shown in Figure 5 reveals a better reactivity of S2 glass afer soaking in simulated body fluid.
Almost all diffraction spectra are identical to those shown in Figure 4. The differences are shown for the sample immersed for 14 days (Fig. 5. d), where they highlighted new apatite peaks located at 2.26 Å (2θ = 39.85°), 1.94 Å (2θ = 46,73°), 1.84 Å (2θ = 49.50°), 1.80 Å (2θ = 50.53°), in according with PDF2 card: 00-009-0932. As well, for the sample socked for 7days in SBF a new phase was identified at 2θ = 29.42, assigned to be (104) calcite in according with ICDD – PDF2 card 00-005-0586.

3.2. XRF analysis

Elemental analyses for the presence of SiO_2, CaO and P_2O_5, were made by using X-ray fluorescence spectroscopy.
The results of semi quantitative chemical analysis achieved by XRF technique for the bioactive glass powder derived from sample thermal treated at 600°C, before soaking in simulated body fluid shown that the SiO2, CaO and P_2O_5 contents in the powder are presented in table 3.

Table 3. X-ray fluorescence analysis for sol – gel derived glasses synthesized in this study

Sample	Oxides contents (wt,%)			Loss on ignition, (wt,%)
	SiO_2	CaO	P_2O_5	
S1	52,77	30,98	5,95	10.1
S2	63.55	30.23	5.97	-

4. CONCLUSIONS

In this study, SiO_2 – CaO – P_2O_5 ternary bioactive glass was successfully synthesized via sol – gel method, by using tetraethylorthosilicate (TEOS), triethylphosphate (TEP) and calcium nitrate tetrahydrate, as chemical reagents.

The X- Ray diffraction pattern presented in this paper are characteristic of amorphous silica–lime–phosphate glasses synthesized by sol – gel process and apatite formation at surface of this glasses.

In vitro bioactivity of sol-gel glass powder was achieved after immersion in simulated body fluid at 37°C and pH = 7.4 for glass surface / SBF volume ratio = 0.005 cm^{-1}. Thus, appearance after 3 days of the first peaks at 31.8 2θ and 39.93 2θ reveals formation of apatite on glass surface soaked in simulated body fluid. The appearance of new peaks and increased intensity of existing peaks has been observed after 7 and 14 day of immersion.

XRD spectra reveals a better reactivity of S2 glass afer soaking in simulated body fluid. This phenomenon is explained by the increasing content of silica in the composition of bioactive glass. Apatite formation on the surface of bioactive glasses is proportional to the percentage of silica in the composition of sol – gel derived glasses.

Chemical composition achieved by fluorescence spectroscopy, related to content of SiO2, CaO and P_2O_5 , in case of sample unsocked in simulated body fluid reveals good results related to theoretical composition.

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