

IN VITRO BIOACTIVITY INVESTIGATION OF POROUS CALCIUM SILICATE BIOACTIVE GLASSES PREPARED BY SOL-GEL USING PEG BEADS AS TEMPLATE

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The influence of the poly (ethylene) glycol (PEG) on the structure of calcium silicate bioactive glasses prepared by the sol-gel method was studied. PEG is a thermoplastic polymer having excellent properties i.e. biocompatibility, non-toxicity and water-solubility. The structure of the bioactive glasses prepared was investigated by Fourier transform infrared spectroscopy (FTIR), thermogravimetric and differential thermal analysis (TG/DTG), X-ray diffraction (XRD), scanning electron microscopy (SEM) equipped with Energy Dispersive X-ray Spectrometer (EDX). The results reveal that the use of the PEG (as porogenic organic template) enables the synthesis of porous calcium silicate bioactive glasses.

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1. Introduction

Bioactive glasses are an option as scaffold material for bone tissue engineering due to their recognized biocompatibility, controllable degradation rate, osteoconductive and osteoinductive properties [1, 2]. Ideally, the bioactive glass scaffolds designed for bone repair must have a suitable interconnected pore network, and high mechanical and microstructure stability for vascular ingrowth in vivo [3]. Many attempts have been made to mimic the interconnected framework structure of bone in terms of structural and biological characteristics. Thus, in previous studies, it was found that a minimum pore size of ~100 μm was necessary for bone ingrowth into the channels [4].

The porosity of scaffolds can be increased by adding polymer beads or other organic agents (porogens) such as PMMA microbeads, polyethylene, gelatine, corn, potatoes and rice starches, sucrose that burn out on sintering and leave pores [5, 6]. The burning out method combines low cost, simplicity and versatility [6]. Among the above mentioned porogens, PEG polymer constitutes another widely used porogenic organic template for scaffolds [7, 8, 9]. PEG is a crystalline, thermoplastic polymer having the general formula of $\text{HO}-(\text{CH}_2\text{CH}_2\text{O})_n\text{H}$. With different values of n , this biocompatible polymer has many consumer applications in packaging, cosmetics, pharmaceuticals, biotechnology, having excellent properties i.e. non-toxicity and water-solubility [10, 11, 12, 13].

In this work porous calcium silicate bioactive glasses were prepared by the sol-gel method using the poly (ethylene glycol) (PEG) as an organic template. The PEG was used in order to change the mesoporous structure of the binary calcium silicate glasses [14] and therefore their properties.

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2. Experimental

The initial proportion of the raw materials was: $\text{SiO}_2/\text{CaO}=70/30$ (mol/mol) by mixing distilled water, HNO_3 (65%, Merck), tetraethyl orthosilicate (TEOS) (99%, Fluka) and calcium nitrate tetrahydrate (99%, Sigma Aldrich). 2N HNO_3 is used to catalyse the TEOS hydrolysis during the sol preparation with molar ratios $\text{H}_2\text{O}/\text{TEOS}=12 / 1$ (mol/mol), and volume ratios $\text{H}_2\text{O}/\text{HNO}_3=6/1$ (v/v) [15]. The derived sol after the completion of hydrolysis was cast in a cylindrical container and aged in a drying oven at 60 °C to reach high viscosity near the gel point. Then, the polyethylene glycol (PEG)(Fluka) particles were ground and sieved in size < 300 μm and added into the gel with strong stirring for ½ h as described in a previous our work [16]. After aging for 1 day at 60 °C (wet gel material: WG), the gel was dried in a flask containing of 50:50 % mixture of water/ethanol at 120 °C for 2 days (dried gel material: DG). The dry gel was ground and sieved in size < 63 μm and pressed into a proper mold of 13 mm in diameter and 2 mm in thickness. The disks formed were thermally stabilized in an electric furnace in air atmosphere by heating with 10 °C/min up to 700 °C and kept for 3h (gel- glass material: GG).

The in vitro bioactivity of these glasses was carried out by immersing them in a simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma [17] at 37 °C and pH 7.4 for a reaction time period of 14 days. After 14 days immersion in SBF, the surface of the bioactive glasses was studied by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM) equipped with Energy Dispersive X-Ray Spectrometer (EDX).

FTIR spectra were recorded using a Perkin Elmer Spectrum 2000, on discs prepared by mixing of the sample powder with KBr powder.

TG/DTG curves were recorded using a TG/STDA Mettler Toledo 851 instrument. The sample was heated from 25 to 1100 °C at a constant heating rate of 5 °C/min under N_2 atmosphere.

XRD measurements were performed with a Siemens D5000 X-Ray Diffractometer by using sample of the material as powder.

The SEM/EDX studies were carried out in a FEI Quanta 200 Scanning Electron Microscope (SEM) equipped with an Energy Dispersive X-ray Spectrometer (EDX).

3. Results and discussion

Bioactive glasses- results

FTIR

Fig. 1 shows the FTIR spectra of the glass after aging, drying and stabilization.

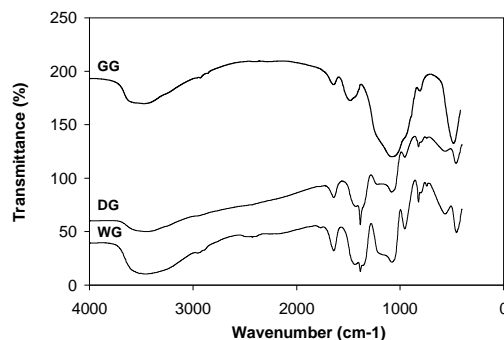


Fig.1. FTIR spectra of materials after different stages in the process: WG (after aging at 60 °C), DG (after drying at 120 °C) and GG (after stabilization at 700 °C).

According to *Fig. 1* and based on the literature, the material WG shows a peak at 445 cm^{-1} which is assigned to the bending modes of the Si-O-Si and O-Si-O bonds [18, 19]. The peak at 819 cm^{-1} corresponds to the stretching mode of the O-Si-O bond [15, 18, 19]. The peak at 945 cm^{-1} corresponds to the Si-O-Ca bonds containing non-bridging oxygen [15, 20]. The peak at 1070 cm^{-1} is attributed to the symmetric stretching vibration of the Si-O-Si bonds [15, 18]. The peak at 1380 cm^{-1} is assigned to the vibration of ionic $(\text{NO}_3)^-$. The vibration of H_2O (vibration due to the OH bond) is at 1631 cm^{-1} [15, 18]. No peaks assigned to organic matter have been observed. The same bands are observed for the material DG [15, 18, 19].

After the stabilization at $700\text{ }^\circ\text{C}$, the material GG shows the peaks of H_2O (vibration due to the OH bond), Si-O-Si and O-Si-O (bending modes), O-Si-O (stretching modes) and Si-O-Si (symmetric stretching modes). The peak at 1380 cm^{-1} due to the vibration of ionic $(\text{NO}_3)^-$ is disappeared [15, 18, 19]. According to literature, the peak at 1470 cm^{-1} corresponds to $(\text{CO}_3)^{2-}$ groups [18]. The presence of carbonate is attributed to a carbonation process of the material due to the atmospheric CO_2 as a consequence of the high calcium content [18].

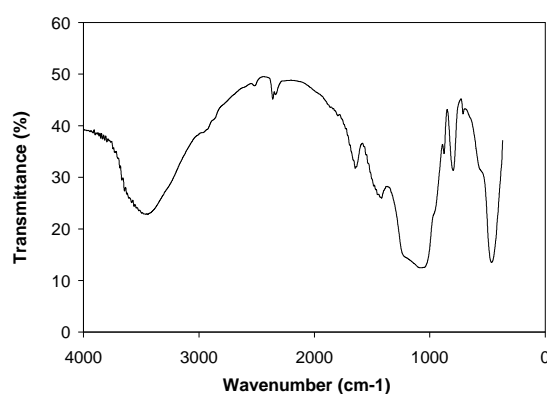


Fig. 2. FTIR spectra of the gel-glass (GG) after 14 days immersion in SBF solution.

According to *Fig. 2* and based on the literature, the material GG after 14 days immersion in SBF shows amorphous phosphate, PO_4^{3-} (peaks at $561, 955$ and 1056 cm^{-1} assigned to the (P-O) vibrational mode), and carbonate, CO_3^{2-} (peaks at $1413\text{-}1440$ and 873 cm^{-1} assigned to carbonate group [19]).

TG/DTG

Fig. 3 and *4* shows the TGA /DTG curves of the glasses after drying and stabilization.

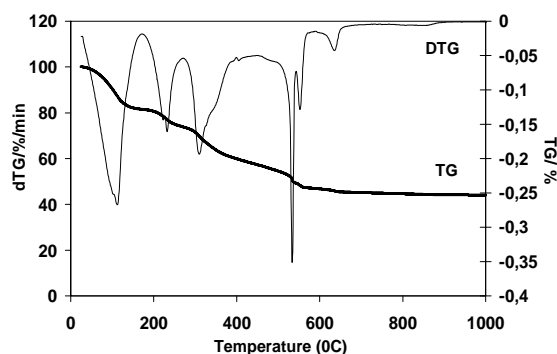


Fig. 3. TG/DTG curves of the glass after drying at $120\text{ }^\circ\text{C}/2$ days.

According to *Fig. 3* the dried material at $120\text{ }^\circ\text{C}$ during its thermal treatment up to $1100\text{ }^\circ\text{C}$ exhibits the following weight losses: 26.1% up to $271\text{ }^\circ\text{C}$, 16.04% between $272\text{ - }443\text{ }^\circ\text{C}$, 8.1% between $497\text{ }^\circ\text{C - }602\text{ }^\circ\text{C}$, and 0.3% between $596\text{ - }753\text{ }^\circ\text{C}$, i.e. the total weight loss is approx. 50.5% . According to literature [15, 19, 22, 23, 24], the weight loss up to $271\text{ }^\circ\text{C}$ is attributed to the

loss of residual water and ethanol and that between 272 - 443 °C corresponds with PEG decomposition [21]. The weight loss between 497 °C έως 602 °C is attributed to the loss of organic (i.e. alkoxy group) and nitrates (in the form of $\text{CaNO}_3 \cdot 4\text{H}_2\text{O}$ and HNO_3) used in the sol preparation [15, 19, 22, 23, 24].

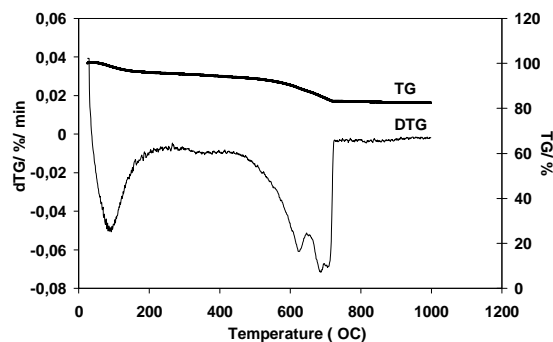


Fig.4. TG/DTG curves of the glass after stabilization at 700 °C/3 hours.

According to Fig. 4, the stabilized material at 700 °C during its thermal treatment up to 1100 °C exhibits the following weight losses: 4.1 % up to 215 °C, 12.5 % between 215 °C - 718 °C and 0.85 % between 718 °C έως 998 °C, i.e. the total weight loss is approx. 17.4 %.

XRD

According to the XRD results (Fig.5), the diffractogram of the stabilized material at 700 °C (GG), confirm its amorphous state as indicative of its internal disorder and glassy nature.

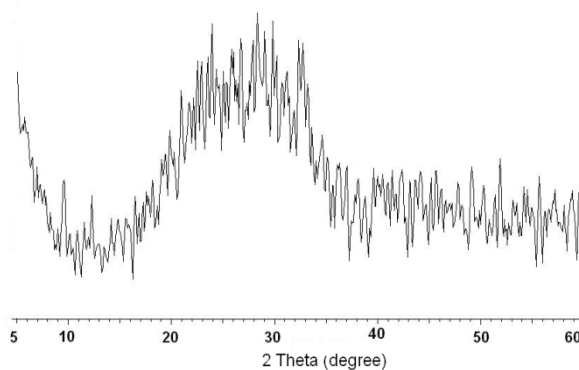


Fig. 5. XRD diffractogram of the gel-glass, GG.

After immersion of the gel-glass (GG) in SBF solution for 14 days, the XRD diffractogram (Fig. 6) indicates the formation of two phases: (211), (002) of apatite and (104), (024) of calcite. [18].

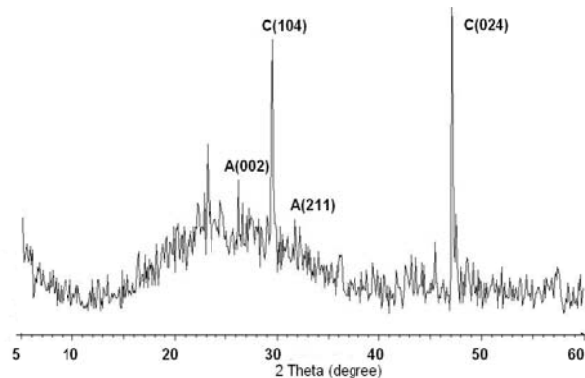


Fig. 6. XRD diffractogram of the gel-glass, GG, after immersion in SBF solution for 14 days.

SEM/EDS

According to Fig. 7(a), (b), (c) based on SEM images, the gel-glass (GG) before immersion in SBF solution has a porous structure. This structure is attributed to porous formed due to the mass loss of the material as gases evolved by combustion of PEG at 280 °C [21] during the thermal treatment. According to the Fig. 7 (a), (b) and (c), the SEM results indicate that the gel glasses have a macroporous network, containing pores mainly between 20 and 100 μm . According to Fig. 7 (d), the EDX results reveal the presence of the Si, Ca and O elements.

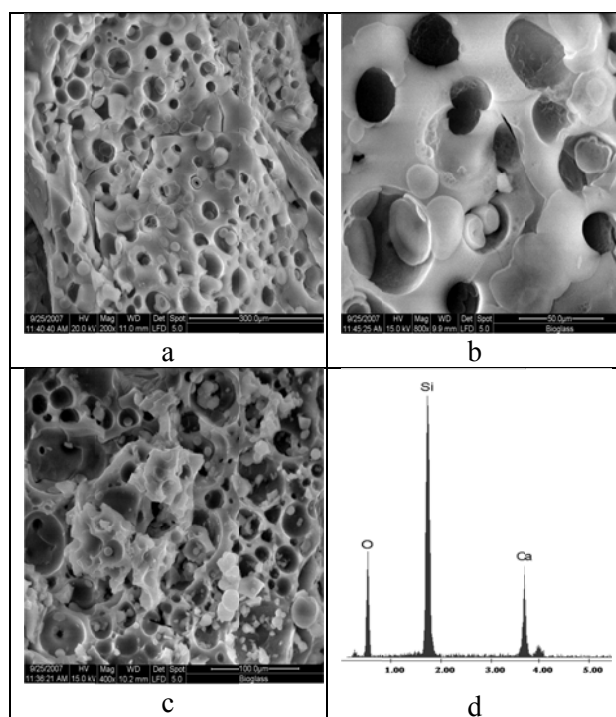


Fig.7. SEM image of the porous gel-glass before immersion in SBF solution (a) magnification 200x; (b) magnification 800x; (c) magnification 400x and (d) EDX spectrum of the porous gel-glass before the immersion in SBF solution.

After 14 day's immersion of the gel glass (GG) in SBF solution, the surface had been covered by clear shaped aggregated hydroxyapatite (HA) particles as shown in Fig. 8 (a), (b) and (c). According to Fig. 8 (d), the EDX results reveal the inclusion of phosphorous and other elements, beside Si, Ca and O, in the composition of the newly formed layer. The crystallized apatite layer

contains also minor quantity of other elements, such as Na, Cl, and K. The atomic ratio of Ca/P was 1.64, which is close to the theoretical value of 1.67 for apatite ($\text{Ca}_{10}(\text{PO}_4)(\text{OH})_2$) [25].

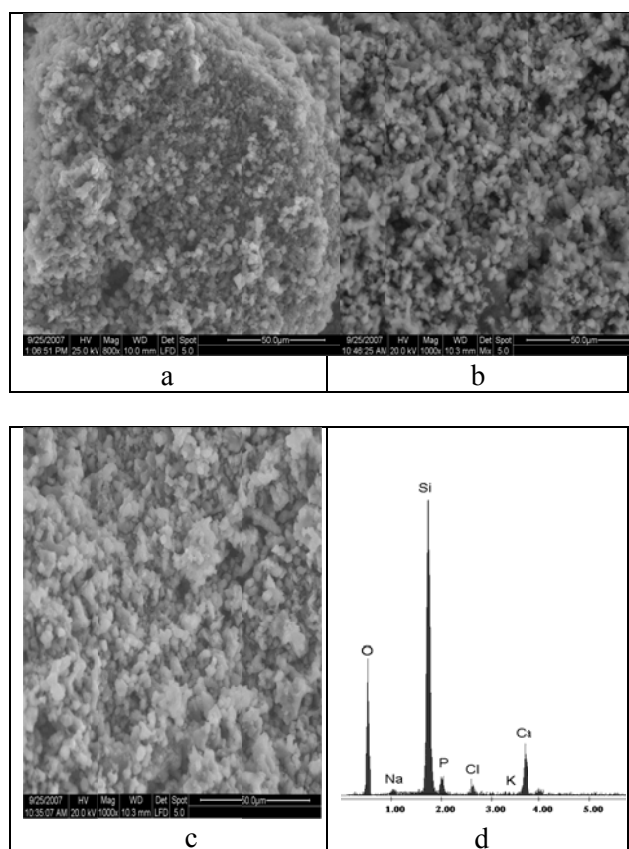


Fig.8. SEM image of the porous gel-glass before immersion in SBF solution (a) magnification 800x; (b) magnification 1000x; (c) magnification 1000x and (d) EDX spectrum of the porous gel-glass after 14 days immersion in SBF solution.

3. Conclusions

Porous calcium silicate glasses have been prepared by using PEG as porogenic organic template which exhibits macroporous network containing pores with diameters mainly between 20 and 100 μm determined from the SEM images. By immersing these porous gel-glasses into the proper simulated body fluid, an apatite layer is formed into the pores and on the surface of the glasses which indicates the bioactivity of this type of glasses. Therefore, these porous calcium silicate bioactive glasses may be used as biomaterials in tissue engineering applications.

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