Synthesis, characterization and evaluation of bioactivity of glasses in the CaO-SiO₂-P₂O₅-MgO system with different CaO/MgO ratios

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Abstract. Bioactive glasses (80Mg, 70Mg, 60Mg and 50Mg) in the system SiO₂-CaO-P₂O₅-MgO were prepared by sol-gel method and then characterized. The structure of the synthesized samples has been studied by X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR) and Environmental Scanning Electron Microscopy (ESEM). In vitro bioactivity tests were performed in Simulated Body Fluid (SBF). The apparent density and wettability variation with time were measured. The in vitro studies showed the formation of an apatite-like layer covering areas of the material surface. The variation in the CaO/MgO ratio has an influence on the chemical durability and bioactivity. The XRD and FTIR analysis revealed that the samples with larger CaO/MgO ratio exhibited better bioactivity. The results showed that the 50Mg glass which has the higher content of CaO/MgO is hydrophilic sample for the two used fluids (water and SBF). The porosity and hydrophilicity increase with increased the rate of CaO/MgO. The surface of bioglasses became rougher with the increased CaO/MgO ratio, which may lead to a decrease in water contact angle.

Keywords: Bioactive glasses, sol-gel, apatite, Magnesium, in vitro bioactivity, apparent density, wettability.

1. Introduction

Bioceramics, including glasses and glass-ceramics, are currently used as implant materials, usually for bone substitution [1]. The first of these materials was developed by Clark and Hench [2] using glasses in the system Na₂O-CaO-SiO₂-P₂O₅. Later other glass systems were studied e.g. by Kokubo et al. [3] and Vogel et al. [4]. A part of the glass-ceramics developed in the last years for biomedical applications is based on the CaO-MgO-P₂O₅-SiO₂ system [3-5]. Previous studies have shown that some glasses and glass ceramics containing Si, Ca and Mg were highly bioactive and could be used for biomedical applications [5]. Metallic ions are essential in human metabolism and are also known to play a critical role in osteogenesis and angiogenesis [6]. They have been considered highly promising for the field of biomedicine [7]. Single inorganic ions such as Calcium (Ca) [8], Phosphorous (P) [9], Silicon (Si) [10], and Magnesium (Mg) [11] are known to be involved in the bone metabolism and to play a physiological role in angiogenesis, growth and mineralization of bone tissue. In particular, metal ions are known to act as enzyme co-factors and therefore influence signaling pathways and stimulate metabolic effects occurring during tissue formation [6]. Mg is an essential minor element in human metabolism that is necessary for the incorporation of calcium (Ca) into body tissues. Also, the simultaneous release of Mg and Ca ions might be beneficial for bone regeneration [12]. In addition, Mg is one of the main substitutes for Ca in biological apatite. Bone, enamel and dentin contain 0.72, 0.44 and 1.23 wt% of Mg respectively [13], therefore, it is expected that the Mg-substituted hydroxyapatite (Mg-HAp) have excellent bioactivity and biocompatibility. Substitution of Mg in the silica glass matrix would modify their stability and improve their mechanical properties [14]. Mg is closely connected to the mineralization of calcified tissues [13] and has an indirect effect on mineral
metabolism [15]. However, the clear role of this ion has not been discovered yet. Mg has been shown to have stimulating effects on new bone formation [11]. Mg is suggested to interact with integrins of osteoblast cells which are responsible for cell adhesion and stability [11]. It seems that Mg directly stimulates the proliferation of osteoblasts with an effect comparable to that of insulin, a well-known growth factor for osteoblasts. On the contrary, Mg depletion affects all stages of skeletal metabolism, results in cessation of bone growth, lowering bone quality, strength and thickness and enhancing bone fertility [16], increased bone resorption and loss in trabecular bone underlining the significant role that Mg plays in bone metabolism [17], decrement of osteoblast and osteoclast proliferation and osteopenia [18]. Synthesis of glasses with low silica amount (25-29 mol %) and high Mg contents (31-36 mol %) have also been reported, suggesting that more improvements at the surfaces and in the glasses can produce potentially attractive bioactive materials [19]. It is noteworthy that replacement of CaO by MgO in the composition of SiO2 glasses would alter their stability and enhance the mechanical properties of the glass [14].

In general, the synthesis of bioactive glass is performed by the traditional melting method, which is regarded as simple and suitable for mass production [20]. However, the evaporation of the volatile component, P2O5, during high-temperature processing has limited this method. In recent years, the sol-gel technique, an alternative approach to the synthesis of bioactive glass has been widely studied [21]. This technique has definitely proved its exceptional potential by providing a possibility of synthesizing a significant number of new materials with high degree of homogeneity, taking place at low temperatures and high purity at a molecular level and with extraordinary physical and chemical properties [22]. The sol-gel reaction is a polymerization process in which a silica precursor hydrolyzes and condenses to form a network. The characteristics of the resulting network depend on some critical parameters of the reaction such as the relative rate of the hydrolysis and the condensation reactions [23], the molar ratio between water and alkoxide [24], the acidic or basic character of the environment [23], the nature [25] and quantity [26] of the solvent in which the sol-gel reaction takes place, and also on whether the hydrolysis takes place in a single or in multiple steps [27]. Many different systems synthesized by sol-gel technique showed bioactivity when immersed in simulated body fluids. Their composition was chosen in the binary system CaO-SiO2 [28], ternary system SiO2-CaO-P2O5 [29] and quaternary system SiO2-CaO-P2O5-MgO [30]. The bioactivity of sol-gel derived bioactive glass (as represented by the induction time of apatite) and their degradability (as represented by the amount of residual glass) are higher than bioactive glass made by the conventional melting method in both in vitro studies [31] and in vivo tests in animal models [32]. In general, higher amount of SiO2 in glass composition induces larger surface area, but lower degree of porosity.

In orthopedic and dental applications, calcium phosphate based ceramics, glass ceramics and bioactive glasses have been used by virtue of their ability to bond directly with bone tissues and to promote bone formation [33]. This property has become known as bioactivity. Hench L.L. demonstrated this bioactive properties for certain glass composition [34]. Dynamic ion exchange and bonding to bone were demonstrated for a certain compositional range with SiO2, Na2O, CaO and P2O5 in specific proportions [35]. But, the composition has to be optimized to give a suitable compromise between bioactivity and solubility [36]. Since biomaterials contact with living tissues, their surface chemistry needs to be optimized to meet the requirements of host tissues, being desirable that they are bioactive [37], i.e., form an interfacial bond with living tissues. Bioactive implants should react chemically with body fluids in a manner compatible with the repair process of the tissues. First, an amorphous calcium phosphate (ACP) rich layer is formed on the surface of the bioactive materials when implanted. The initial ACP crystallizes to hydroxyapatite (HCA) analogous to that present in bones. The HCA crystals, together with collagen fibers form the bonding layer [1]. In general, the bioactivity mechanisms and growth of the layer at the interface deeply depend on the composition of the glass. The biological activity of bioactive glasses is linked to their capability, in aqueous solution, to leach ions from their surface; a porous silica-gel layer is then formed, which will play the part of support for bone-like apatite crystals growth [34]. The development mechanisms of that calcium phosphate-layer lie in the diffusion of calcium and phosphorus ions from the glass and from the aqueous medium to the material surface. As a result, controlling the surface reactions rates and kinetics is of major importance. One of the controversial
topics in the study of bioactive glasses and glass-ceramics is the influence of the MgO content on the properties of these biomaterials. Preliminary results in the system MgO-CaO-P$_2$O$_5$-SiO$_2$ have shown that an increase of the MgO content in the glass could improve the ability to form the calcium- and phosphorus-rich layer [30]. It is a known fact that CaO and MgO are network modifiers but in some reports the role of Mg has been documented with possibility of their role as network former [14]. Substitutions of CaO by MgO in the composition of the silica glasses would modify their stability and would increase the mechanical properties of the glass.

In previous work, glasses in the system CaO-MgO-P$_2$O$_5$-SiO$_2$ were successfully synthesized by varying CaO/MgO ratio using the sol gel method. The bioactive glass is under powder form. The objective of this paper is to understand surface and physico-chemical reactions at the periphery of bioactive glass particles that lead to bioactivity. The degree of in vitro bioactivity was investigated by studying HA formation on the surface of samples after incubation in simulated body fluid (SBF). All the compositions of these glasses were able to form an apatite-like layer similar to that of biological apatites in vitro. Knowledge of the elemental distribution at the bioactive glass periphery is important to understand the physico-chemical mechanisms during interactions with biological fluids. Physical and chemical evaluation of the bioactive glass surface was performed by DRX, FTIR and ESEM. Thus, our measurements and the discussion are focused on Si, Ca, P and Mg elements which are the most important during physico-chemical reactions at the bioactive material periphery. The second objective of this work is focused on the investigation of surface reactivity of glasses to evaluate the effects of CaO/MgO on the bioactivity and chemical durability.

2. Materials and methods

2.1. Materials
The chemicals used for synthesizing the bioactive glass 80Mg, 70Mg, 60Mg and 50Mg were: tetraethyl orthosilicate (TEOS) (Sigma-Aldrich, 99%), triethyl phosphate (TEP) (Merck Schuchardt, 99%), calcium nitrate tetrahydrate (Lobachemie, 98%) and magnesium nitrate (Lobachemie, 98%), nitric acid (Prolabo, 65% - 69%) and deionized water.

2.2. Preparation of the Bioglasses
The chemical composition of 50Mg, 60Mg, 70Mg and 80Mg bioglasses is presented in Table 1. In order to synthesize these bioglasses via sol-gel method, initially, tetraethoxysilane (TEOS) was added to nitric acid, and then placed on a stirrer for the hydrolysis process to be performed. At this stage, nitric acid was used as the sol environment and TEOS was utilized as a source to supply SiO$_2$. Then, triethyolphosphate (TEP) was added to the system as the P$_2$O$_5$ supply, which was then stirred for 45 min. The process was continued by addition of calcium nitrate tetrahydrate powder, which was previously solved in distilled water as the CaO supply, and again a 45 min period of stirring. The last stage was adding of magnesium nitrate (as MgO supply, previously solved in distilled water) and placing the whole system on a stirrer for 1 h for complete hydrolysis reactions to occur. The molar ratio of (HNO$_3$ + H$_2$O)/TEOS = 6. The obtained sol was put in an oven for 3 days at 25°C, in order to approach to the gel state and become suitable for coating on the surface of steel samples. After the final addition, mixing was continued until the gel was formed. The gel was kept in the oven and heated at 70°C for 3 days to remove the residual water and ethanol. During about 52 h the temperature was raised to 150°C slowly and then the gel was calcined for 3 additional hours at 700°C to stabilize the glass and eliminate residual nitrate.
Table 1. Chemical composition of the bioglasses (mol %)

| Sample | SiO\textsubscript{2} | CaO | P\textsubscript{2}O\textsubscript{5} | MgO |
|--------|----------------|-----|----------------|-----|
| 80Mg   | 80            | 13  | 4              | 3   |
| 70Mg   | 70            | 22  | 4              | 4   |
| 60Mg   | 60            | 30  | 4              | 6   |
| 50Mg   | 50            | 39  | 4              | 7   |

2.3. X-ray diffraction investigations

X-Ray diffraction (XRD) analysis were performed on an X'Pert Pro X-ray diffractometer with a CuK\textsubscript{α} radiation (λ= 1.518 Å). Data were collected from 2\textdegree range [10-60\textdegree].

2.4. Structure analysis of bioglasses using infrared absorption spectroscopy

Infrared absorption spectra of the samples were obtained at room temperature between 400 cm\textsuperscript{-1} and 4000 cm\textsuperscript{-1} with a Fourier transform infrared spectrometer (VERTEX 70 FT-IR), operating with a 4 cm\textsuperscript{-1} resolution.

2.5. In vitro bioactivity tests

In the last years it has been observed that the calcium phosphate-rich layer also forms on the surface of bioactive ceramics when they are soaked in synthetic solutions simulating physiological plasma. For the in vitro study of bioglass, Hench et al. used the so-called Tris-buffered solution (Tris-buffer), i.e., distilled water buffered with tris-hydroxymethylaminomethane [38]. For the in vitro study of the glass ceramic A-W, Kokubo et al. proposed in 1990 the Tris-buffered simulated body fluid (SBF) No. 9 [39] with an ion concentration nearly equal to that of human blood plasma (Table 3). Since, unlike Tris-buffer alone SBF contains calcium and phosphorous ions it can be used to study the in vitro bioactivity of a wider variety of materials. Therefore, the in vitro studies in SBF have become very popular as preliminary tests on new candidates to implant materials. The assessment of in vitro bioactivity was carried out by soaking the bioglass powders in 40 mL of an acellural simulated body fluid, proposed by Kokubo et al. [39], at 37°C in sterile polyethylene containers. The SBF solution has a composition and concentration similar to those of the inorganic part of the human plasma. The fluid was prepared by dissolving reagent-grade NaCl, KCl, K\textsubscript{2}HPO\textsubscript{4}, MgCl\textsubscript{2}, CaCl\textsubscript{2}, and Na\textsubscript{2}SO\textsubscript{4} into distilled water and buffering at pH 7.4 with hydrochloric acid at 37°C (Table 3). The soaking periods were 7, 14 and 21 days. After being soaked, the samples were rinsed with acetone and dried in air at room temperature. The dried sample was investigated with XRD, ESEM and FTIR.

Table 2. Reagents for preparing the SBF

| Order | Reagent       | Amount     |
|-------|---------------|------------|
| 1     | NaCl          | 7.996 g    |
| 2     | NaHCO\textsubscript{3} | 0.350 g  |
| 3     | KCl           | 0.224 g    |
| 4     | K\textsubscript{2}HPO\textsubscript{4}•3H\textsubscript{2}O | 0.228 g |
| 5     | MgCl\textsubscript{2}•6H\textsubscript{2}O | 0.305 g  |
| 6     | 1M-HCl        | 40 mL      |

(About 90 % of total amount of HCl to be added)

| Order | Reagent       | Amount     |
|-------|---------------|------------|
| 7     | CaCl\textsubscript{2} | 0.278 g  |
| 8     | Na\textsubscript{2}SO\textsubscript{4} | 0.071 g  |
| 9     | (CH\textsubscript{3}OH)\textsubscript{3}CNH\textsubscript{2} | 6.057 g |
Table 3. Ion concentrations (mM) of SBF and human blood plasma

| Ion     | Simulated Body Fluid | Blood plasma |
|---------|----------------------|--------------|
| Na⁺     | 142.0                | 142.0        |
| K⁺      | 5.0                  | 5.0          |
| Mg²⁺    | 1.5                  | 1.5          |
| Ca²⁺    | 2.5                  | 2.5          |
| Cl⁻     | 148.8                | 103.0        |
| HCO₃⁻   | 4.2                  | 27.0         |
| HPO₄²⁻  | 1.0                  | 1.0          |
| SO₄²⁻   | 0.5                  | 0.5          |

2.6. Apparent porosity measurements

Arthur method was employed to obtain the porosity \( (P_0) \) of bioactive glass using distilled water and xylen. Porosity \( (P_0) \) of samples was obtained employing the relation (1) as given below:

\[
P_0 = \frac{(m_2 - m_1)}{(m_3 - m_1)} \times \frac{\rho_{\text{water}}}{\rho_{\text{xylen}}}
\]

where \( m_1 \) is the weight of sample in air, \( m_2 \) and \( m_3 \) are the weights after soaking respectively in xylen and water, \( \rho_{\text{water}} \) is the density of water and \( \rho_{\text{xylen}} \) is the density of xylen.

2.7. Contact angle measurements

In order to evaluate the hydrophilicity of the synthetized samples (50Mg, 60Mg, 70Mg and 80Mg), the wettability of pellets prepared was measured using a contact-angle goniometer by the sessile drop method (GBX-France). The pellets were prepared using a compression molding method. The bioactive glass powders were compression molded in a stainless steel mould into compact disks with the dimension of 13 mm in diameter and 2 mm in thickness. The compression molding press was operated at a pressure of 10 MPa. The experiment was conducted using the sessile drop method at room temperature in an air atmosphere and with distilled water and simulated body fluid solution as liquid. A drop (~ 1μL) of liquid is placed on the glass disk, fixed on a prepared plate of substratum, and the image is immediately sent via the camera to the computer for analysis. Water contact angles were measured after a delay of 5s to ensure the equilibration of the droplet. The values of contact angle represent the average of three measurements drops at different sites in one sample and final results were presented by the average of measured drops.

3. Results and discussions

3.1. XRD analysis of bioglass

Figure 1 showed the XRD patterns of the bioglasses powders (50Mg, 60Mg, 70Mg and 80Mg) prepared by sol-gel method and calcined at 700°C. In the XRD patterns of the bioglasses no diffraction maxima were observed (only those attributable to the substrate) indicating that the bioglasses were amorphous. According to Pariente et al. [40], the sample almost takes amorphous state indicative of the internal disorder and glassy nature of these materials.
3.2. Biocompatibility Analysis in SBF

The XRD patterns of the bioglass samples (50Mg, 60Mg, 70Mg and 80Mg) after 1, 7 and 21 days immersion in SBF are presented in Figure 2. The formation of an apatite phase was confirmed by observation of HAp characteristic peaks which developed with the length of immersion time. After immersion in SBF solution, the XRD patterns of (50Mg, 60Mg, 70Mg and 80Mg) showed some differences in crystallization. The obtained results demonstrated the formation of the Ca-P layer on the surface of all glasses after 21 days of immersion. A crystalline peak of hydroxyapatite (HAp) at 31.7° 2θ corresponding to the (211) reflection was observed for 50Mg and 60Mg after 14 days of immersion in SBF. Also, other peaks of apatite appeared at 26° and 46° which corresponds to the (002) and (203) HAp reflections. After 21 days of immersion, the appearance of the reflections (002) and (211) indicated the crystallization of carbonated hydroxyapatite (HCA) formed on the surface of 50Mg and 60Mg. The reflections (211) and (203) confirmed the formation of hydroxyapatite (HAp) partially crystallized on the surface of samples (70Mg and 80Mg). Peak intensities of majority of an apatite phase slightly decrease by decreasing the CaO/MgO as shown in Figure 2, indicating the decrease in crystals growth that may be due to ability of MgO to increase the viscosity of silicate glasses by decreasing the non-bridging oxygen in the glass due to which, rate of diffusion and mobility of ions decreased during crystallization process. Therefore, the crystalline character of an apatite phase increases with increasing the CaO/MgO ratio. This result shows that the crystalline character of HCA in the 50Mg sample has a higher than the other samples. The crystalline character of HAp in 80Mg and 70Mg was decreased due to the lower dissolution rate of the glass. In fact, the decrease of CaO/MgO ratio in the glasses decreases the crystalline character of the newly formed apatite layer. Figure 2 shows the appearance of HCA \([\text{Ca}_{10}(\text{PO}_4)_{6}(\text{CO}_3)_2(\text{OH})_2]\) on the surface of both samples 50Mg and 60Mg after 14 days of immersion whereas in 70Mg and 80Mg, HAp phase appears after 21 days of immersion due to low CaO/MgO ratio indicating that bioactivity of the glass increases with increasing the CaO/MgO ratio. This result shows that the crystalline character of HCA in the 50Mg sample has a higher than the other samples. The crystalline character of HAp in 80Mg and 70Mg was decreased due to the lower dissolution rate of the glass. In fact, the decrease of CaO/MgO ratio in the glasses decreases the crystalline character of the newly formed apatite layer. Figure 2 shows the appearance of HCA \([\text{Ca}_{10}(\text{PO}_4)_{6}(\text{CO}_3)_2(\text{OH})_2]\) on the surface of both samples 50Mg and 60Mg after 14 days of immersion whereas in 70Mg and 80Mg, HAp phase appears after 21 days of immersion due to low CaO/MgO ratio indicating that bioactivity of the glass increases with increasing the CaO/MgO ratio. This result shows that the samples 50Mg and 60Mg are more bioactive than 70Mg and 80Mg. The rate of HAp formation on 70Mg and 80Mg was slower than that of 50Mg and 60Mg. The main effect of the decrease of CaO/MgO ratio in the glass is that it slows down the rate of the calcium phosphate layer formation. The retardation in the formation of the layer on the surface of powders could be attributed to one or both of these effects: (i) the decrease of the solubility of the glass, (ii) the influence of the Mg\(^{2+}\) leached to the solution that, as already reported, decreases the rate of formation of certain calcium phosphates [41, 42]. About the first effect, some authors indicate that the replacement of CaO by MgO in the composition of a glass can increase their toughness, as a consequence of the higher Mg–O bond energy compared with the Ca–O [43]. Regarding the second effect, it has been proven that incorporation of magnesium ion in calcium phosphates reduce the crystallization and markedly delay the transformation of amorphous calcium
phosphates to more stable apatite phases [41, 42]. The low CaO content in the samples 70Mg and 80Mg reduces its dissolution but do not inhibit its bioactivity. Figure 2 showed that the 50Mg glass which has the higher content of CaO/MgO is the most bioactive sample. Therefore, it can be seen that the high content of CaO/MgO in the glass increases the growth rate of a calcium phosphate-rich layer on its surface and implies higher porosity facilitating the apatite nucleation from the very first stages of the essay. This result is in agreement with Perez-Pariente J. et al. [30]. Finally, it can be deduced that the variation in the CaO/MgO ratio has an influence on the bioactivity of the synthesized glasses, on the type of the apatite phase formed on their surface and on the degree of the crystalline character of this phase. The analysis of the synthesized sample (50Mg, 60Mg, 70Mg and 80Mg) by XRD showed that the 50Mg sample is the most bioactive glass whose the carbonated hydroxyapatite layer formed on its surface has the highest crystalline character.

Figure 2. XRD patterns of bioactive glass 50Mg, 60Mg, 70Mg and 80Mg before and after soaking for a period of 7, 14 and 21 days in SBF solution

3.3. FTIR evaluation of bioactivity of the glasses

FTIR has been used to identify the formation of apatite layer over the samples 50Mg, 60Mg, 70Mg and 80Mg (Figure 3). Table 4 shows the functional groups and chemical bonds formed on the surface of glass samples during immersion in SBF solution. The FTIR spectra of all the samples exhibited bands located in the range of 450-480, 725-800 and 1000-1200 cm\(^{-1}\) corresponding to the bending mode of Si–O–Si, vibrational mode of symmetric stretch of Si–O–Si, and vibrational mode of asymmetric stretch of Si–O–Si, respectively. The band centered at 3424 cm\(^{-1}\) is attributed to OH\(^{-}\) absorption while 1641 cm\(^{-1}\) is a weak water absorbed band [44]. The precipitation of Ca–P layer was confirmed by the presence of the main phosphate band at about 600-650 cm\(^{-1}\), which is the characteristic band of phosphate in its crystalline phase [45]. Moreover, the presence of the carbonate band at about 880 and 1500 cm\(^{-1}\) indicated the formation of a newly formed hydroxy-carbonate apatite (HCAp) on the surface of the samples [46]. For 80Mg and 70Mg, the bands in range 720-840 cm\(^{-1}\) are
related to Si–O bridging oxygen bonds (NBO). 1000-1100 cm\(^{-1}\) are associated with Si–O–Si vibrational modes [47]. The spectra of 80Mg and 70Mg showed the presence of a band in range 3200-3424 cm\(^{-1}\) associated to OH\(^{-}\) absorption and revealed the disappearance of the IR band around 1400-1530 cm\(^{-1}\) and 800-890 cm\(^{-1}\) which confirms absence of CO\(_3^{2-}\). This result assured the formation of hydroxyapatite on the sample surface of 70Mg and 80Mg. The bands in range 500-560 cm\(^{-1}\) are associated with P–O bending (crystal) which can be assigned to the presence of hydroxyapatite in crystalline phase. Weak bands related to the Mg–O were observed at 418-420 cm\(^{-1}\), which may be due to the incorporation of a small amount of Mg in the formed hydroxyapatite. For 50Mg and 60Mg, several new peaks emerge at 1400-1530 cm\(^{-1}\) and 800-890 cm\(^{-1}\), which can be attributed to the presence of CO\(_3^{2-}\) [48], and the peak at 560-600 cm\(^{-1}\) is assigned to P–O bend in amorphous calcium phosphate. This suggests the onset of incorporation of CO\(_3^{2-}\) into HA. After soaking for 7 days, the bands appear in the region 1100-900 cm\(^{-1}\) and 860-940 cm\(^{-1}\) suggesting the disruption of the NBO bonds due to leaching of Ca\(^{2+}\) and dissolution of soluble silica at the glass interface during the period of immersion in SBF solution [49]. After 14 days of immersion, the bands between 1120-950 cm\(^{-1}\) increase in number which may be due to increased concentration of Ca\(^{2+}\) on the glass surface as a result of uptake from SBF solution. Additionally, the band becomes broader and develops a second band at 1561-1567 cm\(^{-1}\), while the peak at 547-573 cm\(^{-1}\) splits into two sharp modes at 554-660 cm\(^{-1}\), which are characteristic of apatite crystalline phase [50] indicating that HCA now dominates the apatite phase as suggested earlier. As immersion days reached 21 days, the twin bands at 1427 cm\(^{-1}\) and 1567 cm\(^{-1}\) fuse into one and become more intense showing the precipitation of HCA on the glass surface. This result confirms that obtained by DRX. For all samples, the position and intensity of the reflection bands are changed with time of immersion. The position of bands associated with the phosphate band, PO\(_4^{3-}\) antisymmetric bend, (600-650 cm\(^{-1}\)) and Si–O– Si symmetric stretch (725-800 cm\(^{-1}\)) shifted towards higher values (700-750 cm\(^{-1}\) and 800-850 cm\(^{-1}\), respectively), and their intensity decreased. Also, the presence of lowest CaO/MgO ratio in glass composition decreases the intensity of the phosphate band in its crystalline phases [46]. The FTIR spectra of 70Mg and 80Mg showed that the intensity of the phosphate band decreases with decreasing the CaO/MgO ratio. In fact, inorganic ions like Mg\(^{2+}\) is suppressant for the crystallization of apatite through the reduction of the crystal size of the apatite, and lowest CaO/MgO ratio lead to the formation of amorphous calcium phosphate [51]. This phenomenon promotes the dissolution rate of the apatite precipitates. It is notable that the Mg may be able to enter the forming hydroxyapatite nuclei and thus inhibits their evolution to tiny apatite crystals, because this element can’t be accommodated in the hydroxyapatite structure. Mg\(^{2+}\) substitute into the apatite lattice causes changes in its physicochemical properties. Apatite substituted with Mg results in a Ca deficient apatite and may be amorphous Ca-phosphate (Ca,Mg\(_6\))(PO\(_4\))\(_6\) [52]. In fact, inorganic ions such as Mg\(^{2+}\) suppress the crystallization of apatite and favor the formation of amorphous Ca phosphate. These phenomena can promote a greater dissolution of the apatite precipitates in the studied samples (70Mg and 80Mg). Therefore, with regards to the above explanation, the XRD patterns and FTIR spectra indicate some small amounts of Ca phosphate crystals (Mg-hydroxyapatite) formation with poor crystallinity meaning that the Bragg peaks and FTIR bands are not sharp enough.

In general, the formation mechanism of HCA includes some specific steps which highlight the following phenomena: a hydrated silica layer is formed on the surface of bioactive glass before the precipitation of Ca-P species, and the silanol groups are specific sites for apatite nucleation [53]. A well-detailed sequence of reactions occurring at the surface of bioactive glasses has been previously described in the literature [54]. Indeed, it is possible to identify the effect of the addition of different elements on the surface reactivity of bioactive glasses. The bands located at 470 cm\(^{-1}\) (Si–O–Si bend) and 725-800 cm\(^{-1}\) (Si–O–Si symmetric stretch) are related to the amount of silica present in the newly formed layer. The band at 725-800 cm\(^{-1}\) is specially related to the formation of silica-like layer and its intensity becomes low with the lowest CaO/MgO ratio. The intensity of the band at about 470 cm\(^{-1}\) (Si–O–Si bend) also decreased [46]. In fact, if the formation of silanol groups is inhibited, the formation of the SiO\(_2\)-rich layer will also be inhibited. The crystallization of the Ca–P layer results the formation of carbonated hydroxyapatite (HCA). Here, the appearance of the band data range of 600-650 cm\(^{-1}\) in FTIR spectra, characteristic band of phosphate in the crystalline phase, confirmed the
crystallization of the newly formed apatite layer. The FTIR spectra of 50Mg showed the appearance of this band, which means that the increase in the CaO/MgO ratio promotes the crystallization of Ca–P precipitates. It can be deduced that the variation of the CaO/MgO ratio has an impact on the type of the formed apatite phase and on its crystallization. It is important to point out Bigi et al. ascertained that the decreased the CaO/MgO ratio inhibits the crystallization of precipitated HAp through the reduction of crystal sizes of apatite [55]. According to the study of synthetized samples (50Mg, 60Mg, 70Mg et 80Mg) by XRD and FTIR analyzes, it was found that the presence of a low CaO/MgO in 70Mg and 80Mg leads to the formation of a less stable and poorly crystalline hydroxyapatite, which has the tendency to dissolve again. While the presence of a high CaO/MgO in 50Mg and 60Mg allows to obtain a stable and a well crystallized carbonated hydroxyapatite (HCA).

Figure 3. Fourier transform infrared transmittance spectra of bioactive glasses 50Mg, 60Mg, 70Mg and 80Mg before and after soaking for a period of 7, 14 and 21 days in SBF solution
Table 4. Correlation between wavenumber at which transmittance bands emitted and functional groups in bioactive glasses after immersing in SBF

| Wave number (cm⁻¹) | Published data [56, 57] | Vibration mode |
|--------------------|--------------------------|----------------|
| This work          |                          |                |
| 80Mg               | 70Mg                     | 60Mg           | 50Mg           |
| 7 j                | 419.67                   |                |                |
| 14 j               | 418                      | 418            |                |
| 21 j               | 419                      | 419            |                |
| 14 j               | 1421.64                  | 1417.77        | 1567.51        |
| 21 j               | 1416.48                  | 1412.64        |                |
| 14 j               | 1054.86                  | 1036.06        | 1003.44        |
| 21 j               | 1028.20                  | 1015.70        | 1019.77        |
| 7 j                | 1045.86                  | 1042.90        | 1018.55        |
| 14 j               | 1037.07                  | 1015.67        |                |
| 21 j               | 1028.20                  | 1015.70        |                |
| 7 j                | 875.64                   | 850            |                |
| 14 j               | 871.02                   | 872.73         |                |
| 21 j               | 794.33                   | 792.16         | 791.14         |
| 14 j               | 789.50                   | 786.30         | 790.45         |
| 21 j               | 785.25                   | 782.50         | 785.80         |
| 7 j                | 561.33                   | 555.50         |                |
| 14 j               | 557.90                   | 547.12         |                |
| 21 j               | 556.83                   | 548.62         |                |
| 7 j                | 441.17                   | 439.73         |                |
| 14 j               | 438.24                   | 437.91         |                |
| 21 j               | 446.61                   | 448.65         |                |
3.4. pH changes during immersion in SBF
The variations in pH values are measured to understand the dissolution behavior of synthetized bioglass (50Mg, 60Mg, 70Mg and 80Mg) in the in vitro environment. Changes in pH of the SBF solution after immersing the bioactive glass for 7, 14 and 21 days are shown in Figure 4. It is observed that pH of SBF increases to a large extent (7.4 to 8.36) in case of 50Mg and (7.4 to 8.29) in case of 60Mg due to greater concentration of Ca$^{2+}$ ions in this both samples and its large exchange with H$^{+}$ or H$_3$O$^+$ ions in SBF [59] that results in the formation of HCA after 14 days of immersion. Whereas in case of 70Mg and 80Mg, pH of SBF increases but not so quickly that may be due to less amount of CaO/MgO ratio in these samples because pH drop depends on the amount of CaO/MgO. The lowest the amount of CaO/MgO ratio, the lower the pH drop [42] that may be due to ability of MgO to increase the viscosity of silicate glasses by decreasing the non-bridging oxygen in the glass due to which, rate of diffusion and mobility of ions decreased during crystallization process. During the first seven days of immersion, the pH increases more gradually for all cases, because part of the released calcium is used to form CaO-P$_2$O$_5$ rich film, decreasing the Ca release kinetics. A smaller decrease in pH was observed and then increased until the end of the test. According to several studies [60, 61], this increase in pH is associated with a rapid ionic exchange between Mg$^{2+}$ and Ca$^{2+}$ ions from the bioglass and H$^+$ from the SBF. This ionic exchange leads to the formation of silanol groups (Si-OH) on the surface through the reaction shown in Figure 6a, inducing apatite nucleation. The pH variation of the bioactive glass is in agreement with Siqueira et al. [47]. The Ca$^{2+}$ released to SBF and the increase in pH are in agreement with the mechanism proposed by Kokubo for HCA formation on the surfaces of bioactive glasses [62].

![Figure 4. pH of SBF solution that was taken before and after soaking of bioactive glasses for a period of 7, 14 and 21 days](image)

3.5. Changes in SBF composition
Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) was used to measure the concentrations of Ca, P, Si and Mg ions in SBF to study the dissolution behavior of samples in more detail. Figure 5 shows the cumulative variation of Si, Ca, P and Mg ionic concentrations with the soaking time in SBF for samples 50Mg, 60Mg, 70Mg and 80Mg. It is mentionable that the Ca$^{2+}$ concentration is controlled by both the release of Ca$^{2+}$ from the substrate and the formation of phosphate or apatite. As can be observed in all cases, Ca$^{2+}$ concentration in the solution increased during the first 14 days of soaking and then decreased continuously till the last day of immersion (21 days) (Figure 5). The decrease in the Ca$^{2+}$ concentration in SBF is attributed to the rapid growth of the apatite nuclei formed on surface of the glass that overcame the release rate of Ca ions to the solution [28]. As is observed, the lower the Ca content is in the glass composition, the lower the increase of Ca$^{2+}$ concentration in the SBF. However, variation of P concentration in SBF was different for each composition of glass. For 80Mg, P concentration reached a maximum after 7 days of soaking in SBF and decreased then continuously until the end of the test (21 days). While for (70Mg, 60Mg and
50Mg), P concentrations decreased gradually up to 21 days. Figure 5 showed that the silicon concentration released in SBF has increased rapidly during the first 7 days of immersion and then decreased during the 14 days of immersion and after it increased gradually until the end of immersion (21 days). It was observed that the concentration of SiO$_4^{\text{4-}}$ ions for 70Mg and 80Mg was slightly increased compared to the other samples 50Mg and 60 Mg. This result confirms that the samples 70Mg and 80Mg were less bioactive and have higher specific surface areas. The increase in the concentration of SiO$_4^{\text{4-}}$ can be directly associated to the dissolution of the bioglass. The bioglass degradation began immediately after its first contact with the medium through the reaction between water and the SiO$_4$ tetrahedral units from the silicate chains. The hydration-induced disruption of these silicate chains causes the release of soluble isolated SiO$_4^{\text{4-}}$ or even small chains to the medium. The increase in pH is also known to affect its dissolution by promoting several reactions between Si-OH and OH $\to$ [61]. As can be observed in all cases, Mg concentration in the solution increased during the time of immersion. It is found that concentration of Ca and Mg ions increases in SBF in first 14days (Figure 5) that is attributed to exchange of Ca or Mg ions from glass with H$^+$ or H$_2$O$^+$ ions from SBF and as a result, silanol group (Si-OH) is formed on the glass surface due to reaction of Si with OH ions that act as nucleation agent for an apatite phase formation. Continuous decrease in concentration of Ca and P ions into the SBF may be attributed to transfer of these ions from SBF to the immersed sample showing the formation of HCA layer on 50Mg and 60Mg as conformed by XRD and FTIR. Concentration of Si greatly increases in SBF showing the high dissolution rate of part of Si from Si-OH group that helps in formation of calcium phosphate layer. In cases of 70Mg and 80Mg, Ca and P ion concentration increases in SBF and after that it starts decreasing gradually showing the formation of HAp. This delay in HAp formation may be attributed to the less ratio of CaO/MgO in 70Mg and 80Mg. Concentration of Si increases in 70Mg and 80Mg but not so quickly due to less CaO/MgO ratio, which makes the rate of exchange of ions between this samples and solution is very slow and therefore reduces the bone bonding mechanism (Figure 5).

These variations reflect the formation and the growth of an apatite layer at the bioactive glass periphery. Growth of the amorphous Ca-P rich film is by incorporation of soluble calcium phosphates. The calcium ions might increase the degree of supersaturation of the surrounding body fluid and cause precipitation. The precipitation conditions favor the formation of bone apatite like crystals. However, apatite nucleation can be triggered with the presence of the silica gel like layer. Clark A.E. and Hench L.L. [63] proposed that layer formed by condensation of silanol bonds is responsible for the nucleation of apatite. Apatite formation on the surface of materials is a material-dependent and mainly chemical phenomenon. The bioactive surface of the bioactive glass implant serves as a template for amorphous apatite precipitation from surrounding fluids. Formation of this apatite layer represents bioactivity properties of bioactive glasses. In vivo, this layer will permits the creation of an interfacial bonding zone between bone tissues and the implanted materials [64].

One of the accepted mechanisms that explain the precipitation of apatites onto bioglasses is the rapid entrapment of Ca$_{2^+}$ by the hydrated silica-rich layer. As previously described, the pH increases after the initial release of Ca$_{2^+}$ ions, leading to the development of a negative charge on the surface. This negative charge is able to easily attract Ca$_{2^+}$ to the surface, again changing the surface charge and generating the conditions necessary to entrap PO$_{4}^{3-}$. Therefore, the precipitation of a calcium phosphate layer starts at the same time as the increase in pH. Indeed, the Ca$_{2^+}$ concentration in the medium quickly decreased by the end of the 14 days in our experiments (Figure 5). In a similar way, for samples 50Mg and 60Mg, the Ca$_{2^+}$ and PO$_{4}^{3-}$ concentration also decreased and the concentration of Mg$_{2^+}$ are sharply increased, corroborating the formation of carbonated hydroxyapatite (HCA) as suggested by the DRX and FTIR analyses (Figures 1 and 2 respectively). While for samples 70Mg and 80Mg, the concentration of Ca$_{2^+}$ and PO$_{4}^{3-}$ has decreased and it was observed a small increase in Mg$_{2^+}$, which may be due to the incorporation of a small amount of Mg in the formed hydroxyapatite. For 50Mg and 60Mg, the Ca$_{2^+}$ and PO$_{4}^{3-}$ precipitations exhibit distinct dynamics: while the Ca$_{2^+}$ is abruptly precipitated on the 14 days, PO$_{4}^{3-}$ are gradually precipitated throughout the immersion period. According to Harding et al. [65], the PO$_{4}^{3-}$ groups will predominate on the apatite surface during the precipitation process. This has a tendency to stabilize the surface charge during the crystal growth, as
PO₄³⁻ is adsorbed by the Ca²⁺ sites. Therefore, after the rapid capture of Ca²⁺ ions by the reactive silica gel layer, the PO₄³⁻ ions are adsorbed, stabilizing the surface charge.

Figure 5. Si, Ca, P and Mg concentrations in SBF solution that was taken before and after soaking of bioactive glasses for a period of 7, 14 and 21 days

3.6. Porosity measurements

Porous bioactive glasses are potentially useful materials for orthopedic applications. Their porosity is an important characteristic as it allows vascular penetration and subsequent neo-bone growth [66]. A high surface-to-volume ratio may make these materials biodegradable [67] and bioactive, which allows deposition of an apatite-like layer when in contact with body fluid. Porosity of scaffolds is also important. Generally, large pores (tens to hundreds of micrometers) are needed for cell and tissue in growth, micron-sized pores may contribute to cell adhesion, and very small pores (nm) may play a role in protein binding [68]. Application of the sol-gel method allowed us to obtain glasses with four different compositions in the SiO₂–CaO–P₂O₅–MgO system. It was observed that, using the same synthesis method, the porosity in the system SiO₂–CaO–P₂O₅–MgO increased in function of the CaO content [30]. An increase in the CaO content of the glasses also caused an increase in their porosity (Figure 6). Higher porosity facilitated the apatite nucleation on the sample surface during the first 14 days of the in vitro test for 50Mg and 60Mg. This result is in agreement with Pérez-Pariente et al. [30]. The high Ca²⁺ content led to high porosity in our glass, probably as a consequence of Ca(NO₃)₂ decomposition during stabilization treatment. The massive Ca²⁺ release led to an additional increase in porosity. Generally speaking, as the SiO₂ content of the glasses increased, the pores mean size and porosity decreased (Figure 6); on the contrary, the surface area increased. The ability of a substrate to induce the growth of a biologically active apatite-like layer does not depend solely on its composition, but also on the intrinsic parameters of the surface where such growth will take place. In our case, a complex relationship between composition and texture of the samples and their bioactivity was be expected, because those glasses which release more calcium to the solution (giving rise to higher supersaturation of the medium) are at the same time the glasses with lowest surface area for nucleation and growth of the apatite phase. On the other hand, a higher porosity will also have a beneficial effect.
on apatite formation, since it facilitates the transportation of ions from the substrate to the growing apatite crystals through the liquid phase.

![Figure 6](image)

Figure 6. MgO concentrations as a function of porosity of the bioactive glass 50Mg, 60Mg, 70Mg and 80Mg.

3.7. Water and SBF contact angle

Because wettability of biomaterials is of interest in biomedical applications involving cell-biomaterial interactions [69], the wettability of the surfaces of different synthetized bioglasses (50Mg, 60Mg, 70Mg and 80Mg) were measured. Relatively more wettable surfaces with lower contact angle (θ) are termed hydrophilic surfaces, while less wettable surfaces with higher θ are hydrophobic surfaces. Although these are relative terms, the criterion of θ=65° to differentiate between the two regimes has been suggested [70]. As shown in Figure 7, contact angle values for all synthetized bioglasses were <65°, being considered, thus, hydrophilic materials. The surfaces of the obtained bioglasses (50Mg, 60Mg, 70Mg, and 80Mg) were evaluated by contact angle measurements. The values obtained revealed an increase of hydrophilicity of the bioglass with increased the rate of CaO/MgO. The results showed that the bioglass 50Mg is the most hydrophilic sample for the two used fluids (water and SBF) because it has the lowest value of the contact angle. This sample has the highest porosity. For this purpose, bioglass 50Mg would have a rougher surface than other samples (60Mg, 70Mg and 80Mg), which may lead to a decrease in water contact angle [71]. Figure 7 showed that the contact angles of the synthesized samples 50Mg, 60Mg, 70Mg and 80Mg measured with water were significantly higher than those measured with the SBF, which might be explained by the fact that the SBF is an aqueous solution composed of several constituent, thus undergoes various mechanisms of adsorption on the surface of the synthesized materials. It has been recognized that hydrophilic surfaces are generally favorable for short-term cell adhesion and proliferation for osteoblastic cells and that hydrophilic surfaces not only support homogeneous spatial human foetal osteoblast (hFOB) cells growth and mineral deposition but also increase the quantity and quality of hFOB cell mineralization, relative to hydrophobic surfaces [72]. For this reason, the increase in hydrophilicity obtained by inorganic particle addition to the polymer matrices investigated here will potentially lead to a better osteoblastic adhesion, proliferation and spreading, which is relevant for the intended application of the composites in bone regeneration.
3.8. Morphology
Recently, supramolecular chemistry has allowed the emergence of a new generation of advanced mesoporous biomaterials with high surface area, high porosity, and uniform pore channels, which show enhanced bioactivity and have potential for use in the fabrication of porous scaffolds for bone regeneration[73]. In this study, four different bioglasses were fabricated by using the sol-gel method. Previous studies have shown that increasing the surface area of the biomaterials might greatly accelerate the kinetic process of apatite deposition and, therefore, enhance bone-forming bioactivity [74]. Some researchers show the excellent properties of glasses having Mg [75]. Deficiency in Mg has been reported to have injurious effects on skeletal metabolism [18], causing cessation of bone growth, lesser bone quality, strength and thickness and increase bone fragility [16]. It is worth mentioning that replacement of CaO by MgO in the composition of SiO$_2$ glasses would alter their stability and enhance the mechanical properties of the glass [14]. Mechanical properties are found to be dependent on microstructure, grain size and crystalline phase [76].

MEB micrographs of synthesized and thermally treated samples at 700°C that could be used to study the microstructure, size, morphology and homogeneity of samples are shown in Figure 8 and Figure 9 for samples 50Mg, 60Mg, 70Mg and 80Mg respectively. As pictured, the prepared and thermally treated samples (80Mg, 70Mg, 60Mg and 50Mg) have different morphologies, depending on the chemical compositions of the gels. It is obvious from these figures that the particle size ranges in micro size. It can be related to method from which the bioglass has been synthesized. It can also be concluded that morphology of samples changed completely with the rate doped MgO. The surface of 50Mg and 60Mg is very rougher than the surface of 80Mg and 70Mg samples. These observations are in good agreement with other ceramic materials in the system SiO$_2$-CaO-P$_2$O$_5$-MgO [77, 78].

The synthetized bioglass (80Mg, 70Mg, 60Mg and 50Mg) exhibited a macroporous structure with open interconnected pores. The pores appeared almost irregular ranging from tens to hundreds of micrometers (Figure 8 and Figure 9). The bioglass 50Mg is more porous, High-magnification SEM images further revealed that a number of small pores were distributed across the macropore walls. The porosity of 50Mg bioglass prepared by sol gel method was around 45%. The variation of the rate CaO/MgO shows an ability to enhance the rheological property of bioglass. It was found that the surface of bioglass became rougher with the increased rate CaO/MgO. It is probably caused by the fact of agglomeration during drying. However, the obtained rough surface is believed to be suitable for cell adhesion and proliferation.

Figure 7. Contact angle measurements for the different fluids for the synthetized glasses
Figure 8. ESEM images of the bioglasses 70Mg and 80Mg prepared by the sol-gel method

Figure 9. ESEM images of the bioglasses 50Mg and 60Mg prepared by the sol-gel method
Conclusion
Bioglass powders in the system SiO$_2$-CaO-P$_2$O$_5$-MgO, with different MgO content have been successfully synthesized by the sol-gel technique and calcination at 700°C. The in vitro study showed that powders could induce apatite phase formation on their surface after soaking in SBF. The variation in the CaO/MgO ratio has an influence on the chemical durability and bioactivity. According to the study of synthesized samples (50Mg, 60Mg, 70Mg et 80Mg) by XRD and FTIR analyzes, it was found that a low CaO/MgO in 80Mg and 70Mg leads to the formation of a less stable and poorly crystalline hydroxyapatite (HAp), which has the tendency to dissolve again. While a high CaO/MgO in 50Mg and 60Mg allows to obtain a stable and a well crystallized carbonated hydroxyapatite (HCA). Also, it was observed that the 50Mg glass which has the higher content of CaO/MgO is the most bioactive sample. A higher CaO content in 50Mg implies higher porosity facilitating the apatite nucleation on its surface from the very first stages of the essay, whereas a higher SiO$_2$ content increases the surface area of samples. The morphology of samples, depending on the chemical compositions of the gels, changed completely with the CaO/MgO ratio. The results showed that the 50Mg glass which has the higher content of CaO/MgO is hydrophilic sample for the two used fluids (water and SBF). The porosity and hydrophilicity increase with increased the rate of CaO/MgO. The variation of the CaO/MgO shows an ability to enhance the rheological property of bioglass. It was found that the surface of bioglass became rougher with the increased CaO/MgO ratio. The obtained rough surface is believed to be suitable for cell adhesion and proliferation.

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