

SOL GEL MODIFIED DERIVED CaO-MgO-SiO₂ CERAMIC GLASS SYSTEM: PREPARATION AND IN VITRO CHARACTERIZATION

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Keywords: synthesis, sol-gel, *bioactive glass-ceramic*, *hydroxyapatite*, *cytotoxicity*

1 General Introduction

Bioactive glasses and glass-ceramics have been extensively studied in the biomedical field due to their interaction with the physiological environment when implanted. This interaction stimulates a response from the body, bonding to host tissue. Since 1969, when Hench introduced the first bio-glass (Na₂O-CaO-SiO₂-P₂O₅), many other variations on bioactive glasses and glass-ceramic materials [1], such as synthetic hydroxyapatite (HA) and other calcium phosphates have been synthesized and studied. Glass-ceramics and bioglasses of CaO-MgO-SiO₂ system have showed high capacity of bio-reparation and bioactivity on bone tissue surface by HA formation [2]. Some of ternary CaO-MgO-SiO₂ system ceramics such as akermanite (Ca₂MgSi₂O₇) [3-5], diopside (Ca₂MgSi₂O₆) [6-8], wollastonite (CaSiO₃) [9, 10], and tricalcium silicate (Ca₃SiO₅) [11] have in addition to good mechanical properties [12, 13-17] good bioactivity and cytocompatibility [18]. Although the mechanism of apatite formation has not been completely clarified, studies showed that bioglass and glass-ceramics containing Ca and Si, might induce apatite formation in simulated body fluid (SBF) [19, 20]. Some studies reported that it is essential the creation and nucleation site for HA formation, the simultaneous ionic dissolution of silicates and subsequent formation of Si-OH groups [21-23]. Products containing Ca and Si at specific concentration ranging from bioactive ceramics of CaO-MgO-SiO₂ system follow this mechanism [24]. Chemical composition, surface topography, texture and structure are supposed to play an important role in the surface interaction of glasses and glass-ceramics with the medium from which in vitro apatite formation occurs [25].

The mechanism and factors that governing the properties of several types of bioactive glasses and glass-ceramics are object of several studies. Bioactive ceramics of CaO-MgO-SiO₂ system have been studied because of their mechanical properties and degradation rate can be controlled [26].

Various techniques for bio-glass and glass-ceramic powder preparation, including solid-state reaction [27, 28], spray pyrolysis [29], coprecipitation [30], and sol-gel [31- 33] processes have been reported.

Sol-gel technique involves the synthesis of a solution sol, usually from metal-organic and metal salt precursors followed by formation of a gel, and thermal treatment consisting of drying, organic removal, and sometimes crystallization [34]. Sol-gel processing typically provides higher purity and homogeneous materials at lower treating temperatures [35]. Consequently it has been intensely used for preparing ceramics and glasses for several applications including the field of bioceramics. An investigation of bioactive glass powders by sol-gel processing revealed powders produced by this technique are more bioactive than the melt-derived glasses of the same composition [36]. The high bioactivity of the sol-gel derived materials is related to the textural features of the gels, i.e., pore size and pore volume associated with the large surface area, higher rate of dissolution, and the negative surface charge [37].

Common precursors for silica-based sol-gel glasses include alkoxides such as tetraethylorthosilicate (TEOS), Si(OC₂H₅)₄, for SiO₂ and generally inorganic salts to generate the metal component [38, 39, 40, 41]. Few references use organometallic precursor in sol gel processing synthesis [30, 41].

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In this work, a modified sol-gel process is proposed to synthesize CaO-MgO-SiO₂ bioactive glass-ceramic powder. Non-aggregated SiO₂ aerogel particles were initially prepared by surfactant template sol-gel technique, under HCl acid-catalyzed hydrolysis from Na₂SiO₃, as described previously [42]. Ca and Mg ions HNO₃ dissolved were embedded on the silica particles. By thermal treatment of this material; a glass-ceramic powder was obtained that was characterized by SEM (Scanning electron microscopy) and BET (Brunauer-Emmett-Teller) technique. This powder was compacted and sintered at 1320 °C for obtaining glass-ceramic body. Wollastonite (CaSiO₃), akermanite (Ca₂MgSi₂O₇) and dicalcium silicate (Ca₂SiO₄) crystalline phases were detected by XRD (X-ray diffraction) analysis. This glass-ceramic body submitted to cytotoxicity test, with CHO (Chinese hamster ovary) cells, showed it has no toxic effect. Immersion studies in simulated body fluid (SBF) for different time intervals were carried out. SEM and FTIR (Fourier Transform Infrared) investigations were conducted before and after soaking the material in SBF. After 7 days soaking, deposited crystalline particles of apatite was detected.

2 Experimental Procedures

CaO-MgO-SiO₂ glass ceramic composed of Mol % 43.30 CaO, 10.72 MgO and 45.98 SiO₂ was prepared by a modified sol-gel process. Na₂SiO₃ (20.40 g. L⁻¹), calcium oxide and magnesium oxide dissolved in HNO₃ were used as starting material. In the first step, spherical aerogel silica particles were prepared from Na₂SiO₃ solution, in presence of a surfactant, as described in a previous work [42]. The hydrolysis and condensation reaction for obtaining aerogel silica was performed with HCl. In a typical preparation, 3 g of surfactant was dissolved in 40 mL of ethanol and 40mL H₂O, and then 45 mL of 8.5M HCl was added to the surfactant dissolved solution, resulting in a clear homogeneous mixture. Na₂SiO₃ solution of silica source (60 mL) was added at drop wise to the above mixture with constant stirring at room temperature. After complete addition, the mixture was kept at room temperature. After 4h

a white turbid suspension was obtained. The resulting product was recovered by filtration followed by washing with distilled water; up to no Cl⁻ was detected by AgNO₃ test. Stoichiometric amount of 6M HNO₃ solutions of CaO and MgO (to give CaO-MgO-SiO₂ glass ceramic desired composition) were embedded in silica gel particles. The resulted mixture was ultrasonicated for 60 minutes to disperse the silica particles and to assist the embedment. Then it was dried in a hot plate at 150 °C until the obtaining of a yellow powder which was calcined at 370 °C for 1h and 600 °C for 2h. The resulted white powder was characterized by SEM and specific surface area measurements (BET method). The powder was compacted into discs (10mm diameter and ~ 4mm thickness) and thermal treated at 1320 °C for 3h for obtaining of sintered glass-ceramic body that was identified by XRD. In vitro tests were performed by soaking sintered samples in SBF at 37.0 °C for 7,14 and 21 days. The ratio of the sample surface area to solution volume of SBF was 0.1 cm². mL⁻¹. The SBF solution was prepared according to the procedure described by Kokubo [43]. SBF was prepared by dissolving analytical-grade NaCl, NaHCO₃, KCl, K₂HPO₄.2H₂O, MgCl₂.6H₂O, CaCl₂.2H₂O and Na₂SO₄ in distilled water and was buffered with TRIS (trishydroxumethylaminomethane) and 1M HCl to pH =7.25. The composition of prepared SBF solution and human blood plasma is given in Table 1.

Table 1- Ion concentrations of the SBF (simulated body fluid) and human blood plasma

Ion	Concentration (m Mol.dm ⁻³)	
	(SBF)	Human blood plasma
Na ⁺	142.0	142.0
K ⁺	5.0	5.0
Mg ²⁺	1.5	1.5
Ca ²⁺	2.5	2.5
Cl ⁻	147.8	103.0
HCO ₃ ⁻	4.2	27.0
HPO ₄ ²⁻	1.0	1.0
SO ₄ ²⁻	0.5	0.5

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After the respective soaking time, the ceramic body samples were taken out, washed with Milli- Q water, and dried at room temperature for 24h and they were characterized by FTIR. In vitro cytotoxicity was performed with Chinese hamster ovary cell line (CHO-k1) by the indirect method as recommended by ISO 10993 parts 5 (ISO 2009) and previously described [44]. The cells were maintained in RPMI 1640 medium supplemented with antibiotics-antimicotic solution (100 units.mL⁻¹ penicillin, 100 µg.mL⁻¹ streptomycin and 0.025µg.mL⁻¹ amphotericin, 2mM glutamine, and 10% fetal bovine serum), at 37° C in a humidified 5% CO₂ atmosphere. For sub culturing and for experiments, cells were harvested using 0.05% trypsin and 0.02% EDTA at pH 7.4 phosphate-buffered solution. The sintered ceramic body samples were sterilized by gamma radiation at 25kGy, and immersed in RPMI 1640 medium at 37° C for 48 hours under gently shake for extract preparation, at final concentration of 1cm².mL⁻¹. The extract was filtered with pore membrane 0.45µm and used pure and diluted until 6.26% with RPMI 1640 medium. Cytotoxicity test was performed in 96 well microplates seeded with 3000 cells per well and extracts dilutions from 100 to 6.25%. The microplates were incubated for 72 hours at 37°C in a humidified 5% CO₂ atmosphere. The cell viability was measured by adding 20µL of MTS/PMS (20:1) solution and incubated for 2 hours at 37°C in the humidified 5% CO₂ incubator. The micro plates were read in a spectrophotometer reader at 495 nm. The test was compared with a negative control of alumina extract and a positive control of phenol 0.3% in saline 0.9% solution. The cell viability was determined by the relation:

$$CV(\%) = \left(\frac{OD_{sample}}{OD_{control}} \right) \times 100$$

Where: *CV* = cell viability (%); *OD_{sample}* = optical density of the sample and dilutions; *OD_{control}* = optical density of the cells control in the test.

The experimental procedure flow sheet is showed in Fig. 1.

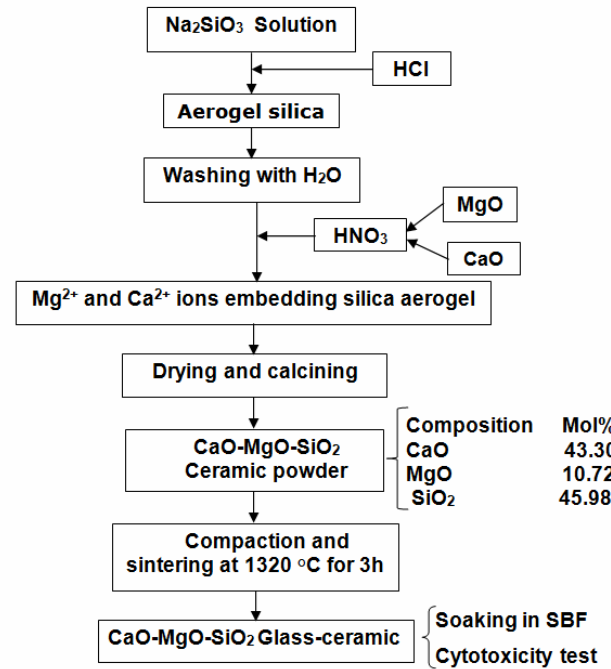


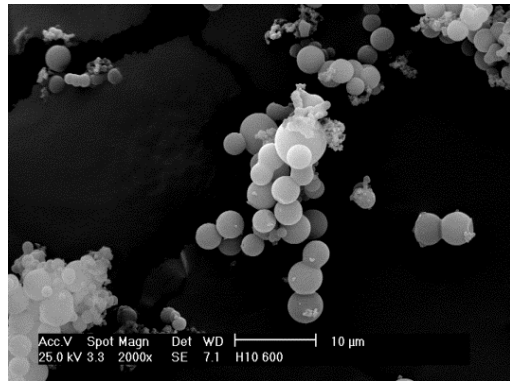
Fig. 1- Experimental procedure flowsheet of CaO-MgO-SiO₂ glass ceramic preparation

3 Results and Discussion

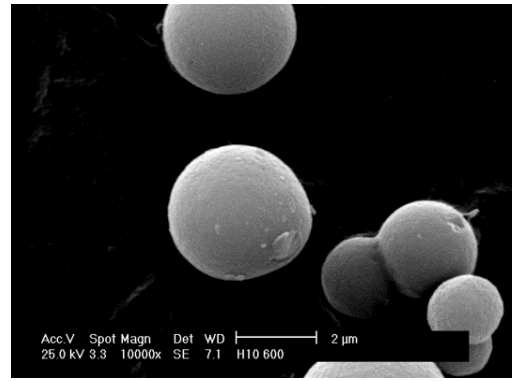
In Fig. 2, typical SEM images of prepared aerogel silica (calcined at 600 °C) before (Fig. 2 (a)) and after embedding Ca and Mg silica calcined at 600 °C (Fig. 2(b)) are showed.

Fig.2 (a) shows silica particles with spherical shape. It can be verified that the particle sizes are below 10µm. In Fig. 2(b) it can be noticed some precipitates on the surface of the spherical particles. It is best observed by comparing Fig.3(a) with Fig.3(b) where high magnification images are seen. Those precipitates are constituted of Ca and Mg oxides that were obtained after thermal treatment at 600°C of the embedded Ca and Mg spherical silica particles. From Fig.3(b) it observes that the precipitates have no regular shape.

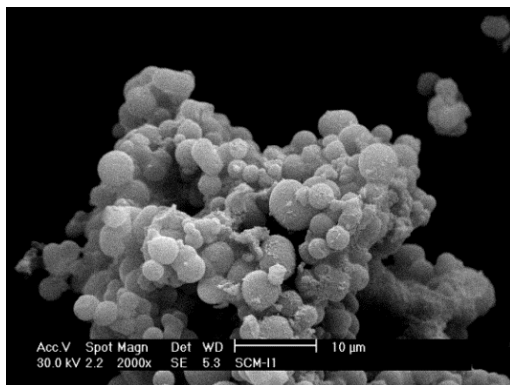
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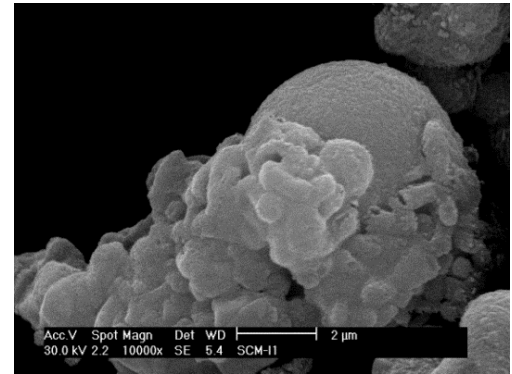
(a)



(a)



(b)



(b)

Fig. 2- SEM images of aerogel silica (calcined at 600 °C) before (a) and after (b) embedding Ca and Mg silica, calcined at 600 °C

Fig.3- SEM high magnification images of aerogel silica (calcined at 600 °C) before (a) and after (b) embedding Ca and Mg silica, calcined at 600 °C.

Results obtained by BET technique revealed that the high specific surface area of 675.26 m².g⁻¹ (typical aerogel (from 500 to 1,200 m².g⁻¹)) of the obtained silica aerogel (before embedding Ca and Mg), has decreased to 391.55 m².g⁻¹ for the Ca and Mg embedded silica. This occurred because of the porosity (the main reason for the high specific surface area of silica aerogel) and the outside surface of initial silica aerogel were filled by embedding Ca and Mg (CaO and MgO after calcination), as consequence a decreasing of the specific surface area is observed.

In Fig. 4, XRD patterns peaks of Wollastonite, akermanite and dicalcium silicate were detected in the compacted powder sample sintered at 1320 °C for 3h.

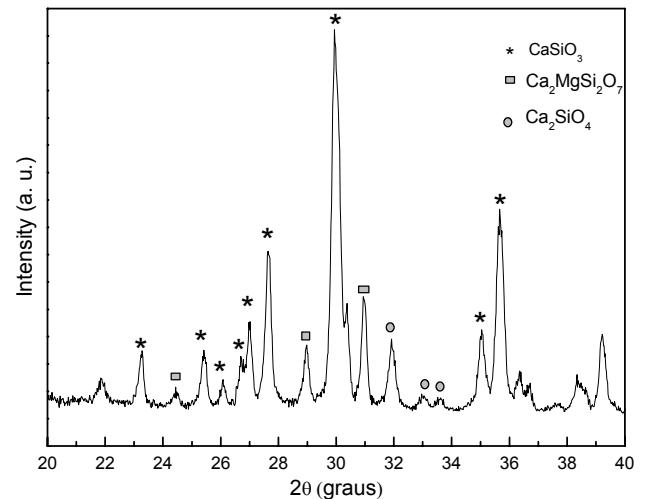


Fig. 4- XRD pattern of the CaO-MgO-SiO₂ glass-ceramic sintered at 1320 °C for 3h.

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FTIR spectra of the sintered samples soaked in SBF for 7, 14 and 24 days are showed in Fig. 5.

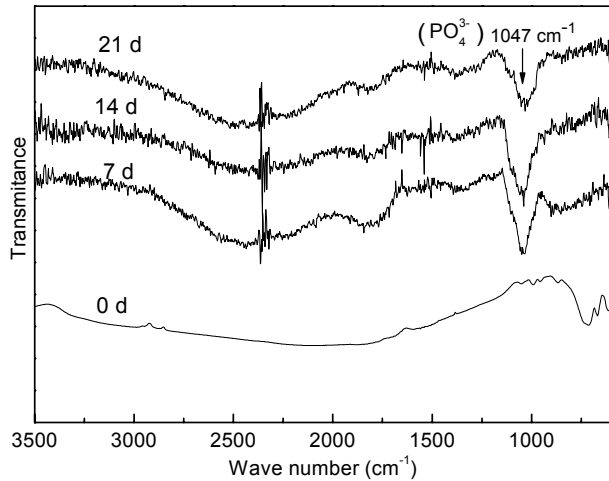
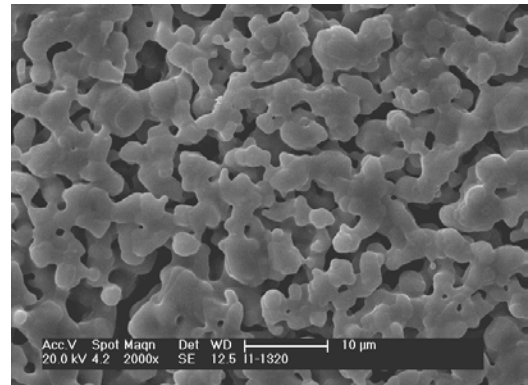


Fig.5- FTIR spectra of sintered material surface before and after 7, 14 and 21 days soaking in SBF, 0d, 7d, 14d and 21d.

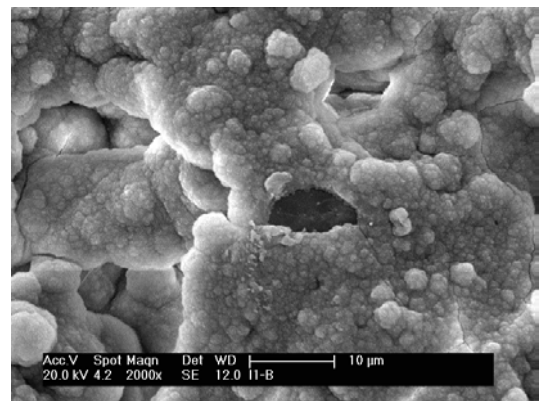
Phosphate absorption band at about 1047 cm⁻¹ is observed on spectra of the samples soaked for 7, 14 and 21 days, which indicates the formation of apatite layer on the surface of the immersed samples [45]. On the other hand this absorption band increases from the sample of 7days to the sample of 14 days. But it virtually decreases for the sample of 21 days. This might indicates that apatite layer was formed and settled on the surface of the samples of 7 and 14 days respectively. For the sample of 21 days, the apatite layer formed on its surface, perhaps, was being dissolved over the time.

Fig.6 shows SEM micrographs of the glass-ceramic before (a) and after 14 days (b) soaking in SBF. In Fig. 6(a) a continuously connected crystalline grains with open pores microstructure of sintered body is observed. In Fig. 5(b), particles with nano microspheres morphology [46] uniformly deposited on the surface are seen.

As confirmed in Fig. 7, the prepared glass-ceramic did not show cytotoxicity. According to ISO 10993-5, when the cell viability of the non-diluted extract in the indirect method is higher than 70%, the sample can be considered non cytotoxic. The cell viability of the non-diluted extract was 97.5%, by reason of this, the samples were considered non cytotoxic.



(a)



(b)

Fig.6- SEM micrographs of sintered CaO-MgO-SiO₂ glass-ceramic body surface: before (a) and after (b) 14 days soaking in SBF.

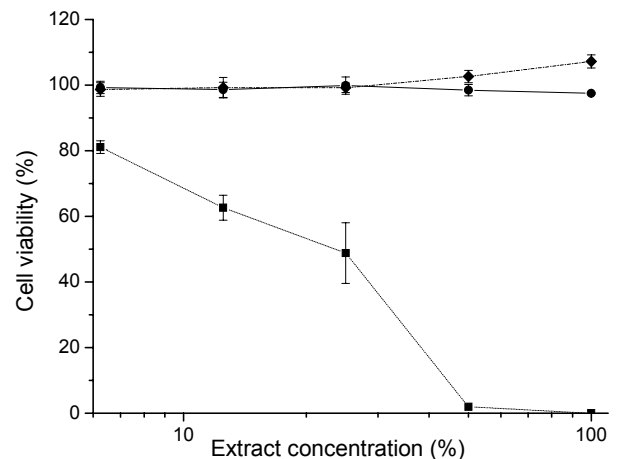


Fig.7 - Cytotoxicity test with CHO: Cell viability as a function of extracts dilutions from 100 to 6.25% - (●) CaO-MgO-SiO₂ glass ceramic, (◆) alumina extract, (■) positive control of phenol 0.3% in saline 0.9% solution.

4 Conclusions

A new modified sol gel method to preparing CaO-MgO-SiO₂ ceramic powder was presented. Use of low-cost Na₂SiO₃ is attractive to substitute the usual high-cost TEOS as Si source. Glass-ceramic body obtained from prepared powder and sintered at 1320 °C presented Wollastonite (CaSiO₃), akermanite (Ca₂MgSi₂O₇) and dicalcium silicate (Ca₂SiO₄) crystalline phases. According to ISO 10993-5, the prepared material can be considered non cytotoxic. The bioactivity evaluation of the ceramic sintered body revealed after 7 days soaking of the glass-ceramic body in SBF, apatite particles were formed on the surface, confirmed by FTIR spectra.

Acknowledgements

Authors thank to FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo), for financial support provided for this research work.

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