Bioactivity of a $CaO-SiO_2$ Binary Glasses System

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Five glasses in the $CaO-SiO_2$ binary system with different silica content (50−90% in mol) have been prepared by the sol−gel method. The referred glasses have been characterized by thermogravimetric and differential thermal analysis (TG/DTA), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), and energy dispersive spectroscopy (EDS) showing clear differences in composition and specific surface and porosity between those glasses with low SiO_2 content (50−70% in mol) and those with high SiO_2 content (80−90% in mol). The in vitro bioactivity study of all glasses prepared were carried out by soaking in a simulated body fluid (SBF) at 37 °C. The FTIR, XRD, SEM, and EDS analysis of the surface of these glasses after the in vitro assays reveal the formation of a hydroxycarbonate apatite (HCA) layer. The formation process of this layer on the glass is a function of the glass composition. The rate of formation increases in those glasses with lower SiO_2 (50−70% in mol).

Introduction

In 1969, Hench et al. synthesized the first bioactive material,[1] which was a glass of the system $Na_2O-CaO-P_2O_5-SiO_2$ obtained by melting and rapid quenching. The research performed during the last three decades has proved that other ceramic materials can also bond to bone. This is the case of certain compositions of glasses, glass−ceramics,[2,3] and sintered hydroxyapatite.[4]

A common feature for all bioactive materials is that, when in contact with physiological fluids, a calcium phosphate rich layer is formed on their surface.[1−6] During the first stage, an amorphous calcium phosphate layer is formed which, upon nucleation and growth of an apatite-like phase, becomes crystalline, giving rise to nanocrystals of hydroxycarbonate apatite (HCA), similar to those formed by the bone. These nanocrystals, combined with collagen fibers, form the layer that bonds the bioactive material with living tissues.[5] The formation mechanism of the bioactive glass to living tissue bonding is very complex, with cellular processes involved. However, in vitro studies can explain a possible mechanism, which allows one to understand the HCA layer formation under such conditions. Hench[5] proposed that, when immersing a bioactive glass in a fluid, a release of the metallic ions to the solution takes place almost instantaneously; as a consequence, silanol (Si−OH) groups are formed on the glass surface. This process is followed by a series of polycondensation reactions, which form siloxane bonds (Si−O−Si). The whole process arrives to the formation of a silica gel layer with high surface area, which promotes the apatite nucleation. Hench extrapolates the initial mechanism to the in vivo conditions when proposing that both layers, silica gel and HCA, determine the adsorption positions of cellular growth factors generated by macrophages and stem cells, which finally lead to the bone growth.

Other authors have also confirmed that the silica gel layer formed on the surface of bioactive glasses and glass-ceramics plays a fundamental role in the nucleation and crystallization of HCA. As a consequence, in recent years, several research groups have developed new types of glasses by sol−gel method for application as biomaterials. Sol−gel glasses in the ternary systems $CaO-SiO_2-P_2O_5$[7−14] and $Na_2O-CaO-SiO_2$[15] and the quaternary system $CaO-P_2O_5-SiO_2-MgO$[16−18] have been prepared and characterized.

In a previous study,[19] our group synthesized a bioactive glass by sol−gel method in the $CaO-SiO_2$ system, with composition of 80 SiO_2/20 CaO (in mol %). The

(1) Hench, L. L.; Splinter, R. J.; Allen, W. C.; Greenlee, T. K. *J. Biomed. Mater. Res.* **1971**, *2*, 117.
(2) Ohura, K.; Nakamura, T.; Yamamuro, T.; Kokubo, T.; Ebisawa, Y.; Kotoura, Y.; Oka, M. *J. Biomed. Mater. Res.* **1991**, *25*, 357.
(3) Kokubo, T.; Shigematsu, M.; Nagashima, Y.; Tashiro, M.; Nakamura, T.; Yamamuro, T.; Higashi, S. *Bull. Inst. Chem. Res., Kyoto Univ.* **1982**, *60*, 260.
(4) Jarcho, M.; Bolen, C. H.; Thomas, M. B.; Bobick, J.; Kay, J. F.; Doremus, R. H. *J. Mater. Sci.* **1976**, *11*, 2027.
(5) Hench, L. L. *J. Am. Ceram. Soc.* **1991**, *74*, 1487.
(6) Kokubo, T., Yamamuro, T., Hench, L. L. Eds. *Handbook of Bioactive Ceramics*; CRD Press: Boca Raton, FL, 1990; Vol. 1, p 41.
(7) Li, R; Clark, A. E.; Hench, L.L. *J. Appl. Biomater.* **1991**, *2*, 231.
(8) Pereira, M. M.; Clark, A. E.; Hench L. L. *J. Biomed. Mater. Res.* **1994**, *28*, 693.
(9) Laczka, M.; Cholewa, K.; Lazca-Osyczka, A. *J. Alloy Compd.* **1997**, *248*, 42.
(10) Vallet-Regí, M.; Romero A. M.; Ragel C. V.; LeGeros R. Z. *J. Biomed. Mater. Res.* **1999**, *44*, 416.
(11) Vallet-Regí, M.; Izquierdo-Barba, I.; Salinas A. J. *J. Biomed. Mater. Res.* **1999**, *46*, 560.
(12) Peltola, T.; Jokinen, M.; Rahiala, H.; Levänen, E.; Rosenhold, J. B.; Kangasniemi, I.; Yli-Urpo, A. *J. Biomed. Mater. Res.* **1999**, *44*, 12.
(13) Vallet-Regí, M.; Arcos, D.; Pérez-Pariente, J. *J. Biomed. Mater. Res.* **2000**, *51*, 2328.
(14) Vallet-Regí, M.; Ramila, A. *Chem. Mater.* **2000**, *2*, 961.
(15) Wilson, J.; Pigott, G. H.; Schoen F. J.; Hench L. L. *J. Biomed. Mater. Res.* **1981**, *15*, 805.

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Table 1. Composition of Glasses

sample	% SiO_2	% CaO
50S50C	50	50
60S40C	60	40
70S30C	70	30
80S20C	80	20
90S10C	90	10

referred study confirmed the bioactive behavior of this glass, a very simple binary system when compared with the ternary and quaternary bioactive systems previously known; no phosphorus was included in its composition, and the presence of only two components allowed a better distribution of them. From all results obtained, the most relevant was to prove that the inclusion of phosphorus in the bioactive glass composition was not necessary because the apatite-like phase developed takes phosphorus from the simulated body fluid. This research open new perspectives in the search for new bioactive glasses in the $CaO-SiO_2$ system; namely, to study the 50−90% compositional range (in mol) of SiO_2 in order to know the limits of bioactivity of such glasses, looking for differences in reactivity when in contact with physiological fluids. This has been the purpose of the present work.

Experimental Section

Preparation and Characterization of the Glasses. Five glasses, with compositions presented in the Table 1, were prepared by hydrolysis and polycondensation of tetraethyl orthosilicate (TEOS) and hydrated calcium nitrate ($Ca(NO_3)_2 \cdot 4H_2O$) in a 2 N solution of HNO_3 in H_2O. HNO_3 was used to catalyze the TEOS hydrolysis, using a molar ratio of (H_2O/TEOS = 8). After the addition of every reactant, the solution was stirred for 1 h. The sol was cast in cylindrical Teflon containers at room temperature, to allow the hydrolysis and polycondensation reactions, until the gel was formed. For aging, the gel was kept in the sealed container and heated at 70 °C for 3 days.

The drying of the gel was carried out in the same container by replacing the previous lid with one featuring a hole 1 mm in diameter, to allow the leak of gases, and heating the gel at 150 °C for 2 days. The dry gel was ground for 60 min and sieved, taking the grains ranging in size from 32 to 63 μm. Fractions of 0.5 g of powder were compacted at 50 MPa uniaxial pressure and 150 MPa isostatic pressure at room temperature to obtain disks of 13 mm in diameter and 2 mm in height. These disks were sintered at 700 °C for 3 h. The sintering temperature to obtain the glass was determined by thermogravimetric and differential thermal analysis (TG/DTA) of the dried gel. These analysis were carried out in Seiko Thermobalance TG/DTA 320 from 25 to 1150 °C at 5 °C/min in air.

Specific surface area and porosity of the disks after the thermal treatment at 700 °C for 3 h were measured by N_2 adsorption in a Micromeritics ASAP 2010 instrument. Specific surface area was obtained from BET method. Pore size distribution was determined by BJH method from the desorption branch of the isotherm.

The surfaces of the disks were studied by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and scanning electron microscopy coupled with energy dispersive spectroscopy (SEM-EDS).

Table 2. Ion Concentration of Simulated Body Fluid and Human Plasma (in mol/L)

ion	simulated body fluid	human 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	103.8
HCO_3^-	4.2	27.0
HPO_4^{2-}	1.0	1.0
SO_4^{2-}	0.5	0.5

XRD patterns were obtained in a Philips X'Pert MPD diffractometer using Cu Kα radiation in $\theta-2\theta$ scans and grazing incidence 2θ scans ($\theta = 1°$).

FTIR spectra were registered in a Nicolet-Magna IR spectrometer 550, on pellets prepared by mixing 1 mg of materials scraped from the glass surface with 250 mg of KBr and compacting at 740 MPa for 2 min.

The SEM-EDS study was made in a JEOL 6400 Microscope-LINK AN 10000 system. For the SEM study, the pieces were coated with a film of gold. For EDS analysis, different pieces were coated with carbon to avoid the overlap of one peak of gold with the K line of phosphorous.

In Vitro Tests. The assessment of in vitro bioactivity was carried out by soaking the disks, mounted vertically in a platinum scaffold in 45 mL of an acellular simulated body fluid (SBF), proposed by Kokubo et al,[20] at 37 °C in sterile polyethylene containers. The SBF solution has a composition and concentration similar to those of the inorganic part of human plasma, as shown in Table 2. The fluid was prepared by dissolving reagent-grade NaCl, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$, and Na_2SO_4 into distilled water and buffering at pH 7.25 with tris(hydroxymethyl)amminomethane. $(HOCH_2)_3CNH_2$. and hydrochloric acid at 36.5 °C. The SBF solution had previously been filtered with a 0.23 μm Millipore System, and all operation/manipulations of the pieces and SBF were done in a laminar flux cabinet Telstar AV-100 to avoid microorganism contamination. The soaking periods were 1.5, 3, 6, and 24 h and 3, 7, and 15 days. After being soaked, the pieces were rinsed with deionized water and acetone and dried in air at room temperature.

The in vitro bioactivity of the sol−gel glasses was evaluated by studying the variations of ionic concentration of solution with the time, and the changes in the morphology, composition, and crystalline phases formed on the surface of the disks.

Analysis of Ionic Concentration in SBF. Calcium ionic concentration in SBF and the pH were measured by electrode ion selective measurement using an Ilyte Na^+, K^+, Ca^{2+}, and pH system. Silicon and phosphorus concentrations were determined for complex formation and UV−vis spectroscopy in a Beckman DU-7 UV−vis spectrometer.

Study of the Apatite Layer Formation on Disk Surfaces. The formation of the apatite layer on the disk surfaces after different soaking periods in SBF was monitored by XRD, FTIR, and SEM-EDS.

Results

Characterization of the Glasses. Five series of glasses were prepared in the $CaO-SiO_2$ binary system by the sol−gel method. The gel formation at room temperature takes place after 72 h, with the exception of glass 90S10C, which gelled after 24 h. Those gels treated at 150 °C are white and opaque, except glass 90S10C, which is translucent.

Figure 1 shows the TG/DTA curves of the gels after drying at 150 °C for 2 days and grinding. All samples

(16) Vallet-Regí, M.; Salinas A. J.; Román, J.; Gil, M. *J. Mater. Chem*. **1999**, *9*, 515.
(17) Pérez-Pariente, J.; Balas, F.; Román, J.; Salinas A. J.; Vallet-Regí, M. *J. Biomed. Mater. Res.* **1999**, *47*, 170.
(18) Perez-Pariente, J.; Balas, F.; Vallet-Regí, M. *Chem. Mater.* **2000**, *12*, 750.
(19) Izquierdo-Barba, I.; Salinas A. J.; Vallet-Regí, M. *J. Biomed. Mater. Res.* **1999**, *47*, 243.
(20) Kokubo, T.; Kushitani, H.; Sakka, S.; Kitsugi, T.; Yamamuro, T. *J. Biomed. Mater. Res.* **1990**, *24*, 721.

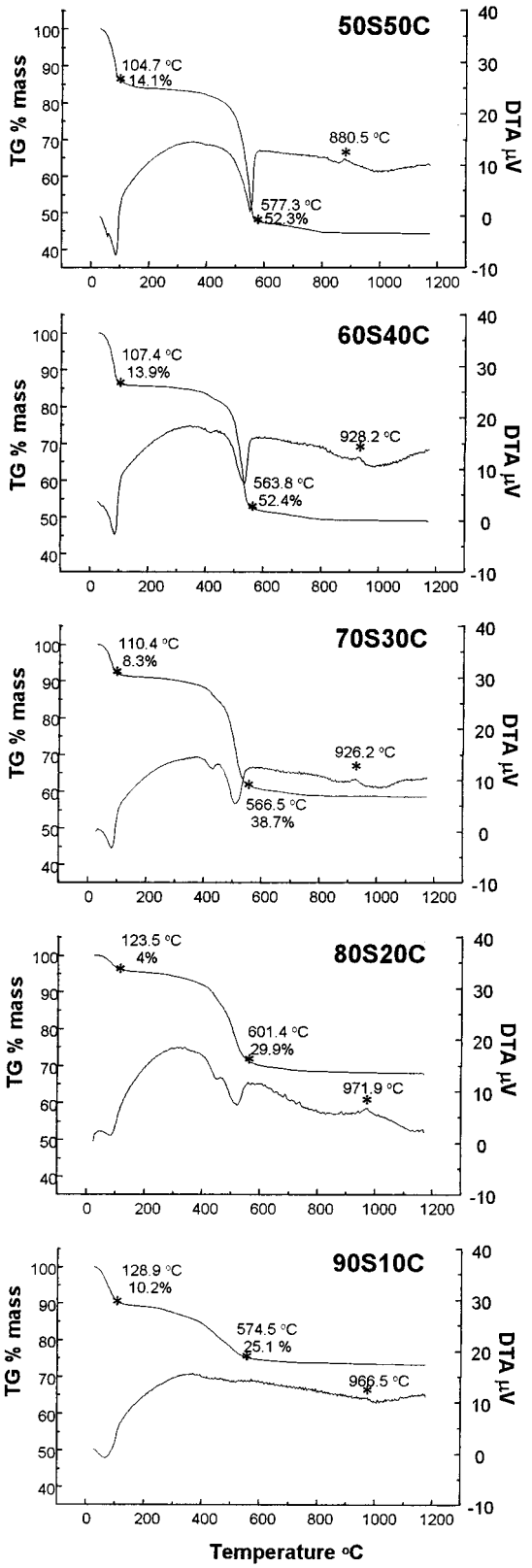

Figure 1. TG and DTA curves of the gel powders of 50S50C−90S10C systems after being dried at 150 °C.

show an initial endothermic process in the range of 104−129 °C, depending on the gel composition, which was attributed to a residual loss of water and ethanol, corresponding to a weight loss of 4−14%. The temperature at which such a process takes place increases with the decrease in initial amount of $Ca(NO_3)_2$. A second endothermic process occurs at a temperature between 560 and 602 °C, with a weight loss of 25−52%; then, the weight remains constant up to 1150 °C. This last process is attributed to the loss of the nitrates used in the sol preparation.[21] Finally, an exothermic process is detected between 880 and 972 °C, attributed to the crystallization of $CaSiO_3$ (β-wollastonite), as was confirmed by the XRD analysis of the residue of TG/DTA. This process is hardly noticeable in the 90S10C series, probably due to the lower $Ca(NO_3)_2$ initial content of these glasses.

Table 3. Textural Parameters for Glasses: Specific Surface Area and Pore Size

sample	$S_{BET}/m^2\,g^{-1}$	dp/nm
50S50C	30	26
60S40C	41	24
70S30C	126	15
80S20C	154	12
90S10C	186	6

These results led us to determine that the optimal sinterization temperature of glasses in the $CaO-SiO_2$ system must be comprised between 700 °C, where nitrates groups are completely eliminated, and 900 °C, where the β-wollastonite crystallizes. To obtain materials with larger surface area and porosity, a treatment at 700 °C for 3 h of such gels, previously compacted, was chosen. The disks so obtained exhibit very different properties regarding their porosity and surface area (Table 3) as functions of their SiO_2/CaO ratio. Larger surface areas and smaller pore sizes are detected for increasing SiO_2 contents and decreasing CaO contents.

The FTIR study of the obtained samples show a large band comprised between 1100 and 1000 cm^{-1} (Figure 2), which, together with the 480−467 cm^{-1} band, can be assigned to the vibration of the Si−O−Si bond. The vibration due to the OH bond is observed for all samples at 1647−1631 cm^{-1}. Those glasses with higher calcium contents (50S50C−70S30C) show bands corresponding to CO_3^{2-} groups between 1490 and 1410 cm^{-1}. This band is very intense for 50S50C glasses and hardly perceptible for 70S30C glasses. The presence of carbonate is attributed to a carbonation process of the material due to the atmospheric CO_2[22] as a consequence of the high calcium content.

The X-ray diffraction study confirms that all glasses prepared are amorphous, although a very wide diffraction maximum can be observed between 28 and 34° 2θ for glasses 50S50C−70S30C, two maxima at 28−34 and 22.5° 2θ for glass 80S20C, and one maximum at 22.5° 2θ for glass 90S10C. The diffraction maximum between 28 and 34° 2θ can be attributed to the incipient presence of different crystalline phases of calcium silicates, such as wollastonite, whereas the maximum at 22.5° 2θ could be due to a higher SiO_2 content in the glass (see Figure 3, t = 0h).Scanning electron microscopy has been used to study the morphology of the particles forming these glasses. All pieces were conformed under identical conditions, and they are all formed by particles of irregular shape and size.

Bioactivity Assays in SBF. *Variations in SBF*. The calcium concentration in the medium varies with the calcium content of the glasses soaked in SBF (Figure

(21) Duval, C. *Inorganic Thermogravimetric Analysis*; Elsevier: New York, 1963; p 274.
(22) Hayashi, T.; Saito, H. *J. Mater. Sci.* **1980**, *15*, 1971.

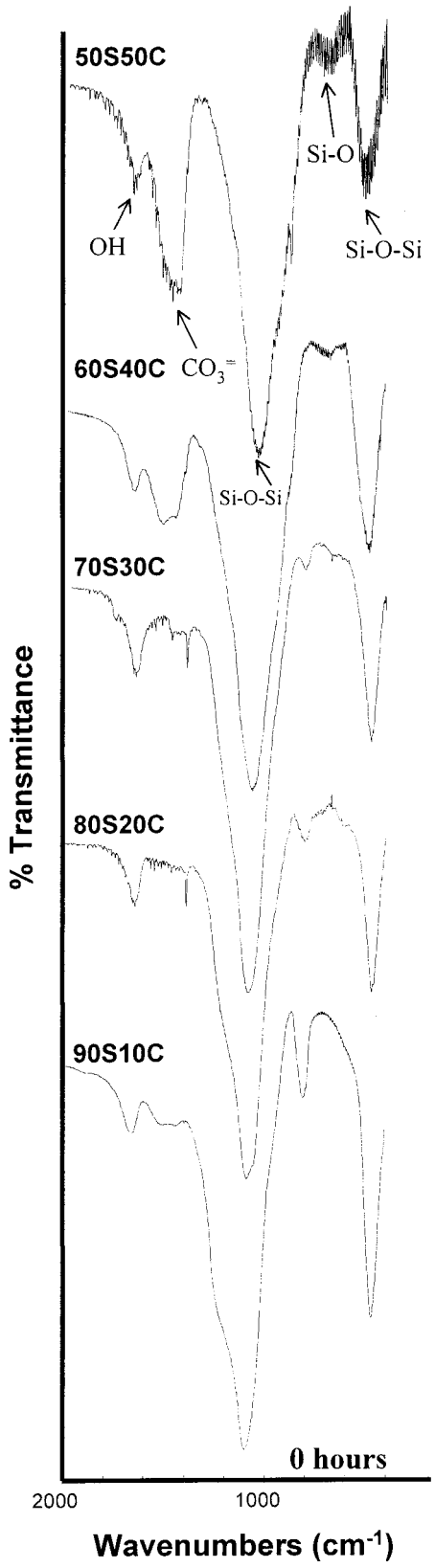

Figure 2. FTIR spectra of the glasses.

4). Two different types of behavior can be distinguished among these glasses: one for glasses with higher calcium content (50S50C−70S30C) and another one for lower calcium contents (80S20C−90S10C). In all cases, the calcium concentration in the medium increases rapidly during the first hours of soaking in SBF. For those glasses with higher calcium content, a maximum value in calcium concentration is reached after approximately 24 h, decreasing afterward until values similar to those of the initial SBF are obtained after 7 days of assay. For larger time periods, the calcium concentration keeps decreasing.

The situation is somewhat different when soaking the glasses with low calcium content: after a rapid increase calcium concentration of the SBF during the first 6 h of assay, a linear increase of ionic concentration is detected for the 90S10C glass. Therefore, the graphical representation of the calcium exchange in this glass can be divided in two stages: a fast, 6-hour step in which a variation from 110 to 287 ppm takes place, followed by a slow stage with a 53 ppm increase in 168 h.

Regarding the phosphorus, all glasses have shown similar behaviors, taking phosphorus from the SBF after the first 1.5 h. Analysis performed at different times indicates that the glass absorbs phosphorus, decreasing its concentration in the fluid.

The pH values increase from initial range 7.3 to 7.5 after 1.5 h of assay, reaching a maximum value of 8.1 after 7 days.

Modifications in the Glass Surfaces after Soaking in SBF. The infrared spectra of all glasses show an intense band between 1100 and 1040 cm^{-1}, attributed to an asymmetric vibration of the Si−O−Si bond, as well as the band detected between 478 and 441 cm^{-1}. The band shown at 814−793 cm^{-1} corresponds to a symmetric vibration of the Si−O bond. After soaking for 1.5 h in SBF (Figure 5a), glasses 50S50C-80S20C displayed a band at 570 cm^{-1} that corresponds to the antisymmetric vibration of the P−O bond in amorphous calcium phosphate. Such a band does not appear in glass 90S10C until after 3 h of assay (Figure 5c). This band evolves to give rise to a doublet between 601 and 568 cm^{-1}, which can be observed in all cases after 7 days of assay. Several authors assign this doublet to crystalline calcium phosphate.[23−26] Series 50S50C−70S30C shows a band that corresponds to the carbonate group already present in the unreacted material. It is also present in soaked glasses of series 80S20C and 90S10C. The FTIR spectra of all glasses prepared after soaking in SBF for 1.5 h are shown in Figure 5a. Parts b and c of Figure 5, for illustrative purposes, show the evolution of apatite formation on the 70S30C and 90S10C glass surfaces for different assay times.

The X-ray diffraction analysis of the five types of glasses under study reveal clear differences between them in terms of their respective calcium content. Once again, two kinds of behavior can be detected. The glasses with lower calcium content (80S20C−90S10C) do not yield any sort of information in the early stages of assay; only after soaking in SBF for 15 days can the initial formation of an apatite-like phase be detected, showing the (002) and (211) reflections (Figure 3a).

(23) Elliott, J. C. *Structure and Chemistry of the Apatites and Other Calcium Orthophosphates;* Studies in Inorganic Chemistry, Elsevier: Amsterdam, 1994; Vol. 18, p 59.
(24) Ohtsuki, C.; Kokubo, T.; Yamamuro, T. *J. Non-Cryst. Solids* **1992**, *143*, 84.
(25) LeGeros, R. Z. Calcium phosphate in oral biology and medicine. In *Monographs in Oral Science*; Myers, H. M, ed.; Karger: Zurich, 1991; p 114.
(26) Li, P.; Ohtsuki, C.; Kokubo, T.; Nakanishi, K.; Soga, N.; Nakamura, T.; Yamamuro, T. *J. Mater. Sci.: Mater. Med.* **1993**, *4*, 127.

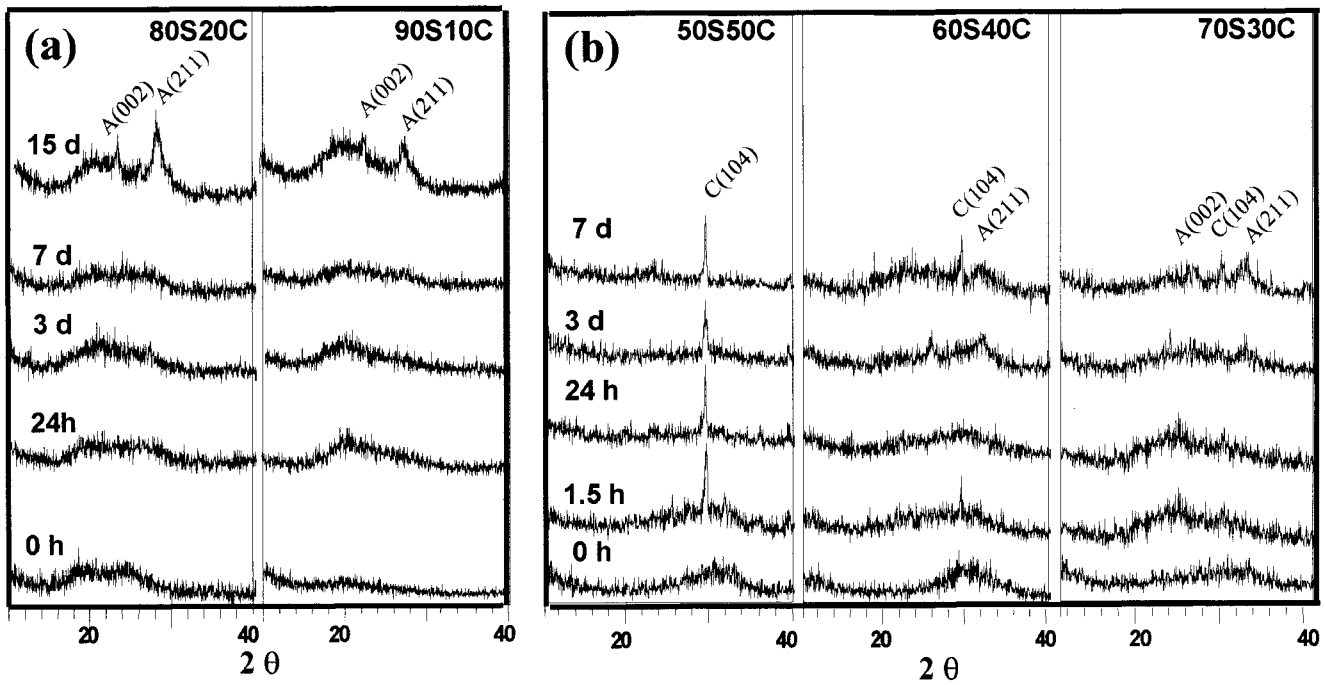

Figure 3. XRD patterns of (a) glasses 80S20C and 90S10C before and after soaking for 24 h and 3, 7, and 15 days in SBF and (b) glasses 50S50C−70S30C before and after soaking for 1.5 and 24 h and 3 and 7 days in SBF (A = apatite, C = calcite).

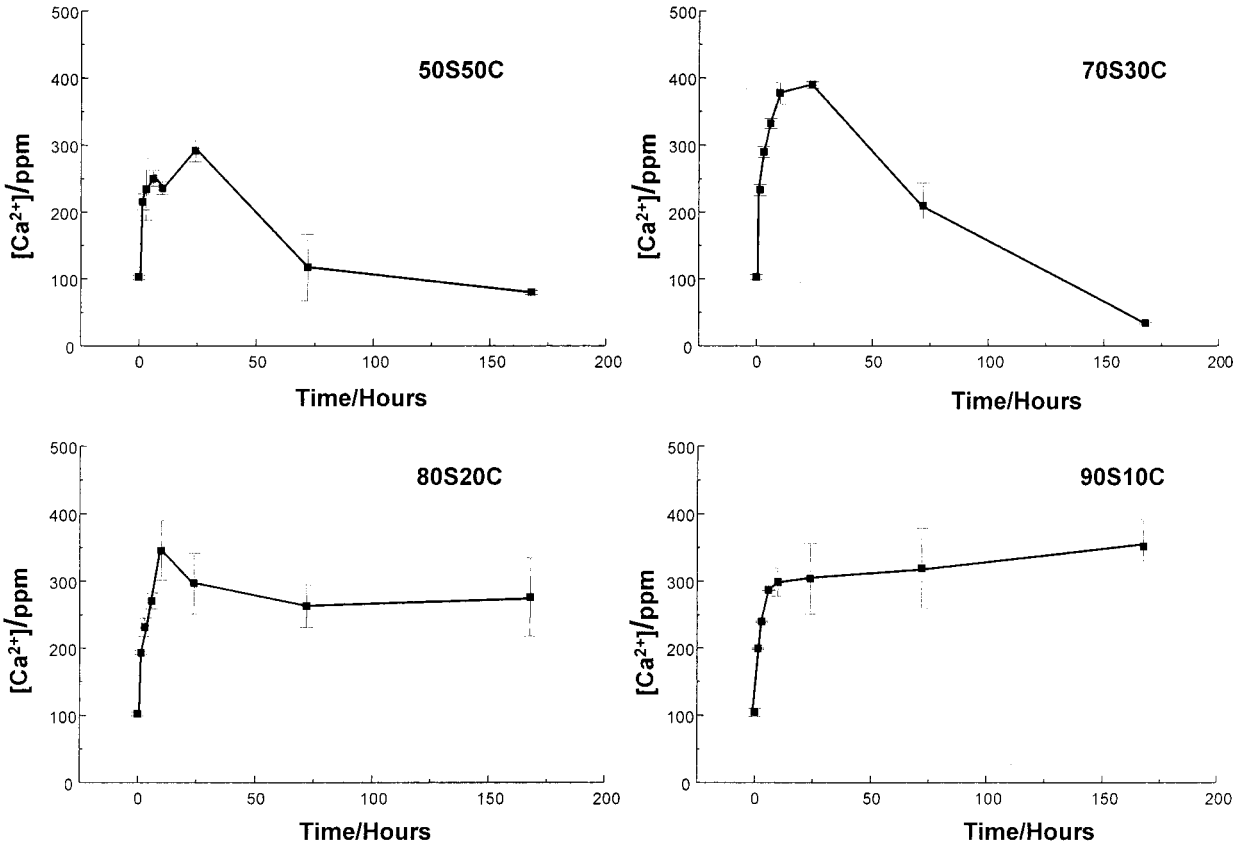

Figure 4. Variation of calcium content (ppm) with soaking time in SBF.

On the other hand, glasses with higher calcium contents (50S50C−70S30C, Figure 3b) give different results. After soaking for 3 days in SBF, the results obtained indicate the formation of two phases: reflections (211) of apatite and (104) of calcite can be detected; glass 50S50C contains a larger proportion of this calcite-like phase, while both phases are almost equal in proportion in glass 60S40C, and the apatite-like phase turns to be predominant in glass 70S30C. Therefore, after soaking, calcite is the main phase in glasses with high calcium content, and the situation changes when the calcium content decreases, with apatite as the main phase in the newly formed layer.

The scanning electron microscopy study of all glasses after soaking in SBF for 1.5 h reveals the formation of a surface layer, made of spherical particles with diam-

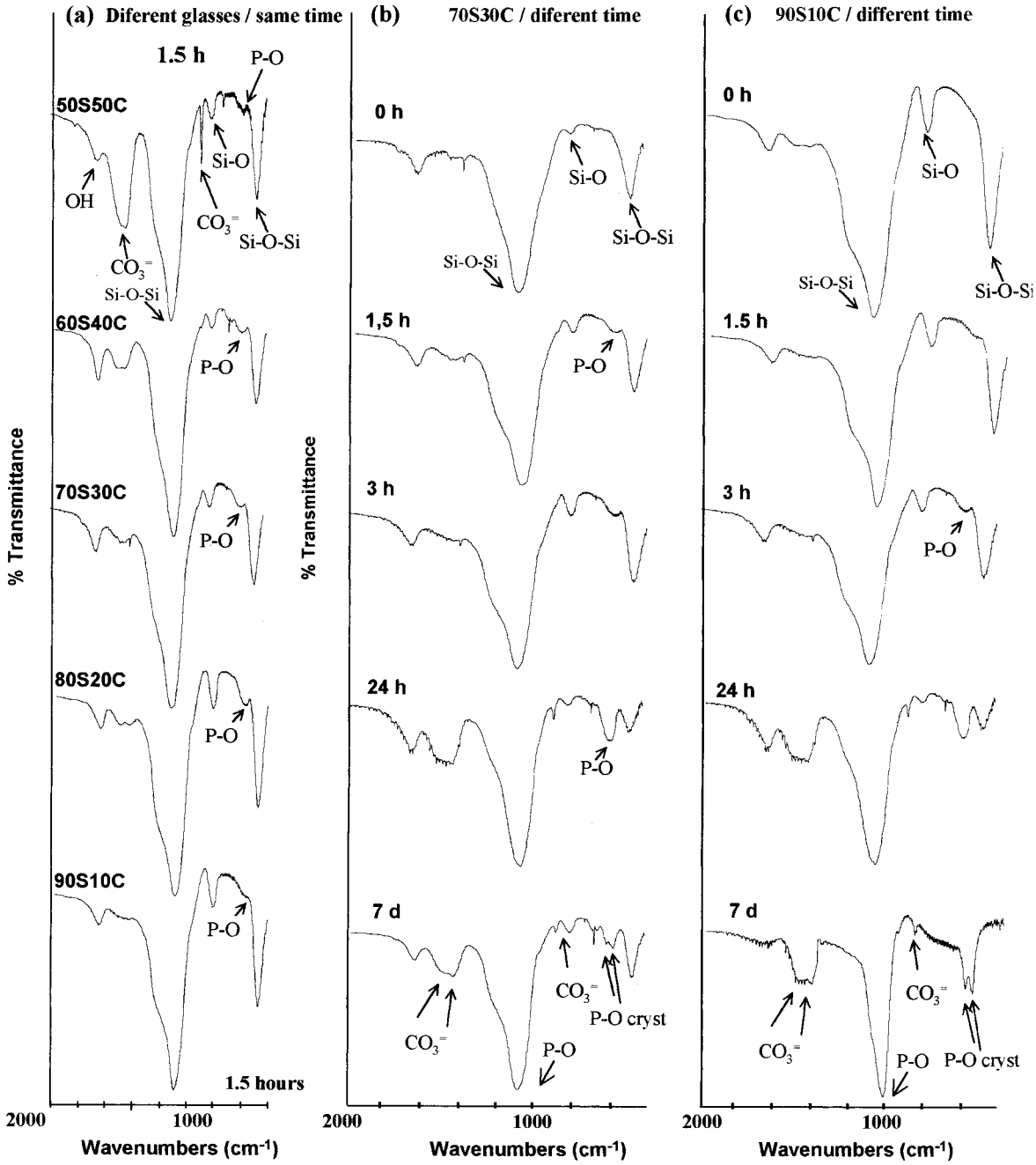

Figure 5. FTIR spectra of (a) all glasses soaked in SBF for 1.5 h and for glasses (b) 70S30C and (c) 90S10C before and after soaking for 1.5, 3, and 24 h and 7 days in SBF.

eters below 1 μm (Figure 6).The evolution of such newly formed layer can be monitored at higher periods of in vitro assay. After 3 h of treatment, the situation previously described remains unchanged, but after 6 h, a more complete coating of the glass surface is observed. When the glass was treated for 24 h in SBF, the layer is more compact and the diameter of the spherical particles exceed 1 μm. After 3 days, such spheres are larger than 3 μm and it can be observed that, in turn, they are formed by aggregates of elongated particles. The situation remains unchanged after 7 days of soaking, although the formed layer is more compact and the spherical aggregates of elongated particles are larger, covering the whole surface of the glass. For illustrative purposes, Figure 7 shows such evolution in series 70S30C for different times of assays.

The EDS studies reveal, in the five series of glasses, the inclusion of phosphorus in the composition of the newly formed layer since the first 1.5 h of assay (Figure 6). The phosphorus present on the newly formed layer proceeds from the SBF solution. It also shows an increase in the calcium content and a decrease in the silicon content as a function of the soaking time. Figure 7 shows the EDS results for the series 70S30C.

The Ca/P molar ratio, determined by EDS on the surface of the samples after 7 days of assay ranges between 1.51 and 1.61, with the exception of the 90S10C glasses, which exhibit a ratio equal to 0.90. This series shows very little variations on the above ratio after 6 h of assay, reaching a Ca/P ratio of 1.40 after 15 days of assay.

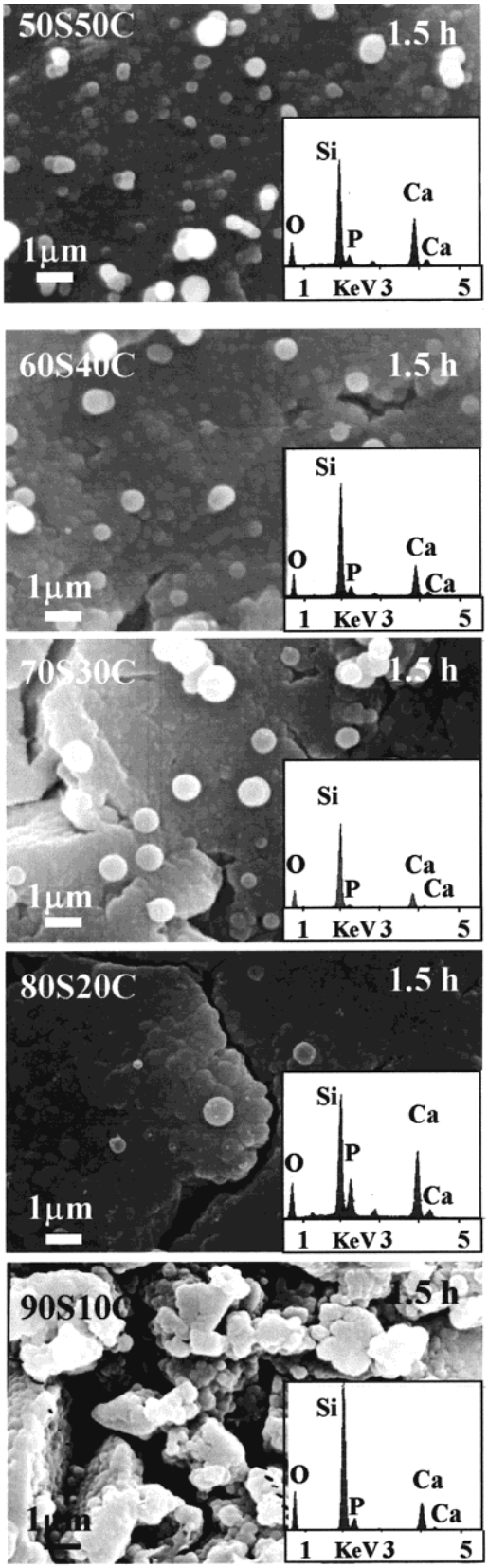

Figure 6. SEM micrographs and EDS patterns of the glasses surfaces after soaking in SBF for 1.5 h.

It can be observed by SEM that the thickness of the formed layer is approximately 1−3 μm. Figure 8 shows a tilted cross-section of the 80S20C glass soaked in SBF for 15 days, displaying in greater detail the layer of spherical agglomerates of particles.

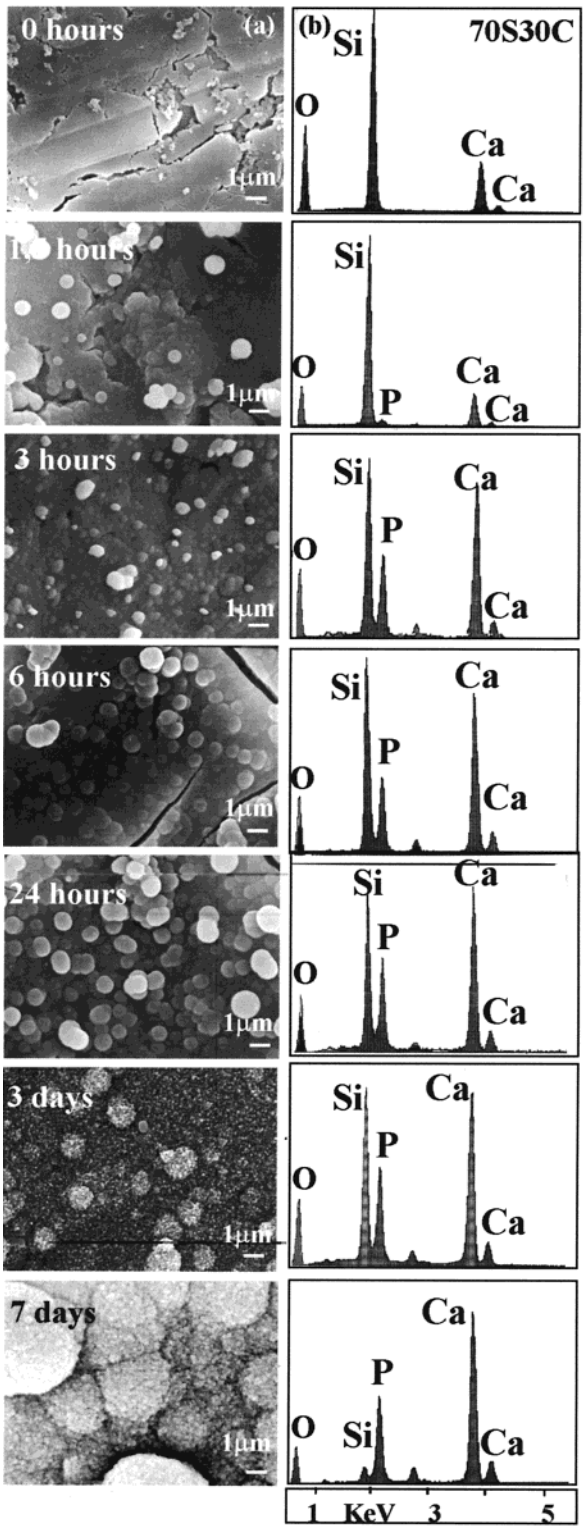

Figure 7. SEM micrographs and EDS patterns of the 70S30C glass surface before and after soaking in SBF for different periods of time.

Discussion

Five new series of glasses have been prepared in the binary system $CaO-SiO_2$ by the sol−gel method, obtaining materials with different properties as function of the composition. A larger surface area and smaller pore size are obtained for higher SiO_2 contents and lower CaO contents.

The XRD study of the unreacted glasses allows to detect the majority presence of calcium silicates in those

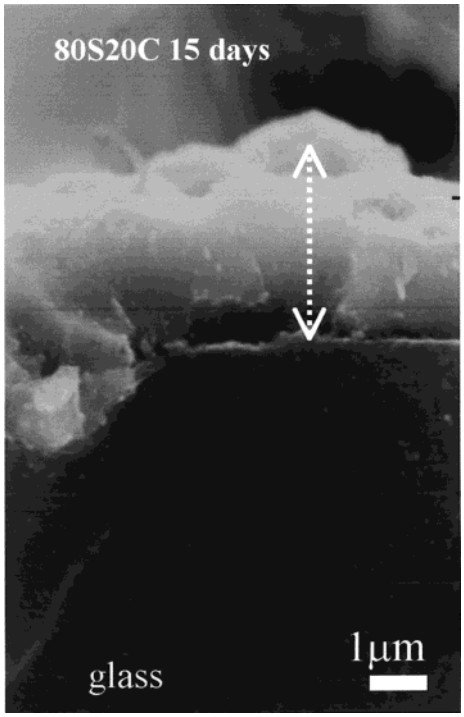

Figure 8. SEM micrograph of a cross section of the glass 80S20C after 15 days in SBF.

glasses with higher calcium content (50S50C−70S30C), whereas for glasses with lower content (80S20C−90S10C), both calcium silicates and SiO_2 are present; this last phase is in much higher proportion in series 90S10C.

These differences in their textural (surface and porosity) and compositional properties give rise to important variations in their in vitro behavior. The chemical analysis of the SBF during the soaking process of each glass, performed at different times, as well as the EDS study of the glass surface after each soaking time in SBF, reveals the inclusion of phosphorus in the surface layer. The phosphorus content of solution starts to decrease after 1.5 h of soaking, and the composition of the newly formed layers reflect this exchange (Figures 6 and 7). This is indicative that the presence of phosphorus in the glass composition is not an essential requirement for the calcium phosphate layer formation.

On the other hand, for all the series under study, an increase of the calcium concentration in SBF is observed during the first few hours of assay as a consequence of hydrolysis of the SiOCa groups. For longer periods of assay, clear differences appear between the series and on the formation of the apatite-like layer on the glass surface, depending on the calcium content of each glass.

If we assume that the soaking process of $CaO-SiO_2$ glasses in SBF gives rise to the release of calcium ions to the fluid, an exchange with protons of the solution and the increase in silanol groups on the glass surface, the percentage of such groups will depend on the amount of silicates in the glass. According to the literature,[5,6,26,29] the silanol groups can induce the apatite nucleation.

The higher calcium silicate content glasses (50S50C−70S30C) give rise to a marked calcium release during the first 24 h of assay; afterward, this concentration decreases until a similar calcium concentration to that in original SBF is reached, after 7 days of assay. The decreases in the calcium concentration in SBF is attributed to the growth of the apatite nuclei formed of surface of the glasses. Once the apatite nuclei were formed, they grew spontaneously by consuming the calcium and phosphate ions from the surrounding fluid. Due to its lower surface area and higher pore size, this calcium released to the fluid must belong to the glass surface.

The higher calcium content of these glasses enhances the growth rate of the apatite-like layer, which can be detected by FTIR, XRD, SEM, and EDS after the first hours of assay. However, the simultaneous presence of calcite on the surface is also observed by XRD. The amount of calcite is higher in those glasses with higher initial calcium contents. The surface growth of calcite can be attributed to the existence of small $CaCO_3$ nuclei on the initial glass, detected by FTIR.

The glasses with lower calcium content (80S20C−90S10C) provoke a rapid increase of calcium concentration in SBF during the first 6 h of assay; after this first stage, the calcium concentration in SBF linearly increases for glass 90S10C, while it reaches calcium levels similar to those of SBF when soaking glass 80S20C, after 15 days of assay. The lower calcium silicate content of these glasses allows a lower proportion of silanol groups, formed by the release of calcium ions to the SBF. This fact, combined with their larger surface area and smaller pore size, which controls the calcium release process,[28] would support the lower growth rate of the apatite-like layer on these glasses.

This is in agreement with the calcium phosphate formation on silica gel glasses.[27−29] An induction period preceded the onset of calcium phosphate on the silica gel for a given concentration of SiOH groups. Therefore, the length of the induction period is related to the rate of hydrolysis on the surface, which in turn depends on the silica structure. The interaction of the SiOH groups with calcium phosphate solution is believed to make a significant contribution to the initiation of heterogeneous nucleation of apatite.

It can be summarized that the in vitro study of the five glasses prepared allows one to confirm their reactivity with SBF and, as a result, the surface growth of an almost amorphous layer with composition $Ca-P-CO_3^{2-}$, which is very similar to the mineral component of biological carbonate apatite. Therefore, we can consider these five glasses as bioactive; that is, they react chemically with physiological fluids enabling the growth of a phase similar to the mineral component of the bone. The differences in their textural and compositional properties give rise to important variations in their behavior as bioactive material. Both together determine the in vitro bioactivity.

Each of the techniques used in these assays, individually, would not allow one to confirm which is the phase formed on the surface of these bioactive glasses, due to the nearly amorphous nature of the formed layer. The low crystallinity hinders its precise X-ray characteriza-

(27) Pereira, M. M.; Clark, A. E.; Hench, L. L. *J. Am. Ceram. Soc.* **1995**, *78*, 2463.
(28) Pereira, M. M.; Hench, L. L. *J. Sol-Gel Sci. Technol.* **1996**, *7*, 59.
(29) Li, P.; Ye, X.; Kangasniem, I.; de Blieck-Hogervorst, J. M. A.; Kein, C. P. A. T.; de Groot, K. *J. Biomed. Mater. Res.* **1995**, *29*, 325.

tion; SEM and EDS yield information that, by itself and without further techniques, would be far from conclusive. The chemical analysis reveals the ion exchange that takes place in the soaking process, which again would be incomplete. FTIR allows one to monitor from the very first hours of assay the formation of P−O and C−O bonds.

It is the combination of all these techniques, with their combined results, which allows one to affirm that the phase formed is a hydroxycarbonate apatite that is similar in composition and crystallinity to the biological apatite.

Conclusions

All gel glasses studied in the $CaO-SiO_2$ binary system are bioactive, growing a carbonate apatite layer on their surface, taking phosphorus from the SBF.

The in vitro behavior of these glasses indicates that the presence of phosphorus in the glass composition is not an essential requirement for the development of the HCA layer. In this case, the layer is formed from the phosphorus present in the in vitro assay solution.

The growth rate of this layer mainly depends on the glass composition. Glasses with lower SiO_2 content and higher CaO content exhibit higher apatite layer growth rates.

Nevertheless, the amounts of samples studied allow us to speculate that such growth rates could also be related to the surface area and porosity of the glasses. Glasses with larger surface area and smaller pore size exhibit decreasing growth rates of the apatite layer, due also to the higher SiO_2 content of the glass.

In glasses with higher calcium content, a calcite layer grows simultaneously with the amorphous calcium phosphate, after the first 1.5 h of assay.

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