



# The influence of the phosphorus content on the bioactivity of sol–gel glass ceramics

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## Abstract

The aim of this work was to study the influence of the phosphorus on the crystallization and bioactivity of glass-ceramics obtained from sol–gel glasses. For this purpose two sol–gel glasses with a similar composition but one of them containing  $P_2O_5$  (70%  $SiO_2$ ; 30%  $CaO$  and 70%  $SiO_2$ ; 26%  $CaO$ ; 4%  $P_2O_5$ , mol%) were prepared. Pieces of these glasses were treated at temperatures ranging between 700°C and 1400°C for 3 h. The obtained materials were characterized by XRD, FTIR, SEM-EDS and the biaxial flexural strength was determined in samples heated at 1100°C. In addition, an in vitro bioactivity study in simulated body fluid (SBF) was carried out.

The results showed that phosphorus plays an important role in the crystallization of the glasses: it induced the crystallization of calcium phosphate phases, the stabilization of the wollastonite phase at high temperature as well as the crystallization of  $SiO_2$  phases at low temperatures. Moreover, the presence of phosphorus produced a heterogeneous distribution of defects in the pieces and, therefore, the flexural strength of samples containing this element decreased. Finally, glass-ceramics obtained from glasses containing phosphorus showed the fastest formation rate of the apatite layer when soaked in SBF.

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## 1. Introduction

Bioactive materials are characterized by their intimate bond with living bone through the formation of a hydroxyapatite interface layer [1]. A similar layer is reported to form on the surface of these materials after soaking in liquids with an ionic composition similar to the human blood plasma [2]. Since Hench and co-workers developed the first bioactive glass [3], several types of silicate glasses, glass-ceramics and calcium silicates [4–6] have been studied as biomaterials for repair and replacement of hard tissues. The results have shown that materials containing  $CaO$  and  $SiO_2$  are bioactive, and were found to bond to living bone [7,8]. However, most melt-derived glasses and glass-ceramics contain other elements, in more complex compositions [9]. The use of the sol–gel technique allowed to obtain

glasses with the simplest composition ( $SiO_2$ – $CaO$ ) which, in addition, resulted markedly more bioactive than melt-derived glasses with the same composition [5,8].

A few studies dealing with the sintering and crystallization of bioactive melt-derived glass have been reported. These studies showed that reheating of such glasses might turn a bioactive glass into an inert material or have very little or no adverse effect on their bioactivity [10–12]. Taking into account that by using the sol–gel technique it is possible to prepare bioactive glasses with the simplest composition, and that these glasses are more reactive than melt-derived glasses, the possibility to obtain bioactive glass-ceramic by thermal treatments of sol–gel glasses was studied in a previous work [13]. The results showed that after thermal treatments at temperatures ranging from 700°C to 1400°C, all the obtained materials (glasses and glass-ceramics) were bioactive. The bioactive behaviour was related with the initial composition of the glasses, the phase composition after thermal treatment and with the

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sintering process. In addition, the crystallized fraction after thermal treatment and the bioactivity seemed to be related with the phosphorus content. However, this influence could not be clearly asserted due to the very different compositions of the studied samples. Therefore, in this work, glasses with a similar composition but one of them containing phosphorus were prepared in order to study the influence of the phosphorus content on the crystallization, mechanical properties and bioactivity of obtained glass-ceramics.

## 2. Materials and methods

### 2.1. Glass-ceramics preparation and characterization

The nominal composition of the studied materials is shown in Table 1. The glasses were synthesized as previously described [14,15]. Tetraethyl orthosilicate, triethyl phosphate and tetra hydrated calcium nitrate were used as precursors of SiO<sub>2</sub>, P<sub>2</sub>O<sub>5</sub> and CaO, respectively. Cylindrical pieces ( $\phi$ —13 mm,  $h$ —2 mm) of dry gels (milled and sieved, 32 to 63  $\mu$ m) were obtained by uniaxial (55 MPa) and isostatic pressure (150 MPa). The pieces were heated to 700°C (5°C/min) for 3 h and subsequently heated to temperatures ranging between 800°C and 1400°C (5°C/min) for 3 h. The samples were characterized by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy coupled with energy-dispersive spectroscopy (SEM-EDS) using a JEOL 6400 Microscope-Oxford Pentafet super ATW system. For the EDS analysis the samples were coated with carbon.

The pellets were milled, in order to obtain the XRD patterns of samples, and the powder analysed in a Philips X'Pert MPD diffractometer (Cu K <sub>$\alpha$</sub>  radiation) in the range 5–120° 2 $\theta$  with step size of 0.02° and a time per step of 10 s. The phase quantification was made by the Rietveld method [16] using the X'Pert Plus software (Philips). FTIR analyses were made on a Nicolet Nexus spectrometer on pellets prepared mixing 1 mg of the samples and 250 mg of KBr (IR grade).

The mechanical assays were carried out in samples calcined at 1100°C. The biaxial flexural strength was measured by using the ball-on-3-ball configuration on 12 specimens of 12.6-mm diameter and 2.2-mm high in sample 70S26C4P and 12.2-mm diameter and 2.1-mm

high in sample 70S30C. An MTS Bionix<sup>®</sup> 858 Test system with a charge cell of 1 kN was used. Test Star II<sup>®</sup> for general control and TestWorks4<sup>®</sup> for data acquisition were used as software.

### 2.2. In vitro assay in SBF

The in vitro bioactivity study was performed by soaking the pieces in the simulated body fluid (SBF No. 9) proposed by Kokubo et al. [2] at 37°C for 3 h, 1, 3 and 7 days. The changes in Ca<sup>2+</sup> concentration and pH of the SBF were determined by using an Ilyte Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, pH system. Upon soaking in SBF, the surface of the pieces was analysed by SEM-EDS and glancing-angle XRD.

## 3. Results

### 3.1. Samples characterization

Figs. 1 and 2 show the XRD patterns of samples heated at different temperatures and Table 2 shows the results of the crystalline-phase quantification. The materials treated at 700°C and 800°C showed XRD patterns typical of amorphous materials, but those corresponding to samples 70S26C4P-700/800 showed a wide diffraction maximum at 32° 2 $\theta$  attributable to an apatite-like phase [17].

Glass crystallization was observed in both samples from 900°C but the crystalline-phase composition was very different.

In samples 70S30C the crystallized phases were similar when treated at temperatures from 900°C to

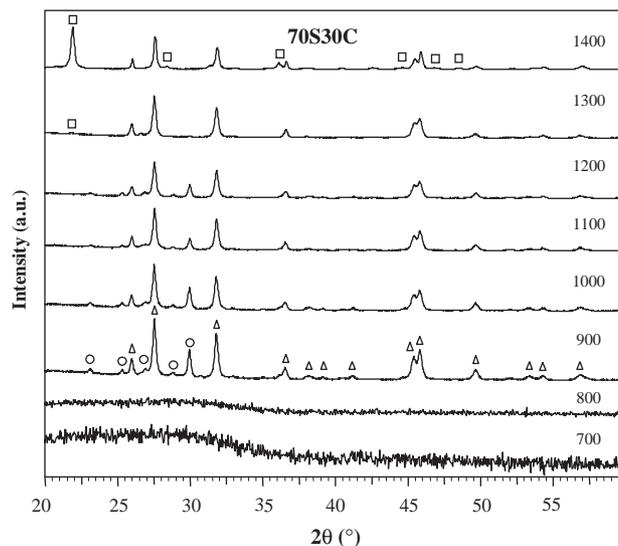


Fig. 1. XRD patterns of samples 70S30C treated at different temperatures (○ = Wollastonite; △ = Pseudowollastonite; □ = Cristobalite).

Table 1  
Nominal composition of synthesized glasses (mol%)

	SiO <sub>2</sub>	CaO	P <sub>2</sub> O <sub>5</sub>
70S30C	70	30	—
70S26C4P	70	26	4

1200°C, consisting of a mixture of wollastonite [18] (W) and pseudo-wollastonite [19] (PsW) (polymorphs of  $\text{CaSiO}_3$ ), being PsW the majority phase. At 1300°C only PsW and a little amount of cristobalite [20] (Ct,  $\text{SiO}_2$ ) were observed. At this temperature the W-phase was transformed into PsW, which is the most stable phase at high temperature. At 1400°C the Ct content increased significantly.

In samples containing phosphorus (**70S26C4P**) the phase composition was much more complex than in samples **70S30C**. At 900°C the main phase was apatite [17] (Ap), while quartz [21] (Q,  $\text{SiO}_2$ ), silicon pyrophosphate [22] (SiP,  $\text{SiP}_2\text{O}_7$ ) and W were also present. At 1000°C the composition was similar to that observed at 900°C, but the amount of Ap-phase decreased whereas

that corresponding to Q increased. At 1100°C these phases were also present but in this case an increase of W content was observed. At 1200°C in addition to W, Ap and Q, tricalcium phosphate [23] ( $\text{TCP } \alpha\text{-Ca}_3(\text{PO}_4)_2$ ) appeared. At 1300°C, besides those phases already mentioned, the Ct-phase [20], polymorph of  $\text{SiO}_2$ , was observed. However, at 1400°C some phases were not present (W, Ap and Q) and besides TCP and Ct, the PsW-phase and another polymorph of  $\text{SiO}_2$  (Tridymite, T) [24] appeared. It is worth noting that in both series the results of the crystalline-phase quantification do not match the original compositions until the highest temperature heat treatments (1400°C). This result indicates that in all the heat treatments below 1400°C there is a considerable amount of amorphous phase present.

Fig. 3 shows the FTIR spectra of series **70S30C** and **70S26C4P** treated at different temperatures. The FTIR spectra of samples treated at 700°C and 800°C showed the typical bands for sol-gel glasses with these compositions. The band at  $1090\text{ cm}^{-1}$  corresponds to the Si–O–Si asymmetric stretching mode, whereas the one at  $792\text{ cm}^{-1}$  is associated with a Si–O–Si symmetric stretching. The band at  $476\text{ cm}^{-1}$  is assigned at the Si–O–Si symmetric bending mode and the shoulder at  $950\text{ cm}^{-1}$  is related to the Si–O–Ca vibration mode [25]. The FTIR spectra of samples **70S26C4P** at 700–800°C showed in addition a doublet at  $569, 603\text{ cm}^{-1}$ , which is associated with the stretching vibration of phosphate groups [26]. The FTIR spectra of sample **70S26C4P-900** was similar to the samples treated at 700°C and 800°C, but two additional bands, assigned to SiP and W, were observed.

At higher temperatures, in both series, new bands were observed. In samples **70S30C** these bands appeared at  $1091, 988, 938, 920, 809\text{--}800, 718, 644, 563, 472$  and  $432\text{ cm}^{-1}$ . The FTIR spectra of samples **70S30C** heated

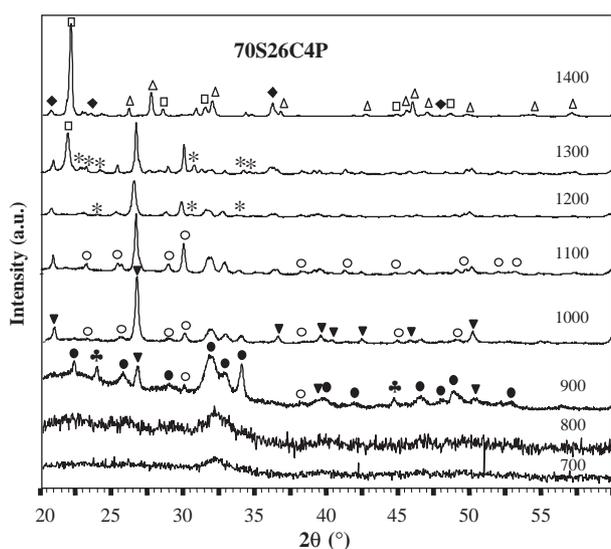


Fig. 2. XRD patterns of samples **70S26C4P** treated at different temperatures (● = Apatite; ▼ = Quartz; ○ = Wollastonite; ♣ = Silicon pyrophosphate; \* = TCP; ◆ = Tridymite; □ = Cristobalite; △ = Pseudowollastonite).

Table 2  
Crystal phase analysis of the glass-ceramics (wt%)

		PsW	W	Ct	Ap	Q	SiP	TCP	T
<b>70S30C</b>	<b>900</b>	77	23						
	<b>1000</b>	79	21						
	<b>1100</b>	80	20						
	<b>1200</b>	76	24						
	<b>1300</b>	98		2					
	<b>1400</b>	59		41					
<b>70S26C4P</b>	<b>900</b>		21		63	3	13		
	<b>1000</b>		25		49	27			
	<b>1100</b>		33		45	21			
	<b>1200</b>		24		24	26		27	
	<b>1300</b>		25	25	6	21		23	
	<b>1400</b>	30		48				18	4

PsW: Pseudo-wollastonite, W: Wollastonite, Ct: Cristobalite, Ap: Apatite, Q: Quartz, SiP: Silicon pyrophosphate, TCP:  $\alpha$ -Tricalcium phosphate, T: Tridymite.





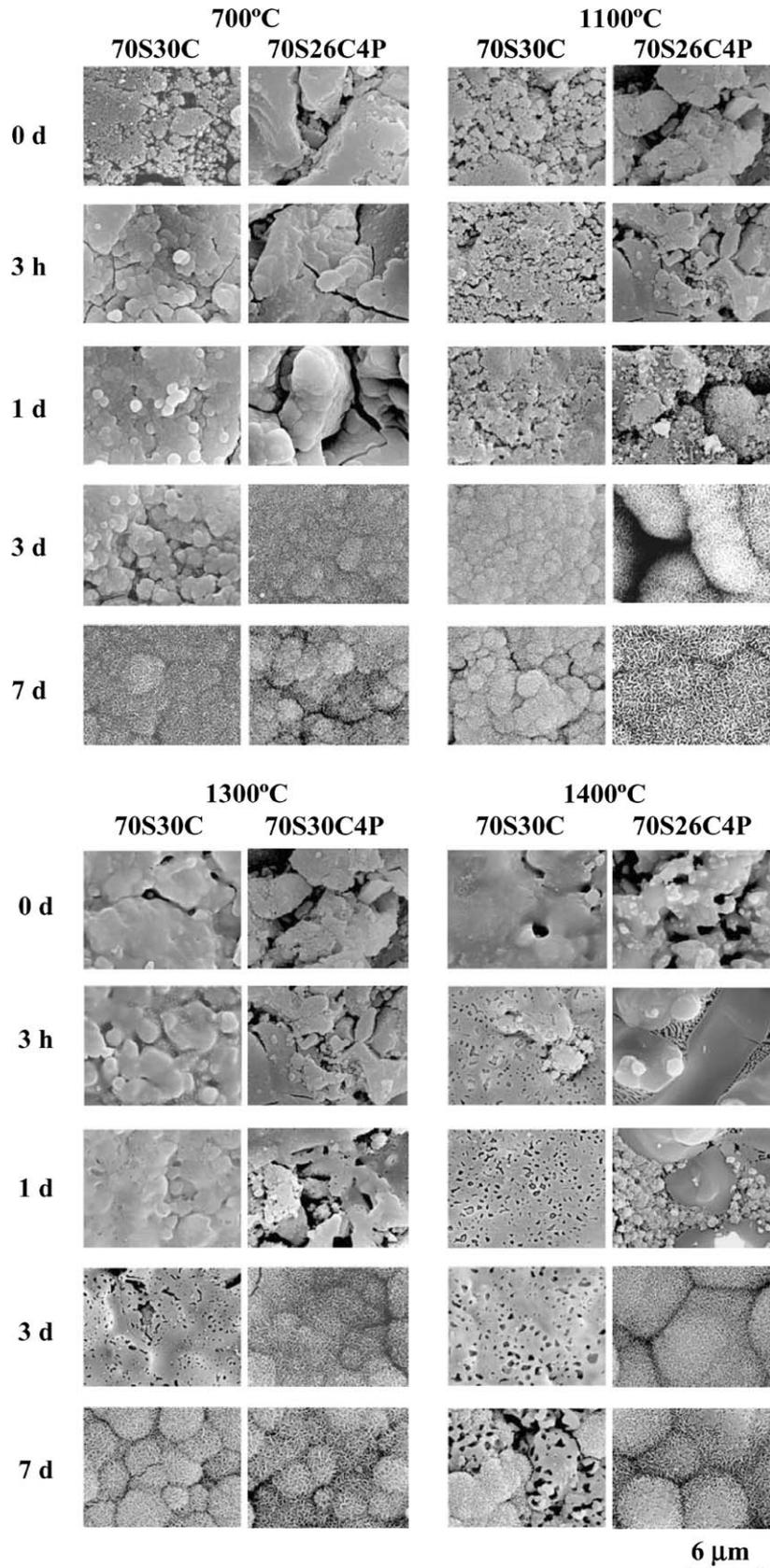


Fig. 5. SEM micrographs of samples 70S30C and 70S26C4P heated at different temperatures before (0d) and after soaking in SBF for 3 h, 1 day, 3 days and 7 days.



Table 4  
Flexural strength,  $S$ , and Weibull coefficient,  $m$ , of samples calcined at 1100°C

	$S$ (MPa)	$m$
<b>70S30C-1100</b>	$31 \pm 3$	12,5
<b>70S26C4P-1100</b>	$21 \pm 5$	4,0

of the thermal treatment, the crystallization of a higher number of phases (Ap, SiP, Q, T) than in samples **70S30C**. In addition, the presence of phosphorus stabilized the W-phase that was the only phase of  $\text{CaSiO}_3$  observed in this sample, until thermal treatments at 1300°C, and PsW was not present until 1400°C. On the contrary, in samples **70S30C** PsW was the main phase from 900°C and a total transformation of W into PsW occurred at 1300°C. This fact was also observed in a previous work, in samples containing the same amount of phosphorus but lower  $\text{SiO}_2$  content (**55S41C4P**: 55% $\text{SiO}_2$ -41% $\text{CaO}$ -4% $\text{P}_2\text{O}_5$ , mol%) [13].

On the other hand, in samples containing phosphorus the crystallization of  $\text{SiO}_2$  (Q) occurred at lower temperatures (900°C) and at 1400°C the polymorphs present were T and Ct, whereas in samples without phosphorus the only  $\text{SiO}_2$ -phase observed was Ct and it occurred at 1300°C.

Comparing the phase composition of samples **70S26C4P** with those observed in samples with the same phosphorus content previously studied (**55S41C4P**) [13], it was observed that in the latter the number of crystallized phases was lower than in sample **70S26C4P**, since that phases Ap, SiP, Q and T were not observed. These results suggest that the influence of phosphorus in the phase's crystallization increases with the  $\text{SiO}_2$  content.

It is worth noting that in samples with higher silicon content, silicon pyrophosphate was detected by XRD. The presence of this phase has not been observed in melted glasses in the system  $\text{Na}_2\text{O}$ - $\text{CaO}$ - $\text{SiO}_2$ - $\text{P}_2\text{O}_5$  [28], where phosphorus remains as phosphate in the amorphous phase. This fact could be related with the route to incorporating phosphorus. Another interesting result is the formation of apatite in samples with higher silicon content, whereas in those with lower silicon content tricalcium phosphate was formed. This could be related with the presence of OH groups in the gels, needed to form apatite. Taking in to account that the OH content directly depends on the  $\text{SiO}_2$  content, the number of hydroxyl groups must be higher in samples containing 70 mol% of  $\text{SiO}_2$ , which would favour the hydroxyapatite crystallization. OH content decreases with increasing temperatures, and the hydroxyapatite transforms to tricalcium phosphate.

Regarding the mechanical properties, it was observed that the flexural strength,  $S$ , and the Weibull coefficient,

$m$ , (Table 4) were higher in samples without phosphorus than in samples **70S26C4P**. The Weibull coefficient is related with the defects distribution that induces breakage of the specimens. A comparatively low Weibull coefficient points out a heterogeneous distribution of defects. These results show that the presence of phosphorus provokes a non-homogeneous distribution of defects and therefore lower mechanical properties.

The bioactive behaviour of samples was also influenced by the phosphorus content. In this sense, it was observed that the layer formation rate in this glass-ceramics was higher in samples containing phosphorus. In addition, although in the glasses treated at 700°C the formation of the apatite layer was observed at the same time in both samples, the needle like aggregates were formed faster in samples containing phosphorus.

The positive effect on the bioactivity observed in samples containing phosphorus must be related with the crystallization of calcium phosphates that could act as nucleation agents increasing the kinetics of new layer formation. This effect could not be related with other crystallized phases because in this samples (**70S26C4P**) the crystallization of  $\text{SiO}_2$ - and W-phases, instead of PsW, would induce an adverse effect on the bioactivity and not the contrary. Although most of the bioactive glass-ceramics reported contain phosphorus in their compositions [9,29], on the basis of the results obtained in the present work, we can state that the presence of phosphorus is not necessary for a bioactive behaviour, although the presence of this element, even in a small degree, increases the bioactivity.

Leaving aside the phosphorus influence on the bioactivity, other aspects influencing the bioactive behaviour were also observed. One of them is the PsW content in the samples, since this is the most soluble phase present in the crystallized samples. In a previous work [13] it was shown that changes in the  $\text{Ca}^{2+}$  concentration and pH of SBF and, therefore, the apatite layer formation rate, were related with the amount of PsW. This fact was clearly observed in samples **70S26C4P** where the changes in the SBF were higher for the sample treated at 1400°C, which contains PsW, in spite of the sintering process.

The sintering process played an important role on the bioactivity in heated samples **70S30C**, where the PsW-phase was the main phase. It was observed that the ionic changes in the SBF and the layer formation rate decreased with the sintering temperature; although samples calcined at 1300°C had the highest PsW content. In these samples, the sintering process (Fig. 5) seems to hinder the dissolution process of PsW, mainly from the bulk, and therefore delays the formation of the apatite layer. The great decrease in the bioactivity observed for the sample treated at 1400°C could be related, besides the sintering process, to the large

crystallization of Ct, which is less reactive as observed by EDS after 7 days (Table 3).

In sample **70S26C4P-1400** the Ct content was as high as in sample **70S30C-1400**; in the former, however, the increase of  $\text{Ca}^{2+}$  and pH was higher. This could be attributed to its surface morphology, in which many holes of considerable size were observed (Fig. 5) making it more reactive.

Another interesting result was observed in sample **70S26C4P-1400** where the segregation of phases took place. It was observed that the growth of the apatite layer occurred preferentially on the phosphorus and P<sub>5</sub>W-rich areas, and at higher times the layer grew and covered the less reactive zones, corresponding with SiO<sub>2</sub>-phases.

## 5. Conclusions

Glasses and glass-ceramics were obtained by thermal treatment of gels with compositions 70SiO<sub>2</sub>-30CaO and 70SiO<sub>2</sub>-26CaO-4 P<sub>2</sub>O<sub>5</sub> (mol%). The presence of phosphorus in the raw glasses induced the crystallization of a higher number of phases, stabilized the wollastonite-phase at high temperature, and induced the crystallization of calcium phosphate (apatite and tricalcium phosphate) and SiO<sub>2</sub>-phases at low temperatures.

The bioactivity of samples was related with the phosphorus content. This element was not indispensable for the bioactivity, but the apatite layer formation rate was higher when phosphorus was present in the composition. The phosphorus content produces a heterogeneous distribution of defects in the samples, therefore worsening their mechanical properties.

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