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A novel hybrid material with Calcium and Strontium release capability.

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Abstract

The preparation of PDMS-TEOS-CaO hybrid materials by sol-gel techniques has been widely described in previous works. Calcium nitrate is the most common source of calcium used in these preparations. However, to remove possible toxic nitrate by-products a thermal treatment is necessary at temperatures above 500 ºC, which leads to the degradation of the polymeric components of the hybrids. Strontium has already shown some promising results in the therapeutic area, being used in cases of osteoporosis and low bone density. In this study a new potential bioactive hybrid material was prepared, by sol-gel techniques, using calcium acetate as a novel calcium source. Also, for the first time, incorporation of strontium in a PDMS-TEOS hybrid system was evaluated. Samples were characterized before and after immersion in Kokubo's Simulated Body Fluid (SBF) by SEM, EDS, ICP and FT-IR spectroscopy.

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1. Introduction

The Sol-gel process is a useful method for the preparation of pure inorganic and pure organic/inorganic hybrid materials with controlled composition. Nowadays Polydimethylsiloxane-Tetraethylorthosilicate (PDMS-TEOS) hybrid systems are being investigated as bone formation promoter materials and as drug delivery systems. They are also used for the preparation of scaffolds for cell's adhesion and proliferation. [1, 2]

Although other sources of calcium for hybrid organic/inorganic materials are under research [3, 4], the most common source of calcium in osteoinductive biomaterials is still calcium nitrate. However, nitrates have been referred as potentially harmful due to toxicity issues related to the formation of nitrate derived by-products.[5, 6] To prevent this, a thermal step using temperatures above 500º C is essential, leading to thermal degradation of the materials prepared.

Strontium has recently showed promising results as a bone formation promoter, being involved in the bone remodeling process, reducing osteoclast activity and enhancing the replication of osteoblasts. It is also used in the form of strontium ranelate to increase the densification of bone in osteoporotic patients. [7, 8]

2. Experimental Procedure

2.1 Sample preparation

Tetraethylorthosilicate (TEOS, Sigma-Aldrich), polydimethylsiloxane (PDMS, Sigma-Aldrich) silanol terminated (550 g mol-1 average molecular weight), calcium acetate monohydrate ($Ca(CH_3CO_2)_2.H_2O$, Sigma-Aldrich) and strontium acetate ($Sr(CH_3CO_2)_2$, Sigma-Aldrich), were used as raw materials for the preparation of the hybrids. The composition of the materials, in molar ratio, and the samples notation are shown in Table 1. Samples were named "CaxSry" being x= $Ca(CH_3CO_2)_2.H_2O$/TEOS and y = $Sr(CH_3CO_2)_2$/TEOS, both in units of 1/100 mol.

Table 1

Experimental procedure was performed by adding TEOS, PDMS and H_2O to either $Ca(CH_3CO_2)_2.H_2O$ or $Sr(CH_3CO_2)_2$ dissolved in H_2O. Afterwards, isopropanol was added and the medium was acidified with HCl.

The prepared solutions were then stirred for 5 hours.

Mixtures were aged for 24 h at room temperature and placed in an oven at 60 ºC during a week for gelation. After that the obtained gels were dried at 150 ºC for 24h.

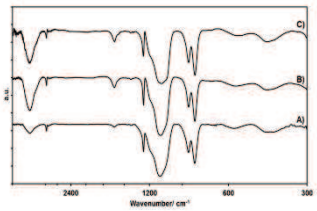
2.2 Samples characterization

Samples dried at 150 ºC were investigated by FT-IR spectroscopy, SEM and EDS. FT-IR spectra were recorded with a 4.0 cm^{-1} resolution in the 350-4000 cm^{-1} range. Structure and elemental analysis were performed by SEM (Hitachi SU-70 and S4100) and EDS (Rontec) with an accelerating voltage of 25 kV.

Calcium-phosphates precipitation and strontium release were evaluated *in vitro* by immersion of the produced materials in SBF for 3 and 7 days. Surface of the samples after soaking was observed by SEM and analysis of the immersion fluid was performed by ICP (Jobin–Yvon JY70 Plus spectrometer).

3. Results

3.1 Structure of the hybrids

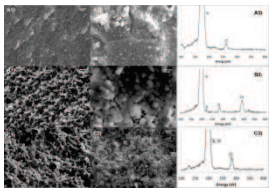


All samples exhibit infrared bands in the 569-3469 cm^{-1} region, which were previously reported by other authors as being present in hybrid materials of the PDMS-TEOS system. [9, 10]
The presence of bands at ca. 560 cm^{-1}, 802 cm^{-1} and 1070 cm^{-1} are assigned respectively to Si-O-Si vibration in 4-fold siloxane rings, symmetric stretching and asymmetric stretching

modes.[11, 12] Bands obtained at *ca.* 414 cm^{-1} and *ca.* 847 cm^{-1} have been assigned to hybrid SiO_2 (Q units) – PDMS (D units) structures [10, 13] and reported as $D_{(Q)}$ units.[14] Bands related with the presence of organic PDMS groups (1263 and 2964 cm^{-1}) and with the presence of O-H groups (1620 and 3402 cm^{-1}) were detected.

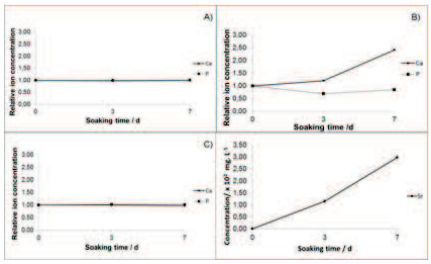
3.2 Formation of Calcium-phosphate aggregates

Samples dried at 150 ºC were observed by SEM before and after immersion in SBF. In fig. 2 images of the samples before soaking and after 7 days of immersion in SBF are presented. The respective EDS spectra are also shown.



After soaking of the samples in SBF for 7 days the presence of calcium phosphate aggregates on the surface of Ca10Sr0 is clearly detected. In samples without calcium in their composition (Ca0Sr0 and Ca0Sr10) no aggregates were observed.

After immersion in SBF for 7 days EDS analysis shows the presence of Si and Cl (due to the HCl used). Sample Ca10Sr0 presented peaks corresponding to Ca and P confirming the precipitation of calcium phosphate aggregates. EDS peaks of Si and Sr are overlapped in Ca0Sr10 spectrum.



ICP results shows that variation of Ca or P concentration in the SBF medium (relative to the concentration of these elements in the initial SBF solution) did not occur for samples without calcium acetate. Release of Ca ions from sample Ca10Sr0 is observed after 3 days of

immersion, increasing through time. Also a slight decrease in the concentration of P in SBF is observed for this sample.

ICP analysis of Ca0Sr10 confirmed the release of Sr to the medium after 3 days of immersion.

4. Discussion

The synthesis of hybrid materials in this study was confirmed by the presence of bands at 414 and 847 cm^{-1} in the FT-IR spectra of the prepared samples.

Surprisingly no C-O vibration modes, common in acetate based materials, were detected by FT-IR. The authors suggest that the highly acidic medium used in the synthesis of the materials could lead to the protonation of the carboxylic group of calcium and strontium acetates and to the formation of acetic acid. The acetic acid reacts with the excess 2-propanol present in the solution originating an ester (2-propyl ethanoate), which has a boiling point lower than the temperature at which the materials were subjected.[15]

The results obtained by SEM, EDS and ICP are in agreement with the accepted mechanism of formation of calcium phosphates in calcium-containing sols produced at room temperature.[16] Samples Ca0Sr0 and Ca0Sr10 do not contain Ca in their composition and so the concentration of this element in the SBF solution does not reach the threshold necessary to induce calcium phosphates crystallization. However, samples that contain calcium released it to the SBF solution over time, increasing its ionic concentration and leading to the precipitation of calcium-phosphate. Calcium-phosphate is not homogeneously deposited, being dispersed through the sample's surface in the form of aggregates.

It must be noticed that although SBF solution has been used to evaluate the capability of the materials synthetized to form calcium-phosphate, it presents several limitations as a technique to evaluate the material's bioactive potential.[17, 18] Strontium ionic concentration in the SBF medium increases during time, achieving a concentration of 116 $mg.L^{-1}$ after only 3 days and 298 $mg.L^{-1}$ at 7 days . The amount of Sr present is well above the minimum value that, according to several authors, can induce osteoblasts activity and inhibit the osteoclasts bone resorption but much lower than the one reported as cytotoxic.[19, 20]

5. Conclusion

In the present work hybrid materials with incorporation of Ca and Sr were successfully obtained by sol-gel procedures.

In vitro deposition of calcium-phosphate aggregates occurred for the samples containing calcium acetate. The preparation of a hybrid material with the capability to form calcium-phosphate precipitates, using a new non-toxic calcium source, was achieved.

Strontium release was observed for the samples prepared with strontium acetate, being the concentrations at 3 and 7 days within the adequate range to induce osteoblasts activity and inhibit osteoclasts related bone resorption.

To the authors' knowledge the work that is being developed reports for the first time the synthesis of a hybrid material from a non-toxic calcium source, exhibiting a strontium release capability that is within the therapeutic doses required for clinical applications as promoter of osseointegration.

Acknowledgments

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References

[1] Prokopowicz M. Correlation between physicochemical properties of doxorubicin-loaded silica/polydimethylsiloxane xerogel and in vitro release of drug. Acta Biomater. 2009;5:193-207.

[2] Fernandez MR, Casabona MG, Anupama VN, Krishnakumar B, Curutchet GA, Bernik DL. PDMS-based porous particles as support beds for cell immobilization: Bacterial biofilm formation as a function of porosity and polymer composition. Colloids and Surfaces B-Biointerfaces. 2010;81:289-96.

[3] Prokopowicz M, Zeglinski J, Gandhi A, Sawicki W, Tafail SAM. Bioactive silica-based drug delivery systems containing doxorubicin hydrochloride: In vitro studies. Colloids and Surfaces B-Biointerfaces. 2012;93:249-59.
[4] Yabuta T, Bescher EP, Mackenzie JD, Tsuru K, Hayakawa S, Osaka A. Synthesis of PDMS-based porous materials for biomedical applications. J Sol-Gel Sci Techn. 2003;26:1219-22.
[5] Ellis G, Adatia I, Yazdanpanah M, Makela SK. Nitrite and nitrate analyses: A clinical biochemistry perspective. Clin Biochem. 1998;31:195-220.
[6] Hunault CC, Lambers AC, Mensinga TT, van Isselt JW, Koppeschaar HPF, Meulenbelt J. Effects of sub-chronic nitrate exposure on the thyroidal function in humans. Toxicol Lett. 2007;175:64-70.
[7] Marie PJ. Strontium ranelate: New insights into its dual mode of action. Bone. 2007;40:S5-S8.
[8] Ortolani S, Vai S. Strontium ranelate: An increased bone quality leading to vertebral antifracture efficacy at all stages. Bone. 2006;38:19-22.
[9] Chen Q, Miyata N, Kokubo T, Nakamura T. Bioactivity and mechanical properties of PDMS-modified CaO-SiO2-TiO2 hybrids prepared by sol-gel process. J Biomed Mater Res. 2000;51:605-11.
[10] Tellez L, Rubio J, Rubio F, Morales E, Oteo JL. FT-IR study of the hydrolysis and polymerization of tetraethyl orthosilicate and polydimethyl siloxane in the presence of tetrabutyl orthotitanate. Spectroscopy Letters. 2004;37:11-31.
[11] Rubio F, Rubio J, Oteo JL. A FT-IR study of the hydrolysis of tetraethylorthosilicate (TEOS). Spectroscopy Letters. 1998;31:199-219.
[12] Yoshino H, Kamiya K, Nasu H. IR study on the structural evolution of sol-gel derived SiO2 gels in the early stage of conversion to glasses. Journal of Non-Crystalline Solids. 1990;126:68-78.
[13] Babonneau F, Thorne K, Mackenzie JD. Dimethyldiethoxysilane/tetraethoxysilane copolymers: precursors for the silicon-carbon-oxygen system. Chemistry of Materials. 1989;1:554-8.
[14] Iwamoto T, Morita K, Mackenzie JD. Liquid-State Si-29 Nmr-Study on the Sol-Gel Reaction-Mechanisms of Ormosils. Journal of Non-Crystalline Solids. 1993;159:65-72.
[15] Ortega J, Gonzalez C, Pena J, Galvan S. Thermodynamic study on binary mixtures of propyl ethanoate and an alkan-1-ol (C-2-C-4). Isobaric vapor-liquid equilibria and excess properties. Fluid Phase Equilibr. 2000;170:87-111.
[16] Ohtsuki C, Kamitakahara M, Miyazaki T. Bioactive ceramic-based materials with designed reactivity for bone tissue regeneration. J R Soc Interface. 2009;6:S349-S60.
[17] Bohner M, Lemaitre J. Can bioactivity be tested in vitro with SBF solution? Biomaterials. 2009;30:2175-9.
[18] Pan HB, Zhao XL, Darvell BW, Lu WW. Apatite-formation ability - Predictor of "bioactivity"? Acta Biomater. 2010;6:4181-8.
[19] Bonnelye E, Chabadel A, Saltel F, Jurdic P. Dual effect of strontium ranelate: Stimulation of osteoblast differentiation and inhibition of osteoclast formation and resorption in vitro. Bone. 2008;42:129-38.
[20] Erol M, Özyuğuran A, Özarpat Ö, Küçükbayrak S. 3D Composite scaffolds using strontium containing bioactive glasses. Journal of the European Ceramic Society. 2012.

Figure Captions

Fig. 1. Infrared Spectra for the samples prepared. (A) Ca0Sr0 (B) Ca10Sr0 (C) Ca0Sr10.

Fig. 2. SEM-EDS results for the samples immersed in SBF (A1) Ca0Sr0 0 days (A2) Ca0Sr0 7 days (A3) Ca0Sr0 7 days (B1) Ca10Sr0 0 days (B2) Ca10Sr0 7 days (B3) Ca10Sr0 7 days (C1) Ca0Sr10 0 days (C2) Ca0Sr10 7 days (C3) Ca0Sr10 7 days.

Fig. 3. Ca and P relative ion concentration for the samples immersed in SBF at 0, 3 and 7 days (A) Ca0Sr0 (B) Ca10Sr0 (C) Ca0Sr10 (D) Sr concentration in SBF medium.

Table 1 Composition of the samples prepared.

PDMS/TEOS = 0.18, H_2O/TEOS = 5 and HCl/TEOS = 0.225 molar content.

Notation	**Composition (in molar ratio)**	
	$Ca(CH_3CO_2)_2.H_2O$/ TEOS	$Sr(CH_3CO_2)$/ TEOS
Ca0Sr0	0	0
Ca10Sr0	0.10	0
Ca0Sr10	0	0.10

Figure 1 - revised

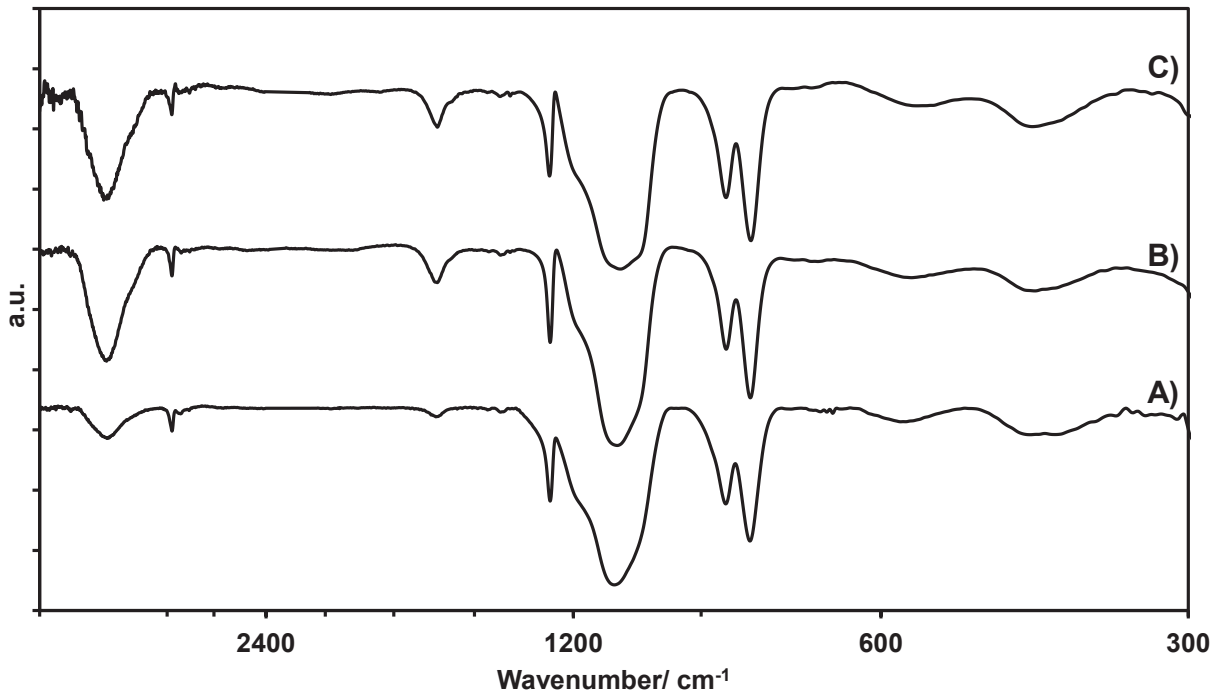


Figure 2 - revised

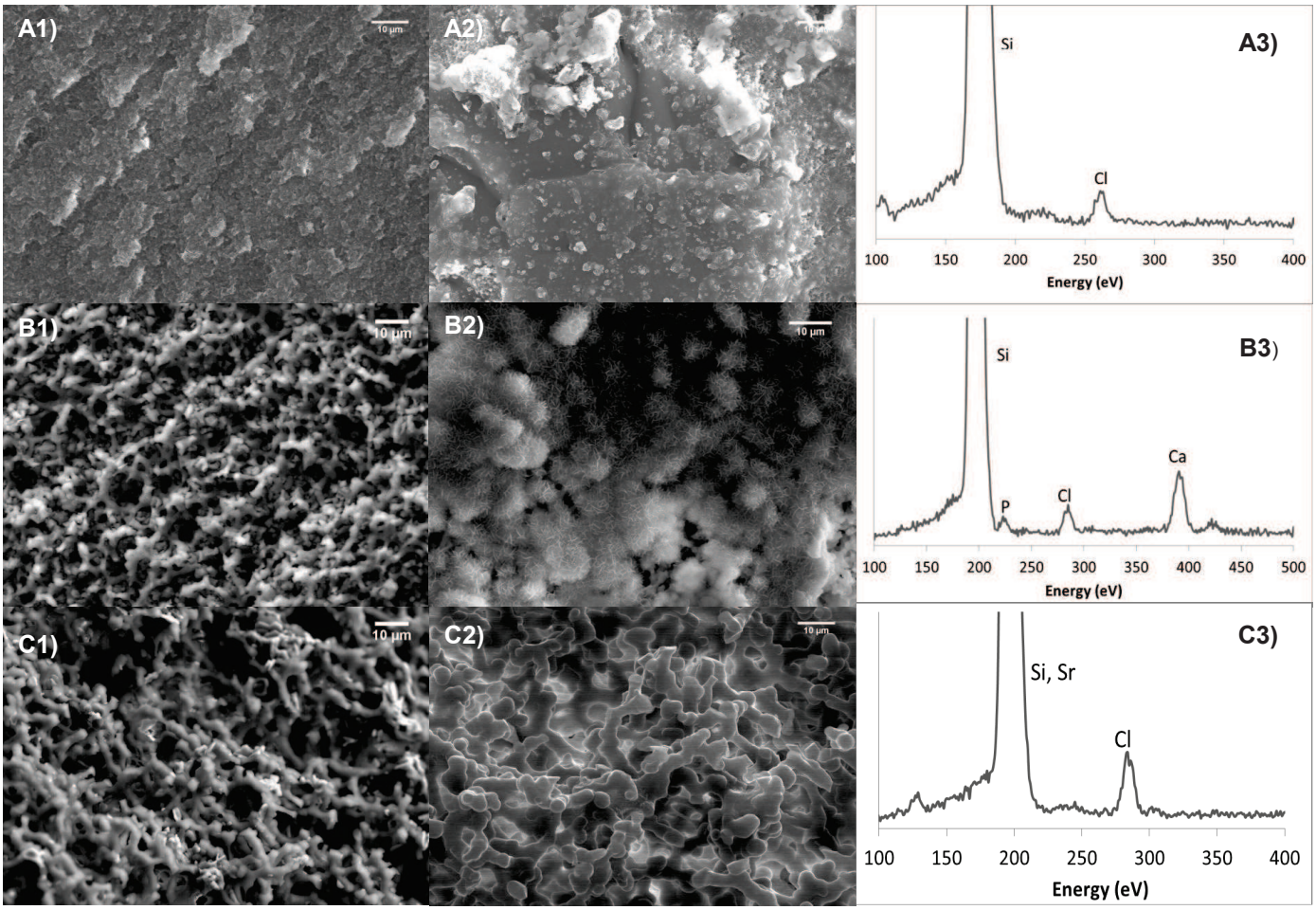
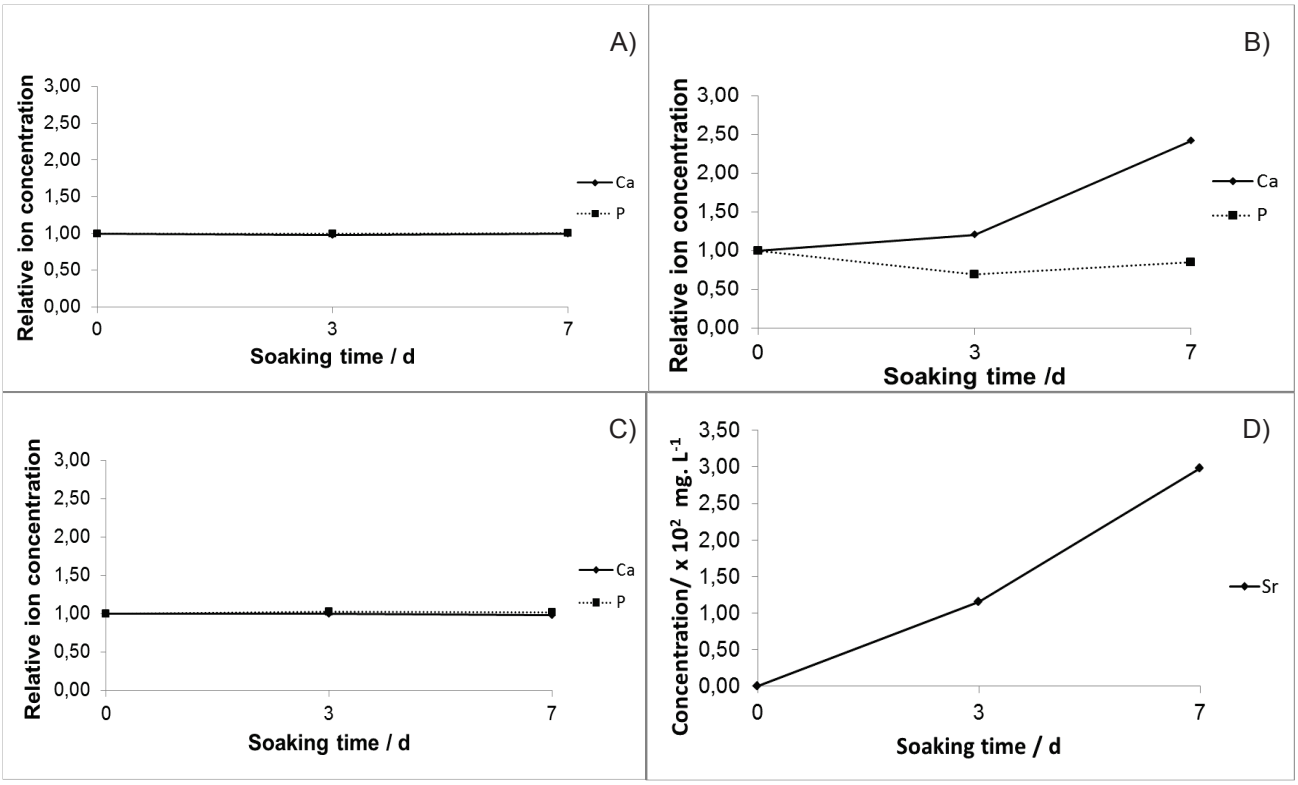


Figure 3


A)
Relative ion concentration
3,00
2,50
2,00
1,50
1,00
0,50
0,00
0
3
7
Soaking time / d
Ca
P
B)
Relative ion concentration
3,00
2,50
2,00
1,50
1,00
0,50
0,00
0
3
7
Soaking time /d
Ca
P
C)
Relative ion concentration
3,00
2,50
2,00
1,50
1,00
0,50
0,00
0
3
7
Soaking time / d
Ca
P
D)
Concentration/ x 10² mg. L⁻¹
3,50
3,00
2,50
2,00
1,50
1,00
0,50
0,00
0
3
7
Soaking time / d
Sr