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Effect of magnesium content on the *in vitro* bioactivity of CaO–MgO–SiO_2–P_2O_5 sol–gel glasses

M. Vallet-Regí,* A. J. Salinas, J. Román and M. Gil

Departamento de Química Inorgánica y Bioinorgánica, Facultad de Farmacia, Universidad Complutense, E-28040 Madrid, Spain

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Three glasses in the system CaO–MgO–SiO_2–P_2O_5, with high silica content (80 mol% SiO_2) and 0, 3 and 7 mol% of MgO, respectively, have been prepared by the sol–gel method. The *in vitro* bioactivity of pellets, prepared by compacting the glass powders, was assessed by determining the changes in surface morphology and composition after soaking in a simulated body fluid (SBF) for up to 14 days at 37 °C. Formation of a calcium phosphate rich layer on the glasses surface was followed by scanning electron microscopy (SEM), X-ray energy dispersive spectroscopy (EDS) and X-ray diffraction (XRD). Three effects of MgO in the glass upon the newly formed layer were detected: (i) it slows down the rate of formation of the layer, (ii) it increases the thickness of the layer and (iii) a whitlockite-like phase appeared together with the apatite-like phase formed on the surface of bioactive materials when exposed to physiological solutions.

Introduction

From the early 1970s when Larry Hench discovered that glasses containing 45% SiO_2, 24.5% CaO, 24.5% Na_2O and 6% P_2O_5 (weight%) form a mechanically strong bond with bone,[1] it has been proven that other ceramics also show this bone bonding ability. Examples include certain compositions of glasses and glass ceramics[2–5] as well as sintered hydroxyapatite.[6] All of them are called bioactive ceramics. Implants made of bioactive materials react chemically with body fluids in a manner compatible with the repair processes of the tissues. Thus, the formation of a fibrous capsule is prevented by the adhesion of the repairing tissues and the failure possibilities of the prostheses decrease.

All bioactive materials form on their surface, when exposed to physiological solutions, an amorphous calcium phosphate layer that crystallises to hydroxycarbonate apatite (HCA). In bioactive silicate glasses and glass-ceramics, a high surface area silica gel layer, formed by partial network dissolution and surface polycondensation reactions, is previously formed. Both layers provide adsorption sites for cellular growth factors generated by macrophages and stem cells.[7]

The recognition that the silica gel layer plays a role in HCA nucleation and crystallisation led to the development, at the beginning of the 1990s by Hench *et al.*[8,9] of a new family of bioactive glasses in the system CaO–P_2O_5–SiO_2, produced *via* sol–gel. *In vitro* studies proved that the increase in the rate of HCA formation, compared with glasses obtained by conventional melt processing, is due to the inherent microporosity and high-surface area of sol–gel glasses. However, in a recent paper it was shown that there was a decrease in the mechanical properties of bone when bone defects were filled with particulates of sol–gel glass instead of with particulates of a melt bioactive glass.[10] Thus, it seems that the high porosity and surface area that enhance the bioactivity of the sol–gel glasses produce a weakening in the mechanical properties of the material. Therefore, material with the high rate of HCA formation of the sol–gel glasses but with improved mechanical properties is of interest for use in bone grafts where certain mechanical requirements are needed.

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. For this reason, in a previous paper[11] we studied the synthesis conditions of sol–gel glasses in the system CaO–MgO–P_2O_5–SiO_2. In all cases, the P_2O_5 content was 4% (mol%) and MgO was included at the expense of the CaO content. In the *in vitro* bioactivity study, soaking the glasses in SBF, proposed by Kokubo *et al.*[12] at 37 °C, an apatite-like layer similar to that of biological apatites was formed for all the compositions.

Our aim in this paper is to study if the presence of MgO, in this family of bioactive glasses, affects the rate of formation of the hydroxycarbonate apatite layer, the thickness of such a layer and the possible formation of secondary phases which compete in stability with the apatite-like phase.

Experimental

(1) Preparation of the glasses

Three glasses, with compositions presented in Table 1, were prepared by hydrolysis and polycondensation of tetraethyl orthosilicate (TEOS), triethyl phosphate (TEP) and $Ca(NO_3)_2 \cdot 4H_2O$ and/or $Mg(NO_3)_2 \cdot 6H_2O$. 1 M HNO_3 was used to catalyse the TEOS and TEP hydrolysis, using a molar ratio of $(HNO_3+H_2O)/(TEOS+TEP)=8$. The sol was cast into sealed cylindrical Teflon containers. Gelation was carried out at room temperature within 7 days. The gels were aged at 70 °C for 3 days and then dried at 150 °C for 52 h. For the drying the containers lids were replaced by other ones with a hole of 1 mm diameter to prevent gas build up.

Discs of 13 mm diameter and 2 mm height were formed using fractions of 0.5 g of ground dried gels (particle size 32–68 μm) compacted at 55 MPa uniaxial pressure and 150 MPa isostatic pressure, at room temperature. According to the results obtained from the TG/DTA analysis of dried gels, performed in a Seiko thermobalance TG/DTA 320, the discs were stabilised by heating in air at 700 °C for 3 h.

Table 1 Composition of the glasses 80S and 80S3M and 80S7M (in mol%)

Sample	%SiO_2	%P_2O_5	%CaO	%MgO
80S	80	4	16	—
80S3M	80	4	12.9	3.1
80S7M	80	4	9	7

(2) *In vitro* tests

In vitro tests were carried out by soaking the stabilised discs of glass, vertically mounted in a special platinum scaffold, in 45 ml of SBF at 37 °C, in sterilised polyethylene containers. Previously, SBF solution was filtered with a 0.23 μm Millipore System, and all operations/manipulations were carried out in a laminar flux cabinet to avoid microorganism contamination. Solutions were renewed every 60 h.

The *in vitro* bioactivity of the sol–gel glasses 80S, 80S3M and 80S7M was evaluated by studying the variations of ionic concentrations of the solution with time, and the changes in the morphology, composition and crystalline phases formed on the surface of the discs, after soaking in SBF for times ranging from 0 to 14 days.

Ca concentration in solution and pH were measured using an ILyte $Na^+K^+Ca^{2+}$ pH system. P and Mg concentrations were determined for complex formation using a Beckman DU-7 UV-VIS spectrophotometer.

The glass discs were taken out of solution at 1, 3, 7 and 14 days and the surface of pieces were studied by X-ray diffraction (XRD) and scanning electron microscopy coupled with energy dispersive spectroscopy (SEM–EDS) analysis. The XRD study was performed on a Philips X'Pert MPD diffractometer, using Cu-Kα radiation. SEM–EDS analyses were made using a JEOL 6400 microscope-LINK AN10000 System.

Results

An XRD study of as-received samples confirmed that all of them were amorphous, since no diffraction peaks were detected, an SEM study showed that the surface of the discs was formed by irregular aggregates with very diverse sizes and the analysis by EDS showed good agreement with the nominal composition of the glasses given in Table 1. After soaking the discs in SBF, changes in both the ionic concentration of solution and the surface of the pieces of glass were observed.

(a) Changes in SBF composition

Fig. 1 shows the cumulative variation of Ca, P and Mg ionic concentrations and variation of pH with the soaking time in SBF for samples 80S, 80S3M and 80S7M. As can be observed in all cases, P concentration in the solution increased during the first hours of soaking and then decreased continuously. pH Variation with time was also similar for the three samples, increasing up to 7.9 during the first 4 days of immersion and then no significant change took place. However, variation of Ca and Mg concentration in SBF was different for each composition of glass. For 80S, Ca concentration reached a maximum after 4 days of soaking in SBF and did not change significantly until the end of the test, while for 80S3M and 80S7M Ca and Mg concentrations, after a maximum at 4 days, gradually decreased up to 14 days. As is observed, the lower the Ca content is in the glass composition, the lower the increase of Ca^{2+} concentration in the SBF.

(b) Changes in the surface of the glasses

After soaking in SBF, the surface of the discs (80S, 80S3M and 80S7M) was covered by a layer formed of spherical-shaped particles.

Fig. 2 shows the SEM micrographs of 80S, 80S3M and 80S7M after 7 days in SBF. The XRD patterns (10 to 70° 2θ) of samples are also included. As can be observed, the spherical particles are formed by numerous needles and the size of such particles decreases when the MgO content in the glass increases (1 μm for 80S7M and 10 μm for 80S).

The XRD pattern of the glass 80S, after 7 days soaking in SBF, shows several diffraction maxima at 26, 32, 39.8, 46.7, 49.4 and 53.1° 2θ that correspond, respectively, to the reflections (002), (211), (310), (222), (213) and (004) of an apatite phase. For the samples 80S3M and 80S7M, in addition to the maxima mentioned, a new group of diffraction maxima at 28, 31.2, 34.7 and 53.1° 2θ was observed that can be assigned to the reflections (214), (0 2 10), (220) and (330) of a whitlockite phase.

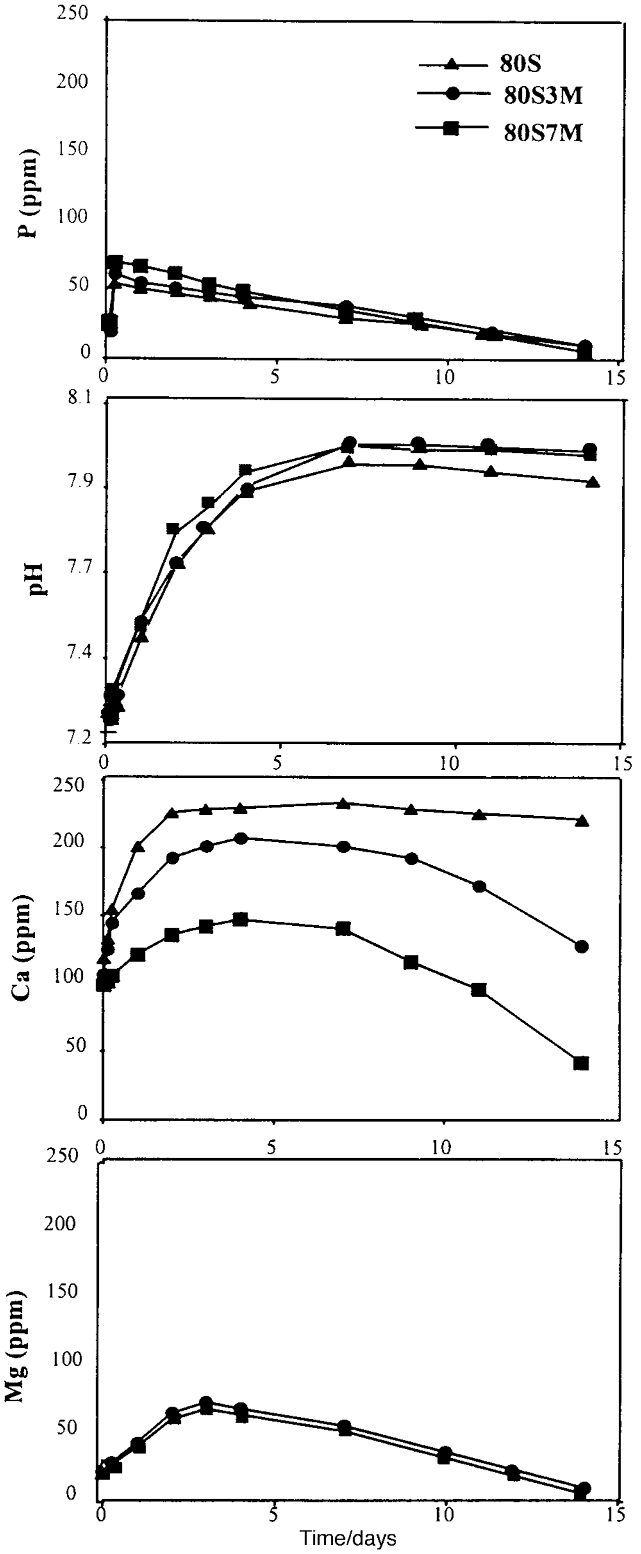

Fig. 1 Variation of pH and phosphorus, calcium and magnesium concentration (ppm) in the SBF with soaking time, for samples 80S, 80S3M and 80S7M.

In Fig. 3, the SEM micrographs of tilted cross sections of 80S and 80S3M after 7 days in SBF are presented. The EDS

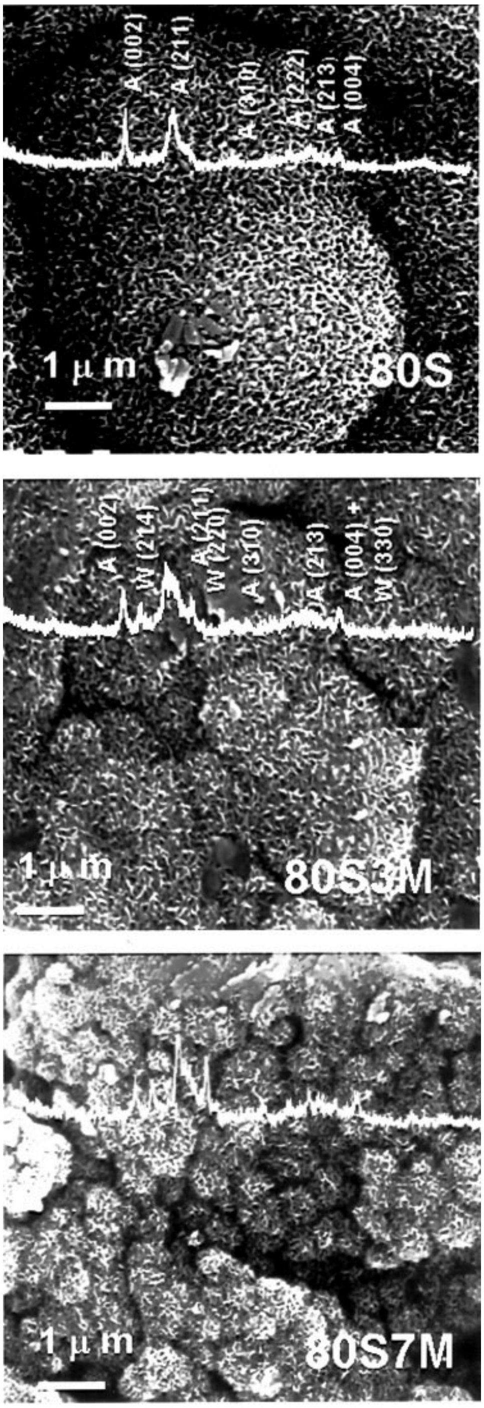

Fig. 2 SEM micrographs and XRD patterns (10 to 70° 2θ) of 80S, 80S3M and 80S7M samples after 7 days in SBF (A = apatite, W = whitlockite).

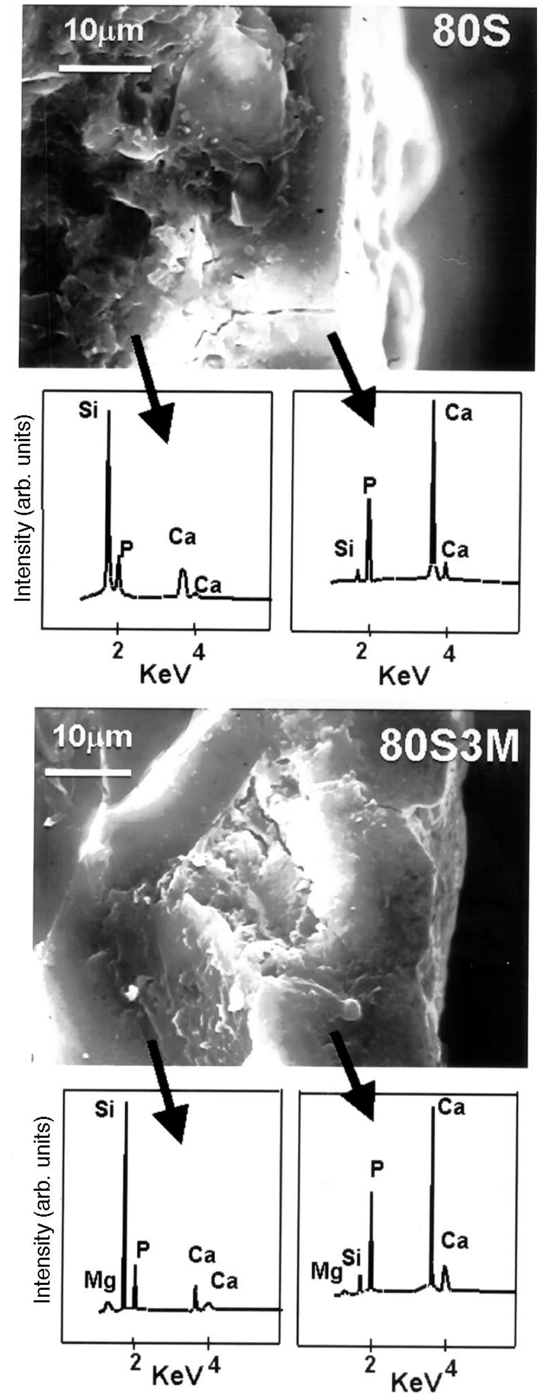

Fig. 3 SEM micrographs and EDS profiles of the tilted cross section of glasses 80S and 80S3M, after 7 days in SBF.

profiles of the inner part of the glasses and the newly formed layer are also included. In both samples, high concentrations of Ca and P were observed in the layer, and a small amount of Mg was also detected. For the 80S sample the layer thickness was about 10 μm and no significant increase was observed after 14 days in SBF. However, for samples 80S3M and 80S7M, the layer thickness was about 15 μm after 7 days in SBF and 30 μm after 14 days in SBF.

Discussion

The observed variations with time of the pH and the calcium concentration in SBF, for the 80S sample, are in agreement with the nucleation and growth mechanism of an apatite-like layer on Na_2O– or $CaO–SiO_2$ bioactive glasses, proposed by Hench and Kokubo.[7,13]

For samples 80S3M and 80S7M the Ca^{2+} release to the SBF is lower than that observed for sample 80S as a consequence of the lower content of CaO in these glasses. However, observed variations of pH with time are analogous for all the samples. This could be explained taking into account that for glasses containing magnesium, a leaching of Mg^{2+} takes place together with the Ca^{2+} release to the SBF (Fig. 1). Thus, for samples 80S3M and 80S7M, interchange between the Mg^{2+} of the glass and the H_3O^+ of the solution simultaneously with that of Ca^{2+} can be proposed. The decrease in Ca^{2+} and Mg^{2+} concentrations in the SBF after reaching maximum values indicates that such ions could be incorporated in the layer formed on the surface of the glasses.

Results obtained by SEM–EDS showed that a calcium phosphate rich layer, formed of spherical particles, fully covered the glasses surface when soaking in SBF. An XRD study confirmed that such a layer corresponded to an apatite phase in 80S, and to apatite and whitlockite phases in 80S3M and 80S7M. The higher the MgO content of the glasses the lower is the rate of the calcium phosphate layer formation and the higher is the thickness of this layer.

The retardation in the formation of the layer on the surface of discs 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.[14,15]

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.[16] So, glasses containing MgO, *i.e.* 80S3M and 80S7M, should be less reactive in aqueous solution and the leaching ions rate should be lower. Following the nucleation and growth mechanisms of an apatite-like layer proposed by Hench and Kokubo[7,13] this would result in a lower rate of formation of the layer, as actually occurs.

Regarding the second effect, it has been proven that traces of magnesium ion reduce the overall rate of seeded calcium phosphate crystallisation and markedly delay the transformation of amorphous calcium phosphates to more stable apatite phases.[14,15] This effect also agrees with the experimental results obtained in this paper with the glasses 80S3M and 80S7M, in which a release of Mg^{2+} to the SBF was observed (Fig. 1).

On the other hand, for glasses containing magnesium, an increase in the thickness of the newly formed layer was observed by SEM (Fig. 3). This result agrees with the decrease of Ca^{2+} and Mg^{2+} concentration in SBF after four days of soaking for samples 80S3M and 80S7M. This is indicative of the incorporation of such ions into the layer as was observed by EDS (Fig. 3), and with the presence of a whitlockite-like phase together with the apatite-like phase on the layer detected by XRD (Fig. 2).

To understand the differences observed between glasses with and without magnesium, it must be taken into account that pure calcium-whitlockite, $\beta\text{-}Ca_3(PO_4)_2$, cannot be directly obtained from aqueous solutions. Only magnesium-substituted whitlockites, $\beta\text{-}(Ca,Mg)_3(PO_4)_2$, can be prepared by precipitation from solutions containing Mg^{2+} in a Mg/Ca molar ratio = 0.25–0.5.[17] Thus in sample 80S the rapid release of Ca^{2+} to the solution decreases the Mg/Ca molar ratio favouring the formation of an apatite-like layer as a unique phase present on the layer. However, in samples 80S3M and 80S7M, the lower Ca^{2+} release, a consequence of the lower calcium content of these samples, combined with the Mg^{2+} release, as was shown in Fig. 1, produces an increase in the Mg/Ca molar ratio present in the solution. This could justify the presence of a magnesium-substituted whitlockite-like phase on the layer together with the usual apatite-like layer formed on other bioactive materials.

Conclusion

Three glasses in the system $CaO–MgO–SiO_2–P_2O_5$, with different MgO content have been prepared by the sol–gel method. All of them show *in vitro* bioactivity. However, the presence of magnesium in the composition of the glass has produced the following effects.

1. It slows down the rate of formation of the apatite layer for MgO contents above 7 mol%.
2. In samples containing MgO, a whitlockite-like phase together with the apatite-like phase is present in the newly formed layer.
3. An increase in thickness of the layer was detected for MgO-containing sol–gel glasses.

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References

1 L. L. Hench, R. J. Splinter, W. C. Allen and T. K. Greenlee, *J. Biomed. Mater. Res.*, 1971, **2**, 11.
2 H. Bromer, K. Deutscher, B. Blenke, E. Pfeil and V. Strunz, *Science of Ceramics*, 1977, **9**, 219.
3 T. Kokubo, S. Ito, M. Shigematsu, S. Sakka and T. Yamamuro, *J. Mater. Sci.*, 1985, **20**, 2001.
4 W. Vogel, W. Holand, K. Nauman and J. Gumel, *J. Non-Cryst. Solids*, 1986, **80**, 34.
5 O. H. Anderson, G. Liu, K. H. Karlsson, L. Niemi, J. Miettinen and J. Juhanoja, *J. Mater. Sci: Mater. Med.*, 1990, **1**, 219.
6 M. Jarcho, C. H. Bolen, M. B. Thomas, J. Bobick, J. F. Kay and R. H. Doremus, *J. Mater. Sci.*, 1976, **11**, 2027.
7 L. L. Hench, *J. Am. Ceram. Soc.*, 1998, **81**, 1705.
8 R. Li, A. E. Clark and L. L. Hench, *J. Appl. Biomater.*, 1991, **2**, 231.
9 M. M. Pereira, A. E. Clark and L. L. Hench, *J. Biomed. Mater. Res.*, 1994, **28**, 693.
10 D. L. Wheeler, R. G. Hoellrich, S. W. McLoughlin, D. L. Chamberland and K. E. Stokes, in *Bioceramics*, *10*, ed. L. Sedel and C. Rey, Elsevier, Oxford, 1997, p. 349.
11 M. Vallet-Regí, F. Balas, M. Gil, E. Nogueroles, A. Romero, J. Román, A. J. Salinas and C. V. Ragel, in *Non-crystalline Nanoscale Materials*, *Nanostructured and Non-Crystalline Materials*, ed. J. Rivas and M. A. López-Quintela, World Scientific, Singapore, 1998, p. 55.
12 T. Kokubo, H. Kushitoni, S. Sakka, T. Hitsugi and T. Yamamuro, *J. Biomed. Mater. Res.*, 1990, **24**, 331.
13 T. Kokubo, *An. Quim. Int. Ed.*, 1997, **93**, S49.
14 M. H. Salimi, J. C. Heughebaert and G. H. Nancollas, *Langmuir*, 1985, **1**, 119.
15 W. Kibalcyc, J. Christoffersen, M. R. Christoffersen, A. Zielenkiewicz and W. Zielenkiewicz, *J. Crystal Growth*, 1990, **106**, 355.
16 J. M. Fernández Navarro, in *El vidrio*, Colección Textos Universitarios no. 6, C.S.I.C. Madrid, 1991, p. 140.
17 R. Z. LeGeros, in *Calcium Phosphates in Oral Biology and Medicine. Monographs in Oral Science Vol. 15*, ed. H. M. Myers, Karger, Zurich, 1991, p. 15.

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