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MAGNESIUM INFLUENCE ON BIOACTIVITY OF SILICATE GLASSES PREPARED BY DIFFERENT SOL-GEL ROUTES

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ABSTRACT. Xerogels of SiO_2, SiO_2-CaO, SiO_2-MgO and SiO_2-CaO-MgO compositions were prepared following the acid catalysed and quick alkali mediated sol-gel routes. The samples were characterized by X-ray diffraction, scanning electron microscopy and elemental composition energy-dispersive X-ray spectroscopy both before and after 8 days immersion in simulated body fluid. The results indicate that the calcite formation is favoured on calcium containing samples with up to 15 mol % MgO prepared by quick sol-gel route. Above this concentration magnesium inhibits the calcite formation and implicitly the expected bioactivity.

Keywords: *sol-gel, magnesium, bioactivity*

INTRODUCTION

The sol-gel synthesis method is a low temperature preparation technique largely applied to obtain bioactive glasses [1-4]. Si-Ca-based bioactive glasses are investigated for more than four decades, and the first results were reported by Hench and co-workers [1]. The acid and quick set sol-gel techniques provide accessible way to obtain bioactive glasses [3] which can be used for bone repair or regeneration [5]. The sol-gel route is a synthesis technique based on inorganic polymerisation reactions of metal alkoxide and metal salt precursors [6, 7]. The “quick-set” process was developed [8] to obtain rapid gelation by the pH adjustment of the acid sols with dilute ammonium hydroxide that reduces at room temperature the gelation time from several days to a few minutes.

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Bioactivity is a property of the materials to form a bond with a living tissue [4] and this ability is related to the development of an apatite layer when these materials are immersed in physiological solutions [4,9].

Magnesium is an important element for the human body, and magnesium ions play a significant role in the qualitative changes of bone matrix [10, 11]. Nevertheless further investigations concerning the magnesium ions effect on the bioactivity properties are needed.

In the present study are reported the results related to the bioactivity tested *in vitro*, in simulated body fluid, for six compositions prepared following the acid catalysed and quick alkali mediated sol-gel routes. The main objective was to investigate the magnesium influence on the bioactivity of the synthesized samples.

RESULTS AND DISCUSSION

The structural properties of the xerogel samples (Table 1) were analysed by X-ray diffraction (XRD). The XRD patterns are presented in Figure 1. Before incubation in simulated body fluid (SBF) at 37^{o}C, both acid catalysed (A) and quickly gelled (Q) samples show a predominant amorphous structure denoted in the 2θ range by a broad peak recorded between 15-35°. As can be observed from diffractograms, during SBF immersion calcium carbonate crystals in form of calcite (PDF # 60-7239) were developed on the surface of some samples. Crystalline calcium carbonate can adopt several structures differing in the lattice parameters of the crystal. The atomic structure with the lowest lattice energy, and hence the most stable, is calcite. After 8 days soaking in SBF the results prove that the growth of calcite crystals was not yet achieved on pure SiO_2 samples and on the samples with the highest amount of magnesium, i. e. 20 mol % MgO, whether they were prepared by acid or quick gelation route. At the same time one remarks that up to 15 mol % MgO the addition of magnesium to calcium containing silicate samples brings forward their bioactivity. The self-assembling of calcite occurs among the first stages of bioactivity of glass-ceramics [12]. Concurrently, the presence of carbonates on samples surface would further promote the crystallization of the carbonated hydroxyapatite. Hydroxyapatite has a similar crystal structure and close lattice match to calcite. This structural compatibility is expected to favor nucleation of hydroxyapatite and permit the formation of a coherent, possibly epitaxial, layer of hydroxyapatite on the surface of calcite [13].

For investigation of morphological properties scanning electron microscopy (SEM) images were taken before and after immersion in SBF (Figures 2 and 3). The morphologies depend on the chemical composition and the preparation route of the samples. The glasses prepared by Q route appear more porous in comparison with the samples of similar composition prepared by A route. After SBF immersion clear changes are observed on the samples

surface. The quickly gelled samples present higher bioactivity. Magnesium together with calcium enhances the samples bioactivity but only whether MgO content does not exceed 15 mol % MgO concentration. Higher magnesium content inhibits the calcite formation.

In Figures 2 and 3 are also presented the compositions determined from energy dispersive X-ray spectroscopy (EDS) measurements before and after immersion in SBF. The EDS results support the bioactivity of the matrices.

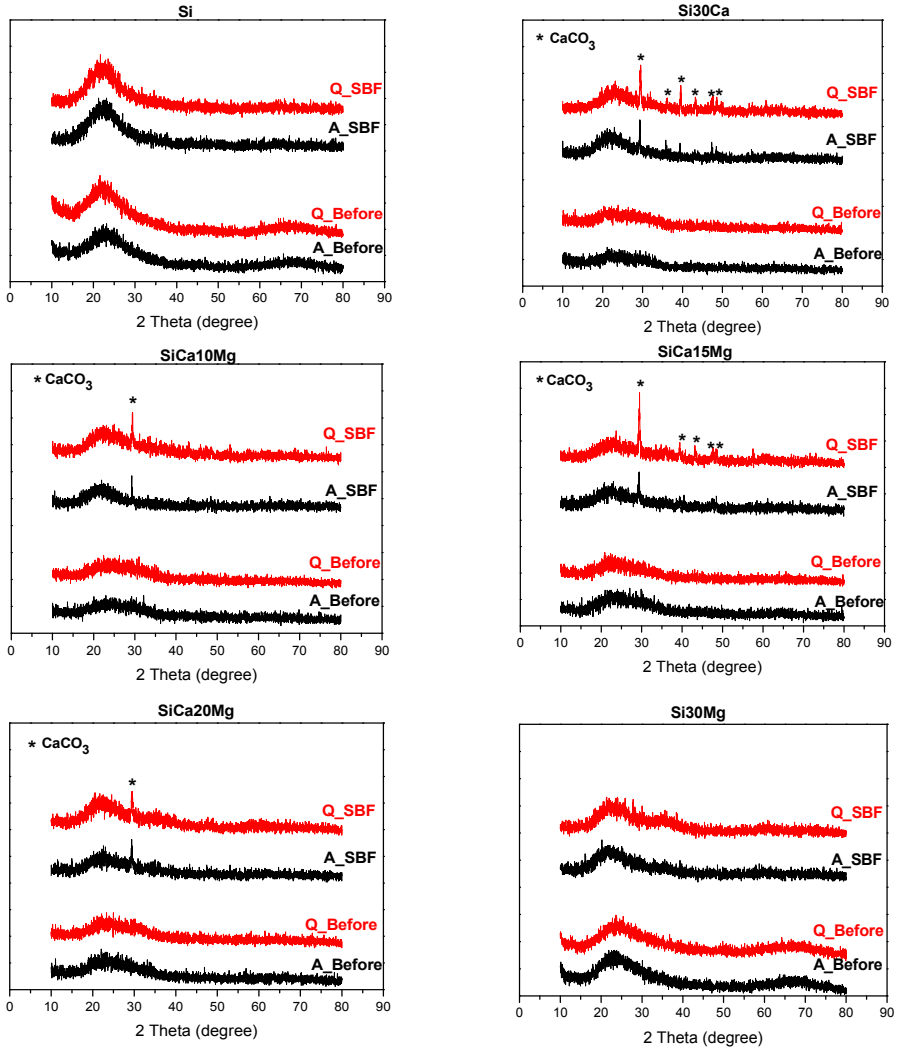
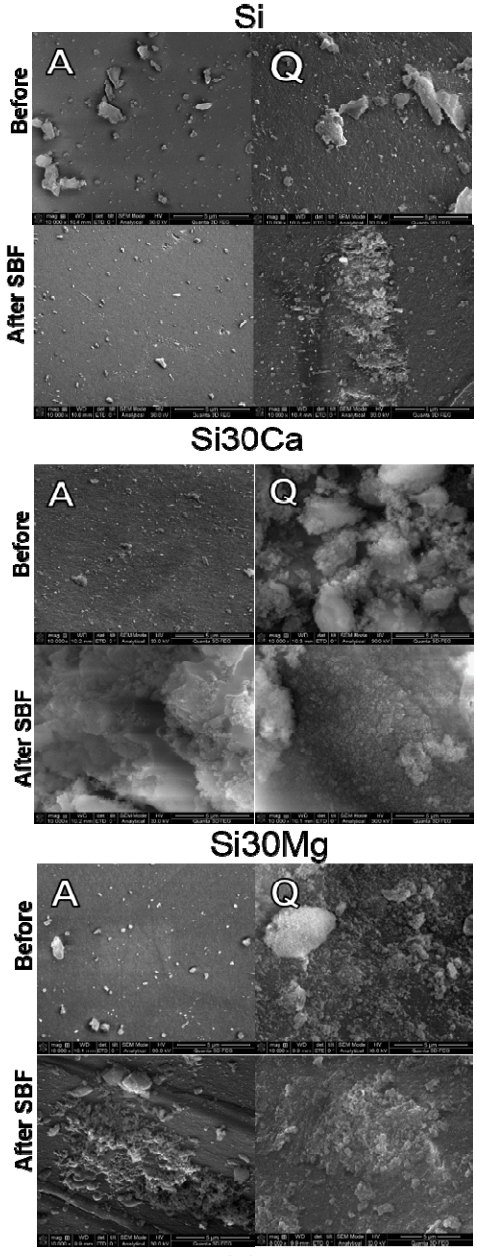
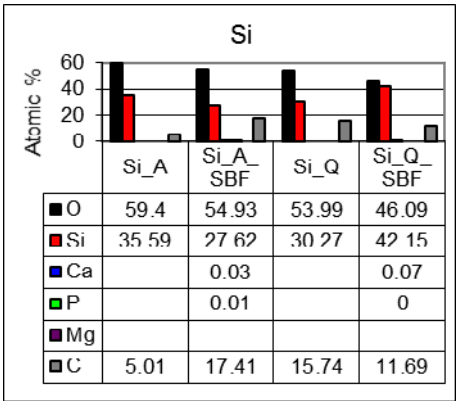
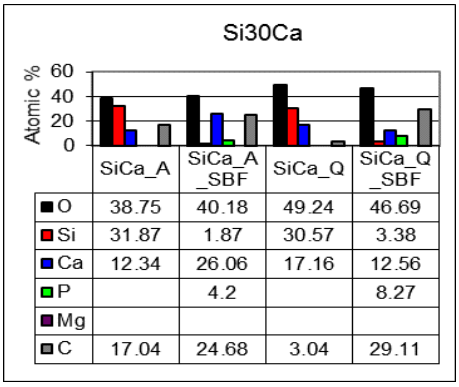


Figure 1. X-ray diffractograms recorded before and after 8 days SBF immersion of the investigated samples

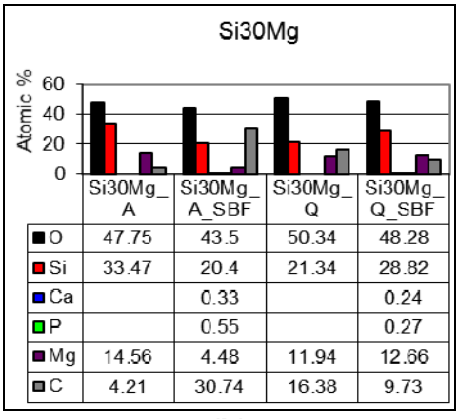




	Si_A	Si_A_SBF	Si_Q	Si_Q_SBF
O	59.4	54.93	53.99	46.09
Si	35.59	27.62	30.27	42.15
Ca		0.03		0.07
P		0.01		0
Mg				
C	5.01	17.41	15.74	11.69



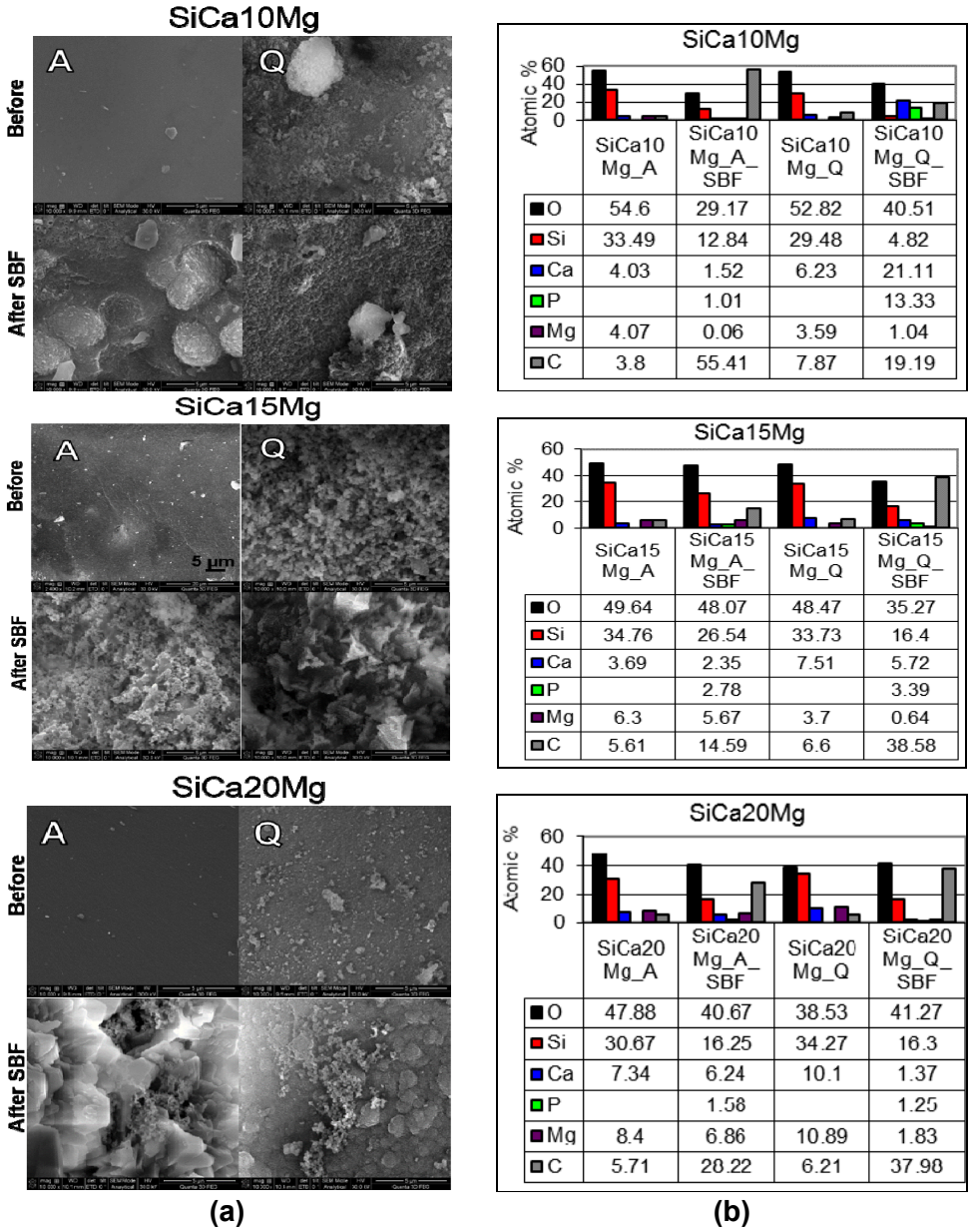
	SiCa_A	SiCa_A_SBF	SiCa_Q	SiCa_Q_SBF
O	38.75	40.18	49.24	46.69
Si	31.87	1.87	30.57	3.38
Ca	12.34	26.06	17.16	12.56
P		4.2		8.27
Mg				
C	17.04	24.68	3.04	29.11



	Si30Mg_A	Si30Mg_A_SBF	Si30Mg_Q	Si30Mg_Q_SBF
O	47.75	43.5	50.34	48.28
Si	33.47	20.4	21.34	28.82
Ca		0.33		0.24
P		0.55		0.27
Mg	14.56	4.48	11.94	12.66
C	4.21	30.74	16.38	9.73

(a) **(b)**

Figure 2. SEM images (a) and EDS results (b) for acid catalysed (A) and quickly gelled (Q) Si, Si30Ca and Si30Mg samples before and after immersion in SBF. Scale bars correspond to 5 μm.



SiCa10Mg

Atomic %

	SiCa10 Mg_A	SiCa10 Mg_A_ SBF	SiCa10 Mg_Q	SiCa10 Mg_Q_ SBF
O	54.6	29.17	52.82	40.51
Si	33.49	12.84	29.48	4.82
Ca	4.03	1.52	6.23	21.11
P		1.01		13.33
Mg	4.07	0.06	3.59	1.04
C	3.8	55.41	7.87	19.19

SiCa15Mg

Atomic %

	SiCa15 Mg_A	SiCa15 Mg_A_ SBF	SiCa15 Mg_Q	SiCa15 Mg_Q_ SBF
O	49.64	48.07	48.47	35.27
Si	34.76	26.54	33.73	16.4
Ca	3.69	2.35	7.51	5.72
P		2.78		3.39
Mg	6.3	5.67	3.7	0.64
C	5.61	14.59	6.6	38.58

SiCa20Mg

Atomic %

	SiCa20 Mg_A	SiCa20 Mg_A_ SBF	SiCa20 Mg_Q	SiCa20 Mg_Q_ SBF
O	47.88	40.67	38.53	41.27
Si	30.67	16.25	34.27	16.3
Ca	7.34	6.24	10.1	1.37
P		1.58		1.25
Mg	8.4	6.86	10.89	1.83
C	5.71	28.22	6.21	37.98

Figure 3. SEM images (a) and EDS results (b) for acid catalysed (A) and quickly gelled (Q) SiCa10Mg, SiCa15Mg and SiCa20Mg samples before and after immersion in SBF. Scale bars correspond to 5 µm.

Excepting the pure SiO_2 quickly gelled sample, phosphorus is detected on all other samples after immersion in SBF. For Si30Ca, SiCa10Mg and SiCa15Mg compositions phosphorus content on the samples quickly gelled by addition of ammonium hydroxide is higher than for the samples which were only acid catalysed. Generally, the magnesium content decreased after immersion in SBF and suggests a release of magnesium ions in simulated body fluid. Carbon presence on all samples before immersion in SBF is primary due to the ubiquitous carbon contamination. After immersion in SBF the carbon content considerably increased, excepting Si_Q and Si30Mg_Q samples, in good agreement with the occurrence of calcite crystallites evidenced by XRD analysis. The values of Ca/P ratio for SiCa10Mg_Q (1. 58) and SiCa15Mg_Q (1. 69) are close to that of hydroxyapatite (1. 67) and denote the enhanced bioactivity potential of these samples, expecting the further nucleation of hydroxyapatite on the surface covered with calcite after 8 days immersion in SBF.

CONCLUSIONS

Pure silica and silicate xerogels with different contents of CaO and MgO acting as glass network modifiers were prepared via acid and quick gellation sol-gel method. The samples treated at 600°C for 6 hours have a predominant amorphous character. After 8 days incubation in simulated body fluid the XRD analysis evidenced the development of calcium carbonate calcite phase on all calcium containing samples. Calcite formation denotes a first stage preceding the nucleation of hydroxyapatite related to samples bioactivity. Scanning electron microscopy and energy dispersive X-ray spectroscopy (EDS) data support the XRD results. In the case of quickly gelled samples the bioactivity indicia are better than for the samples obtained following the one step acid catalysed route. Magnesium inhibits the calcite formation whether MgO content exceeds 15 mol % but up to this concentration the addition of magnesium to calcium containing silicate samples appears to promote their bioactivity.

EXPERIMENTAL SECTION

SiO_2, SiO_2-CaO, SiO_2-CaO-MgO and SiO_2-MgO samples were prepared via sol-gel method following (i) the acid catalysed route (noted A), and (ii) the quick alkali mediated route (noted Q). The compositions of the prepared samples are given in Table 1.

Table 1. The samples symbol related to composition and preparation way.

Symbol	Composition (mol %)
Si_A / Si_Q	100 SiO_2
Si30Ca_A / Si30Ca_Q	70SiO_2·30CaO
SiCa10Mg_A / SiCa10Mg_Q	70SiO_2·20CaO·10MgO
SiCa15Mg_A / SiCa15Mg_Q	70SiO_2·15CaO·15 MgO
SiCa20Mg_A / SiCa20Mg_Q	70SiO_2·10CaO·20 MgO
Si30Mg_A / Si30Mg_Q	70SiO_2·30 MgO

The reagents used for glass synthesis were tetraethoxysilan $SiC_8H_{20}O_4$ (TEOS) supplied by Merck– precursor for SiO_2, Ca $(NO_3)_2$·4 (H_2O) supplied by Lar-Ner, and Mg $(NO_3)_2$·6 (H_2O) supplied by Penta. For preparation of pure SiO_2 samples TEOS, ethanol, distilled water and nitric acid were continuously stirred (pH ~ 2) and the mixture was allowed to react for 30 minutes under continuous stirring for the acid hydrolysis of TEOS. Then a half of the solution was left for gelation (Si_A), and in the other half 2 mL 1 M ammonia was added drop wise (pH ~ 8) under continuous stirring for quick gelation (Si_Q). For the multicomponent samples the precursors of CaO and MgO, i. e. Ca $(NO_3)_2$·4 (H_2O) and Mg $(NO_3)_2$·6 (H_2O), were dissolved in distilled water, and added to the 2 pH TEOS solution under continuous stirring. The samples Si30Ca_A, Si30Ca_Q, SiCa10Mg_A, SiCa10Mg_Q, SiCa15Mg_A, SiCa15Mg_Q, SiCa20Mg_A, SiCa20Mg_Q, Si30Mg_A, Si30Mg_Q were obtained following the same procedure as for pure SiO_2. In case of multicomponent samples obtained by quick gelation 4 ml 1 M ammonia for Si30Ca_Q and Si30Mg_Q, 7. 5 ml 1 M ammonia for SiCa10Mg_Q, 11 ml 1 M ammonia for SiCa15Mg_Q, and 12 ml 1 M ammonia for SiCa20Mg_Q was added. The samples prepared by A route had different gelation times: 2 days Si_A, 5 days Si30Ca_A, 5 days SiCa10Mg_A, 4 days SiCa15Mg_A, 6 days SiCa20Mg_A, and 10 days for Si30Mg_A. The gelled samples were dried for 24 hours at 110°C. The dried gels were calcinated at 600°C for 6 hours.

The structural properties of 600°C treated samples were analysed with a Shimadzu XRD-6000 diffractometer, using Cu Ka radiation (λ= 1. 5418 Å), with Ni-filter with a speed of 2°/ min, in the 10–80° range (2θ). Scanning electron microscopy (SEM) images were taken with a FEI Quanta 3D FEG dual beam microscope for morphological properties investigation. Before SEM imaging the samples were covered with a 5nm gold layer in a Q150R ES rotary pumped sputter coater in argon atmosphere. Chemical analysis of local area was carried out by energy dispersive X-ray spectroscopy (EDS) measurements performed on the same microscope.

In vitro investigation of the calcinated samples was performed in simulated body fluid (SBF) prepared according to Kokubo's protocol [14]. 40 mg of sample were immersed in 10 ml SBF at 37°C and kept under static conditions for 8 days. The SBF was renewed in the fourth day. After SBF immersion the samples were filtered on filter paper and washed three times with bidistilled water and after that dried at 37 ºC in air for 24 hours.

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