



Mechanism of calcium lixiviation in soda-lime glasses with a strong biocide activity

J.S. Moya ^{a,*}, B. Cabal ^a, J. Sanz ^a, A.C. da Silva ^b, S. Mello-Castanho ^b, R. Torrecillas ^c, F. Rojo ^d

^a Instituto Ciencia de Materiales de Madrid (ICMM-CSIC), Cantoblanco, 28049 Madrid, Spain

^b Instituto de Pesquisas Energéticas e Nucleares (IPEN-CNEN), SP-CP 11049, São Paulo, Brazil

^c Centro de Investigación en Nanomateriales y Nanotecnología (CINN), Consejo Superior de Investigaciones Científicas (CSIC), Universidad de Oviedo (UO), Principado de Asturias, Parque Tecnológico de Asturias, 33428 Llanera (Asturias), Spain

^d Centro Nacional de Biotecnología (CNB-CSIC), Cantoblanco, 28049 Madrid, Spain

ARTICLE INFO

Article history:

Received 25 August 2011

Accepted 25 November 2011

Available online 2 December 2011

Keywords:

MAS-NMR spectroscopy

Calcium lixiviation

Biocide activity

Soda-lime glasses

ABSTRACT

In a recent work, it has been proved that soda-lime glasses, belonging to the $\text{SiO}_2\text{-Na}_2\text{O-CaO-B}_2\text{O}_3$ system with a high content of calcium oxide (15 to 20 wt.%), are efficient biocides against Gram positive, negative bacteria and yeast. In the present work we discuss the possible mechanism responsible for the biocide action of these series of glasses against *Escherichia coli* microorganisms. The local structure of these glasses has been investigated by MAS-NMR spectroscopy. The importance of the strength with which calcium is retained in the glass structure has been pointed out. This strength decreases as the condensation degree of tetrahedral in the glass structure decreases, as a consequence the biocide activity also increases.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Soda-lime glasses are constituted by silica tetrahedron segments, where differences are expressed in terms of the coordination number and polyhedra condensation. In glasses, local structure is defined by polyhedra condensation of tri or tetravalent cations (former cations), the monovalent or divalent cations contributing to decrease polyhedra condensation (modifier cations) [1–5]. In the case of Si^{4+} tetrahedra, different tetrahedral Q^n species (n bridging oxygens), are due to the depolymerization induced by alkali or alkali earth [Na^+ or Ca^{2+}] cations. In the case of B^{3+} cations, tri and fourfold coordinations can be produced, increasing the amount of tetrahedrally coordinated cations with the incorporation of monovalent or divalent cations. In these glasses, alkali earth cations (R^{2+}) promote the silica tetrahedra interconnection through non bridging oxygens (NBO). In this case, R^+ or R^{2+} cations participate to the charge compensation of structural segments through ionic Si-O-R^+ or $\text{Si-O-R}^{2+}\text{-O-Si}$ bonds. In this case, Ca^{2+} should favor a bigger interconnection of structural segments than Na^+ cations.

Elementary chemical mechanisms responsible for silicate aqueous corrosion are well known [6, 7]. They include hydratation, exchange of protonated species (H^+ , H_3O^+) with alkali or earth alkali ions (interdiffusion), and the hydrolysis of the covalent bonds, followed by a possible configuration of the glass network into another rearrangement. The relative importance of different configurations depends on both the glass composition and the corrosion conditions.

In a recent work, Moya et al. [8] have proven that several soda-lime glasses, belonging to the $\text{SiO}_2\text{-Na}_2\text{O-CaO-B}_2\text{O}_3$ system with high content of calcium oxide (15 to 20 wt.%), are efficient biocides versus Gram positive, negative bacteria and yeast. In addition, these glasses can be considered as “green” antimicrobials with no adverse effect on the environment. The important role of calcium in the biocide activity of glasses has been pointed out. However, the mechanism through which the lixiviation of Ca^{2+} takes place is still unclear. In the present work we tried to clarify the possible mechanism operating during the biocide action of the glass against microorganisms e.g., *Escherichia coli*.

2. Experimental section

2.1. Materials

Two borosilicate glasses, with and without Al_2O_3 (labeled as GAIB and GB, respectively), with different SiO_2 content but with the same B_2O_3 concentration and $\text{Na}_2\text{O/CaO}$ ratios were prepared. For a comparative purpose, a typical window soda-lime glass with high silica content (labeled as G0) was used. The glasses were prepared by melting appropriate mixtures of reagent grade SiO_2 (Cuarzos Industriales S.A., Santiago de Compostela), $\alpha\text{-Al}_2\text{O}_3$ (Taimei Chemical Co. Ltd., Japan), H_3BO_3 , Na_2CO_3 , and CaCO_3 (Sigma Aldrich). The starting materials were mixed thoroughly and heated in platinum crucibles at 850 °C for 1 h to favor decarbonation of samples. Subsequently, they were melted at 1400 °C for 1 h, and then quenched by dipping into water. All the obtained glasses were found to be transparent (see the inset of Fig. 1).

* Corresponding author.

E-mail address: jsmoya@icmm.csic.es (J.S. Moya).

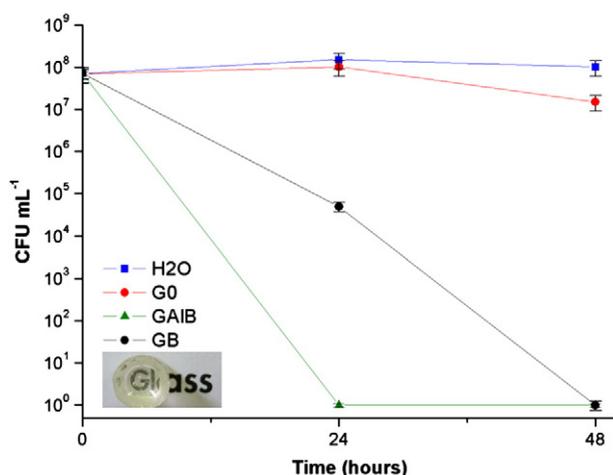


Fig. 1. Colony forming units (CFU mL⁻¹) of *E. coli* versus time. Inset shows the transparent appearance of the glass GB. Similar pictures were obtained for the glasses G0 and GAIB.

Table 1
Chemical composition (mol%) of the glasses.

Sample	Chemical Composition (mol%)						
	SiO ₂	B ₂ O ₃	Na ₂ O	CaO	K ₂ O	MgO	Al ₂ O ₃
G0	71.76	–	14.95	8.30	0.08	3.93	0.95
GAIB	42.50	6.84	21.16	22.03	0.32	–	7.15
GB	52.19	6.54	20.22	21.05	–	–	–

2.2. Experimental techniques

Glasses were milled down to 30 μm by using a planetary agate ball mill. Table 1 gives the actual composition of glasses measured by ICP. The local structure of glasses was investigated by NMR spectroscopy. ²⁹Si, ²⁷Al and ¹¹B MAS-NMR spectra were collected at 79.49, 104.26 and 128.38 MHz on a Bruker MSL-400 spectrometer. In MAS-NMR experiments, samples were rotated at 10 kHz around an axes inclined 54°44' with respect to the external magnetic B₀ field. When the central region was analyzed, frequency filters used were 100 kHz, but when whole spectra were analyzed frequency filters were increased to 1 MHz. To improve the signal/noise ratio, S/N, the number of accumulations was increased to 400, 40 and 50, respectively.

2.3. Biocide tests

The biocide tests were developed in triplicate as follows: 1) a single colony of *E. coli* W3110 was inoculated in liquid Luria Bertani (LB) media

and incubated overnight at 37 °C; 2) 10 μL of this culture was added to 1 mL fresh media and cultured at 37 °C for 6 h. 3) subsequently, 75 μL of an aqueous suspension of glass powders (200 mg mL⁻¹) was added. A glass free media was used as control. The survivors from each culture were tested every 24 h after plating serial dilution. After biocide testing, calcium concentration in the supernatant liquid, obtained by filtering and centrifugation, was determined by ICP using a Perkin Elmer optical emission spectrometer model Optima 2100 DV.

3. Results and discussion

The results obtained from biocide tests (Fig. 1) showed that the aluminoborosilicate glass (GAIB) reduces completely the number of colonies of *E. coli* as early as 24 h, achieving a logarithm reduction higher than 7, indicating a safe disinfection. However, no biocide activity was deduced for the soda-lime glass (G0) in the conditions in which experiments were carried out. An intermediate situation was obtained for the borosilicate glass (GB), diminishing three orders of magnitude the CFU parameter for 24 h (logarithm of reduction ~3). After 48 h the biocide activity of both glasses (GAIB and GB) was found to be the same. The pH was measured at the end of the growth. All samples showed similar pH to the one of the fermentation broth, (not higher than pH 8).

It has also been found that the calcium concentration in the supernatant liquid after 48 h of biocide test were ≈ 30 ppm for the G0 glass and > 200 ppm for both GB and GAIB glasses. These results are in agreement with those obtained previously [8]. In order to get insights and to rationalize the operating mechanism explaining the biocide activity of studied compositions, the structure of different glasses were analyzed by NMR. ²⁹Si MAS NMR patterns of these glasses are shown in Fig. 2.A. The spectrum of the soda-lime glass (G0) is formed by an asymmetric broad band centered around -94 ppm attributed to Q⁴ structural units of silicon (4 NBO). This band shifts to -80 ppm in GAIB, indicating the presence of abundant Q² species (i.e., 2 NBO and 2 BO). An intermediate situation is obtained for GB; in this case the central band centered around -85 ppm was mainly assigned to Q³ silicate species (i.e., 1 NBO and 3 BO).

Results obtained in ²⁹Si MAS-NMR spectra can be explained taking into account the increasing concentration of calcium in the glasses. High concentration of network modifiers like Ca²⁺ has been reported to have a greater effect on 'depolymerizing' the borosilicate network [9]. This depolymerization lowers network covalency, as a consequence of the conversion of Si–O–Si into Si–O–Ca or Si–OH linkages, thereby increasing the concentration of nonbridging oxygen atoms and the amount of exchangeable cations. This depolymerization makes that Si detected signals shift toward lower chemical shift values when calcium increases in glasses.

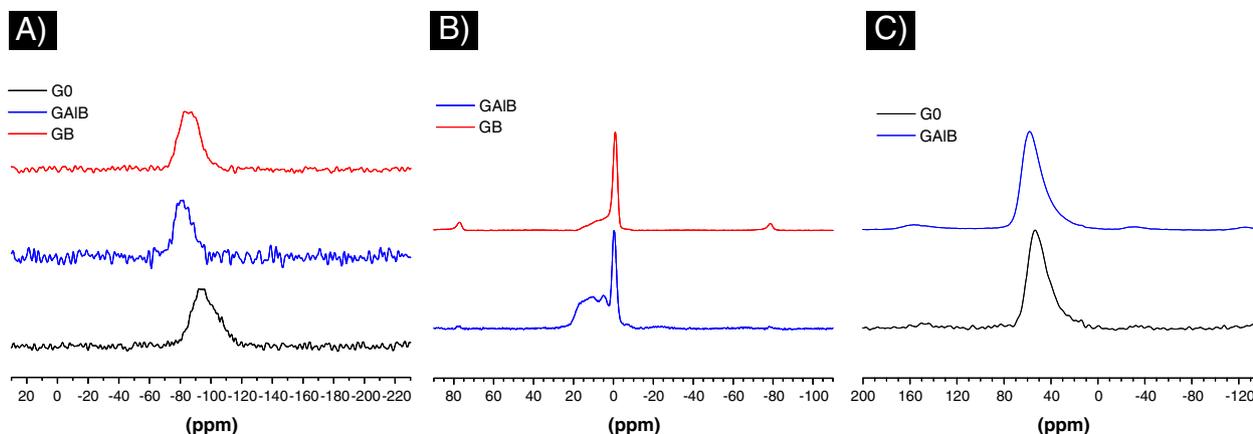


Fig. 2. A) ²⁹Si MAS NMR, B) ¹¹B MAS NMR and C) ²⁷Al MAS NMR patterns of glass samples.

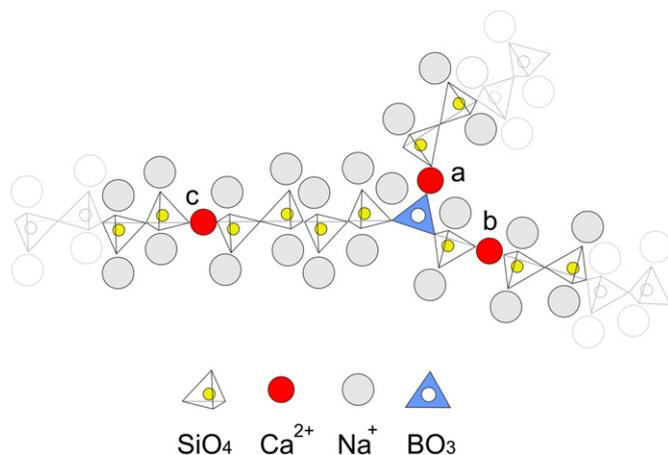


Fig. 3. Different possible interactions of calcium cations in the glass structure.

Fig. 2.B shows the ^{11}B MAS NMR patterns of the glasses. In this case, trigonal and tetrahedral borate species were detected. The fraction of trigonally coordinated boron structural units (BO_3) increasing appreciably in the GAIB sample. In this glass, several tetrahedral components could be present.

Fig. 2.C shows the ^{27}Al MAS NMR patterns. In the analyzed spectra, an asymmetric tetrahedral band was detected in the central component of spectra. In the analyzed glasses, B and Al tetrahedra are favored, participating in tetrahedral Si condensation of former cations. This fact makes that other alternative coordinations (octahedral Al and trigonal boron) are not favored, decreasing dramatically the intensity of associated components in the analyzed glasses.

From the obtained results, it can be inferred that the substitution of AlO_4 by SiO_4 in the glass network, as it happens in GB with regard to GAIB, apart from small modification on the connectivity of the silicon structural units (mainly Q^3 versus Q^2), decreases dramatically the fraction of trigonally coordinated boron in glasses.

The kinetics of calcium lixiviation is strongly conditioned by the amount of NBO available in the glass structure. For instance, in the case of G0, calcium cations are associated with the four-fold coordinated aluminum to compensate the negative excess charge. This interaction is much stronger than the one that takes place in GAIB or GB glass, where calcium acts in asymmetric arrangements such as $[\text{SiO}_4]:\text{Ca}:[\text{BO}_3]$ (denoted **a** in Fig. 3). Therefore, higher is the fraction of trigonally coordinated BO_3 structural units, higher is the amount of NBO, and consequently the lixiviation of calcium is favored.

When a glass particle closely interacts with the bacteria/yeast cell membrane, its bactericidal activity is strongly related to its capability to lixiviate Ca^{2+} at the glass–particle interface, that leads to the membrane depolarization and the subsequent death of the cell (as

analyzed in a previous work [8]). In this regard, Ca^{2+} ions weakly retained at weak tetrahedral interconnections (e.g., with BO_3) are necessary to ensure a high biocide activity in a short period of time, i.e., <24 h, as indicated in Fig. 1. The glass with higher biocide activity is the one that displays a higher fraction of Ca^{2+} in **a** positions (see the schematic view of Fig. 3). This is the case of GAIB glass.

4. Conclusions

The local structure of soda-lime glasses, belonging to the SiO_2 – Na_2O – CaO – B_2O_3 system was investigated by MAS-NMR spectroscopy. ^{29}Si , ^{27}Al and ^{11}B MAS-NMR spectra indicated the formation of condensed tetrahedral networks constituted by Q^2 and Q^3 units, where tetrahedral boron and aluminum are incorporated.

It has also been stated that strength with which calcium is retained decreases as the condensation degree of tetrahedral decreases from Q^3 to Q^2 in the analyzed glasses. Consequently, in glasses with higher biocide activity, tetrahedral segments are linear and part of calcium ions participates on the stabilization of segments arrangement. This presumably facilitates Ca^{2+} lixiviation and biocide activity.

Acknowledgments

Authors are grateful for support received from ITMA (Asturias, Spain), MICINN (MAT2010-19837-CO6 project), MATERYENER S2009 PPQ-1626 program, and CNPq-FAPESP (Brazil).

References

- [1] Schneider J, Mastelaro VR, Panepucci H, Zanotto ED. ^{29}Si MAS-NMR studies of Q^n structural units in metasilicate glasses and their nucleating ability. *J Non-Cryst Solids* 2000;273:8–18.
- [2] Avramov I. Diffusion coefficient of foreign particles in glass forming melts. *J Non-Cryst Solids* 2008;354:1537–40.
- [3] Gedeon O, Liska M, Machacek J. Connectivity of Q-species in binary sodium-silicate glasses. *J Non-Cryst Solids* 2008;354:1133–6.
- [4] Cailleteau C, Weigel C, Ledieu A, Barbox P, Devreux F. On the effect of glass composition in the dissolution of glasses by water. *J Non-Cryst Solids* 2008;354:117–23.
- [5] Lu H, Dieckmann R. Sodium tracer diffusion in glasses of the type $(\text{CaO}:\text{Al}_2\text{O}_3)_x(\text{SiO}_2)_{1-x}$. *J Non-Cryst Solids* 2007;353:2528–44.
- [6] Bouyer F, Geneste G, Ispas S, Kob W, Ganster P. Water solubility in calcium aluminosilicate glasses investigated by first principles techniques. *J Solid State Chem* 2010;183:2786–96.
- [7] Hernández JA, Oteo JL. Reacciones de la superficie del vidrio con soluciones acuosas. *Bol Soc Esp Ceram Vidrio* 1982;21:81–97.
- [8] Moya JS, Esteban-Tejeda L, Pecharrómán C, Mello-Castanho SRH, da Silva AC, Malpartida F. Glass powders with a high content of calcium oxide: a step towards a “green” universal biocida. *Adv Eng Mater* 2011;13(No. 6):B256–60.
- [9] Wu J, Stebbins JF. Effects of cation field strength on the structure of aluminoborosilicate glasses: high resolution ^{11}B , ^{27}Al , ^{23}Na MAS NMR. *J Non-Cryst Solids* 2009;355:556–62.