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

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In a recent work, it has been proved that soda-lime glasses, belonging to the SiO2–Na2O–CaO–B2O3 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.

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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 polyhedral condensation (modifier cations) [1–5]. In the case of Si4+ tetrahedra, different tetrahedral Q4 species (n bridging oxygens), are due to the depolymerization induced by alkali or alkaline earth [Na+ or Ca2+] cations. In the case of B3+ 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 (R2+) promote the silica tetrahedra interconnection through non bridging oxygens (NBO). In this case, R+ or R2+ cations participate to the charge compensation of structural segments through ionic Si−O−R+ or Si−O−R2+−O−Si bonds. In this case, Ca2+ 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 hydration, exchange of protonated species (H+, H2O+) 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 SiO2–Na2O–CaO–B2O3 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 Ca2+ 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 Al2O3 (labeled as GALb and GB, respectively), with different SiO2 content but with the same B2O3 concentration and Na2O/ CaO ratios were prepared. For a comparative purpose, a typical window soda-lime glass with high silica content (labeled as GO) was used. The glasses were prepared by melting appropriate mixtures of reagent grade SiO2 (Cuazros Industriales S.A., Santiago de Compostela), α-Al2O3 (Taisei Chemical Co. Ltd., Japan), H3BO3, Na2CO3, and CaCO3 (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).
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 (GAlB) 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 (GAlB 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 GAlB 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. 2A. 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 GAlB, 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 depolimerization 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. 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 GAlB.](image1)

<table>
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<th>Table 1 Chemical composition (mol%) of the glasses.</th>
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<tr>
<td>Chemical Composition (mol%)</td>
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<td>Sample</td>
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<tr>
<td>G0</td>
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<td>GAlB</td>
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![Fig. 2. A) 29Si MAS NMR, B) 11B MAS NMR and C) 27Al MAS NMR patterns of glass samples.](image2)
Fig. 2.C shows the $^{27}$Al 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 GAlB sample. In this glass, several tetrahedral components could be present.

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 GAlB sample. In this glass, several tetrahedral components could be present.

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 SiO$_4$:Ca:[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$_2$O–CaO–B$_2$O$_3$ 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.

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