The influence of local structure and surface morphology on the antibacterial activity of silver-containing calcium borosilicate glasses

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A B S T R A C T

A silver containing series of glasses starting from Ag-free 1.5B2O3 · SiO2 · CaO parent glass and ending with 3 mol% Ag2O was synthesized by the conventional melt quenching method and analyzed in order to correlate the composition and the structural peculiarities with the antibacterial effects. The structure and surface morphology of samples were characterized by X-ray diffraction (XRD), infrared (FTIR) and Raman spectroscopy, Scanning Electron Microscopy (SEM), X-ray photoelectron spectroscopy (XPS) was used to determine the nature of chemical bonding and the surface composition of the samples. The results indicate that all samples are vitreous and their local structure — mainly built by tetrahedral [BO4], triangular [BO3 and B2O5] borate species (O representing an oxygen atom bridging two boron atoms) and silicate Q1, Q2, Q3 units — is reactive to the silver oxide addition. Based on XPS data, both Ag+ and Ag0 ions are present on the surface of detected silver nanoparticles. Ag nanoparticles (AgNPs) embedded in glasses were evaluated for their antimicrobial activity against the gram-positive and gram-negative bacteria, i.e. Staphylococcus aureus and Escherichia coli bacteria. The antibacterial effect of investigated samples increases simultaneously with the Ag2O content and AgNP dimension. The proposed mechanism is discussed in relation with the local structure, Ag+/Ag0 ratio and AgNP dimensions.

In the field of tissue engineering, the scaffolds inserted in the human body could exhibit biocompatibility as well as antibacterial and/or antiviral character. In these terms, bioglasses doped with silver ions are worth investigating keeping in view the antimicrobial properties of silver. Over the last several decades, the use of silver-containing medicines prescribed by physicians decreased dramatically as the use of antibiotics increased. Recently, mutant bacteria and viruses have been found to be more aggressive and unresponsive to classical drugs, so that the silver ions, well known for their biocidal effects since antiquity, could be a solution. Silver has been investigated in vitro and in vivo for use in wound dressing, catheters, dental applications as well as a coating agent for medical devices and accessories. Moreover, it has been shown that materials containing nanoparticles of silver are even more appropriate for biological applications since the nanosilver has anti-inflammatory effects and improves wound healing. Earlier reports described that nanosilver was found to have wide antimicrobial effects. However, further studies must be done to understand the properties and behavior of Ag nanoparticles (AgNPs) as they are synthesized, applied, and developed in a particular environment.

In the present work, we propose to characterize bioactive calcium–borosilicate glasses containing AgNPs. Samples following the formula xAg2O (100 − x) [1.5B2O3 · SiO2 · CaO] with 0 ≤ x ≤ 3 mol% were synthesized by the conventional melt quenching method. A recently published study on some of these samples (0.7 ≤ x ≤ 3 mol% Ag2O) reported on the presence of dispersed, roughly spherical AgNPs at the...
sample surface, as indicated by transmission electron microscopy; in addition, the increase of AgNP size with increasing Ag$_2$O content was related to the changes in the electronic structure of samples, as measured by XPS and confirmed by theoretical calculation [16]. The aim of the present work was therefore to complete the picture and shed more light on the complex antibacterial behavior of glasses from B$_2$O$_3$–SiO$_2$–CaO system with different Ag contents with a special emphasis on local structure, AgNP dimension, dose- and interaction time-dependent effects of the obtained powder samples on different bacteria in vitro.

2. Experimental procedure

2.1. Glass preparation

The studied glasses have the composition expressed by the formula $x$Ag$_2$O (100 – $x$) [1.5B$_2$O$_3$ · SiO$_2$ · CaO] with $x$ = 0; 0.3; 0.5; 0.7, 1 and 3 mol%. They were prepared using the conventional melt quenching method described in detail elsewhere [16]. Appropriate quantities of reagent grade AgNO$_3$, H$_3$BO$_3$, SiO$_2$ and CaCO$_3$ were used as precursors.

2.2. Methods of glass characterization

The sample structure was investigated by X-ray diffraction using a standard Bruker X D8 Advance diffractometer with a monochromator of graphite for CuK$_\alpha$ ($\lambda$ = 1.54 Å). The diffractograms were recorded in 2θ range from 10° to 90° with a speed of 1°/min.

The morphology was investigated with a scanning electronic microscope (SEM) – FEI QUANTA 3D FEG dual beam – in high vacuum mode using EDT (Everhart Thornley Detector) and an accelerating voltage of 30 kV. In order to amplify the secondary electrons signal a cover of 5 nm thickness was performed with Pt–Pd into Agar Automatic Sputter Coater, in Ar atmosphere.

For Fourier transform infrared (FTIR) measurements identical amounts of glasses were mixed with KBr in order to obtain thin pellets containing approximately 1.05 wt.% glass powders. The pellets thickness was about 3 mm. The spectra were recorded at room temperature in the 350–4000 cm$^{-1}$ range with a 6100 Jasco spectrometer with a maximum resolution of 0.5 cm$^{-1}$ and signal/noise ratio 42,000:1.

Raman spectra were acquired from similar quantities of powdered sample using an Equinox 55Bruker spectrometer with Fra 106/S Raman module in 180° scattering geometry and Nd-YAG laser with...
1064 nm. For FTIR and Raman measurements the error in peak positions are of 0.5 cm$^{-1}$.

X-ray photoelectron spectroscopy (XPS) analysis was performed using a SPECS system, with appropriate computer controlled data collection. The base pressure in the analysis chamber spectrometer was of high resolution spectra all peaks were deconvoluted and of the data was carried out with Casa XPS software[17]. For the analysis of high resolution spectra all peaks were deconvoluted and fitted using Shirley background and GL(30) line shapes, i.e. a combination of Gaussian (70%) and Lorentzian (30%) line shapes. The element relative concentrations (at.%) were determined with errors less than 0.2%.

### 2.3. Antibacterial activity tests

Antibacterial activity of Ag-containing glasses was tested on gram-positive bacterium Staphylococcus aureus (1190 R) resistant to antibiotics and gram-negative bacterium Escherichia coli (ATCC 25922) not resistant to antibiotics. 30 mg of Ag containing glasses have been mixed with the bacteria in liquid culture (10$^4$ cells/ml). 100μl of culture were plated on solid medium (Muller Hinton agar) and incubated overnight at 37 °C. Negative control was performed in the same way but without glasses. The CFU (Colony Forming Units) were counted the next day.

### 3. Results

#### 3.1. Sample characterization

All samples were obtained in vitreous state, the X-ray diffraction patterns consisting in two broad peaks (Fig. 1). The presence of a second minor band in the diffractograms indicates the presence of two vitreous phases in the investigated samples, a common phase separation phenomenon in the case of oxide glasses containing two network formers.

Fig. 2 represents the micrographs of some investigated samples. The sample surfaces are heterogeneous, and the morphology changes significantly with the silver addition.

The infrared spectra (Fig. 3) of samples are very sensible to the silver addition. The main absorption bands and their assignment are present in Table 1. The local structure of glasses is dominated by silicate and borate units with a high tendency of depolymerisation once silver ions are introduced in the composition. For samples containing more than 0.5 mol% Ag$_2$O, an absorption band characteristic to Ag–O linkage vibration was detected.

Raman spectra (Fig. 4) complete the FTIR data and additionally reveal the presence of silicate units with 4 bridging oxygen atoms in Q3 units Diborate groups and stretching vibrations of a single NBO in SiO$_4$ tetrahedroon (SiO$_2$O$^-$: Q$^2$). Vibration of oxygen atoms or BØ3 triangles bridging two boroxol rings BØ$_2$O$^-$ triangles linked to other borate triangular units.

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**Table 1**

FTIR and Raman band assignments.

<table>
<thead>
<tr>
<th>Peak position [cm$^{-1}$]</th>
<th>Assignment according Refs[19–26].</th>
<th>Raman</th>
</tr>
</thead>
<tbody>
<tr>
<td>450 501</td>
<td>$\nu$(O–B–O) and (Si–O–Si) rocking motion</td>
<td>Si–O–Si, B–O–B, B–O-Si linkage vibration</td>
</tr>
<tr>
<td>475 655</td>
<td>$\nu$(BO$_2$O$^-$) and Ag–O bond vibration</td>
<td>$\nu$(Si–O–Si) in (Si$_2$O$_2$O$^-$: Q$^3$)</td>
</tr>
<tr>
<td>690 726 785</td>
<td>$\nu$(B–O–B) from pentaborate groups and $\nu$(B–O–Si)</td>
<td>$\delta$(Si–O–Si) in (SiO$_2$O$^-$: Q$^3$)</td>
</tr>
<tr>
<td>945 973 1000</td>
<td>$\nu$(B–O) in BO$_4$ units from diborate groups and asymmetric $\nu$(Si–O$^-$) in Q$^3$ units</td>
<td>Symmetric breathing vibration of six-member rings with one or two BO$_3$ replaced by BO$_4$ tetrahedral and $\nu$(Si–O–Si)</td>
</tr>
<tr>
<td>1048 1098</td>
<td>$\nu$(B–O) in BO$_4$ from tri- and penta-borate groups</td>
<td>Si–O$^-$ from bridging oxygen in structural units that contain NBOs (Q$^2$, Q$^3$)</td>
</tr>
<tr>
<td>1245 1270 1410 1428</td>
<td>Asymmetric $\nu$(B–O) from orthoborate groups</td>
<td>Diborate groups and stretching vibrations of a single NBO in SiO$_4$ tetrahedroon (SiO$_2$O$^-$: Q$^3$)</td>
</tr>
<tr>
<td>1470</td>
<td>Vibration of borate triangles</td>
<td>Vibration of oxygen atoms or BO$_3$ triangles bridging two boroxol rings</td>
</tr>
<tr>
<td>1410 1428</td>
<td>$\nu$(B–O) from borate groups</td>
<td>BØ$_2$O$^-$ triangles linked to other borate triangular units</td>
</tr>
</tbody>
</table>

Abbreviations: $\delta$: bending vibrations, $\nu$: stretching vibration, NBO: non bridging oxygen atom.
Based on XPS results, the surface elemental composition of the as-prepared samples was determined and some of them are listed in Table 2. The increase in the concentration of Ag as determined by XPS is consistent with the Ag2O content according to the nominal samples composition. In order to elucidate what type of Ag species are present on the samples surface, high resolution XPS spectra of Ag3d were analyzed. Ag3d spectra (Fig. 5) showed the presence of both Ag0 and Ag+ species at the samples surface. Using the Gaussian–Laurenzian (GL30) peak fitting, and a Shirley background, the Ag3d XPS spectrum could be deconvoluted into two doublets, one located at lower binding energy (dash–dot line) characteristic of Ag0, and the other at higher binding energy (solid gray line), characteristic of Ag+. This suggests that Ag species exist in the forms of Ag+ and Ag0 in the Ag-doped matrix.

### 3.2. Antibacterial activity

The glass powders with no silver and 0.7 mol% Ag2O had no antibacterial activity with either of the two strains of bacteria (Figs. 6, 7). Glass sample containing 1% Ag2O presented a slightly detectable antibacterial activity with both bacterial strains. This antibacterial activity could be observed only after 6 h of incubation (Figs. 6, 7). Glasses containing 3% Ag2O showed a higher antibacterial activity, but this activity is different for the two bacterial strains. In E. coli these particles reduce the number of cells by 60% after 1 h and no bacteria were observed after 2 and 6 h; for the S. aureus this reduction is only 50% after 6 h.

### 4. Discussion

Generally, the silver ions are present in the materials obtained by melt techniques in three forms: Ag0, Ag+ or Ag2+ highly influenced by the glass composition and time/temperature melting parameters. The solubility of Ag2O in glass melts is an important factor for the effective production of Ag-containing glasses since silver is known as an element difficult to insert homogeneously in a glass composition. In 1.5B2O3 · SiO2 · CaO host glass composition, the silver solubility was good, so that the obtained samples are vitreous.

The borate-based glasses structure consists of both BO3 and BO4 units strongly influenced by the preset composition and synthesis procedure and parameters [18]. On the other hand silica based glasses have the local structure mainly built by interconnected SiO4 tetrahedral units described in terms of Qn distribution, where \( n = 0 \ldots 4 \) is the number of bridging oxygen atoms. The presence of B2O3 in the structure of

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**Table 3**

<table>
<thead>
<tr>
<th>X (mol%)</th>
<th>Ag+ B.E. (eV)</th>
<th>%</th>
<th>Ag0 B.E. (eV)</th>
<th>%</th>
<th>Ag+/Ag0</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7</td>
<td>374.2</td>
<td>33.4</td>
<td>374</td>
<td>4.4</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>368.4</td>
<td>55.6</td>
<td>368</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>375.2</td>
<td>23.1</td>
<td>374</td>
<td>15.3</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>368.3</td>
<td>38.5</td>
<td>368</td>
<td>23.1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>374.9</td>
<td>12.5</td>
<td>374</td>
<td>25</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>368.6</td>
<td>20.9</td>
<td>368</td>
<td>41.6</td>
<td></td>
</tr>
</tbody>
</table>

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Fig. 5. The high-resolution Ag3d spectrum of samples with \( x = 0.7, 1 \) and 3. Dash–dot line at lower binding energy indicates the Ag0 component and the solid gray line, at higher binding energy, the Ag+ component. Dashed lines are guide for the eyes.

Fig. 6. Antibacterial activity of Ag containing glasses (0, 0.7, 1 and 3 mol% of Ag2O) on Escherichia coli cells. NC—negative control. The results are the average of three experiments and the error bars represent the standard deviations.

Fig. 7. Antibacterial activity of Ag containing glasses (0, 0.7, 1 and 3 mol% of Ag2O) on Staphylococcus aureus cells. NC—negative control. The results are the average of three experiments and the error bars represent the standard deviations.
Ag2O as well as certain time of incubation is needed for causing a clear antibacterial behavior. On silver oxide addition to the glass parent composition, the B–O–B linkages break progressively and a new absorption band characteristic of Ag–O linkages vibration was detected [22]. The Si–O–Si band at 450 cm$^{-1}$ shifts to higher wavenumbers and becomes more defined as the concentration of Ag2O in the glass increases. Therefore, the absorption band at 475 cm$^{-1}$ can be attributed to vibration of various Q$^0$ silicate units containing non-bridging oxygen atoms, suggesting some changes in the silicate network. Complementary, Raman analysis of the investigated glasses indicates a major contribution of silica-based units in the glass network and indicates a great stability of silicate units to the silver addition. The Raman spectra are dominated by the band from ~501 cm$^{-1}$ associated with various vibrational modes of cations–oxygen bridges [19,21], three types of bridging bonds being detected in this case: Si–O–Si [21,23], B–O–B [19–21,23], and B–O–Si [21,26]. Raman bands centered at ~726, 1001 cm$^{-1}$ (Fig. 4) prove that glass network consists of silicate units with 2 and 3 non-bridging oxygen atoms (Q$^2$ and Q$^3$). For samples containing more than 0.7 mol% Ag2O, Q$^2$ silicate units were detected simultaneously with the increase of Q$^2$ type units. For the investigated system the FTIR spectroscopy is more sensitive to the boron linkages vibration while Raman is more responsive to silicon–oxygen tetrahedrons of Q$^2$ and Q$^3$ types; (ii) on silver addition the borate network is destroyed with the generation of non-bridging oxygen atoms that help in the rearrangement of silicon ions in Q$^2$ and Q$^3$ units. Through silver addition, the local structure around boron atoms is depolymerized, while the silicon atoms are disposed in a more ordered network. The coexistence of two vitreous networks is also reflected by X-rays diffraction patterns where two broad peaks are present all over the compositional range. Based on XPS data the amount of silver distributed on the surface increases with the gradually addition of Ag2O in the sample composition (Table 2) while the Ag$^+$/Ag$^0$ species ratio decreases (Table 3).

Antibacterial tests (Figs. 6 and 7) revealed that a certain content of Ag2O as well as certain time of incubation is needed for causing a clear antibacterial effect which indicates that the killing process is not caused simply by ionic silver released from particles. This observation is confirmed by XPS data which showed a significant decrease in Ag$^+$/Ag$^0$ ratio (Table 3) on silver oxide addition which indicates that indeed not only the Ag$^+$ distributed on the sample surface is responsible for antibacterial behavior.

For the 0.7 ≤ x ≤ 3 mol% Ag2O samples, previous TEM measurements have shown that the detected AgNPs are spherical and their size slightly increases with increasing the Ag2O content [16]. The mean size of isolated circular AgNPs for the sample with 3 mol% Ag2O is d ~ 9 nm (±0.2 nm), the highest for the investigated samples [16]. Thus, higher Ag2O content may result in a reduced Ag$^+$ ion release into the medium, due to the lower surface area to volume ratio. The best antibacterial behavior observed for sample with highest Ag2O content (3 mol%) but large AgNP size and low concentration of Ag$^+$ (Table 3) suggest that the cell destruction processes probably involved silver ions from inner layers of the material. However, the ability of silver ions to move through the surface when the sample is attacked by bacteria looks to be strongly related to silver oxide content, the presence of AgNPs and the surface morphology of samples. SEM images show that progressive addition of silver oxide to the calcium borosilicate glass matrix changes the sample surface (Fig. 2). A harsh, compact surface characterized the 0.3 mol% sample while for 3 mol% Ag2O a bright, wave like surface was evident. Overall, the structural and morphological features of sample with 3 mol% Ag2O eases the migration of Ag$^+$ ions through surface and/or helps the supplementary ionization of silver from surface placed nanoparticles when is “attacked” by bacteria.

The difference in the bactericidal effect of the two strains of 3 mol% Ag2O glass particles was expected since the gram-positive bacteria are less susceptible to Ag$^+$ than gram-negative bacteria mainly due to the peptidoglycan particularities [27]. The killing time of E. coli bacteria, of 2 h, is reasonable taking into account the level of silver oxide content.

5. Conclusions

The connection between structural peculiarities, surface composition, morphology and antibacterial effect in melt-derived Ag2O–B2O3–SiO2–CaO bioactive glasses was studied. Small sized silver nanoparticles were developed on the samples. The silver oxide addition leads to gradual borate network depolymerization simultaneously with the increase of short range order degree of silicate network. Moreover, increasing the Ag2O content, the samples contain less Ag$^+$ ions on the surface but present a better antibacterial effect. We propose for these opposite behaviors two possible mechanisms determined at sample contact with the bacteria, i.e. Ag$^+$ ions stocked inside move through the surface and/or supplementary silver ions from surface placed nanoparticles are formed. These processes are present for certain quantities of silver oxide (higher than 0.7 mol%), for silver nanoparticles smaller than 10 nm and with a smooth wave like surface. Even for samples with higher silver oxide content (3 mol%) the inhibitory action is clearer for gram-negative bacteria (E. coli) and it is less noticeable for the gram-positive bacteria (S. aureus). The Ag$^+$ most likely acts on the cell membranes and as a result the cell membrane is lysed. The results observed on the two different types of bacteria most likely are due to differences in their membrane structure. Moreover, our results have demonstrated that for the same sample this interaction is dependent not only on the amount of Ag$^+$ available to be in contact with bacteria but also on the time of incubation. However, possible different dissolutions, specific particle effects, aggregation and uptake kinetics remain subjects for further studies, with more sensitive analytical techniques.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jnoncrysol.2014.08.004.

References


