Antibacterial effect of $ZnO-B_2O_3$ Matrix Doped with Silver Ions on Gram Positive Bacteria Cultures

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Abstract

In this work, vitreous oxidic structures from the $xAg_2O\cdot(100-x)[45ZnO55B_2O_3]$ system were prepared, with a high content of silver oxide $0\leq x\leq 7$ mol%. The start substances were mixed in appropriate proportions and melted at 1230 °C, then undercooled at room temperature. The samples were obtained in the form of disks with a width between 2 and 5 mm by pressure between two stainless steel plates.
The capacity of interaction with bacterial culture was investigated in agar environment through the method of dilution for gram positive bacteria *Staphylococcus aureus* and *Streptococcus spp. in order to* use them for medical purposes.
The most intense effect was registered in the case of *Staphylococcus aureus*, and the samples which do not contain silver, turned out efficient against both investigated strains.

Keywords : glass, antibacterial, gram positive

1. Introduction

Once with the increase of the resistance of the microorganisms to antibiotics and the adaptation of the pathogenic germs, the necessity to prepare compositions to destroy the organism without the possibility to adapt appeared and became more and more stringent. Even though the mechanism of action is not clearly elucidated, the preparation of new components with antibacterial properties is increasingly more necessary.
The transitional metals gold (Au), silver (Ag), zinc (Zn) proved their utility in various medical applications from inhibitors of the angiogenesis [1] to compounds with antibacterial properties [2, 3].
Silver is used in different ionic Ag^+ forms, metallic or colloidal, introduced only in the surface that comes in contact the bacterial environment through the method of ionic exchange [4] or undercooling of the melt [3]. Being contained in the internal structure of the vitreous material or by the sol-gel method [5] of preparation of samples at room temperature.
The oxidic B_2O_3 base glasses are not difficult to prepare, the boron acid being a classic vitreous network former, does not need a very high temperature to decompose.
The zinc oxide turned out to be a good inhibitor of the growing of several species of bacteria [6] being used nowadays for the preparation of baby creams. In the case of ZnO the inhibition capacity is attributed to its roughness as well as to the formation of reactive species of oxygen on their surface [7]. The obtaining of vitreous structures with a high content of A_2O is another issue that is hard to solve because silver cannot be incorporated homogenously in vitreous matrices though the method of melting.
The inclusion of the metallic oxides in vitreous oxidic matrices is a structural goal that leads to the obtaining of compounds with new physical properties that can be used in the medical fields. In the case of ZnO it was proved that that granulation under which is used may have different effects. The use of inorganic oxides in

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small quantities an antimicrobial agent has also the advantage that these microelements are essential for people. [7]

2. Materials and methods

The oxidic glasses from the $xAg_2O\cdot(100-x)[45ZnO55B_2O_3]$ system were obtained using as start materials P_2O_5, H_3BO_3, $CaCO_3$, Ag_2O with a chemical purity of 99+. The oxides were mixed in appropriate proportions, homogenized and melted at 1230 °C, founded and pressed between stainless steel plates.

For the filtration of the experimental points the Origin v8.1. was used. The vitreous character of the samples was tested though diffraction of X-rays using a diffractometer. Detailed experimental descriptions should be restricted to X ray powder diffraction data were obtained with Bruker D8 Advanced Diffractometer equipped with Si monochromator.

The antibacterial efficiency of the studied compounds was tested on gram positive germs (standard germs *Staphylococcus aureus* ATCC 6538 P, *Bacillus spp.*) through the method of diffusion in 2% agar gel. The agar pored on a Petri plates with a diameter of 12 cm, was sown with the bacterial inoculum, prepared from the 24 hour culture on the agar, diluted in physiological serum at the density of tube 1 on Mc Farland scale. Immediately after sowing wells with a diameter of 3,5 mm were made, in which the compounds were placed, in a concentration of 15%, in a quantity of 40 µl/well. Each compound was twice tested. The diameters of the inhibition areas were read after an incubation of 24 hours at 37°C, being expressed in mm.

3. Results and discussion

The effect of the oxidic glasses from the $xAg_2O\cdot(100-x)[45ZnO55B_2O_3]$ system for $0 \leq x \leq 7$ mol% over the gram positive *Staphyloccocus aureus* bacteria is presented in Fig. 1.

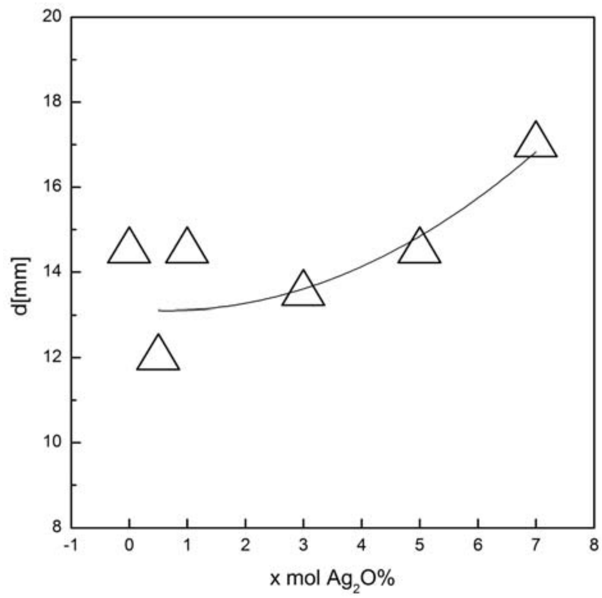

Figure 1 the antibacterial effect of the vitreous structures from the Ag_2O-ZnO-B_2O_3 system on the *Staphyloccocus aureus* bacterium

As it can be seen the inhibition area increased once with the molar content of Ag_2O of the samples.

The medium inhibition diameter is contained in the interval $d\in[12;17]$ mm.

This type of bacterium is sensitive to the oxidic glasses that do not contain silver ass well (Fig.1). The inhibition area in the case of the glass ZnO-B_2O_3 base is approximately equal with the one determined by the sample for x=1 that contains 1mol% Ag_2O.

The experimental points were filtered with a grade 2 polynomial. The studies compounds have greater efficiency in the case of gram negative bacteria the maximum area of inhibition being of 20 mm.

The inhibition area of bacteria growth depends on the molar content of Ag_2O from the samples and the process is not at the point of saturation.

The growth of the *Staphyloccocus aureus* ATCC 6538 P germ is inhibited proportionally with the increase of the molar content of Ag_2O matrix ZnO-B_2O_3, the relation between the minimum and maximum diameter is d_{max}/d_{min}=1.41 while the relation between the molar concentrations of the silver oxide at the ends of the compositional interval is of 1:14 and the zinc oxide remains constant.

The difference between the inhibition areas induced by the $xAg_2O\cdot(100-x)[45ZnO55B_2O_3]$ vitreous system into the cultures of *Bacillus spp*.

Is not significant with the modification of the content of silver oxide in the surface.

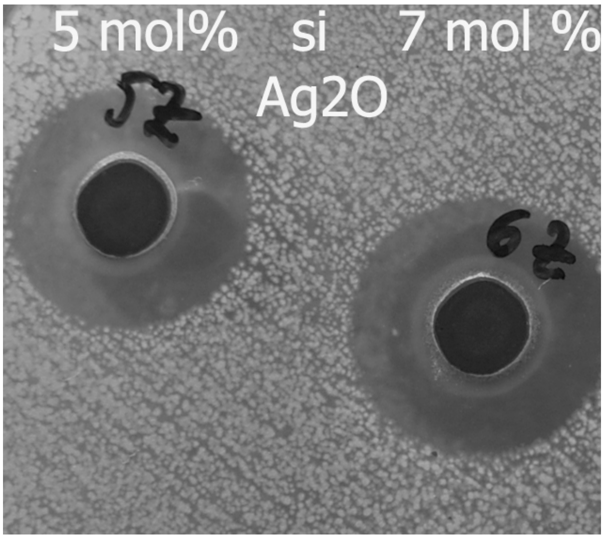

Figure 2 the apparition of circular inhibition areas in the case of the *Bacillus spp.* for x=5 and 7 mol % Ag_2O in matrix ZnO-B_2O_3.

Difference between x=5 and respectively 7 mol% is not significant

In the case of *Bacillus spp. The* diameter of the inhibition circle increases slightly once with the increase of the molar content of Ag_2O in matrix ZnO-B_2O_3.

Therefore, for the investigated germ $d \in [10;13.25]$ mm.

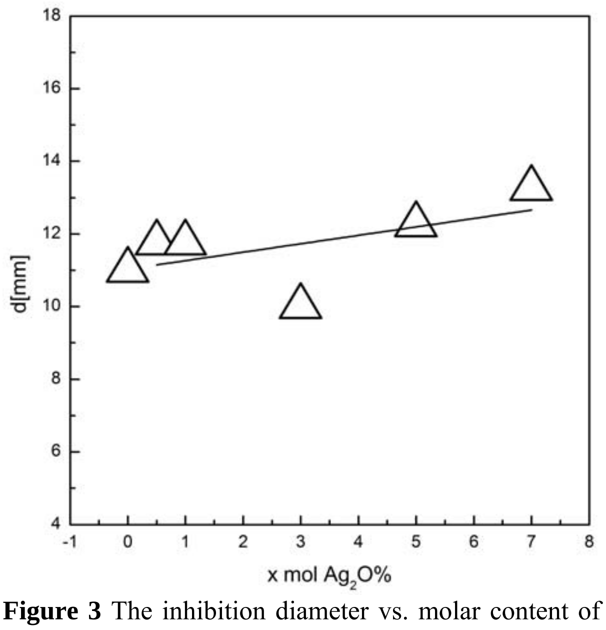

Figure 3 The inhibition diameter vs. molar content of de Ag_2O from the vitreous matrix ZnO-B_2O_3 in the case of Bacillus spp.

Sample x=0 which does not contain any silver has an inhibitive effect on a diameter of d=11 mm. even if there re some residual colonies the diameter of inhibition of this sample is larger than that of sample 3 with 3 mol % de Ag_2O. ZnO in small concentrations ($c \leq mM$) can have an antibacterial effect [7] and therefore some species developed intra and extra cellular mechanisms of control of the Zn^+ diffusion.

In the present work it was also investigated (Fig. 4) the dependence of the antimicrobial effect of the granulation of the powder in the case of sample $1Ag_2O \cdot 99[45ZnO55B_2O_3$.

The diameter of inhibitions is d ~11.75 mm and does not represent an outstanding inhibitor effect. This value belongs to the interval in which their diameters of inhibition can be found for the sample with usual granulations from the same system.

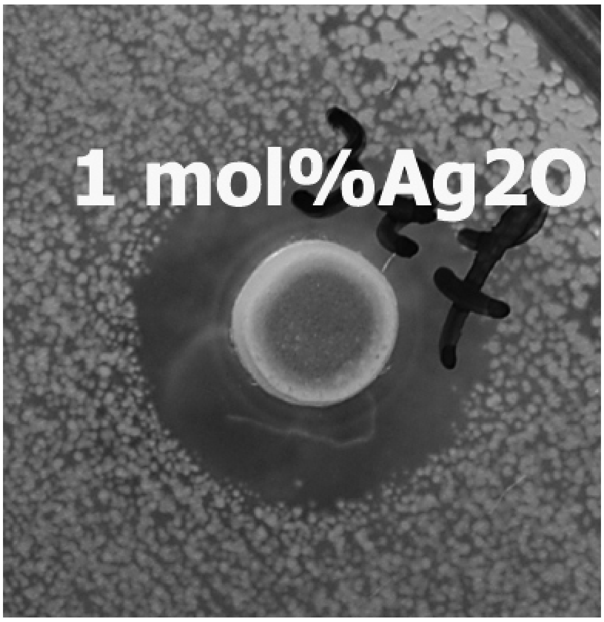

Figure 4 the areas of inhibitions created by the vitreous sample $1Ag_2O \cdot 99[45ZnO55B_2O_3]$ with fine granulation ($d \leq 45$ μm) in the *Bacillus spp*. Culture.

4. Conclusions

Homogenous vitreous materials were obtained, with transparent aspect and color specific to the metallic ions introduced and state of oxidation with a very high concentration of silver oxide. The materials prepared through the method of subcooling of melt turned out to be very efficient in the inhibition of the growth of the gram positive *Staphylococcus aureus* si *Bacillus spp.* bacteria. The capacity of inhibition increases with the molar concentration of Ag_2O in the investigated oxidic matrix.

The start $45Zn-55B_2O_3$ matrix presents an increased capacity of inhibition in the case of the two gram positive investigated bacteria strains.

By comparison of the areas of inhibitions generated at the same molar concentrations of silver in the $xAg_2O(1-x)[45ZnO55B_2O_3]$ it can be observed that the effect is significantly more intense in the case of the first investigated germ (*Staphylococcus aureus*) this one being more sensitive at the action of the two transitional metals.

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