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The study of the effect of silver contained in the B_2O_3-CaO-P_2O_5 on *Candida albicans*

Răzvan Stefan, Nicodim Fiţ

Veterinary Medicine Faculty, University of Agricultural Science and Veterinary Medicine, Manastur street, no.3-5, 400372, Cluj-Napoca, Romania;
rstefan@usamvcluj.ro

Abstract. The present paper studied the action of the oxidic glass from the xAg_2O (100-x)[$42B_2O_3 24CaO 34P_2O_5$] system with $0 \leq x \leq 9$ mol% on the fungi *Candida albicans* through the method of diffusion on agar. The powder manifests an inhibitory effect on all the tested strains, the c_3 strain being totally inhibited, the growth in plate being almost inexistent. For the c_1, c_2 and c_4 strains the inhibitive effect depends on the x molar concentration of A_2O in the structure of the glass, the biggest diameter was of 24 mm and it was measured for the c_2 strain. The diameter of the inhibition areas is proportional with the concentration of Ag_2O in the vitreous samples.

Keywords: glass, silver, antibacterial, *Candida albicans*

INTRODUCTION

The phosphorus oxide is a classic former of vitreous net being in competition with the boron oxide for the construction of a net of the investigated compounds. The phosphorus pentoxide P_2O_5 forms tetraedric structures (Brow, 2000 , Moguš-Milanković *et al.*, 2005) connected by tips denominated tetraeders Q^n, where n=0, 1, 2 and 3 and depending on the number of oxygen atoms that link the atoms of phosphorus from neighboring structures. The most used modifier of vitreous net is calcium oxide, playing a part in the change of the melting temperature, hardness, refraction index, chemical durability and the studied material.

The boron oxide forms vitreous nets alone and within a large compositional interval in combination with other oxides and it situates in trigonal BO_3 groups and respectively tetragonal BO_4 included in complex structural groups. (Kamitsos *et al.*,1987 , Varsamis *et al.*,2000): boroxol, diborate, pentaborate, triborate, metaborate etc. depending on the modifier concentration or of other oxides from the vitreous matrix.

Considering the large number of atomic species contained by the glass, is made of complex structural units that may contain B – O – P links (Ducel *et al.*,1993) between the structural groups deriving from the 2 formers. The zinc oxide is used in medical applications as an antibacterial agent (Xu *et al.*,2008) and antifungal (Aleš Panáček *et al.*,2009) and it is difficult to be introduced into the vitreous systems.

In the present thesis the effect of the xAg_2O (100-x) [$42B_2O_3 24CaO 34P_2O_5$] system was investigated for $0 \leq x \leq 9$ mol% prepared through the method of melting sub cooling on the Candida albicans fungus with the purpose to use them in medical applications.

MATERIALS AND METHODS

Samples from the $xAg_2O(100-x)[24CaO \cdot 42B_2O_3 \cdot 34P_2O_5]$ system ($0 \leq x \leq 9$ mol%) were prepared using pure reagent-grade compounds, i.e. H_3BO_3, H_3PO_4, CaO and Ag_2O, in

appropriate ratios. The mixtures corresponding to the desired compositions were melted in air, in sintered corundum crucibles, in an electric furnace at 1200 °C, t=15 min. The melts were quickly undercooled to room temperature by pouring onto stainless-steel plates. The structure of the samples was examined by means of X-ray diffraction using a standard Bruker X D8 Advance diffractometer with a Si monochromator for incident beam in order to obtain only CuKa radiation (k = 1.54 Å).

In the experiment were used four strains of *Candida albicans* isolated from skin lesions from animals in the laboratory of Microbiology of the Faculty of Veterinary Medicine Cluj-Napoca.

Diffusimetrical technique was performed using agar culture medium as Sabouraud dextrose agar (SDA) in Petri plates of 9 cm diameter and strains were cultivated before in Sabouraud broth 48 hours, for them to obtain a suspension of germs in saline medium to an optical density of 0.5 McFarland scale. Subsequently plates were flooded with the *Candida* strain, dried and were radially distributed model in a quantity of 35µl on each well.

The plates were introduced at thermostat for 48 h at $28^0C \pm 1^0C$ and the results were interpreted determining the inhibition area diameters total and partial. The results were expressed in mm.

RESULTS AND DISCUSSION

The method that was used pursues the differentiation of the sensitivity of various (C_1,-C_4) strains of *Candida albicans* at the action of the vitreous oxidic matrix B_2O_3-CaO-P_2O_5 doped with silver ions in various molecular concentrations. It was shown that that dissolved silver has besides the antibacterial effect (Simon *et al.*,2007, Yamamoto *et al.*,1996) an inhibitive effect on fungi as well (Aleš Panáček *et al.*, 2009).

In the case of the c_1 *Candida albicans* strain, the diameter of the inhibitive area measures in plate increases linearly once with the increase of the Ag_2O content in the oxidic matrix.

The total inhibitive diameter is contained within the interval $d \in [10 ; 21]$ mm, with an average increase of 1,2 mm/mol. The line is drawn as a guide for the eyes in order to observe the constant tendency of enlargement of the inhibitive diameter.

Through the method of diffusion in agar we brought out the fact that the diameter of the growth inhibition area of *Candida albicans* strain C_2 depends on the molar Ag_2O in the samples as happens in the case of various bacterial strains (Simon *et al.*,2007 , Xu *et al.*,2008). The inhibition process is saturated for x = 6 mol % silver oxide regardless of how much silver samples contain the inhibited area remains at the same surface. The reliance of the inhibition diameter (on the growth area) on the inhibitor ion is given by the $\Delta d/dc$ =1.6 mm/mol ratio and is the “fastest” inhibition curve.

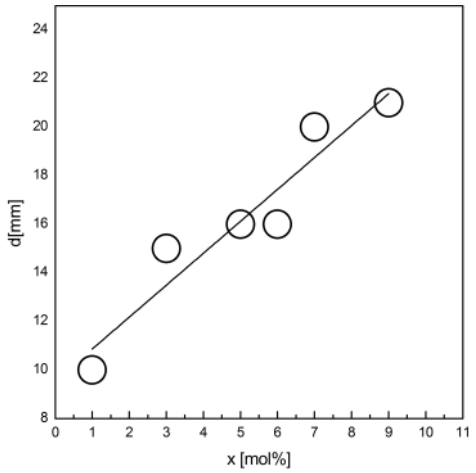

Fig.1. The evolution of the inhibitive diameter of *Candida albicans* (C_1) depending on the content of Ag_2O ions

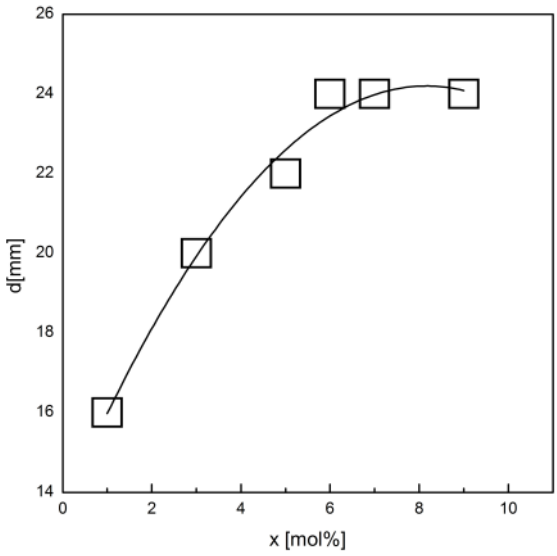

Fig.2. The inhibition diameter vs. the content of de Ag_2O ions in the case of *Candida albicans* (C_2)

Candida albicans C_3 turns out to be the most sensitive at the action of the compounds the inhibition diameter surpassing 35 mm for all the investigated concentrations.

The inhibition diameter in the case of the C_4 (*Candida albicans*) strain depends on the molar concentration. Ag_2O in samples reveals a linear increase of this without the process being saturated in the investigated compositional domain.

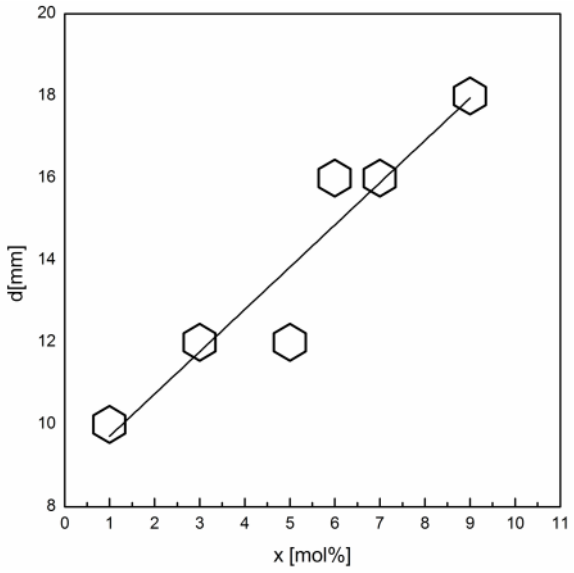

Fig.3. The dependence of the inhibition diameter by the Ag_2O ion content from the $xAg_2O(100-x)$ $[24CaO \cdot 42B_2O_3 \cdot 34P_2O_5]$ system for the C_4 strain.

The (C_4) strain is the less sensitive to the action of the samples the maximum diameter being of 18 mm. also, the action of the compounds can be evaluated through the ratio between the increase of the diameter in total inhibition and the molar percentage of Ag_2O contained in the matrix and is of 1 mm/mol, smaller than in the case of the C_1 strain .

CONCLUSIONS

- The xAg_2O $(100-x)[24CaO \cdot 42B_2O_3 \cdot 34P_2O_5]$ system was prepared through the method of melting under cooling integrating a high molar content of Ag_2O (x= 9 mol%)
- The inhibition degree is due more to the existence of a bigger silver quantity at the surface available for being released in the biological environment, than to the structural modifications that appeared to a bigger quantity of Ag_2O.
- The effect of the oxidic glass from the $xAg_2O(100-x)$ $[24CaO \cdot 42B_2O_3 \cdot 34P_2O_5]$ system depends on the concentration of Ag_2O from the samples.
- The degree of effect also depends on the tested strain, this way, the C_3 strain is the most sensitive, while the Candida albicans (C_4) is the less sensitive to the action of the investigated samples, the maximum inhibition diameter not surpassing 18 mm.
- In the case of the C_2 strain a saturation of the process in ascertained at x = 6 mol%, a higher content of inhibitive agent does not modify the inhibition area.

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