Antibacterial glass-composite coatings for protection of special purpose steel panels

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Abstract. It has been established that the most informative and universal method for determination of biocide properties of vitreous coatings is qualitative method that takes into account the growth level of biotest microorganisms inoculated into liquid nutrient media. It is shown, that biocidity of glass-composite coatings on the basis of glasses of $Na_2O - K_2O - CaO - ZrO_2 - TiO_2 - Al_2O_3 - P_2O_5 - B_2O_3 - SiO_2$ system is determined by the presence of calcium phosphates in them and depends on the type of bactericide filler. The most effective ones by the action on *Pseudomonas aeruginosa* bacterium and *Aspergillus niger* and *Candida albicans* fungiare zinc titanate and Ag^+, to *Escherichia coli*– only zinc phosphate.

1. Introduction

A reliable long-term antibacterial protection of human activities objects, considering existent environmental situation all over the world is an important problem. Its relevance is determined by the necessity of life quality improvement and the amplification of epidemics of different etiology, particularly SARS, pig flu and bird flu, which take away thousands of lives every year, as well as by insufficient effectiveness of known solutions concerning the reduction of pathogenic bacteria reproduction.

Presently, much attention is paid to development and use of biocide (i.e. antibacterial and fungicide) materials on the basis of plastics, special glasses, composite, metallic, polymeric, glass-ceramic and vitreous enamel coatings in different branches of industrial and domestic sectors. Effectiveness and prospectiveness of the use of vitreous enamel coatings as antibacterial ones are due to their substantial advantages in comparison with other materials in relation to the durability against the action of one of the most common conditionally-pathogenic microorganisms – *Escherichia coli*[1].

As it is known [2], vitreous enamel coatings are widely used for protection of versatile purpose metal articles because of the combination of functional and hygienic properties: chemical resistance, mechanical strength, thermal resistance and resistance to bio-corrosion, aesthetic and decorative characteristics.

The rapid evolution of architectural industry demands the development and implementation of competitive universal protective and decorative coatings on steel panels for medical, pharmaceutical and sanitary purposes. Among them the special place is taken by vitreous enamels with antibacterial function.

In spite of the relevancy of production and use of such coatings and availability of the developed enameling industry in Ukraine, large-scale researches in this direction are not being carried out.

Production of biocide glass coatings, beside the compositions synthesis, requires development of methodological approach to establishing their antibacterial and fungicidal functions and adaptation to vitreous enamels of standards on these properties existing for other materials. That is why the purpose of the current work, connected with the development of biocide vitreous enamel coatings, is the development of methodological approach and the synthesis of specified coatings resistant against conditionally-pathogenic bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and micro fungi *Aspergillus niger* and *Candida albicans*.

2. Development of methodological approach

Methodological approach used in synthesis of biocide glass coatings provides the development of evaluation system of physicochemical properties, performance characteristics and structure of vitreous enamels with the use of standard methods [4], as well as antibacterial and fungicidal effects and fungus resistance of obtained coatings.

These properties were determined by the following methods with the use of solid substrate, liquid and gaseous media.

1st method – diffusion (qualitative) which is based on investigation of formation of a test microbe growth deceleration zone around a test specimen with the use of dense agarized nutrient media.

The suppression of growth on the contact section with nutrient medium depends on the diffusion rate of antimicrobial agents to the nutrient agar layer. This method is applicable for migrating compounds only [5, 6].

2nd method – (quantitative) which is based on registration of the growth level of biotest microorganisms, inoculated into liquid nutrient media in the presence of test specimens or without them [7].

3rd method – aerosol infection in optimal conditions for microbes development imitating natural infection of test specimens in air by spores suspension or vegetative cells of test cultures with subsequent account of their growth.

3rd method* – aerosol infection without additional source of nutrition in the so-called “hungry” medium [8, 9].

3. Principles of synthesis of biocide glass coatings

The idea of combination of poly-functional vitreous enamel coatings with bactericidal action of heavy metal cations is at the basis of the development of antibacterial vitreous enamel coatings [3]. Imparting of fungicidal effect to glass coatings is achieved through the incorporation of anti-fungal oligodynamic components in their structure, fungus resistance reflects the resistance of the coatings to bio-corrosion.

The principle of imparting biocide properties to vitreous enamel coatings is based on the factthat heavy metals, due to their chemical properties, affect micro- and macro-organisms. Heavy metals belong to the group of antimicrobial substances with denaturation action having high affinity to sulfur. The cations of these toxic substances react with strictly definite biochemical structures with corresponding structure. Therefore, heavy metals which belong to oligodynamic components firstly block the active center of the biochemical enzyme structure, then bond the –SH groups, and as a result, the enzymes lose their ability to function. Ag^{+}, Hg^{2+}, Cd^{2+}, Cu^{2+}, Au^{3+}, Ni^{2+}, Zn^{2+}, Ti^{4+}, etc., can be used as bactericidal properties carriers.

With the purpose of obtaining biocide glass coatings components, Zn^{2+}и Ti^{4+}cations were chosen as oligodynamic components, having toxic effect on pathogenic organisms, in concentrations not exceeding acceptable migration rates for humans, and for comparison, Ag^{+} which is a well-known bactericide agent.

Bactericide cations were incorporated in vitreous enamel as pre-synthesized nano- and micropowders during frit milling or in the prepared slip.

For the glass matrix synthesis Na_2O – K_2O – CaO – ZrO_2 – TiO_2 – Al_2O_3 – P_2O_5 – B_2O_3 – SiO_2system was chosen. The structure of this glass matrix enables a directional orientation of biocide metal cations and their uniform distribution in the surface layer of the coating. The main factor determining the directional orientation of cations is the presence of calcium phosphates in the glass compositions and in the coatings on their basis. During the heat treatment of glass coatings on the basis of this system in conditions of directed volume crystallization, fine crystals of hydroxyapatite (HAP) are produced, and there structure acts as Zn^{2+}cations transporter [10]. These cations are adsorbed onto the surface of the HAP crystals and partially substitute Ca^{2+}isomorphically in the HAP structure.

4. Results and discussion

4.1. Preparation of glass composite coatings

As an antibacterial nanopowder, zinc orthophosphate was chosen. Itwas synthesized by chemical deposition [11], with the particle size about 100 nm. The fractional composition of $Zn_3(PO_4)_2$ was established with Zeta Sizer – Nano 2000 device. As a biocide component, zinc titanate was chosen, as a compound combining the biocide properties of both Zn^{2+}и Ti^{4+}cations which are increased in the case of their joint action. $Zn_2TiO_4$was obtained by sintering ZnO and TiO_2 powders. The particle sizes in this micropowder were less than approximately 60 µm. In order to add Ag^+ cations, $AgNO_3$ solution was used. To prepare the glass coatings, the glass-forming region of the initial system was chosen, limited by following oxides contents, in mass %: 45–50SiO_2; 25–30 (CaO+P_2O_5); 20–25 (Na_2O + Li_2O); 5 – 10 (TiO_2 + ZrO_2), 5 – 20 (B_2O_3 + Al_2O_3).Twelve compositions of model glasses differing in CaO and P_2O_5 contents and ratioswere synthesized (figure 1). The structure and the phase composition of glasses after melting at 1300 °C, as well as coatings after heat treatment in firing conditions on steel substrate at 820 °C for 3 – 5 minutes were determined by infrared spectroscopy, differential thermal analysis and X-ray diffraction. The crystal phase distribution in glass coatings after heat treatment was investigated with the use of optical and electron microscopy of the specimens.

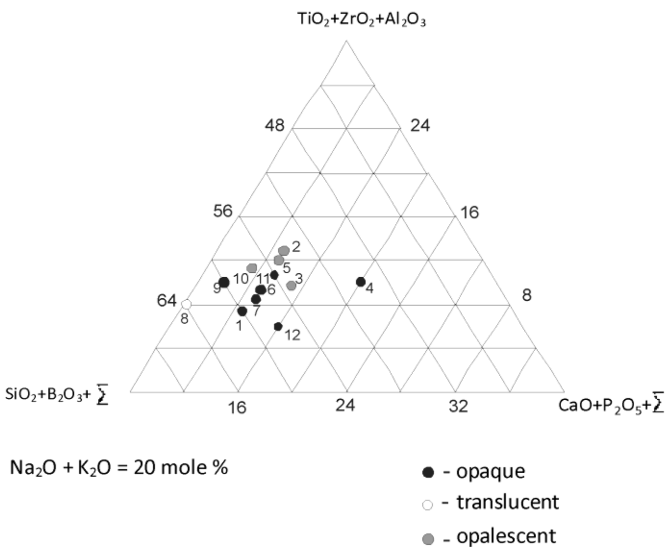
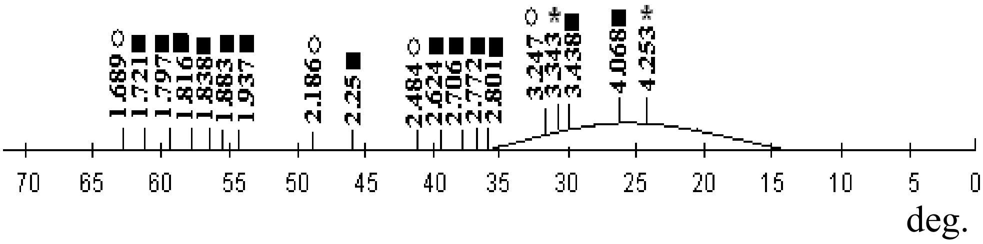


Figure 1. Compositions of model glasses

We have found that a fine-crystalline structure with hydroxyapatite crystals was formed in KF-9, KF-10 and KF-11 glass coatings containing 4 – 5 mole % P_2O_5 and 6 – 10 mole % CaO for CaO/P_2O_5 ratios 1.5, 1.75 and 1.6, respectively. This finding has also been confirmed by thermograms of these materials. In particular, a negligible value of delineated area of endothermic effect at 560 °C, which is a measure of a crystalline phase quantity, and exothermic effect at 680 °C corresponding to the crystallization maximum were observed in case of KF-11 composition. This provides evidence for a small quantity of formed nuclei and, as a result, of minor crystallization in this glass with formation of small hydroxyapatite crystals (as can be seen from XRD and Differential Thermal analyses, figures 2 and 3 respectively) [12].



* – SiO_2quartz; ○ – TiO_2rutile; ■ – $Ca_5(PO_4)_3OH$

Figure 2. XRD diagram of glass coating KF-11

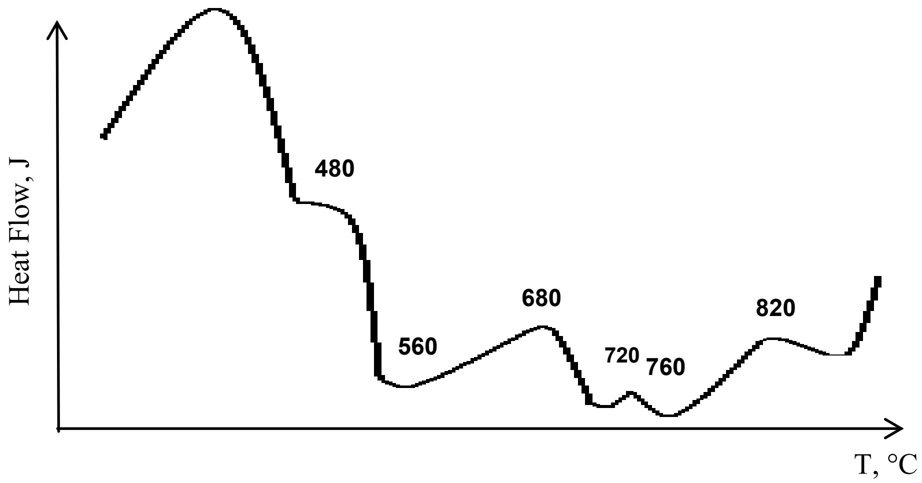


Figure 3. Thermogram of glass coatingKF-11

A uniform distribution of fine 3 – 5 μm hydroxyapatite crystals was found in KF-11 samples, they are formed as a result of liquation processes in initial glasses (figure 4).

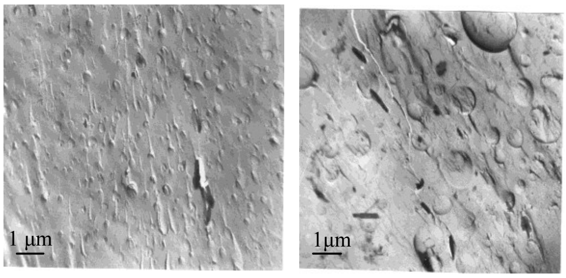


Figure4. TEM microstructure of KF-11 glass

By investigation of physicochemical properties and technical characteristics we have concluded that the glass coating KF-11 on the basis of test glass of the same designation meets the requirements to coatings for protection of special purpose steel panels and has chemical durability of class A (according to EN14483), gloss55 %, thermal coefficient of linear expansion$120 \cdot 10^{-7}$ K^{-1}.

The glass composite coatings KF-11-1, KF-11-2 [13] and KF-11-3 were obtained on the basis of KF-11 glass and biocide powders Zn_2TiO_4, $Zn_3(PO_4)_2$and $AgNO_3$, respectively.

Zinc orthophosphate and zinc titanate were added during wet milling to KF-11 glass in quantities of 1 – 5 mass parts and $AgNO_3$was added in quantity of 0.1 mass part to 100 mass parts of glass. Slip was applied on low-carbon steel 0.7 mm thick specimens with subsequent drying and firing of the coatings at 820 °C.

4.2. Investigation of biocide properties of glass-composite coatings

Investigation of inhibiting properties of tested glass-composite coatings and, for comparison, of the KF-11 coating not containing bactericide metal cations – Zn^{2+}, Ti^{4+} and Ag^{+}– has shown that the effect of their biocide properties on solid substrate, in liquid and gaseous media is different.

With the use of diffusion method by the estimation of growth deterioration zones [5] (change of diameter of inhibiting zone around the disc) it has been found that glass-composite coatings with additions of 0.1 mass parts of silver nitrate and 5 mass parts of zinc titanates how a biocide action only relatively to *Pseudomonas aeruginosa* bacteria and micro fungus *Candida albicans*.

In studying Petri dishes inoculated with *Pseudomonas aeruginosa* and *Candida albicans* cultures with the concentration of 10^7 cells /cm^3, an inhibition zone around the KF-11-3 specimen was noticed(figure 5), and in the case of KF-11-1 specimen it was observed only for *Candida albicans*. The inhibition zone diameter was 14 mm [5] or about4 mm [6]. For lower concentration of inoculant *Candida albicans* culture, of 10^5 cells/cm^3 around KF-11-3 specimen containing Ag^+ the decrease of growth density of microorganisms was visually observed (figure 6). These data provide the evidence of a significant migrating ability of Ag^+ to dense nutrient media. However, migrating ability of Ag^+ needs to be strictly monitored, as silver cations can accumulate in a human organism in significant quantities, causing various diseases.

The experimentally established absence of biocide effect for glass-composite coating containing $Zn_3(PO_4)_2$is explained by insignificant leaching from the glass material of Zn^{2+}. However, in simultaneous Zn^{2+} and Ti^{4+} presence a synergetic effect occurs which positively influences biocide properties of coatings.

The massive lawn growth of microorganism colonies on the entire surface of plates, including the space around all glass-composite coatings was observed on agar plates of all Petri dishes inoculated with *Escherichia coli* and *Aspergillus niger* cultures. Thus, inhibition effect was absent.

Figure 5. Zone of growth inhibition (dark areas) of *Candida albicans* around KF-11 specimen with $AgNO_3$at inoculant density of 10^7 cells /cm^3

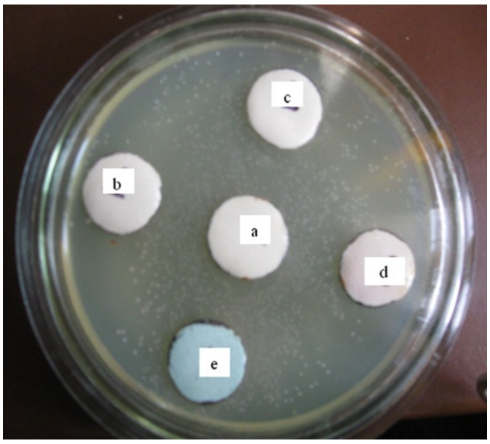


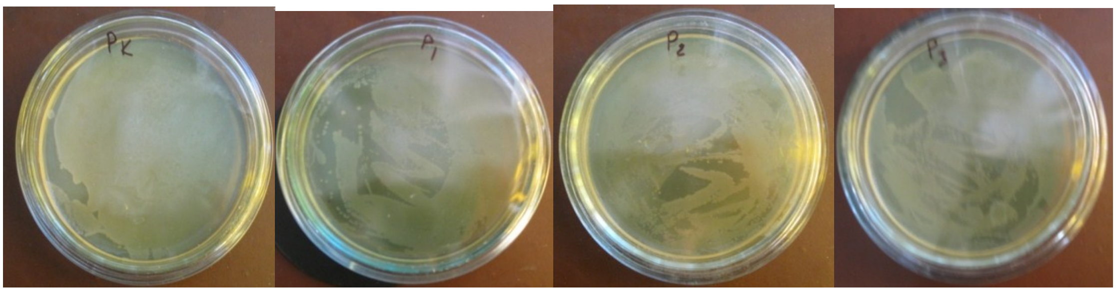
Figure 6. Zones of decreased growth of fungi *Candidaalbicans* – dark areas at the border of **b**, **d** and **e** specimens. White dots at the border of **a** and **c** specimens –*Candida albicans* fungus: **a** – KF-11 (control); **b** – KF-11 with Zn_2TiO_4; **c** – KF-11 with $Zn_3(PO_4)_2$; **d** – KF-11 with $AgNO_3$;**e** – KF-11 with $Cu_3(PO_4)_2$

Tests of the fungicide activity of the glass-composite coating by quantitative method in liquid media have shown that compositions KF-11-1 and KF-11-3 had fungicide properties. This was evidenced by growth decrease of vegetative cells and spores of *Aspergillus niger* and *Candida albicans* fungi. Thus, after seven days of exposure, the concentration of *Aspergillus niger* vegetative cells in nutrient medium of culture without glass-composite coatings has increased almost 20 times. During the same period, in the case of control glass coating KF-11 without bactericide powders the cell concentration has increased 19 times, and in the case of KF-11-2, 16.5 times, whereas KF-11-1 and KF-11-3 compositions have shown intensive inhibiting action on vegetative cells and spores of *Aspergillus niger* fungus; indeed, its concentration has increased only 1.4 and 1.3 times, respectively.

The coatings containing silver and zinc titanate have shown even more significant inhibiting action to *Candida albicans*. After 7 days of exposure in nutrient medium the concentration of fungus *Candida albicans* vegetative cells in these coatings has increased 145 times and in the case of initial glass coating with zinc phosphate KF-11-2 , 140 times whereas for KF-11-1, KF-11-3 it has increased only by a factor of10.

A bactericide activity test of glass-composite coatings in liquid medium has shown that almost all coatings, with the exception of KF-11-2, were indifferent relatively to *Escherichia coli* although as light antibacterial action has been observed to *Pseudomonas aeruginosa*. From figure 7 it is seen that on agar plate in Petri dish, where the inoculation from liquid media was made after the contact of control glass-coating KF-11 with the *Pseudomonas aeruginosa* culture, almost solid growth of colonies was observed. The colonies occupied almost 100 % of surface, showing the absence of bactericide effect. All glass-composite coatings were characterized by insufficient bactericide effect, which was 5 % for KF-11-2 and 15 % for KF-11-1 and KF-11-3.

The fungicide and fungus resistance tests of the same coatings by the aerosol method in artificially modeled growth conditions of *Candida albicans* culture have shown that in presence of an additional nutrient source – glucose – the viability of conidia partially persists for all specimens, including the control ones. In the conditions excluding additional nutrition source, the viability of fungus conidia was quite low at the contact with virtually all glass-composite coatings, except for KF-11-2 specimen, which apparently is used by microbes as a nutrient substrate due to the presence of phosphates.



(a) (b) (c) (d)

Figure 7. Influence of the type of biocide agent on the growth of *Pseudomonas aeruginosa*bacteria: (a) KF-11 (control glass-coating); (b)KF-11with Zn_2TiO_4; (c)KF-11with $Zn_3(PO_4)_2$;(d)KF-11with $AgNO_3$

The lowest number of sprouted vegetative cells was observed for KF-11-3 containing Ag^+ due to significant silver cations leaching form glass coatings. According to [9], the composition of this glass composite cannot be a nutrient medium (neutral or fungi static) for *Candida albicans* conidia and the compositions of glass-composite coatings KF-11-1 and KF-11-2 as well as of the control glass coating KF-11 may induce insignificant development of fungi.

A visual estimate of fungus resistance of glass-composite coating by surface destruction extent of the surface has shown that all tested coatings exhibit no change of color and gloss, no cracks, flaking or bubbles. This indicates that the test glass-composite coatings are resistant against organic acids.

In table 1 a comparative estimation of biocide properties of glass-composite coatings on the basis of KF-11 is given.

Table 1. Comparative estimation of biocide properties of glass-composite coatings on the basis of KF-11 glass

Biotest microorganism culture	Test method	Marking of coating			
		KF-11	KF -11-1	KF -11-2	KF -11-3
Bacteria		Bactericidal			
Escherichia	1	-	-	-	-
coli	2	-	-	+	-
Pseudomonas	1	-	+	-	+
aeruginosa	2	-	+	+	+
Fungi		Fungicidal			
Aspergillus	1	-	-	-	-
niger	2	-	+	-	+
Candida	1	-	+	-	+
albicans	2	-	+	-	+
	3	-	-	-	+
	3*		-	-	+

From the test results of the inhibiting properties of the glass-composite coatings it has been established that their action on solid substrate is determined by the migrating ability of Ag^+, Zn^{2+} and Ti^{4+}cations. Thus, the glass-composite coatings containing $AgNO_3$ and Zn_2TiO_4 as biocide components are characterized by bactericidal effect to *Pseudomonas aeruginosa* and fungicidal effect to *Candida albicans*. The fungicidal activity test of these glass-composite coatings by quantitative method relatively to *Candida albican s*and *Aspergillus niger* has also determined their biocide effect. The bactericidal activity to *Pseudomonas aeruginosa* has been shown for all tested glass-composite coatings. In the case of *Escherichia coli* the effect was observed only for the coating containing $Zn_3(PO_{4)2}$. The test of glass-composite coatings by aerosol method for fungicidal action to *Candida albicans* has shown that only the coatings with $AgNO_3$ additions have inhibiting properties.

5. Conclusions

It has been established that the directional crystallization of calcium phosphates in glasses of Na_2O – CaO – TiO_2 – P_2O_5 – SiO_2 system is the principal condition of structure formation which is necessary for providing of bactericidal metal Zn^{2+} and Ti^{4+}cations orientation in surface layer of vitreous coating.
Quantitative technique of the pathogenic micro-organisms growth determination in liquid medium is the most suitable for assessment of vitreous coatings bactericidal action in service conditions of enamelled steel products in contact with food.
We have shown that glass-ceramic coatings containing zinc titanate and Ag^+cations have the most significant biocide action to *Pseudomonas aeruginosa* bacteria and *Aspergillus niger* and *Candida albicans* fungi whereas *Escherichia coli* is affected by coating with zinc phosphate only.
The principles of synthesis of the biocide glass-ceramic coatings developed by us can be used to create vitreous materials for protection of household and architectural steel products.

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