Journal homepage: www.fia.usv.ro/fiajournal
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XIV, Issue 1 - 2015, pag. 89 – 92

NANODISPERSED COMPOSITE ANTIMICROBIAL MATERIALS BASED ON CALCIUM HYDROXYLAPATITE

***Igor KOBASA[1], Galyna BILOKOPYTA[2], Larysa ARSENIEVA[3]**

[1]*Yu. Fedkovych National University of Chernivtsy, Ukraine,*
[2]*Bukovynian State Medical University, Chernivtsi, Ukraine*
[3]*National University of Food Technologies, Kyiv, Ukraine*
i.kobasa@chnu.edu.ua
* Corresponding author
Received 5th March 2015, accepted 28th March 2015

Abstract: *Some composite materials based on calcium hydroxylapatite and exhibiting antimicrobial activity have been investigated in the context of influence of the synthesis conditions on the phase composition of the materials and their antimicrobial activity against Staphylococcus aureus and Escherichia coli. It has been found that the method of synthesis involving sedimentation from the solutions of* $Ca(NO_3)_3$ *and* $(NH_4)_2HPO_4$ *by ammonium hydroxide results in formation of the hydroxylapatite structures (*$Ca_{10}(PO_4)_6(OH)_2$*) and* $Ca_{2,993}H_{0,014}(PO_4)_2$ *exhibiting high antimicrobial activity against the above mentioned pathogenic microbes.*

Keywords: *calcium hydroxylapatite, antimicrobial activity, Staphylococcus aureus, Escherichia coli*

1. Introduction

Antimicrobial protection is very topical issue related to many branches of today's life, trade, production and other activities. Even though dozens of new antimicrobial agents are being constantly developed and introduced, contagious diseases remain very serious problem affecting millions of people each year [1]. However, many of such diseases can be prevented by using various solutions that depress reproduction of pathogenic microbes. Therefore, any technologies involved in such solutions seem very topical.

There are numerous chemical compounds exhibiting bactericide properties: ozone, permanganates, hydrogen peroxide (all these compounds exhibit so-called oxidative bactericide mechanism involving oxidative destruction and/or deactivation of microbial proteins and ferments) and some metal ions with 'oligodynamic' bactericide activity. These ions can be ranged in the following row according to decrease in their activity: $Ag^+ > Hg^{2+} > Cd^{2+} > Cu^{2+} > Au^{3+} > Ni^{2+} > Zn^{2+}$ [2].

As seen from the recent publications, the nanotechnological solutions are quite attractive and popular because physico-chemical and biological properties of the nanosized materials are quite different from their macrosized analogs [3-5].

Nanoparticles of calcium hydroxylapatites have been recently proposed for development of new composite antimicrobial materials. Such materials can be applied to development and production of antibacterial ceramics, pigments for the dye coverings, packaging materials and so on [6-8]. It is well known that many properties of calcium hydroxylapatite including its antibacterial activity can vary within quite a wide range depending on the synthesis conditions, presence and concentration of some arbitrary admixtures, specific surface area value, lattice structure and defectiveness class and concentration [4, 9, 10].

This paper deals with investigation of antimicrobial activity of some nanosized examples of calcium hydroxylapatite synthesized by sedimentation of calcium nitrate and ammonium hydrophosphate by ammonium hydrate ("wet" method) and by calcination of the dry mixture of calcium carbonate and ammonium hydrophosphate ("dry" method).

2. Experimental

The "wet" method of synthesis assumes obtaining of the calcium hydroxylapatite by its precipitation from the source solution and formation of the amorphous product according to the reaction:

$$10Ca(NO_3)_2 + 6(NH_4)_2HPO_4 + 8NH_4OH = Ca_{10}(PO_4)_6(OH)_2\downarrow + 20NH_4NO_3 + 6H_2O$$

It should run under the temperature 45 ± 5 ºC and pH=11.

The "dry" method of synthesis assumes mixing and grinding of the dry $CaCO_3$ and $(NH_4)_2HPO_4$ followed by calcination at 1000 ºC during 2 hr. The following reaction occurs during the thermoprocessing:

$$10CaCO_3 + 6(NH_4)_2HPO_4 = Ca_{10}(PO_4)_6(OH)_2 + CO_2\uparrow + NH_3\uparrow + 6H_2O$$

The final product of calcination should be ground to the uniformal state.

Antimicrobial activity has been determined by the diffusion ("holes") method according to the standard ISO 27447:2009(E) as described in details below.

The bacterial cultures were grown using the stable nutritional medium and standard bacterial strains of Staphylococcus aureus and Escherichia coli from American Type Culture Collection. The inoculation material was prepared as follows: fresh bacterial colonies were taken after the 18 hours of growing in the sterile physiological medium (0,85 % NaCl) in such a way to ensure formation of the suspension with 0.5 McFarland's density degrees, which is approximately equal to $1\text{-}2\cdot10^8$ CFU/ml. Then the material was ten times diluted forming the 0.05 degrees system and then ten times again to form the 0.005 degrees system. Density of the systems has been determined nephelometrically. The latter (0.005 degrees) system was used for the next investigations. A cotton tampon with the inoculation material was immersed in the sterile solution, extracted and squeezed to remove the excessive solution. Then the inoculation material was seeded on the substrate by triple application of the tampon with rotation 60^0 after each application. The substrate plates were dried and then 50 μl of various hydroxylapatite suspensions were added to investigate their antimicrobial activity. Each probe contained of the 0.1; 1.0; 5.0 and 10.0 % suspensions of two hydroxylapatites: (1) – the "wet" calcium hydroxylapatite; (2) – the "dry" calcium hydroxylapatite. All the suspensions were prepared on the basis of physiological solution. The probes were placed into the incubator and stayed in the oxygen containing medium under 37^0 for 16-18 hours. Then the diameter of the microbes decelerated growth area was measured as the source parameter to evaluate the antimicrobial activity. The standard disk impregnated by novobiocin was used as the reference sample of the antimicrobial activity.

3. Results and discusion

It was found that the phase composition of calcium hydroxylapatites depends on the synthesis conditions. The "wet" material has the composition that combines $Ca_{10}(PO_4)_6(OH)_2$ and $Ca_{2,993}H_{0,014}(PO_4)_2$ while the "dry" material combines $Ca_{10}(PO_4)_6(OH)_2$ and $Ca_3(PO_4)_2$. This difference may be caused by different synthesis and next thermal processing conditions. It will be shown below that the structural composition of calcium hydroxylapatite affects antimicrobial activity of the composite materials comprising this compound.

Analysis of antimicrobial activity of hydroxylapatite samples proves that the diameter of the decelerated growth area of Staphylococcus aureus depends on the synthesis method and concentration of calcium hydroxylapatite (see Fig. 1).

The "wet" hydroxylapatite shows the higher antimicrobial activity than the "dry" compound and activity of all the samples grows with increase in the content of calcium hydroxylapatitein in the suspension.

It was found that antimicrobial efficiency of all hydroxylapatites against the agar colonies of Escherichia coli prevails that against Staphylococcus aureus. The total extinction of the Escherichia coli colonies growing on meat infusion agar has been registered (see Fig. 2). As seen from Fig 1 and Fig. 2, the "wet" hydroxylapatite with concentrations 0.1, 1.0, 5.0 and 10.0 % is active against the standard bacterial colonies of Staphylococcus aureus and Escherichia coli (bacterial suspensions with density 0,005 McFarland degrees).

Antibacterial efficiency is growing with increase in the suspensions concentration. The "dry" suspensions also exhibit some antibacterial activity with concentrations 0.1, 1.0, 5.0 and 10.0 % but its level is lower than that of the corresponding "wet" suspensions.

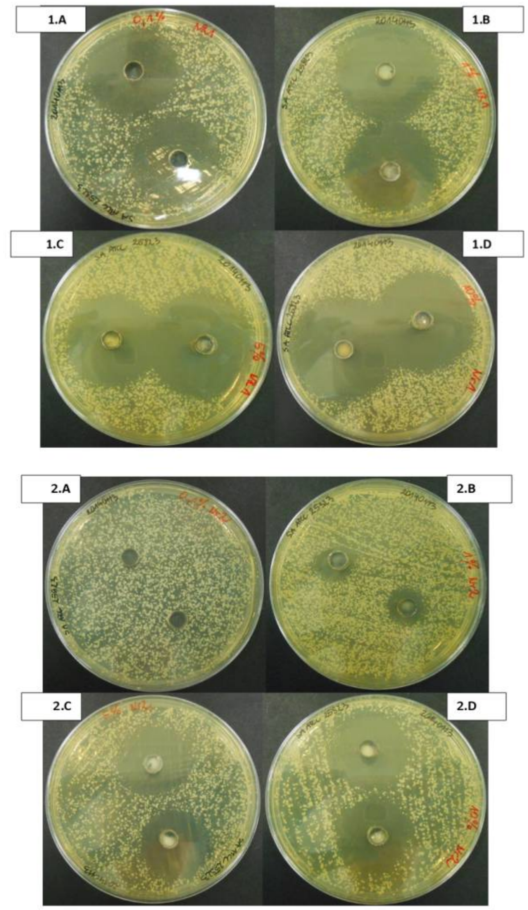

Fig. 1. Sensitivity of Staphylococcus aureus to antimicrobial action of the "wet" (1) and "dry" (2) calcium hydroxylapatite with the following concentrations: A – 0.1, B – 1.0, C – 5.0 and D – 10 %.

For instance, the 0,1 % "dry" suspension does not exhibit any antibacterial activity against Staphylococcus aureus while the effect of similar "wet" suspension is quite tangible (see Fig. 1).

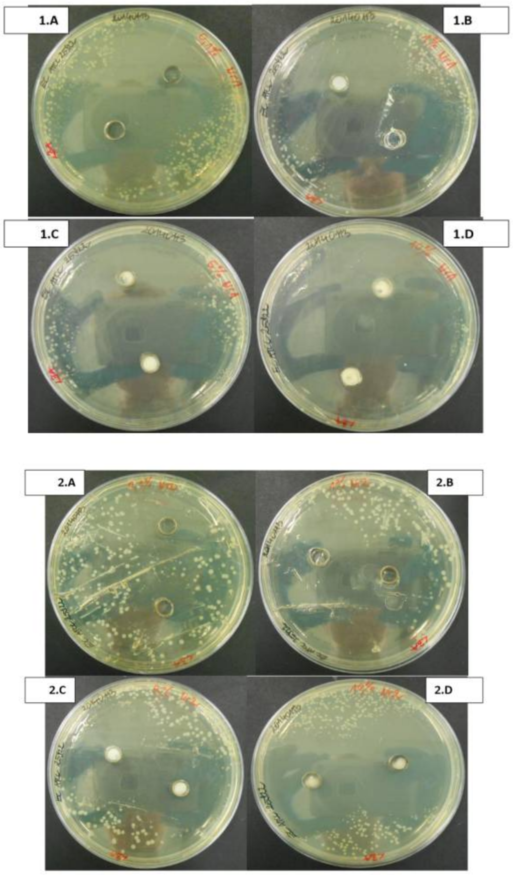

Fig. 2. Sensitivity of Escherichia coli to the "wet" (1) and "dry" calcium hydroxylapatite with concentrations: A – 0.1, B – 1.0, C – 5.0 and D – 10 %

4. Conclusion

Calcium hydroxylapatite materials synthesized through sedimentation from aqueous solution of calcium nitrate and ammonium hydrophosphate by ammonium hydroxide or through calcination of the dry mixtures of calcium carbonate and ammonium hydrophosphate exhibit the antibacterial activity against strains of Staphylococcus aureus and Escherichia coli. The activity of the "wet" material is higher than that of the "dry" samples, which can be used for development of the bactericide composite materials.

5. References

[1] K.E. JONES, N.G. PATEL, M.A. LEVY [et. al.] Global trendsin emerging infectious diseases. Nature. **451**, 990-993 (2008).

[2] E. VOB, C. STÖRCH. Evaluation of bacterial growth on various materials. The 20th International Enameller Congress, Istanbul-Turkey, 15-19 May. 194–210 (2005).

[3] R.Y. PELGRIFT, A.J. FRIDMAN. Nanotechnology as a therapeutic tod to combat microbial resistance. Adv. Drug Deliv. Rev. **65**, 1803-1815 (2013).

[4] I.M. KOBASA, W. STRUS, M.A. KOVBASA. Highly photosensitive titanium dioxide and process for forming the same. US 2008/0146441.

[5] I.M. KOBASA, W. STRUS, M.A. KOVBASA. Method of surface modifying titania using metal and compositions therefrom. WO 2009/052510.

[6] O.V. SAVVOVA, L.L. BRAGINA, E.V. BABICH. Nanostructural calciumsiliconphosphate glasscrystalline materials of medical purpose. Nanosystems, nanomaterials, nanotechnologies. **8**, 903-910 (2010)

[7] O.V. SAVVOVA, L.L. BRAGINA, N.P. SOBOL. Inorganic powder based on calcium phosphate for obtaining of antibacterial glass-enamel covering. Patents and inventions, №91878, 2010.

[8] M. A. RAUSCHMANN, T.A. WICHELHAUS, V. STINAL [et. al.]. Nanocrystalline hydroxyapatite and calcium sulphate as biodegradable composite carrier material for local delivery of antibiotics in bone infections. Biomaterials. **26**, 2677-2684 (2005).

[9] V. STANIC, S. DIMITRIJEVIC, J.ANTIC-STANKOVIC [et. al.]. Synthesis, characterization and antimicrobial activity of copper and zinc-doped hydroxyapatite nanopowders. Applied Surface Science. 256, 6083-6089 (2010).

[10] M.P. FERRAZ, A.Y. MATEUS, J.C. SOUSA, F.J. MONTEIRO. Nanohydroxyapatite microspheres as delivery system for antibiotics: Release kinetics, antimicrobial activity, and interaction with osteoblasts. Biomedical Materials Research Part A. **81A**, 994-1004 (2007).