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NANOSIZED TITANIUM DIOXIDE AS AN ANTIBACTERIAL ADMIXTURE FOR FOOD PACKAGING MATERIALS

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Abstract: *The article deals with research on antibacterial activity of the composite materials based on titanium dioxide. The influence of synthesis condition on phase composition of titania as well as its photocatalytic activity and antibacterial effect against the pathogenic microbes such as Staphylococcus aureus and Escherichia coli have been investigated. It was proved that the structure of nanosized titania samples synthesized by the high temperature hydrolysis of titanium tetrachloride in the air-hydrogen atmosphere at 700-1100 ^{0}C is non-stoichiometric. These samples consist of both anatase and rutile crystalline modifications and exhibit comparatively high antibacterial activity.*

Keywords: *TiO_2, photocatalytic activity, antimicrobial activity, Staphylococcus aureus, Escherichia coli.*

1. Introduction

Contagious diseases set acute challenge for the health care field all over the world despite rapid progress achieved in pharmacy and new drugs research and development [1]. That is why special attention is paid to the search of new technological solutions related to antibacterial protection. In this context, it seems topical to design and develop new non-toxic packaging materials providing protection of food from pathogenic microbes and other adverse effects during the production and storage stages.

Main efforts are being spent to development of the antibacterial composite materials to be used in the bactericide coverings for medical, pharmaceutical, sanitary-technical and household needs. It has been reported that some antibacterial glass/enamel coverings were synthesized on the base of copper and silver compounds [2], titanium dioxide [3] and calcium hydroxylapatite [4, 5]. Other authors reported new antibacterial composite materials containing the ions of some noble or heavy metals Ag^+, Au^{3+}, Hg^{2+}, Cd^{2+}, Cu^{2+} [6] or nanosized particles of silver and gold [7, 8].

TiO_2 nanoparticles have been recently proposed as a source material for synthesis of the composites with high antibacterial activity [9]. Such composites can be used as components of antimicrobial ceramics, lacquer/dye coverings or microbe-proof packaging.

It is known [10-12] that antibacterial and photocatalytic activity (PA) of TiO_2 can vary depending on the conditions of its synthesis, temperature of preliminary treatments, nature and concentrations of the admixtures added to the source synthesis composition, specific surface area, presence or absence of the crystalline inclusions, TiO_2 structure defects, their nature and concentration. Therefore, it is possible to govern photocatalytic and antibacterial activity of TiO_2 through changes in these parameters and obtain either highly or moderately active products.

It is known that anatase is the most photocatalytically active modification of titania (rutile and brookite are two other modifications) [13]. Different crystal lattices, various degree of the lattice imperfectness, presence of some admixtures can be mentioned as the factors causing different photocatalytic activity of various titania modifications. TiO_2 is one of the semiconducting photocatalysts used most widely because of stability of its aqueous mixtures, cheapness, non-toxicity and high activity of the material that is based on strong oxidation efficiency of the photogenerated holes. Besides, TiO_2 is registered as a food additive E171. This interest towards TiO_2 has initiated numerous investigations aimed onto development of the TiO_2-based materials with predefined photocatalytic and antibacterial characteristics, phase composition and texture. Our efforts also followed this direction and were targeted onto synthesis of the TiO_2 material with some required properties.

This paper reports results of investigation of the activity of the anatase and rutile modifications of titania and the non-stoichiometric product containing a mixture of anatase and rutile that has been carried out in order to develop the antibacterial packaging materials.

2. Experimental

Three kinds of the TiO_2 samples were used in this work: (1) nanosized titania synthesized by the high temperature hydrolysis of titanium tetrachloride vapors in the air-hydrogen flame at 700-1100 ^{0}C according to [13], specific surface area (S_{BET}) = 50 m^2/g; the anatase (2) and rutile (3) modifications of TiO_2 with S_{BET} of 20 and 10 m^2/g respectively, which have been synthesized by the low temperature hydrolysis of titanium chloride and sulfate [10]. The phase composition and particles size of all the samples were checked prior other investigations.

The powder surface was analyzed with X-ray Photoelectron Spectroscopy (XPS) also referred to as Electron Spectroscopy for Chemical Analysis (ESCA) using ESCALA™ XI$^+$ X-ray Photoelectron Spectrometer Microprobe. XPS is an elemental analysis technique which is capable of detecting all elements except for H and He and it has a nominal detection limit of ~0.1 atom%. Spectral interferences may prohibit the detection of some elements in relatively low concentrations. Samples were measured at a 90° Take-Off-Angle yielding a sampling depth of ~10 nm. The analysis area was ~500 µm in diameter. Analyses were performed with a monochromatic Al kα x-ray source. The powder particle size analysis was performed with a Field Emission Scanning Electron Microscopy (FESEM) using Hitachi SU70 Electron microscope. FESEM images depict topographic features of the sample surface. FESEM imaging was performed at 2 keV. One hundred particles of each powder were measured to provide an average particle size. Both powders were coated with ~100 Å of gold to facilitate analysis.

A reaction of methylene blue reduction has been used to measure photocatalytic activity of the samples [14]. Antibacterial activity of the samples has been investigated using the diffusion method (also known as the disks method) according to the standard ISO 27447:2009(E) and methods [4]. The bacterial colonies were cultivated from the ATCC standard strains *Staphylococcus aureus* and *Escherichia coli* while a paper disk impregnated with antibiotic novobiocin was used as a control experiment. Antimicrobial activity has been determined by the diffusion ("holes") method according to the standard ISO 27447:2009(E) as described in details below.

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 nanodispersed TiO_2 suspensions were added to investigate their antimicrobial activity. Each sample consisted of 0.1; 1.0; 5.0 and 10.0 % suspensions of the nanosized titanium dioxide. 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 (5 μg) was used as the reference sample of the antimicrobial activity.

3. Results and Discussion

It was found that phase composition and photocatalytic activity of the TiO_2 samples depend on the method used to synthesize each sample (see Table 1).

The pyrogenic TiO_2 obtained by the high temperature synthesis from $TiCl_4$ vapors is a highly defective material that contains both rutile and anatase crystalline modifications. There are many paramagnetic centers in such material, which serve as active photocatalytic centers [15]. The size of these particles ranged between 45 to 174 nm (see Fig. 1).

A mechanism of photocatalytic and antibacterial activity of nanosized TiO_2 depends on the process or origination of the photoexcitated electrons initiated by the light quanta. These electrons can be transferred from the allocated valence band of a semiconductor into its unallocated molecular orbital in the conductivity band. A pair electron-hole (e^-–h^+) is formed as a result of these processes and then further transformations can run in different directions [16]:

- photoexcitation: $TiO_2 + hv \rightarrow e^- + h^+$;
- absorption of electron by oxygen: $O_2 + e^- \rightarrow O_2^{\bullet-}$;
- ionization of water: $H_2O \rightarrow OH^- + H^+$;
- peroxide formation by protonation of the ion-radical: $O_2^{\bullet-} + H^+ \rightarrow HOO^\bullet$.

The hydroperoxide radical formed in the latter process can take part in the following processes: $HOO^\bullet + e^- \rightarrow HO_2^-$ or $HOO^- + H^+ \rightarrow H_2O_2$. Therefore, the processes of oxidation and reduction can run simultaneously on the surface of photoexcitated semiconducting photocatalyst. An electron-hole recombination can take place if oxygen is capable to accept electrons and form peroxide ($O_2^{\bullet-}$), which participates in the follow-up protonation and form hydro peroxide radical ($HOO^{\bullet-}$) and then hydrogen peroxide (H_2O_2).

Particles sizes for the anatase (2) and rutile (3) samples of TiO_2 synthesized by the liquid phase hydrolysis are 0.2 and 0.5 μm simultaneously, these samples are low-defective and exhibit low PA. As it will be explained below, phase composition and structure of TiO_2 also provides a significant influence on antibacterial activity of the titania-based composites. For instance, no antibacterial activity has been found for the TiO_2 samples synthesized by the liquid phase hydrolysis of titanium chloride and sulfate.

Table 1

Photocatalytic activity and phase composition of the TiO_2 samples synthesized by various methods

Synthesis method	Phase composition	PA*, mg/(ml×min×m^2)
Liquid phase hydrolysis of $TiCl_4$	anatase	$1.2\times10^{-2} \pm 0.05$
Liquid phase hydrolysis of $Ti_2(SO_4)_3$	rutile	$1.7\times10^{-3} \pm 0.05$
High temperature hydrolysis of $TiCl_4$ vapour	anatase 50 % + rutile 50 %	1.2 ± 0.09

** - all the data are obtained in the 5-repetitions series and the mean values with standard errors are shown in the column*

As seen from the antibacterial activity investigation results (see Fig. 2), the colony extension inhibition area depends on TiO_2 concentration in suspension.

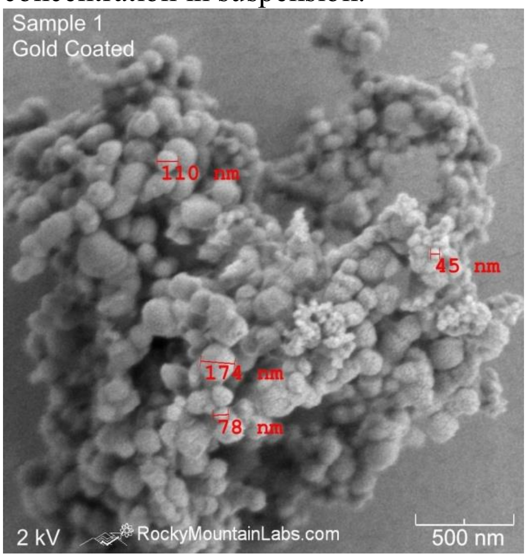


Figure 1. SEM image of the TiO_2 sample.

Antibacterial activity of TiO_2 suspensions containing 1.0, 5.0 and 10.0 wt % of TiO_2 as against the gram-negative microbes ***Escherichia coli*** is higher than that of novobiocin (see Fig. 2, C and D); the novobiocin deceleration area diameter was of 13 mm, while the nanosized TiO_2 suspensions areas were 7, 15, 16 and 20 mm for TiO_2 concentrations 0.1; 1.0; 5.0 and 10 % respectively. Therefore, three later compositions exhibit higher antibacterial activity than antibiotic novobiocin.

Antibacterial activity of TiO_2 suspensions containing 5.0 and 10.0 wt % of TiO_2 against the gram-positive microbes ***Staphylococcus aureus*** is higher than that of novobiocin (see Fig. 2, C and D): the novobiocin deceleration area diameter was 12 mm while the nanosized TiO_2 suspensions areas were 1, 7, 13 and 15 mm for TiO_2 concentrations 0.1; 1.0; 5.0 and 10 % correspondingly. Therefore, two later compositions exhibit higher antibacterial activity than antibiotic novobiocin.

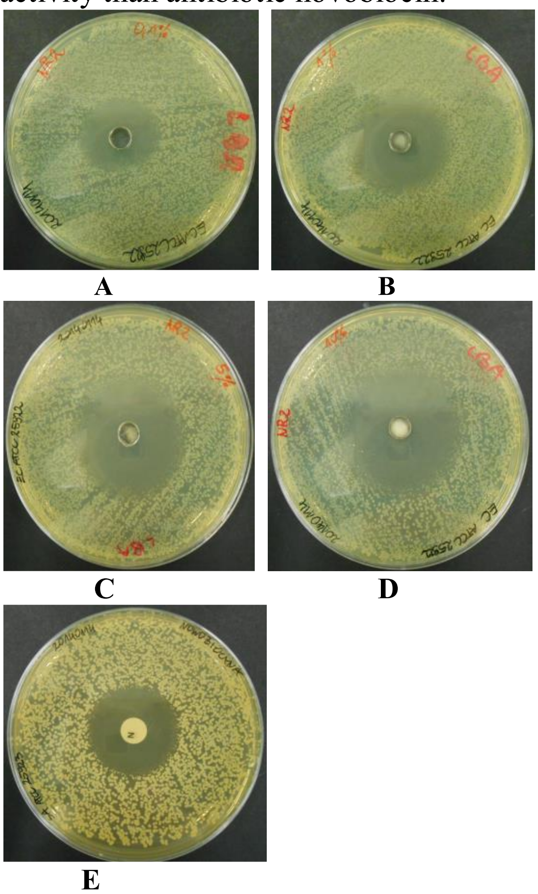
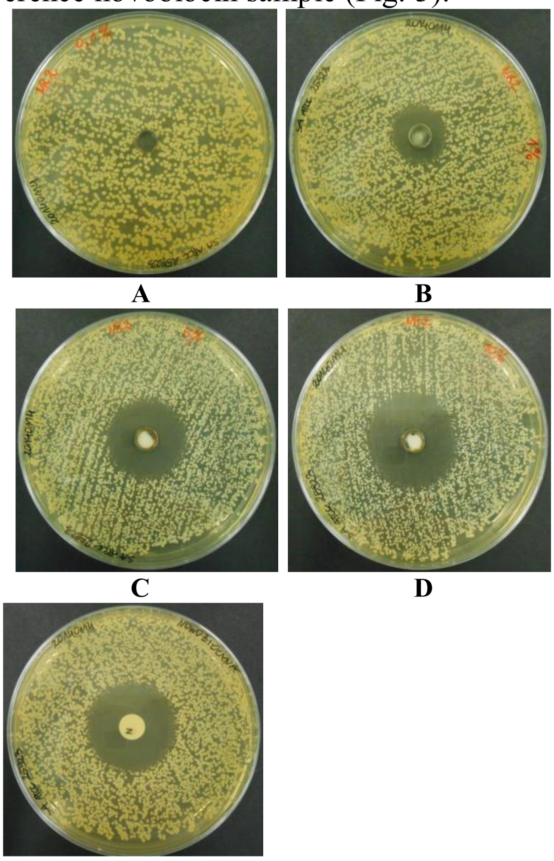


Figure 2. Antibacterial activity of various compositions against *Escherichia coli*: A – 0.1, B – 1.0, C – 5.0 and D – 10 % of TiO_2; E – novobiocin.

The activity rises with increase in the concentration so that antibacterial activity of the 1.0 % TiO_2 suspension is approximately similar to that of the standard sample test performed with antibiotic novobiocin (5 μg) (Fig. 2 E). Moreover, antibacterial activity of the 5.0 and 10.0 % TiO_2 suspensions in relation to *Escherichia coli* (Fig. 2 C, D) is higher than that of novobiocin.
Antibacterial activity of the nanosized titania-based compositions against gram-positive *Staphylococcus aureus* cultivated in the beef-extract agar is less noticeable than the activity against *Escherichia coli*. Only after the concentration of TiO_2 in the suspensions becomes equal to 5.0 %, the activity reaches same value as for the reference novobiocin sample (Fig. 3).



A B C D E

Figure 3. Sensitivity of *Staphylococcus aureus* to the nanodispersed suspensions of TiO_2 with concentrations: A – 0.1, B – 1.0, C – 5.0 i D – 10 %; E – novobiocin.

As seen from analysis of experimental results of the TiO_2 samples antibacterial activity investigation, only nanosized titania reveals some activity against pathogenic organisms. That is why such material can be used in further antibacterial research as a component of special bactericide coverings.
In this context, the antibacterial activity of TiO_2 samples was tested by adding 15 wt % of this source material to acryl-based lacquer/dye compositions. An aqueous suspension of the composition was applied by spraying over the metal surface in such a way to ensure formation of a thin homogeneous layer that covers the entire surface. Then the covering was dried in the open air at the room temperature during 24 hours and used to carry out the antibacterial activity test according to ISO/WD 17094: "Fine ceramics (advanced ceramics , advanced technical ceramics) – Test method for antibacterial activity of semiconducting photocatalytic materials under indoor lighting environment" [17]. All samples were preliminary disinfected by 70 % ethanol and then kept under the following incubation conditions: 4 or 8 hours at 25 ^{0}C, humidity 65 %. Illumination intensity was 1000 lx. All the coverings received by this method ensured comparatively high antibacterial activity against pathogenic microbes *Escherichia coli* and *Staphylococcus aureus*. It was found that 99.4 and 93.7 % of the above colonies respectively were extinguished after 4 hours long contact with the surfaces containing the nanodispersed TiO_2 while 8 hours long contact caused total extinguishing of the colonies of both bacterial species. No antibacterial activity has been revealed in the control experiments with the same coverings containing no TiO_2.
That is why it can be assumed that the nontoxic bactericide packaging materials can be obtained by adding some TiO_2 as a source component.

4. Conclusion

The TiO_2 samples synthesized by high temperature hydrolysis of $TiCl_4$ vapors in the air/hydrogen mixture have shown high antibacterial activity against the standard strains of *Staphylococcus aureus* and *Escherichia coli* (total extinguishing of both microbial colonies has been achieved after the 8 hours long application of the TiO_2 samples).

A comparatively high antibacterial activity has also been registered by the acryl-based lacquer-dye compositions containing some nanosized TiO_2. These materials can potentially find wide applicability in the area of food packaging materials production.

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