EASY-IMPLEMENTING ANTIMICROBIAL POLYPROPYLENE MATERIALS MODIFIED BY SILVER(I) AND COPPER(II) IONS

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Common polypropylene exhibiting no antimicrobial activity was effectively transformed into novel functional material. A surface of polypropylene materials was preliminarily activated via two chemical methods which are the treatment of the sample with water solution of sodium hydroxide in presence of iron(II) sulfate under boiling temperature and treatment with water solution of hydrogen peroxide in presence of iron(II) sulfate (to activate decomposition of the peroxide). Silver(I) and copper(II) ions were immobilized onto the surface of activated polypropylene, giving a set of four hybrid materials. Resulting functional polymers are of continuous and comprehensive antimicrobial activity. An impact of preliminary chemical activation of the polypropylene surface on the antimicrobial activity of the hybrids was shown. The highest efficiency in terms of antimicrobial properties is manifested by nonwoven polypropylene material activated by 20% water solution of chemically pure sodium hydroxide in the presence of iron(II) sulfate under boiling temperature for 2 hours followed by immobilization of silver ions.

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INTRODUCTION

Polymers are of wide application in almost every sphere of humanity. Polypropylene is one of the most widely used and available commercial commodity polymers produced in tons. In order to impart it more functions, one need to functionalize a surface by some active compounds because polypropylene itself has limited application range due to brittleness and hydrophobic nature. There are three common ways to realize the functionalization, namely: plasma treatment that is expensive, but environmental-friendly;[1,2,3] microwave treatment being controversial;[4,5,6]and relatively dirty but cheap chemical activation.[7,8] Resulting surface acquires activated groups being able to form bonds with different compounds that make polypropylene to be a potential carrier. This approach is used, for example, for catalysis of oil sweetening.[9,10] However, one of the most significant polymers application area is medicine.

Use of biomedical polymer materials modified by curative and antimicrobial compounds becomes increasingly common in recent years.[11,12] Practical medicine concluded advisability of using hybrid medicinal drugs being principally (polymer carrier)+(a biologically active substance (BAS)) combination.[13] Chemical modification of polymer surface by BAS requires the initial materials to be biologically inert, mechanically durable and able to link BAS after preliminary activation. Polypropylene (PP) meets all of these requirements. Materials based on it are widely used in medical practice, which for example, are suture materials for microsurgery, surging clothes, dressing and covering means made of non-woven fabric produced using spunbond technology ("Spunbond"), etc.

There are two common and easily available metals exhibiting antimicrobial activity, namely copper and silver.[14,15,16,17] In order to approach obtaining antimicrobial activity being easy and at the same time effective these very two elements were chosen for the work.

We previously obtained PP films functionalized by indomethacin,[18] PP films and nonwoven materials containing immobilized gentamycin.[19] All compounds exhibited antimicrobial activity. Although microorganisms become resistible towards antibiotics, there is no adaptation to antiviral, antifungal and antiseptic action of silver and copper ions. Therefore, the work provides data on PP material's modification by copper and silver ions to impart them antimicrobial properties. The goal was to develop a cheap, easy-implementing and efficient approach for obtaining antimicrobial materials, which could be performed right on the place of probable application, for example, hospital.

RESULTS AND DISCUSSION

PP is one of the most inert polymers that is to say it contains no active functional groups neither on the surface no in the volume. Therefore, it is impossible to anchor antimicrobial substances on the surface of the initial PP. That is why chemical activation of PP surface being part of the entire modification process is of great importance.

Successful activation of polypropylene surface is proved by spectrophotometric studying resulted in samples. Spectrophotometric studies of chemically activated polymer surface by ATR IR technique indicate the formation of absorption bands in the range of 3650–3100 cm^{-1} correspondings to valent vibrations of H-bonded OH-groups upon alkaline etching at elevated temperature (method 1). This is supported by absorption bands in the range of 1680–1600 cm^{-1} related to stretching vibrations of OH-groups. Moreover, this kind of activation leads to the formation of absorption bands in the range of 1710–1650 cm^{-1}

characterizing asymmetrical vibrations of carbonyl groups of modified polymer surface layer as well as absorption band in the range of 1580–1560 cm^{-1} which are attributed to carboxylate-ions.[20,21]

Similar spectral change is observed upon chemical activation of PP by the solution of hydrogen peroxide in the presence of iron(II) salt (method 2). IR-spectra show formation of primary alcohol groups upon activation according to method 2 that is confirmed by absorption bands in the range of 3000–2800 cm^{-1} (stretching vibrations of H-bonded OH-groups) complemented by absorption bands in the range of 1350–1240 cm^{-1} (deformation vibrations of OH-groups). There is also an increase of absorption band in the range of 1180–1160 cm^{-1} intensity corresponding to vibrations of C-(O)H groups. In addition, signal accumulation is observed in the range of 1750–1730 cm^{-1}, which corresponds to asymmetric valence vibrations of the carbonyl group and is confirmed by the amplification of the absorption band signal in the range of 1380–1360 cm^{-1} (deformation vibrations of the carbonyl group).[20,21,22] ATR IR spectra of PP film before treatment and after are shown in Figure S2. Spunbond exhibits similar spectral behavior.

Summarizing the aforesaid, suchlike chemical activation of polymers leads to the formation of the modified surface having a large number of reactive groups. Herewith, the amorphous phase is predominantly etched and globular structures of the crystal phase are redistributed.[23] Such changes of the activated PP surface were observed by AFM. Since the PP films have low surface stiffness, the AFM intermittent contact mode was applied, which allows to significantly reduce the lateral forces between the probe and the sample. Figure 1 provides 3D micrographs of the PP film's surface obtained by AFM method.

Figure 1 demonstrates the chemical activation being able to change the surface morphology. The surface of unactivated PP films is characterized by the presence of large peaks and depressions, some of the concentration areas are of 10-45 μm high and 0.8-2.0 μm diameter (Figure 1, a). Surface morphology is smoothed resulting from chemical activation that is shown in Figure 1, b, where the height of some concentration areas is 0.3-3.0 μm and diameter is 0.1-0.5 μm. The samples activated according to method 2 exhibit resembling characteristics, the height of the concentration areas is 0.2-1.7 μm and diameter is 0.05-0.18 μm (Figure 1, c). AFM data are supported by SEM images given in Figure S1.

Table 1. Physicomechanical characteristics of the initial and activated polypropylene film.

***t*, min**	**The workload at break (Nm^{-1})**	**Elongation at break, %***
Untreated		
0	9.8 ± 0.4	35 ± 1.7
Activated by 30 % aq. H_2O_2		
90	9.4 ± 0.5	32.5 ± 1.5
120	9.5 ± 0.4	32.2 ± 1.7
Activated by 20 % aq. NaOH		
90	7.2±0.9	28.3±1.7
120	7.1±1.0	28±1.7

*Relative to initial sample length equal to 60 mm working speed at break is 100 mm s^{-1} (constant for all samples); error in measuring the working load and elongation at break is not greater than 5 %.

Obtained data suggest a less essential change of the surface in case of chemical activation according to method 2 compared to that one according to method 1. This fact indicates less harsh modification of supramolecular polymer surface, that is in agreement with results of a study of the strength characteristics of activated PP samples given in Table 1 showing treated materials to lose mechanical durability insignificantly.

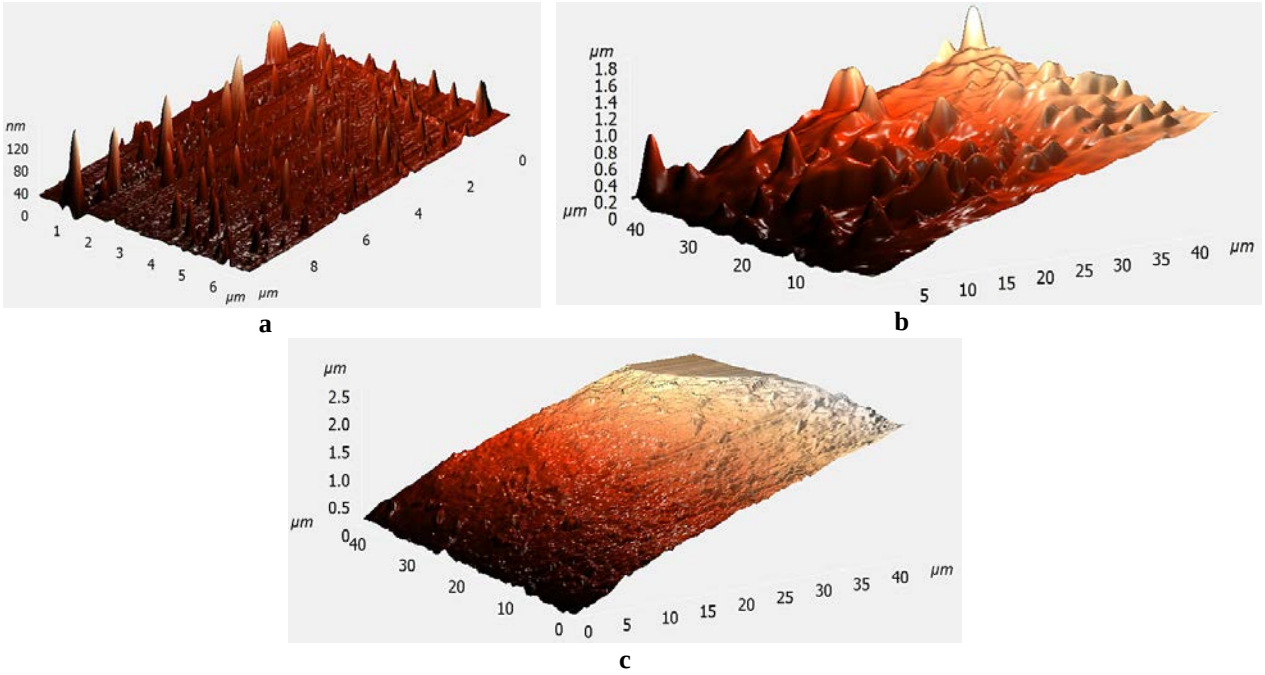

Figure 1. 3D micrographs of the surface according to the size's distribution (AFM data): **a** – initial PP film; **b** – PP film activated according to method 1; **c** – PP film activated according to method 2.

This indirectly suggests smooth morphology's modification without inner structure changes. Chemically activated samples were treated by water solutions of silver(I) and copper(II) nitrates in order to obtain PP materials (film, "Spunbond") being antimicrobial. Since the chemically activated surface of PP materials has active oxygen-containing functional groups and immobilized substances are dissociating, the way of anchoring metal ions onto the surface of polymer-carrier proceeds through the formation of chelate bonds in case of Cu, while Ag is bonded through weak coordination interaction. Proposed mechanism for the process is presented in Figure 2.

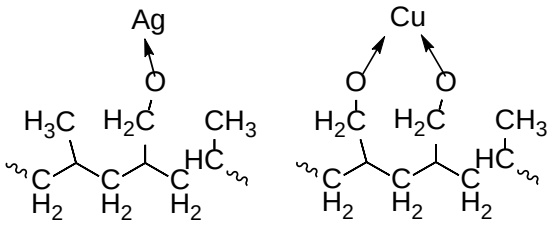

Figure 2. The proposed binding mechanism for Ag and Cu with polypropylene.

Obtained modified PP materials were tested toward antimicrobial activity. Experimental data are presented in Figure 3, Table 2. Conditions A and B used upon antimicrobial experiment were applied in order to find out the antimicrobial effect of weakly-bonded metal ions.

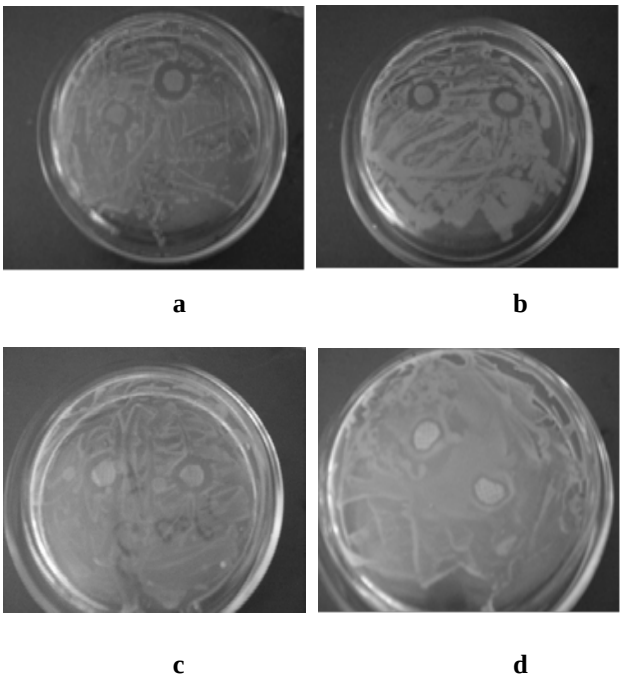

Figure 3. Images of modified "Spunbond" material (activation according to M1 with silver) toward *Staphylococcus aureus*(a), *Bacillus subtilis*(b), *Escherichia coli*(c) and *Pseudomonas aeruginosa* (d).

Table 2 shows unmodified materials to have no antimicrobial properties. The samples containing silver ions are more active than copper-containing ones as a rule that is explained by the difference in action mechanisms of silver and copper ions toward microbial cells.

Nonwoven PP material (Spunbond) is known to have more developed specific surface compared to PP film and thus greater possibility to bind metal ions.[23] This fact is indirectly supported by more pronounced antimicrobial effect observed in the case of Spunbond compared to the film. Storing for 7 days of samples in the medium of infected agar decreases antimicrobial activity but not more than two times. Films activated according to method 2 and modified by copper ions are of exception – they have completely lost their activity on the fifth day. Loss of activity is more pronounced in the case of Spunbond usage. At the same time, some of the films modified by silver ions have not changed antimicrobial activity even after 7-days soaking.

Based on obtained results one can propose the duration of antimicrobial activity of obtained polymer materials is related to the mechanism of metal ions binding by chemically activated PP carrier, the amount of immobilized substance exhibiting antimicrobial properties and the sterilization method of such materials.

EXPERIMENTAL

Following materials,being samples of 25×60 mm size, were used in present work: biaxially oriented commercial film of isotactic polypropylene (PP film) TU RB 00204079.164-97 (RF, Moscow, JSC "Moscow Oil Refinery") (molecular mass 400-700 thousand, thickness 20 μm);non-woven polypropylene material "Spunbond" (Spunbond) grade N-55/2100 (Republic of Belarus, Mogilev, Mogilev Kkhimvolokno OJSC) with specific density of 40 g/m^2, molecular weight 400-700 thousand, thickness 40 μm).

Method 1 (M1). PP material sample of 20×65 mm is treated by wt.20% water solution of sodium hydroxide in the presence of iron(II) sulfate under boiling temperature for 2 hours.

Method 2 (M2). The similar sample is treated by wt.37% water solution of hydrogen peroxide in the presence of iron(II) sulfate (to activate decomposition of the peroxide) under the temperature of 60ºC for 2 hours.

After activation, the samples were thoroughly washed by distilled water (till pH ≅ 5.5 for method 1; negative reaction toward hydrogen peroxide according to iodine-starch paper for method 2). The change in the chemical composition of the surface layer of polypropylene was studied by multiply attenuated total reflection infrared spectroscopy (ATR IR). The measurements were carried out on an "Avatar 360" FT-IR ESP spectrophotometer in the wave-number range of 400–4000 cm^{-1}. The ATR prism of the crystalline zinc selenide was used. The angle of incidence of the beam at the media interface was 45°, the number of reflections was 12. The spectra were recorded with an accumulation of the signal based on the results of 32 scans. The surface structure of modified films was investigated by AFM method (in a semi-contact mode using cantilevers NSG 11) on an atomic force microscope Solver P-47 Pro.

Table 2. Antimicrobial properties of polypropylene materials modified by metal ions (Me).

№	PP material	Me	Experiment condition*	Inhibition level** of test culture growth around the sample, mm			
				St.au.	*E.c.*	*Bac.s.*	*Ps.ae.*
			Initial material				
	Film	-	-	Growth under the sample, g s^{-1}			
	Spunbond	-	-	Growth under the sample			
			Activation according to method 1 followed by Me immobilization				
1	Film	Ag	A	11	13	12	12
2			B	10	10	10	10
3	Spunbond	Ag	A	20	21	19	17
4			B	10	13	10	12
5	Film	Cu	A	10	12	12	11
6			B	g/s	10	10	10
7	Spunbond	Cu	A	12	14	14	13
8			B	10	10	10	10
			Activation according to method 2 followed by Me immobilization				
9	Film	Ag	A	10	10	10	12
10			B	10	10	10	10
11	Spunbond	Ag	A	20	18	15	12
12			B	10	10	10	12
13	Film	Cu	A	10	10	11	12
14			B	g/s	g/s	10	11
15	Spunbond	Cu	A	10	14	10	11
16			B	10	10	10	11

Mechanical tests were carried out on the breaker PM-30-1 according to the known method.[24] The speed of the extension of the clamps of the testing machine is 100 mm min^{-1}, the studies were carried out for a set of 10 samples.

Activated PP materials were treated by water solutions of silver or copper nitrates to impart antimicrobial activity. Immobilization of Ag^{+} and Cu^{2+} ions was performed by means of 2% water solutions of corresponding salts under the temperature of 17 ± 2 ºC for 12 hours. Antimicrobial activity tests of the samples were performed right after immobilization of the ions and washing by distilled water. Improved “agar plate" method on base of meat-peptone agar (MPA) was applied in order to study the antibacterial activity of modified PP materials. The following test cultures being primary causative agents of wound infection were used: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 27853) and *Bacillus subtilis* (ATCC 6633). The microbial load was 10^7 cells ml^{-1}. Each Petri dish of 15 cm diameter was loaded with 20 ml of contaminated with a microorganism environment. Then, test samples of the material having ~10mm diameter were placed onto not hardened MPA under the temperature of +45ºC in the way that the sample was in contact with the substratum.

The dished were maintained under room temperature for 1-2 hours to eliminate time variation upon the sample's loading. After this, the dishes were incubated under the temperature of (36 ± 1) ºC for 24-48 hours. Uneven inhibition zones were observed around test samples of the material after incubation. Diameters of inhibition zones were measured in narrowest place.[25]

Conclusion

In this way, by means of various methods, we found the chemical treatment of polymer to change the morphology of the PP supramolecular surface becoming rough. Based on IR spectra, one can conclude successful activation of the surface via the formation of oxygen-containing groups

binding copper and silver ions. Absence of dramatical loss of physicochemical characteristics (within 5%) suppose studied methods to be applied for obtaining antimicrobial polypropylene materials of biomedical purposes.

The highest efficiency in terms of antimicrobial properties is manifested by Spunbond material activated by 20% water solution of sodium hydroxide in the presence of iron(II) sulfate under boiling temperature for 2 hours followed by immobilization of silver ions.

Hereby, obtained PP materials provide one of the possible ways of biologically active polymers creation that is of great importance and could be recommended for medicine, veterinary or agriculture purposes (disposable medical clothing, suture, plugging, dressing and covering materials, etc.). The synergy of inert carrier and the active substance is quite a simple formula giving outstanding results. Novel materials combine properties of starting ones in order to perform tasks they were made for.

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