Title: New Class of Antimicrobial Agents: SBA-15 Silica Containing Anchored Copper Ions

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Research Article

New Class of Antimicrobial Agents: SBA-15 Silica Containing Anchored Copper Ions

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The paper is about a new class of antimicrobial functional nanomaterials. Proposed compounds are based on SBA-15 porous silica matrices and contain anchored copper ions. Thanks to the immobilization of functional groups the compounds are safer for environment than commonly used disinfectant agents. We prepared and examined silica based materials containing two concentrations of copper-containing groups: 10 and 5%. For the reference we prepared samples containing free-standing CuO molecules in the structure and checked their antimicrobial properties. Antibacterial effect of considered SBA-15-Cu material was tested on *Escherichia coli* bacteria. Antimicrobial tests were applied for the pure form of the material and as modifying agents for plastics. The obtained results showed that the sample with lower concentration of active copper-containing groups has stronger antimicrobial properties than the one with higher concentration of copper. Interestingly, silica containing free-standing CuO molecules has no antimicrobial properties. Considering the obtained results, we can conclude that the most probable antimicrobial mechanism in this case is an oxidation stress. When a plastic modifier is applied the material is enriched with bacterial inhibitory properties. It seems that SBA-15 silica containing low concentration of anchored copper ions is promising in terms of its antibacterial property and biomaterial potential for commercial use.

1. Introduction

It is known that the growth of many bacteria, fungi, and viruses can lead to the development of infectious disease. Thus, there is an ongoing research for the formation of low-cost, efficient, and easy to use antimicrobial surfaces and materials for many applications such as medicine and medical devices, linens and clothing, or coatings for home-used appliances (toilets, sinks, freezers, knives, etc.). Recently a substantial increase of using antimicrobial nanomaterials has been noticed. Metals like silver or copper can be toxic to bacteria, having antimicrobial activities at exceptionally low concentrations. Moreover, unlike other antimicrobial agents they are stable under conditions currently found in the industry allowing for their use as additives. Particularly, copper and silver based compounds have been widely used as antimicrobial agents [1] in many applications in agriculture, healthcare, and general industry. Nevertheless, using metallic copper or silver in industrial applications involves several challenges associated with the nature of the metal itself. Silver is relatively expensive, while copper is susceptible to corrosion process. Anyway, metal nanoparticles have become

more and more popular. As it has been proven, such a form of silver or copper exhibits strong antimicrobial properties [2–4] which becomes stronger with the decrease in their hydrodynamic size [5]. Therefore many research teams are struggling to get the smallest metal nanoparticles [6]. In the case of copper, nanoparticles can be dissolved faster in comparison with larger particles, releasing higher amount of Cu^{2+} ions [7]. Copper biocide behaviour is triggered by the metal reduction potential. Copper plays a role of catalyst and causes one-electron reduction of oxygen atoms [1]. In the presence of either superoxide or other reducing agents such as ascorbic acid, Cu^{2+} can be reduced to Cu^+ catalyzing the formation of hydroxyl radicals from hydrogen peroxide [8, 9]. This can induce an oxidative stress damaging cellular proteins, lipids, and DNA [10–12] or even trigger proinflammatory signaling cascades into the cell that are able to induce its programmed death [13]. Nevertheless, recent studies indicate antibacterial property through a mechanism of damaging bacterial cell membranes by the generation of reactive oxygen species but surprisingly preserving the integrity of bacterial genomic DNA [14].

Moreover some toxic mechanisms can depend on cellular characteristics at the nanoscale and the particle size itself can be crucial in this case [8]. A good example of this mechanism can be the one called "the Trojan horse": direct incorporation of nanoparticles into the cell via endocytotic mechanisms causes subsequently ions release within the cells by nanoparticle dissolution. This results in massive oxidative stress and cell's death [15–17].

It seems that nanoparticles have the potential to extend the range of antimicrobial application of copper. Recently their addition to a polymer matrix to obtain a composite material is very promising. Important advantage of such materials is the extremely low amount of filler needed to achieve desired features. Required amounts can be one or even two orders of magnitude lower than conventional microfillers [18, 19]. For example, copper was impregnated on the surface of cotton fibers, latex, and other polymeric materials [20]. Interesting approach is copper incorporation by plasma immersion ion implantation to produce antibacterial polyethylene surface, as reported in [21]. In [22] authors show the process of ionic crosslinking of alginate chains within the cotton cellulose fibers with Cu^{2+} ions during production of copper alginate-cotton cellulose (CACC) composite fibers.

Interesting study was presented in [23], where chitosan nanoparticle was loaded with copper ions. Authors observed significant enhancement of their features compared to those of chitosan nanoparticles and copper ions used separately.

The antimicrobial mechanism of supported copper nanoparticles is reported similarly to the mechanism of the metal ions [19, 24]: the metal nanoparticle release and accumulation in the bacteria's cell or the metal ions releasing from the particles and their action [11]. Nevertheless majority of authors indicated this second mechanism as leading in antibacterial action of metals on supports [1, 25–28]. In [29, 30] authors confirmed that antimicrobial effect of these composed materials is related to the metal ion releases rather than to the leaching of the whole metal nanoparticles.

The antimicrobial activities of metal nanostructures are mostly restricted to strains of pathogenic microorganisms and rarely extended to other microorganisms [31, 32]. Nevertheless, the impact of nanoparticles on environmental accumulation, especially on microbial communities residing in land fields or bodies of water, which are critical to environmental recycling, is unknown. Therefore, it is vital to limit the migration of antimicrobial agents to the environment. It would be advantageous to design an antimicrobial material that is active only locally with low migration of the agent from the working place.

We propose different approach to the problem using copper-containing antimicrobial units homogeneously distributed inside SBA-15 mesoporous silica matrix [33, 34] and anchored inside pores.

The specimen proposed here shows strong antimicrobial action against *E. coli* bacteria. The biocidal mechanism revealed by copper-containing silica introduced by the authors seems to be different than that proposed elsewhere for copper species known from literature. Moreover they operate locally with significantly limited antimicrobial agent depletion (metal ions anchored to silica surface), so possibility of metal migration outside the active area is highly reduced.

We prepared SBA-15 mesoporous silica containing copper ions bounded inside pores via propylphosphonate units and also SBA-15 containing free-standing copper oxide molecules inside pores as a reference to test this hypothesis. We utilized transmission electron microscopy (TEM), EDX quantitative elemental analysis, and Raman spectroscopy supported by numerical simulations to find characterization of the materials molecular structure. It is worth noting that we show molecular structure's characterization only for SBA-15 containing free-standing copper oxide molecules while silica containing copper ions bounded inside pores via propylphosphonate units were thoroughly analyzed in [35].

Furthermore, we examined the antibacterial properties of considered materials using well established microbiological assay. Discovered antimicrobial properties of the samples enabled us to raise a hypothesis about the most probable bacteria elimination mechanism.

To confirm antimicrobial action mechanism we additionally carried out elemental analysis to show hydrogen content in the species and atomic absorption spectroscopy to evaluate copper ion migration to the medium.

Moreover we examined bacterial inhibitory properties of two plastics modified by functional nanomaterial: high density polyethylene (HDPE) and polyurethane (PU).

2. Materials and Methods

Four batches of samples were investigated with regard of their antimicrobial properties:

  (i) Mesoporous silica SBA-15 containing copper ions bounded inside pores via propylphosphonate units (SBA-prop-POO_2Cu) in concentrations 10% and 5%—main investigated material
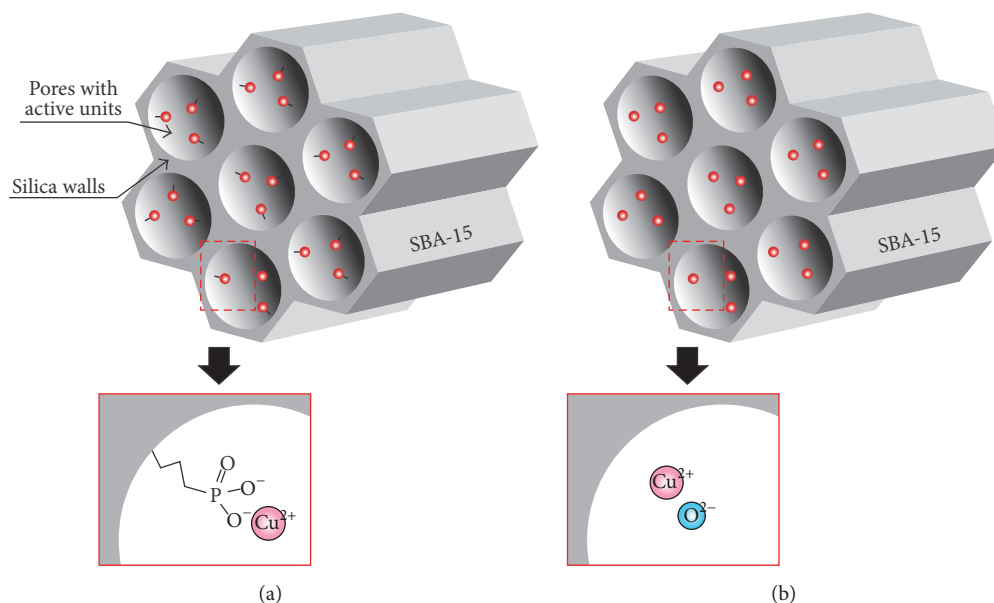
FIGURE 1: Schematic representation of the SBA-15 silica containing copper ions anchored via propylphosphonate unit with magnification of the copper-containing unit anchored to the pore (a) and SBA-15 silica containing free-standing copper oxide molecules (b).

(ii) Mesoporous silica SBA-15 containing nonbounded copper oxide molecules inside pores (SBA-CuO) in concentrations 10% and 5%

(iii) High density polyethylene modified with SBA-prop-POO_2Cu (HDPE-Cu) containing 0.5 and 1% of modifier

(iv) Polyurethane modified with SBA-prop-POO_2Cu (PU-Cu) containing 0.5 and 1% of modifier

The structure of the samples containing anchored copper ions (SBA-prop-POO_2Cu) and free-standing copper oxide molecules (SBA-CuO) can be seen in Figure 1.

For bactericidal tests we used SBA-15 mesoporous silica containing propylphosphonate units (SBA-prop-$PO(OH)_2$) as a reference sample for copper-containing bioactive materials while references for modified plastics were pure HDPE and PU.

In the case of Raman spectroscopy of SBA-CuO, pure SBA-15 silica was a reference to probe a molecular structure.

2.1. Methodology. The main goal of the present work was to examine antibacterial properties of SBA-prop-POO_2Cu and determine the mechanism of bacteria elimination.

Proposed methodology is based on comparison of antimicrobial properties of the sample containing immobilized copper-containing groups (SBA-prop-$PO(OH)_2$) with the sample that contains nonbounded copper oxide molecules (SBA-CuO). We compared samples containing exactly the same molar concentration of copper-containing groups (anchored copper phosphonate or free copper oxide). In both cases the copper ions are coordinated by oxygen atoms. As far as biocidal properties are concerned, the main difference between samples lays in copper-containing units mobility.

In the case of SBA-prop-POO_2Cu migration of copper-containing units is strongly limited due to bounding to silica walls. Thus the probability of bacteria cells penetration is also limited. Opposite situation can be found in the case of SBA-CuO; copper oxide molecules can migrate and penetrate the bacteria cells.

To confirm our statement about limited migration of copper from SBA-prop-POO_2Cu samples, we carried out atomic absorption spectroscopy. The experiment was performed in the following way: the materials that underwent antimicrobial tests (SBA-prop-POO_2Cu 5%, SBA-prop-POO_2Cu 10%, SBA-CuO 5%, and SBA-CuO 10%) were added to the same nutrient broth that was used for antimicrobial tests in amount of 1 g/L (the highest specimens' concentration used for antimicrobial tests; see next text) and rigorously mixed in magnetic stirrer in the temperature of 37°C (the same temperature as for antimicrobial tests). After the time of 1 h, 28 h, 48 h, and 72 h we poured out some amount of suspension and filtered it out using filtering funnel with gradation of 4. The obtained clear solution was investigated under AAS with regard to copper content.

Another difference between the samples lies in the surroundings of copper ions. For SBA-prop-POO_2Cu copper surrounded by six copper ions creates octahedral environment with further oxygen atoms in crystalline lattice (see next chapter). In this case silica pores play the role of oxygen tanks. On the other hand for SBA-CuO specimen copper is coordinated with only two oxygen atoms with no presence of oxygen crystalline lattice. In this case silica pores do not contain significant quantity of oxygen. When pathogens elimination caused by the bacteria cells penetration is considered, we should observe much stronger biocidal properties of SBA-CuO, while in the case of bacteria elimination caused by oxidation stress SBA-prop-POO_2Cu

should be much more efficient. To confirm in explicit way the differences in oxygen content for all samples investigated we carried out additional elemental analysis. The results were juxtaposed with theoretical percentage content of oxygen (calculated on the base of atomic composition) and results for reference samples were mesoporous silica containing propylphosphonate units not activated by copper (SBA-prop-$PO(OH)_2$). During theoretical content calculation we omitted hydrogen atoms in the surface units; it is impossible to estimate their content in the structure. For the reason of low atomic mass of hydroxyl atoms the error caused by this simplification can be neglected.

2.2. Samples Preparation.

Mesoporous silica SBA-15 containing copper ions bounded inside pores via propylphosphonate units (SBA-prop-POO_2Cu) and reference material (SBA-prop-$PO(OH)_2$) were prepared according to procedure described in [35]. Obtained materials contained two molar concentrations of the copper-containing unit: 10% and 5%. For 10% concentration of the phosphonate units proportions between tetraethyl orthosilicate (TEOS) and PhosphonatePropylTriEthoxySilane (PPTS) were 1:9, while for 5% they were 1:19.

Samples containing free-standing copper oxide molecules inside pores were prepared by calcination of the SBA-prop-$PO(OH)_2$ in air in $1100^\circ C$.

Polyethylene sample (Hostalen GC 7260, manufactured by Lyondell and Basell) modified by SBA-prop-POO_2Cu was prepared by mechanical stirring of granulated polymer with modifier and then extruded with the use of continuous extruder. Bioactive agent was added in amount of 0.5 and 1% of mass. Samples were obtained on the extruder KRAUSS MAFFEI KM65-160C1. Applied extrusion parameters for all samples were as follows: maximal pressure in the plasticiser part, 60 MPa; extrusion time, 0.6 s; clamps pressure, 30 MPa; clamps time, 28 s; cooling time, 15 s; dozing time, 6.6 s; form closing force, 650 kN; form temperature, $40^\circ C$; extrusion temperature, $195^\circ C$; screw rotational speed, 250 mm/s. It must be stressed that decomposition temperature of organic moiety in the sample (propyl chain) is $320^\circ C$, while phosphonic acid groups decompose at $320^\circ C$. Thus there is no possibility of modifier decomposition during the process. After this process the material was casted into discs. Granulate of composites was obtained using the rotor mill. Average diameter of the grains was 2 mm.

In order to prepare polyurethane modified by 0.5 and 1% (in weight proportions) of SBA-prop-POO_2Cu we used commercially available two-compound PU resin (MoldStar 321 manufactured by Smooth-on ltd.). In the first step we mixed polyol with appropriate weight of modifier. Next we added isocyanate in exactly the same amount (in weight proportion) as polyol. This was mixed mechanically for about one minute. The mixture was deaerated in the vacuum chamber for 2 minutes under the pressure of 150 μmHg. Then we casted material in the form of ring with diameter of 10 cm and thickness of 1 cm using silicone form. After polycondensation (4 hours) the material was grinded into the grains with diameter about 2 mm.

2.3. Characterization Methods.

TEM imaging was carried out using the FEI Tecnai G2 20 X-TWIN electron microscope, equipped with emission source LaB6, CCD camera FEI Eagle 2K and X-ray microanalyzer EDX.

Raman spectroscopy measurements were carried out at room temperature in the wavelength range from 300 to $4000\,cm^{-1}$. The Raman spectrometer (Nicolet Almega XR) was equipped with a Nd:YAG laser. The experiments were carried out at 532 nm and the laser was operated at a power level of 40 mW. The spectral resolution of the spectrometer was $1\,cm^{-1}$. As SBA-15 silica is a noncrystalline solid, Raman scattering resolution is rather poor. To increase spectrum quality powder was compressed to pellets, so each spectrum was a result of 10 scans.

In order to determinate the oxygen quantity inside samples elemental analysis was carried out with the use of vario EL cube elemental analyzer upgraded to oxygen determination with a pyrolysis in $1150^\circ C$.

The copper content determination was evaluated by atomic absorption spectroscopy (AAS) with use of Shimadzu model AA-680 atomic absorption spectrometer having hollow cathode lamp and a deuterium background with an air-acetylene flame. The lamp current was set to 6 mA. Measurements were carried out in the integrated absorbance mode at 324.8 nm, using slit width of 0.7.

2.4. Numerical Simulations.

For considered samples we performed quantum chemistry simulations to support Raman spectroscopy and identify vibrational modes observed in the Raman spectra. Applied procedure was described in [34]. Considering the fact that SBA-prop-POO_2Cu sample's structural investigation was presented in other papers [34, 35] we limited our presentation to numerical model for SBA-CuO samples only.

We used the bicyclic 5-6-s cluster model [36] that would be applied as a representative cluster model of SBA-15 silica. This system was used as a starting point for our simulations. Copper(II) oxide molecule was placed in the distance of Van der Waals interaction. The multiplicity of SBA-15 model molecule and copper oxide molecule were different. The geometry of the model was fully optimized at the level of B3LYP [37, 38] with the 6-311G(d,p) basis set. After geometry optimization, Raman vibrational modes were calculated. For calculation of harmonic vibrational frequencies the same method and basis set were used.

All of these theoretical calculations were carried out using the GAUSSIAN 09 package [39] with default convergence criteria applied. The assignment of the calculated Raman bands was done on the basis of PED analysis [40, 41] and aided by the animation option of the GaussView 5.0 graphical interface for Gaussian programs [42], which gave a visual representation of the vibrational modes shape. Then VEDA software [43] was applied to proceed with the PED analysis. By combining the results of the visualization with potential energy distribution (PED) we obtained very accurate description of the molecules vibrations.

2.5. Antibacterial Tests.

The antimicrobial activity of the copper-containing silica nanomaterials was tested against
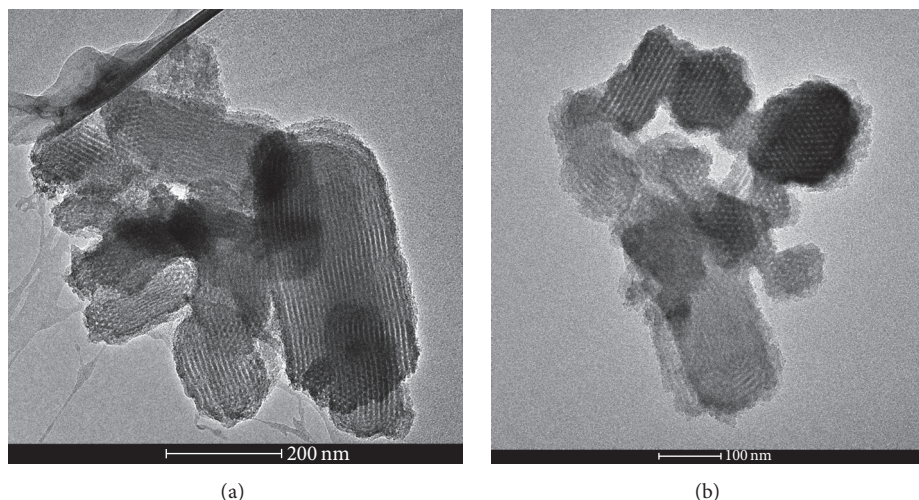
(a)

(b)

FIGURE 2: TEM images of the SBA-15 silica containing free-standing copper oxide molecules (SBA-CuO) observed along the direction of the pore array (a) and perpendicularly to the pore array (b). The grains sizes can be easily estimated as varying mainly from 50 to 300 nm.

the Gram-negative bacteria *Escherichia coli* (ATCC 8099, Rockville MD, USA). To carry out macrodilution test [44] for SBA-prop-POO₂Cu and SBA-CuO powders we prepared ten sets of 10 mL nutrient broth medium containing nanomaterial with concentration from 100 to 1000 μg/mL. Each set was inoculated aseptically with 10^8 CFU/mL of the bacterial suspension. The inoculated sets were incubated at 37°C for 24 h. Each experiment was carried out in triplicate to ensure reproducibility. Turbidity of the media was treated as a measure of bacterial growth. For better evaluation of the bacterial concentration differentiating medium was used (ChromoCult, Merck). The minimum bactericidal concentration (MBC) [45] was evaluated by measuring the turbidity of bacterial strains exposed to different nanoparticles' concentrations. Control experiments were also run parallel to investigate the antibacterial activities of nutrient broth medium with pure silica matrix.

Antibacterial tests for the plastics modified by SBA-prop-POO₂Cu were prepared in different way. In this case we decided to maintain constant conditions (the same amount of nutrient broth medium and modified plastic in the form of pellets and the same concentration of bacterial suspension) for investigated materials: HDPE and PU (both polymers modified with 0.5 and 1% of bactericidal agent and in its pure form as a reference). So we placed 4 grams of samples pellets in the Petri dishes and added 10 mL nutrient broth medium inoculated aseptically with 10^8 CFU/mL of the bacterial suspension. The inoculated sets were incubated at 37°C for 48 h. To estimate inhibitory properties of the sample we counted bacteria colonies in the Petri dishes with samples and compare with results obtained for nonmodified polymers. The mean reported for each polymeric sample was based on ten replicates.

3. Result and Discussion

3.1. Structural Investigation and Molecular Structure Probing. The structure of SBA-prop-POO₂Cu material had been thoroughly investigated in our previous work [35], so we showed investigation results only for SBA-CuO sample.

Main features of the structure were provided by the transmission electron microscopy (TEM). TEM images of the SBA-CuO, observed along the direction of the pore array and perpendicularly to the pore array, can be seen in Figure 2.

As can be easily noticed, TEM microscopy confirms that investigated sample has well ordered, hexagonally arranged pore array pattern nanostructure. Also pore diameter can be roughly measured as about 5 nm. Moreover, any impurities or copper-containing groups agglomerations cannot be seen.

The quantitative EDX analysis was carried out for copper oxide containing silica samples (containing 5 and 10% of CuO) in order to confirm the chemical composition of the samples. The EDX spectrum is shown in Figure 3 and quantification results are presented in the Table 1.

The presence of nickel (Ni) in the EDX spectra is attributable to the preparation technique, in which samples were placed on a nickel grid. The presence of carbon (C) is connected with vacuum chamber contamination (using a carbon tape for placing the grids). Oxygen (O) amount is not reliable, because of air remains in the chamber.

In this case we used EDX measurement for finding proportion between key elements in our samples: silicon and copper. Even in the case of contamination, which is common for this technique, the proportions between the elements listed before remain unchanged and results are reliable.

It is clearly seen that the SBA-CuO samples have almost assumed quantity of copper; the molar ratio of silicon to copper in the case of samples containing 5 and 10% of CuO is equal to 21.69 and 12.20, respectively. Minor deficiency of
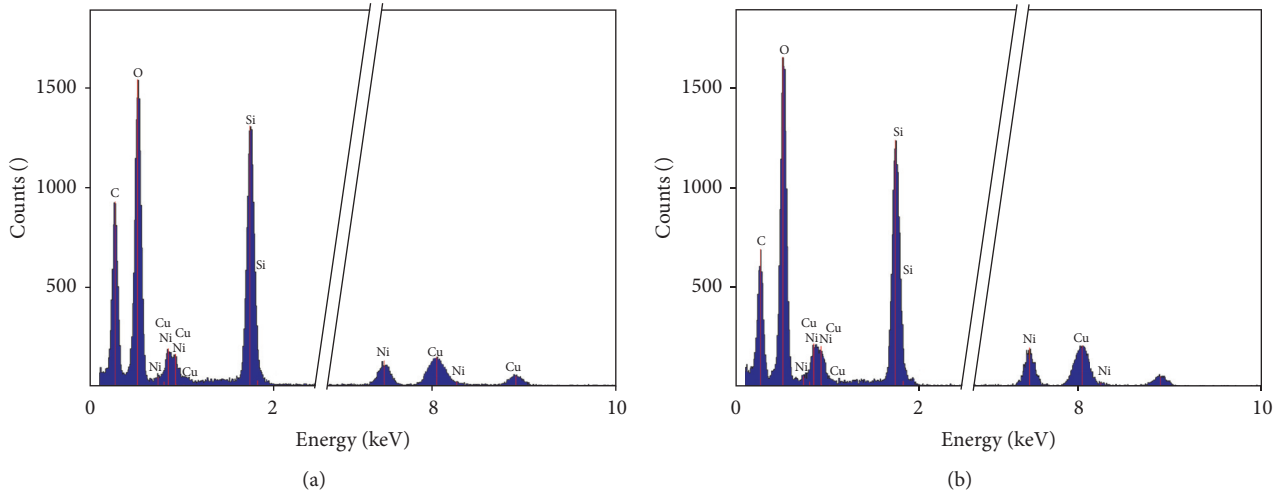
(a)

(b)

FIGURE 3: EDX spectrum of SBA-15 silica containing 5% (a) and 10% (b) free-standing copper oxide molecules. For better visibility region between 2 and 8 keV (no peaks presence) has been cut.

TABLE 1: EDX quantification results of SBA-15 mesoporous silica powder containing 5 and 10% of copper oxide units (SBA-CuO).

Element	Weight%	Atomic%	Uncertainty%
		SBA-CuO 5%	
O(K)	41.89	46.75	0.45
C(K)	21.57	32.06	0.37
Si(K)	30.61	19.46	0.12
Cu(K)	3.19	0.90	0.07
Ni(K)	2.73	0.83	0.05
		SBA-CuO 10%	
O(K)	51.14	56.05	0.51
C(K)	19.22	28.06	0.25
Si(K)	21.90	13.67	0.17
Cu(K)	4.06	1.12	0.08
Ni(K)	3.68	1.10	0.08

copper is probably caused by incorporation of propylphosphonic acid in silica walls in initial material before calcination and also by migration of free-standing copper oxide units.

We did not notice presence of phosphorus, which confirmed the correctness of the calcination process.

To identify bounds inside SBA-CuO samples and unambiguously confirm assumed molecular structure we carried out Raman spectroscopy supported by numerical simulations. Obtained theoretical model allowed vibrations to be identified in explicit way. Juxtaposition of the Raman spectra for model molecule, SBA-15 mesoporous silica (reference material), and silica containing copper oxide molecules can be seen in Figure 4. We present here low frequency spectra (from 200 to 1200 cm^{-1}), only for the range in which we can observe significant Raman features.

We obtained good correlation between theoretical and experimental data, which confirms correctness of DFT simulations. The silica support exhibits Raman features at 370
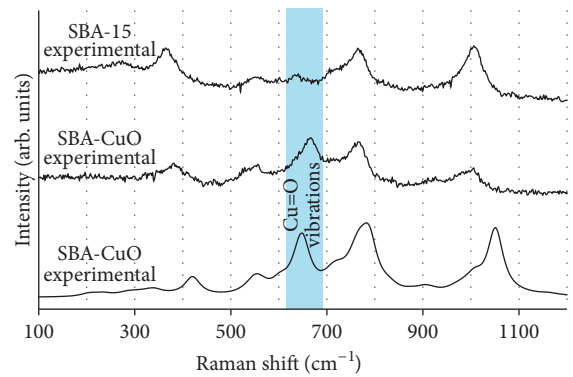

FIGURE 4: The juxtaposition of the Raman spectra for silica containing copper oxide units; theoretic and experimental data and pure SBA-15 mesoporous silica. The vibrational mode characteristic for copper oxide is marked as colour bands.
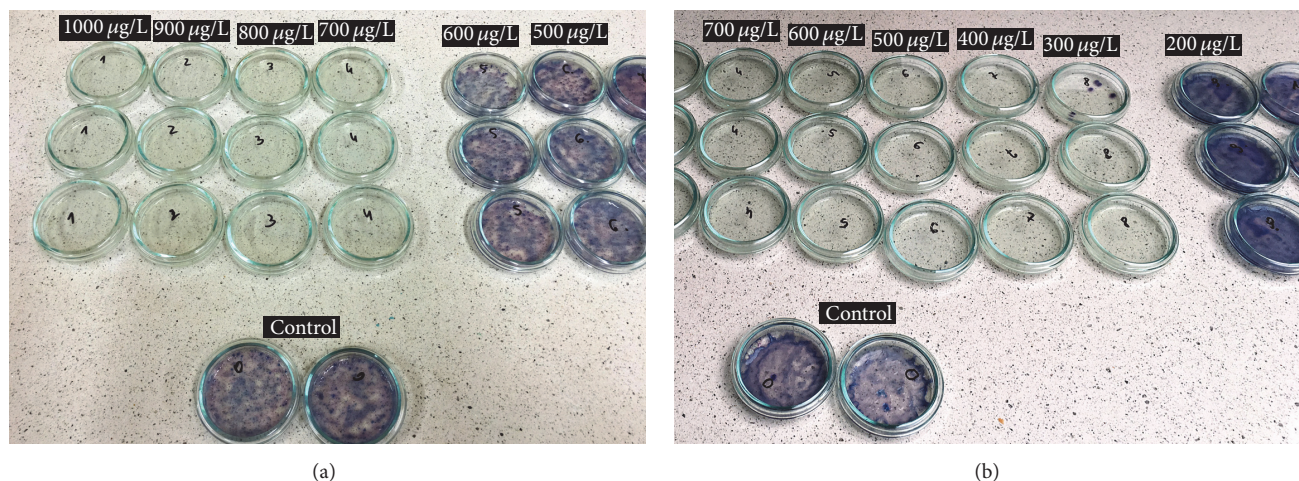
(a)

(b)

Figure 5: Macrodilution antimicrobial tests against *Escherichia coli* bacteria for SBA-15 silica containing anchored copper ions in molar concentration of 10% (a) and 5% (b). Control sample is SBA-15 silica containing pure propylphosphonate units (precursor of SBA-prop-POO$_2$Cu).

and $560\,cm^{-1}$ (deformation of siloxane rings), $770\,cm^{-1}$ (stretching of siloxane bridges), and $1000\,cm^{-1}$ (bending of surface Si-H bounds). Absence of typical vibrational models of hydroxy units ($1200\,cm^{-1}$) [46, 47] is caused by silanation of silica matrix during synthesis process. Applied theoretical model also had no silanol units.

Considering all abovementioned analysis we can be sure that the molecular structures of the investigated powders are in accordance with our expectations.

3.2. Antimicrobial Tests

3.2.1. Antimicrobial Activity of Pure Functional Powders. Antimicrobial activity of copper ions bounded within the pores of SBA-15 silica matrix (SBA-prop-POO$_2$Cu) was analyzed against bacterial strain of *Escherichia coli*. We tested nanomaterials with two molar concentrations of copper-containing units: 10 and 5% (samples named SBA-prop-POO$_2$Cu10 and SBA-prop-POO$_2$Cu5, respectively).

Obtained results were surprising for us. While specimen containing 10% of copper-containing units showed quite strong antibacterial activity, specimen containing 5% of anchored copper ions revealed significantly stronger antimicrobial properties. The results of macrodilution tests was presented in Figure 5. For these tests reference material was SBA-15 silica containing pure anchoring groups, propylphosphonate (control sample).

For the SBA-prop-POO$_2$Cu10 sample MBC was found as 700 μg/mL. This result was comparable to commercially used nanosilver [48] and not much worse than silica-silver core-shell systems [49]. It is worth noting that investigated species included only 10% of functional copper-containing groups (in molar concentration). Taking into consideration the elemental composition of the specimen, MBC of copper ions was 57 μg/mL. This result is better than that obtained for pure copper oxide nanoparticles [50]. Additionally, our material has important property; functional copper-containing units

are immobilized in the matrix and their migration and depleting is strongly limited.

SBA-prop-POO$_2$Cu5 sample has much stronger bactericidal properties than SBA-prop-POO$_2$Cu10 although it contains 2 times lower molar concentration of functional groups. For this sample we obtained MBC 300 μg/mL. Taking into consideration molar concentration of functional groups equal to 5% we obtained MBC equal to 12,4 μg/mL which is comparable with the best available biocidal agents.

The fact of bactericidal activity increasing with decreasing of functional groups concentration is a key to understanding the bacteria elimination mechanism. However the bactericidal action mechanism of metal nanoparticles is still not well understood. In literature we can find various modes of nanoparticles antimicrobial action [4, 51–55]. Interestingly, each mechanism proposed in literature assumes diffusion of the active particle into the cell or direct contact of metal with cell membrane [56]. The mechanism of antimicrobial action of SBA-prop-POO$_2$Cu active material seems to be different. First of all, copper-containing units are placed inside silica pores. Pores diameter is only 4.92 nm [35] which is much lower than the size of *Escherichia coli* bacteria. Moreover, samples used for antimicrobial tests have a form of fine powder in each case, with grains varying mainly from 50 to 300 nm, (see: 2) which is much smaller than bacteria's cell. Thus the direct contact between bacteria cell and copper-containing groups is rather unlikely.

To confirm presented hypothesis, we carried out antibacterial tests for the samples containing nonanchored copper oxide, SBA-CuO (also in this case we investigated samples containing two molar concentrations of CuO molecules: 10 and 5%). Antimicrobial tests sets as for the case of SBA-prop-POO$_2$Cu revealed that both SBA-CuO samples are ambient for *Escherichia coli* bacteria. Even the concentration of 1500 μg/mL (both SBA-CuO10 and SBA-CuO5) does not show any antibacterial properties.

TABLE 2: Juxtaposition of the content of trace copper in the nutrient broth medium after assumed steering time for the samples investigated under antimicrobial tests. Initial suspension contains concentration of the specimen in the broth medium of 1 g/L.

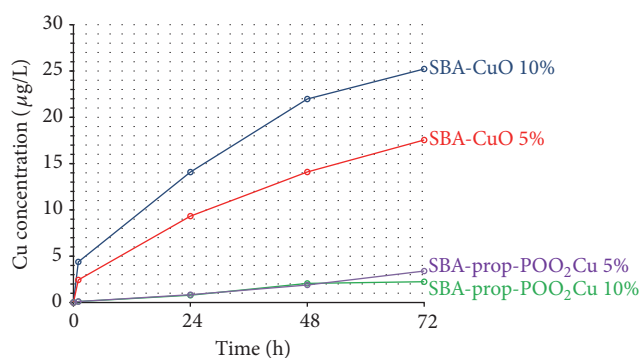| Time [h]	Cu content			
	SBA-CuO 10%	SBA-CuO 5%	SBA-prop-POO_2Cu 5%	SBA-prop-POO_2Cu 10%
0	0	0	0	0
1	4.384	2.439	0.103	0.098
24	14.081	9.324	0.782	0.843
48	21.971	14.093	2.065	1.891
72	25.221	17.546	2.238	3.398

FIGURE 6: The content of trace copper in the nutrient broth medium as a function of steering time for the samples investigated under antimicrobial tests. Initial suspension contains concentration of the specimen in the broth medium of 1 g/L.

TABLE 3: Elemental analysis results indicating percentage content of oxygen in the samples investigated.

Specimen	Oxygen percentage content [%]	
	Theoretical value	Elemental analysis result
SBA-PO(OH)$_2$5%	51.35	52.04
SBA-PO(OH)$_2$5%	49.72	51.39
SBA-CuO 5%	52.45	56.32
SBA-CuO 10%	51.74	57.01
SBA-prop-POO_2Cu 5%	49.04	77.32
SBA-prop-POO_2Cu 10%	45.75	69.21

It is highly probable that copper ions migration into nutrient broth medium is strongly limited in the case of SBA-prop-POO_2Cu, while SBA-CuO can migrate into the medium. Obtained AAS results revealed the level of ions migration into the medium. AAS results were shown in the plot (Figure 6) and additionally juxtaposed in the Table 2.

As can be clearly seen, migration of copper from every species investigated to the medium is rather low. Even in the case of the specimen containing nonbounded copper oxide molecules, after 24 h copper concentration in the nutrient broth medium seems to be too low to reach MIC against *E. coli* bacteria [57]. Nevertheless, for SBA-15 silica containing bounded copper ions (SBA-prop-POO_2Cu) it is significantly lower than that for SBA-CuO. Looking at these results we can say that bacteria elimination mechanism of copper-containing silica is not connected with copper ions release into the broth medium.

This leads to the conclusion that antimicrobial action of such compounds (materials containing homogeneously distributed copper-containing units) is not connected with direct interaction of copper-containing molecules with a bacteria cell.

Therefore we can conclude that antimicrobial action of SBA-prop-POO_2Cu seems to be involved in environment of copper ions, not copper ions themselves. This hypothesis explains well inverted dependence between molar concentration of functional groups and bactericidal activity of the

species. As we showed on the base of magnetic research (not yet published), copper ions inside SBA-POO_2Cu10 create distorted tetrahedral environment; each Cu is surrounded by four oxygen atoms. Unlike SBA-POO_2Cu10, in the case of silica containing 5% of bounded copper-containing units, each copper ion is coordinated by six oxygen atoms, creating distorted octahedral environment. Distortion is caused by propylphosphonate anchoring groups. Such a difference in copper ion's configurations is connected with distance between functional units in species containing different molar concentration of copper-containing groups. In the case of SBA-POO_2Cu10 copper phosphonate groups are relatively close to each other and the most energetically favourable is tetrahedral configuration, whereas the SBA-POO_2Cu5 copper-containing units are placed sparsely which involves octahedral configuration of copper ions. In both cases coordinating oxygen atoms creates local crystalline structure, which can be seen even in SQUID magnetometry. As a consequence, different amount of oxygen is accommodated inside the SBA-15 support pores. Much higher concentration of oxygen can be found in SBA-POO_2Cu5 sample. Also AAS revealed differences in percentage content of oxygen in our sample. The results were juxtaposed with theoretical percentage content of oxygen (taken from structural assumption) and oxygen content in reference samples: mesoporous silica containing propylphosphonate units not activated by copper (SBA-$POOH_2$). Obtained results were juxtaposed in the Table 3.

As can be clearly seen for both reference samples and silica containing copper oxide molecules amount of oxygen
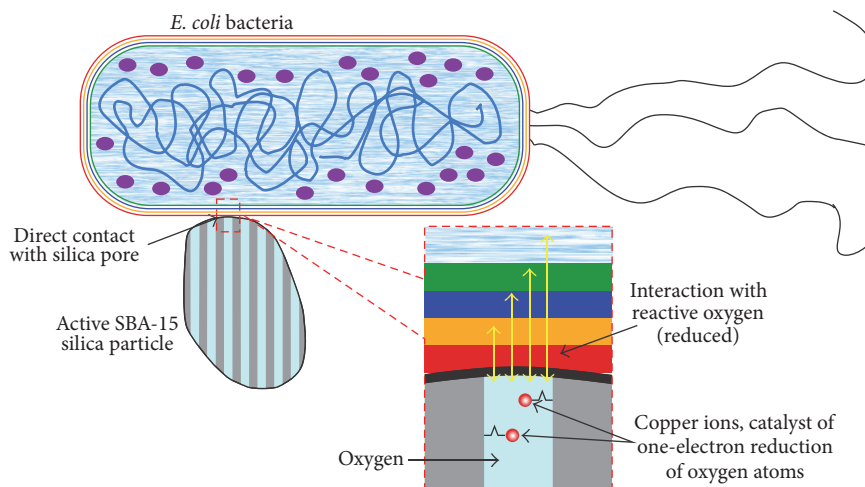
FIGURE 7: Schematic presentation of antimicrobial action of SBA-prop-POO$_2$Cu specimen against *E. coli* bacteria.

seems to be close to theoretically calculated value. On the other hand amount of oxygen for the samples with copper ions bounded via propylphosphonate units is significantly higher than assumed. Moreover, oxygen amount inside sample containing 5% of bounded copper ions is higher than that for the sample containing 10% of copper phosphonate units. Assuming the same structure of the matrix (SBA-15 type silica) we can explain these differences as caused by oxygen trapped inside pores. It seems that this confirms in an explicit way our thesis about the presence of oxygen crystalline lattice inside pores.

Concerning all the above, it seems to be confirmed that bactericidal activity of SBA-prop-POO$_2$Cu is connected with oxygen accumulated inside silica pores.

The antimicrobial mechanism proposed here is a result of direct contact between activated SBA-15 silica grains and bacteria's cell. This causes direct interaction with oxygen stored inside silica pores with bacteria's cell membrane. Copper centres inside silica pores can play a role of catalyst and cause one-electron reduction of oxygen atoms. Presence of superoxide Cu^{2+} that can be reduced to Cu$^+$ catalyzes the formation of hydroxyl radicals from hydrogen peroxide [9]. The hydroxyl radical is the most powerful oxidizing radical reacting with practically every biological molecule [58]. It can cause oxidative damage by abstracting the hydrogen both from an amino-bearing carbon to form a carbon centred protein radical and from an unsaturated fatty acid to form a lipid radical [56]. Thus bacteria's cell membrane has a contact with highly reactive oxygen, which can cause its damage and finally death. Described mechanism was depicted in Figure 7.

The antimicrobial action attributed to SBA-prop-POO$_2$Cu involves a few advantages of these species. Migration and depleting of antimicrobial units are strongly limited, so the proposed biomaterials can operate locally and can be used as disinfectant with long-time local action, unlike antibiotics.

3.2.2. Antimicrobial Activity of Modified Polymers. Antibacterial activity of polymers modified by SBA-prop-POO$_2$Cu5 was shown in Figure 8. After 48 hours of incubation we counted colony forming units in Petri dish. We tested high density polyurethane modified by copper-containing agent in amount of 0.5 and 1% of weight (HDPE-Cu 0.5 and HDPE-Cu 1) and polyurethane modified in the same concentration of biocidal specimen (PU-Cu 0.5 and PU-Cu 1). To estimate inhibitory properties of the plastics we juxtaposed obtained results with activity of pure polymers (high density polyethylene, HDPE, and polyurethane, PU).

As can be clearly seen, modified polymers inhibit growth of the *Escherichia coli* bacteria. In the case of HDPE we obtained $72 * 10^9$ CFU after 48 hours of incubation. Looking at results for modified HDPE we see significant decreasing of the CFU numbers after the same time of incubation. In the case of HDPE-Cu 0.5 it is $40 * 10^9$ CFU (bacterial growth fallen by 44%), while for HDPE-Cu 1 we counted $16 * 10^9$ CFU (bacterial growth had fallen by 77%).

Similar results were obtained for modified polyurethane. Reference sample (PU) showed $68 * 10^9$ CFU after 48 hours of incubation, while modified samples revealed inhibitory properties; we counted $35 * 10^9$ of CF for PU-Cu 0.5 (bacterial multiplication fallen by 49%) and $11 * 10^9$ of CF for PU-Cu 1 (bacterial multiplication fallen by 84%).

Antimicrobial action of polymers filled by SBA-POO$_2$Cu is connected with silica particles placed on the polymers surface. This fact seems to explain why antimicrobial action in this case is weaker; only limited amount of modifier added to polymer can be placed in the surface after polycondensation process.

Nevertheless it is worth noticing that modification of the commercially used polymers with a small amount of proposed antimicrobial nanomaterial containing low concentration of anchored copper-containing units can enrich them with bacterial inhibitory properties. Moreover such modified polymers are thermally stable and modification
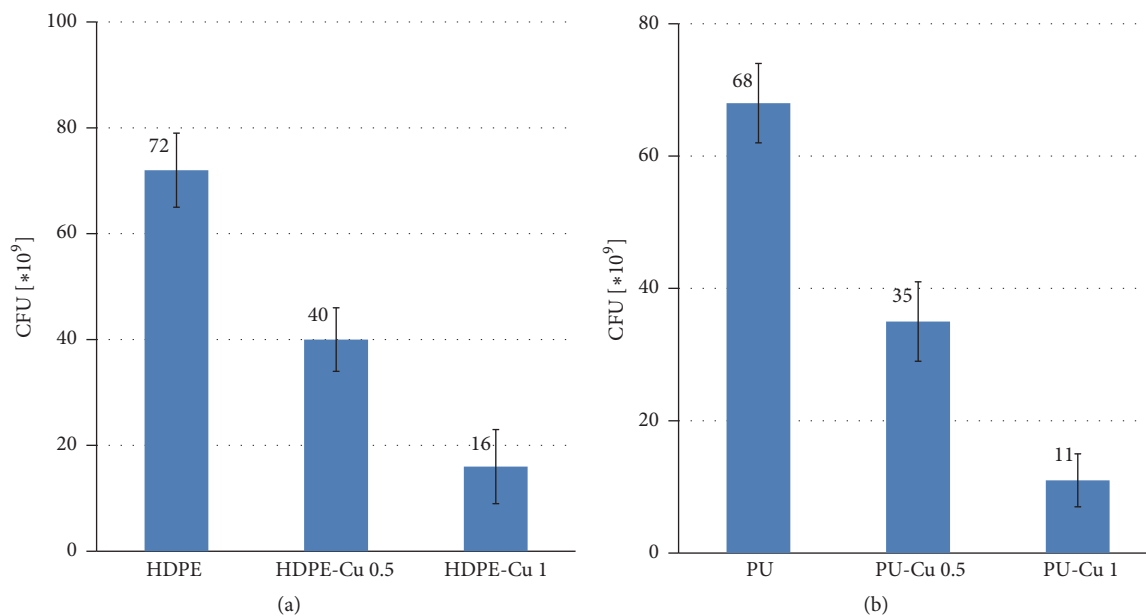
FIGURE 8: Counts of bacteria colony forming units (CFU) (*Escherichia coli*) for polymers modified by SBA-POO$_2$Cu5 in different mass concentration. High density polyethylene (a) and polyurethane (b).

can be carried out in easy way during polycondensation or melting process [59].

4. Conclusion

We presented a new class of antimicrobial nanomaterials: SBA-15 mesoporous silica containing copper ions bounded inside pores via propylphosphonate units. Proposed materials showed strong antibacterial action against *Escherichia coli* bacteria. Moreover, antimicrobial properties increase with decreasing of copper-containing concentration in the SBA-15 support. Taking into consideration the content of copper ions, specimen with the molar concentration of 5% of copper phosphonate units exhibited better antibacterial action than the best commercially available disinfectant materials based on copper or silver.

To reveal the mechanism of its antibacterial action we carried out comparative biological investigations with SBA-15 silica containing free-standing copper oxide molecules. Before biological research we had thoroughly characterized the molecular structure of the species to be sure that their composition is exactly the same as we assumed.

On the base of obtained results and molecular configuration of copper-containing groups we concluded that the mechanism of antimicrobial action is oxidative stress.

We showed that modification of the polymers with copper-containing antimicrobial material enriches them with inhibitory properties towards *Escherichia coli* bacteria.

All of this seems to be really important as far as potential applications of the SBA-prop-POO$_2$Cu are concerned.

Their strong antimicrobial character can be used as the material that destroys pathogens (antimicrobial) or stabilizes bacteria multiplication (biostatic). Anchoring of the functional groups causes the antimicrobial agent migration to be strongly limited. This makes the species safe for ecofriendly microorganisms. Possibility of using them as polymeric materials modifiers opens the doors to application of these materials to dental fillings or manufacturing antimicrobial switchers, parts of beds, or even toilet seats. Also fabrics or medical dressings can be modified by the proposed material. Wide application potential makes our materials extremely interesting both from commercial and from scientific point of view.

Competing Interests

The authors declare that they have no competing interests.

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