VERMICULITE WITH Ag AND Cu USED AS AN ANTIBACTERIAL NANOFILLER IN POLYETHYLENE

VERMIKULIT S Ag A Cu POUŽITÝ JAKO ANTIBAKTERIÁLNÍ NANOPLNIVO V POLYETHYLENU

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Abstract

Vermiculite (Ver) enriched with silver and copper was used as nanofiller to the polyethylene (PE) matrix. Specifically, the low density polyethylene (LDPE) was chosen as a matrix. The samples Ver-Ag,Cu were prepared by shaking of Ver with the aqueous solutions of silver and copper nitrate. The mixtures of the Ver nanofillers and PE were homogenized by melt compounding technique and further thin plates were pressed from stiff matter of PE with Ver nanofiller. The exfoliation of the powdery Ver nanofillers in PE matrix was characterized by the X-ray diffraction analysis of thin plates. Distribution of Ver nanofiller in PE matrix was observed by Light microscopy. The reinforcing effect of nanofillers onto PE matrix was studied by creep experiment. Antibacterial activity of powder Ver-Ag,Cu samples and surfaces of PE/Ver-Ag,Cu samples was tested on the Gram-positive bacteria *Enterococcus faecalis*. All tested PE/Ver-Ag,Cu surfaces showed good antibacterial behaviour after 24 h in comparison to pure PE. The number of colonies decreased from the countless number to several hundred colonies.

Abstrakt

Vermikulit (Ver) obohacený stříbrem a mědí byl použit jako nanoplnivo v polyethylenové (PE) matrici. Konkrétně byl jako matrice vybrán nízko hustotní polyethylen (LDPE). Vzorky Ver-Ag,Cu byly připraveny mícháním Ver s vodnými roztoky dusičnanu stříbrného a měďnatého. Směsi Ver nanoplniv a PE byly homogenizovány postupem míchání taveniny a následně byly ze ztuhnuté směsi PE s Ver nanoplnivem vylisovány tenké destičky. Exfoliace práškového Ver nanoplniva v PE matrici byla hodnocena pomocí Rentgenové difrakční analýzy tenkých destiček. Distribuce Ver nanoplniva v PE matrici byla studována Světelnou mikroskopií. Vyztužující efekt nanoplniv na PE matrici byl studován pomocí krípového experimentu. Antibakteriální působení práškových vzorků Ver-Ag,Cu a povrchů vzorků PE/Ver-Ag,Cu bylo testováno na grampozitivní bakterii *Enterococcus faecalis*. Všechny testované povrchy PE/Ver-Ag,Cu vykazovaly dobré antibakteriální působení po 24 h oproti povrchu čistého PE. Počet kolonií poklesl z nespočetného množství na několik stovek kolonií.

Key words: Vermiculite, Polyethylene, X-ray diffraction, Antibacterial activity

1 INTRODUCTION

Vermiculite belongs to the layered silicates with structure consist from type of layer 2:1, thus, one octahedral sheet is sandwiched between two tetrahedral sheets. Central octahedral cations (mainly Si^{4+}) and tetrahedral cations (mainly Al^{3+}, Fe^{3+}) are usually substituted by cations with lower valency (Al^{3+}, Fe^{3+}, Fe^{2+}, Mg^{2+}, etc.) and a negative charge arising on vermiculite layers from these subtitutions. The interlayer space of vermiculite is occupied by exchangeable hydrated cations which compensate a negative layer charge [1]. Due to structural properties the clay minerals are suitable substrates for various inorganic and/or organic constituents. Vermiculite is occasionally used as silver or copper particles support [2-5].

Polymers with layered silicate nanofillers represent the wide group of the clay mineral-polymer nanocomposites (CPN). The most commonly used clay minerals as a nanofiller are montmorillonite, vermiculite, kaolinite and less often halloysite, hectorite etc. The layered silicates are often modified with various inorganic and organic compounds. The main reason to their modification with organic surfactants is to change their hydrophilic nature to hydrophobic for better compatibility with polymer. These clay mineral nanofillers can improve the mechanical and thermal properties [6-7], anticorrosion properties [8], gas permeability resistance [9] and fire retardancy [10] of polymers. Various preparation procedures of the antibacterial CPN were published [8-9,11-13].

Nigmatulin et al. (2008) prepared Nylon-6/clay nanocomposites by melt extrusion. They used an organically modified montmorillonites as clay nanofillers in mass content of 1, 2, 5 or 10 wt.%. Antibacterial activity was studied on bacteria *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). Authors found that antibacterial activity increased with an increase loading of the organoclay nanofiller. They also observed that antibacterial effect is a function not only of the organic components activity, but also of the nanocomposites structure [12].

Xue et al. (2011) prepared nano-Cu/organo-montmorillonite/LLDPE (Linear Low Density Poly-Ethylene) via melt mixing and melt extruding process. Authors observed that antibacterial effect against bacteria *S. aureus* increased with nano-Cu content [8].

Bruna et al. (2012) studied antimicrobial activity of Cu^{2+}-montmorillonite/LDPE prepared by melt mixing LDPE with 1, 2, 3 and 4 wt.% of Cu^{2+}-montmorillonite. The best antimicrobial effects against *E. coli* was found for CPN containing 4 wt.% of the nanofiller in the LDPE [11].

In this work, vermiculite enriched with silver and copper without organic modifiers was used as a nanofiller to the PE matrix. Structural changes, level of filler dispersed in PE and antibacterial effect of prepared materials were studied.

2 EXPERIMENTAL PART

2.1 Materials

As starting material, vermiculite from Brazil – Santa Luzia (material obtained from Grena, a.s., CZ) was used. Its fraction under 40 μm (labelled as Ver) was prepared by milling in a planetary ball mill, sieving and afterwards used for the experiment. The structural formula $(Si_{3.12}\,Al_{0.88})\,(Mg_{2.32}\,Fe^{3+}_{0.48}\,Ti_{0.05})\,O_{10}\,(OH)_2\,K_{0.23}\,Ca_{0.15}\,Na_{0.07}$ was calculated from the elemental analysis.

Silver nitrate ($AgNO_3$) and copper nitrate ($Cu(NO_3)_2.5H_2O$) with analytical purity (Vitrum, CZ) were used for preparation of Ver with Ag and Cu.

Polyethylene (PE) was prepared from three commercial low density PE (LDPE) in the mass ratio: 33 wt.% of Bralen VA 20-60 powder, 42 wt.% of Bralen VA 20-60 granulated and 25 wt.% of Bralen FB2-17 powder (Slovnaft, SK).

2.2 Samples preparation

Preparation of Ver-Ag,Cu nanofillers

Ver was mixed with 0.1 mol. dm^{-3} aqueous solution of silver and copper nitrate (samples were labelled VerAg and VerCu) and with both of these solutions in molar ratio 1:1 (sample was labelled VerAgCu). Detailed preparation procedure was published in our previous work [5]. The elemental analysis revealed 5.6 wt.% of Ag in VerAg, 3.5 wt.% of Cu in VerCu and 2.1 wt.% of Ag and 1.4 wt.% of Cu in VerAgCu.

Preparation of PE/Ver-Ag,Cu samples

PE samples were prepared by melt compounding at 160°C in Brabender kneader. The prepared mixtures of PE with nanofillers were pressed into the thin plates (125 × 125 × 1 mm) according the same conditions as in our previous works [14,15]. The prepared materials were labelled as PE/VerAg, PE/VerCu, PE/VerAgCu and PE/VerAgCu-b. Samples PE/VerAg, PE/VerCu, PE/VerAgCu contained 7 wt.% of the nanofiller VerAg, VerCu and VerAgCu in PE, and sample PE/VerAgCu-b contained only 3.5 wt.% of the nanofiller VerAgCu.

2.3 Methods

The X-ray diffraction (XRD) patterns of powder samples and composite thin plates were measured on the X-ray diffractometer Ultima IV Rigaku (reflection mode, Brag-Brentano arrangement, CuKα radiation) under ambient temperature, and working conditions 40kV, 40mA, range of angle 2 – 50 ° 2θ.

The images of the samples with spot elemental analysis were obtained by Scanning electron microscopy (SEM) on PHILIPS XL-30 with energy dispersive spectrometer.

Nanoparticles of silver on Ver surface were observed by Transmission electron microscope Jeol JEM 2010, operating at 80–200 kV.

Distribution of vermiculite fillers in PE was observed using the Light microscopy (LM). The images were obtained by Olympus BX51 with camera UC30 at transition mode, using bright field of the polarized light.

The reinforcing effect of Ver filler onto PE matrix was studied by creep testing applying constant stress of app. 1 MPa at temperature 25°C.

2.4 Antibacterial test

Testing of powder Ver-Ag,Cu nanofillers

The antibacterial activity of powder Ver-Ag,Cu samples was studied on Gram-positive (G^+) bacteria *Enterococcus faecalis* (*E. faecalis, CCM 4224*) by the broth dilution method [16]. The minimum inhibition concentration (MIC) was determined, as the lowest concentration of the sample that absolutely stops bacterial growth. The bacterial suspension was prepared by twenty hour cultivation in glucose broth. The density of *E. faecalis* glucose suspension was 1.1×10^9 CFU per ml. The tested samples were prepared as 10% (w/v) aqueous suspensions, further diluted to 3.33%, 1.11%, 0.37%, 0.12% and 0.04%. Detailed procedure of antibacterial test was described in [4].

Testing of PE/Ver-Ag,Cu samples

The antibacterial activity of PE/Ver-Ag,Cu samples was studied on G^+ bacteria *E. faecalis* by the fingerprints method. The bacterial suspension (density 1.5×10^8 CFU per ml) was applied on the surface of the thin plates and also on control pure PE plate (25 µl of suspension on plate 50 × 50 mm). The plates were placed into the laminar box. The fingerprints were made on blood agar in Petri dishes after 24 h, 48 h, 72 h and 96 h of exposure. The Petri dishes were placed into the thermostat at 35°C for 24 h. The numbers of colony forming units (CFU) were recorded. The three fingerprints were made for each plate and the countless number of bacterial colonies was expressed as CN. Detailed procedure was described in [14].

3 RESULTS AND DISCUSSION

3.1 Characterization of materials

The XRD patterns of the studied powder samples in Fig. 1 (green patterns) show basal reflections (002) of Ver that changed after saturation with silver and copper nitrate.

The original Ver (Fig. 1b) showed reflections corresponding to the interlayer space value with the cations surrounding with one layer of water molecules (d = 1.28 nm), two layers of water molecules (d = 1.44 nm) and their mixed-layered hydrate domains (d = 2.38 nm) [17,18].

The XRD pattern of VerAg (Fig. 1c) showed no reflection with d = 2.28 nm and reflection with d = 1.28 nm has shifted to d = 1.20 nm. A new reflection with d = 1.00 nm proved formation of mixtures of less hydrated phases. These changes mean that Ver structure was partially disturbed and probably Ag^+ was intercalated into the Ver interlayer. Besides, Ag is anchored in the form of nanoparticles on the Ver substrate as confirmed by TEM images in Fig. 2c. The XRD pattern of VerCu (Fig. 1e) showed only slight widening of reflections profile. Position of basal reflection d(002) was not significantly changed in VerCu. Nevertheless, value d = 1.42 nm is in agreement with data from literature for Cu-vermiculite [19], thus, Cu^{2+} can be intercalated in the Ver interlayer.

The XRD pattern of PE (Fig. 1a) shows the crystalline region with the reflections, corresponding to the values d = 0.42 nm and d = 0.38 nm, over the amorphous region between 15–25 °2θ [20]. The XRD patterns in Fig. 1 show that the vermiculite fillers structure has not changed significantly after mixing with PE. We can say

that no intercalation or exfoliation of the fillers in PE occurs and only traditional microcomposite structure is formed in prepared samples. Ver fillers are in the form of individual particles distributed in PE matrix without interaction with PE.

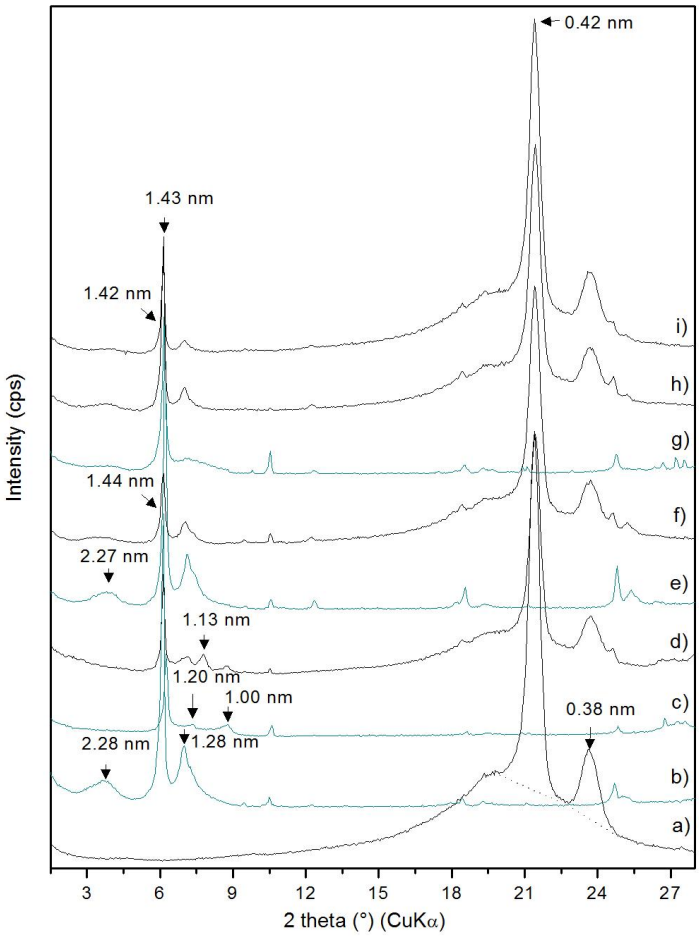

Fig. 1. XRD patterns region with reflections of nanofillers and composites PE (a), Ver (b), VerAg (c), PE/VerAg (d), VerCu (e), PE/VerCu (f), VerAgCu (g), PE/VerAgCu (h) and PE/VerAgCub (i).

The images of the samples VerAg and VerAgCu with spot elemental analysis (Fig. 2) show that characteristic vermiculite lamellar morphology was particularly disturbed mainly in the sample VerAg what confirmed also XRD patterns (Fig. 1). TEM image of sample VerAg (Fig. 2c) shows nanoparticles of silver on Ver surface. The elemental mapping showed that the distribution of Ag and Cu in sample VerAgCu was inhomogeneous (Fig. 3).

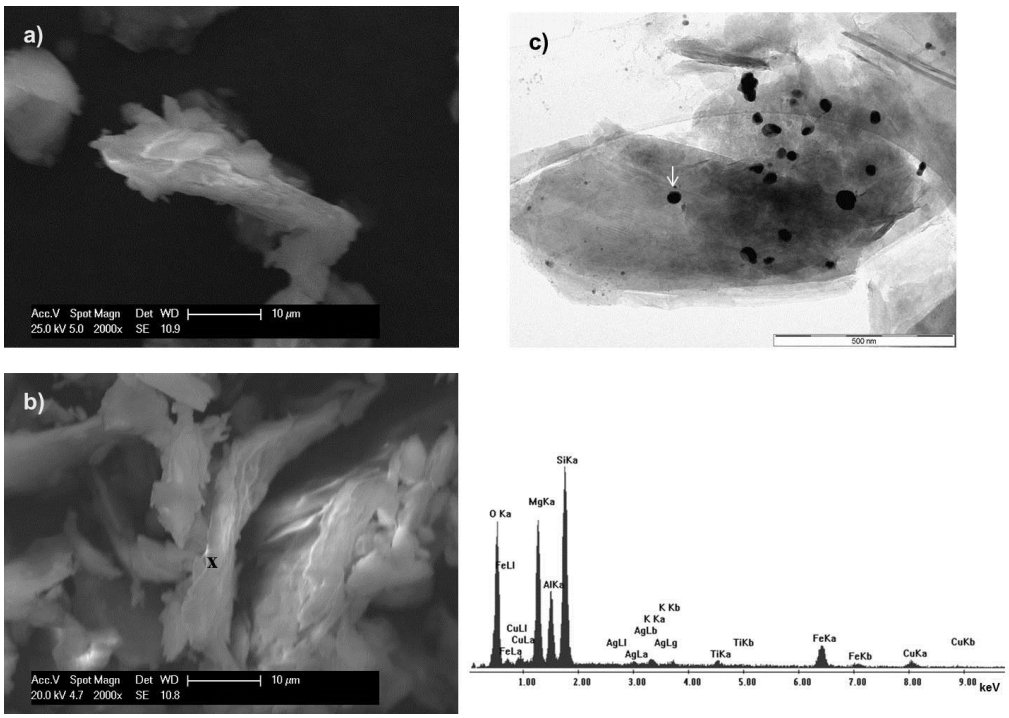

Fig. 2. SEM images of the sample VerAg (a) and VerAgCu with spot elemental analysis (b) and TEM image of sample VerAg with nanoparticles of silver (c).

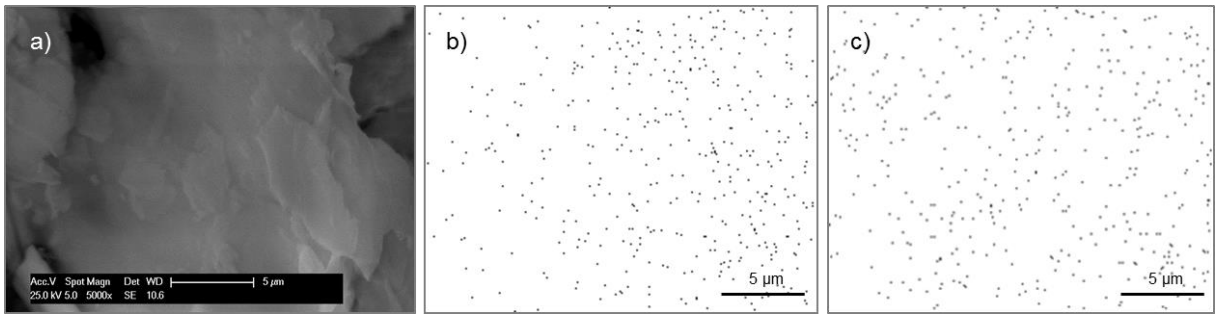

Fig. 3. SEM image of VerAgCu sample (a) with elemental maps of Ag (b) and Cu (c).

The images of samples obtained by LM in Fig. 4 showed no significant differences of the nanofiller particles distribution in PE matrix, except the sample PE/VerAg. The frequency of visible particles was observed with approximate average size in the ranges from 80-60 µm, 60-40 µm, 40-20 µm and under 20 µm (Fig. 5). The clay nanofiller particles in PE formed agglomerates from smaller particles. The largest number of visible particles was in the sample PE/VerAg with higher amount of particles under 20 µm – 75% (Fig. 5a).

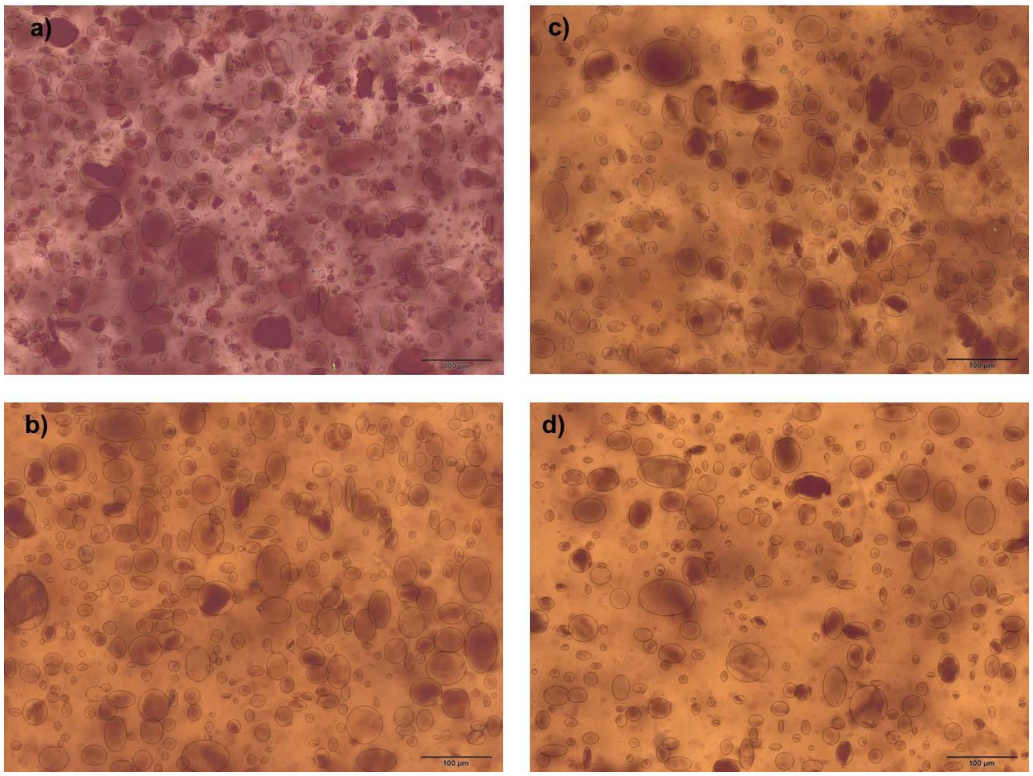

Fig. 4. LM images of composite samples PE/VerAg (a), PE/VerCu (b), PE/VerAgCu (c) and PE/VerAgCu-b (d) with highlight Ver filler particles. The size of the observed area is 710.5 x 527.4 µm.

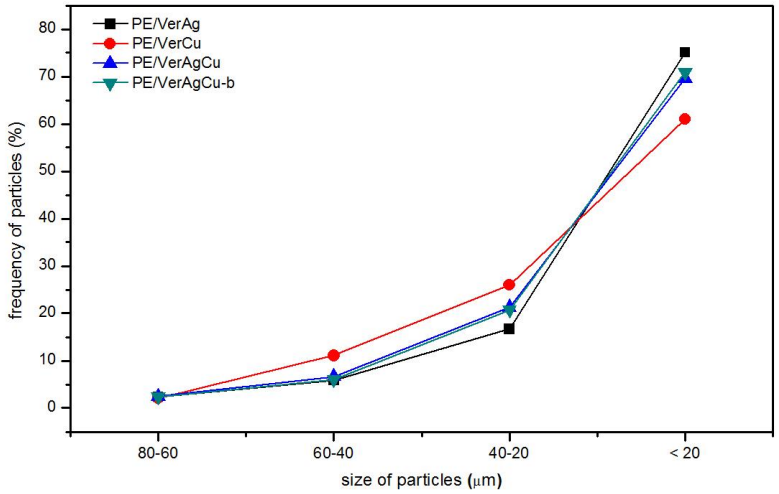

Fig. 5. Frequency of visible particles in the size ranges on the plates observed at the area of 710.5 × 527.4 µm.

3.2 Creep test

Experimental data in Fig. 6 and Tab. 1 compare pure PE matrix with selected PE/Ver-Ag,Cu samples. The results represent time dependence of tensile creep compliance data. These data show significant modification which indicates that the presence of vermiculite based nanofillers increases rigidity of PE matrix. These differences can be quantified also by initial tensile creep modulus representing elastic part of deformation. Values of initial tensile creep modulus in Tab. 1 represent increase about 43% for PE/VerAg, 25% for PE/VerCu and 57% for PE/VerAgCu compared to pure PE. The highest reinforcement effect showed sample PE/VerAgCu.

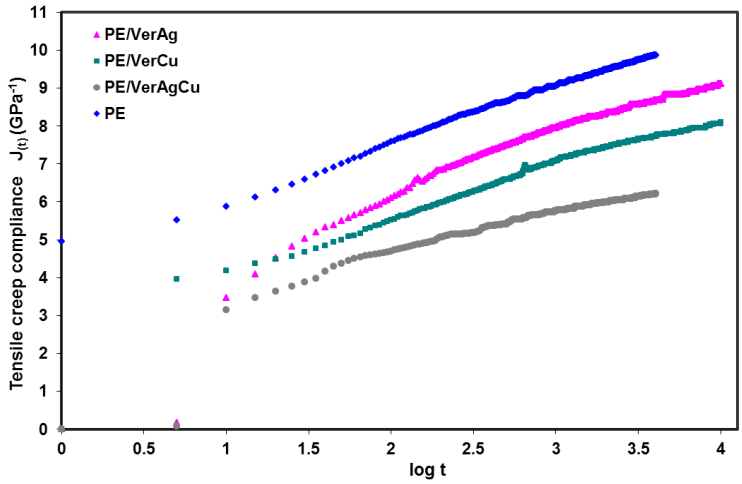

Fig. 6. Tensile creep compliance data for pure PE and PE/ Ver-Ag,Cu samples.

Tab. 1 Values of initial tensile creep modulus $E_{(t)}$ for PE/Ver-Ag,Cu samples.

	PE	PE/VerAg	PE/VerCu	PE/VerAgCu
$E_{(t)}$ (MPa)	202	288	253	317

3.3 Antibacterial test

The results of an antibacterial test in Tab. 2 show MIC values of powder Ver nanofillers. Sample of original Ver had no inhibition effect on bacteria growth. Samples VerAg and VerAgCu showed the MIC value 10% (w/v) after 1 h and 3.33% (w/v) after 24 h. These samples were slightly more effective in comparison to the sample VerCu which showed MIC value 10% (w/v) after 5 h and 3.33% after 48 h. Antibacterial action of Ver-Ag,Cu samples can be attributed to several mechanisms of action of Ag and Cu in samples: damage the bacterial cell membrane, interaction with functional groups in bacteria cell or causing the oxidative stress [21-23].

Tab. 2 Minimum inhibition concentration (MIC) of samples against bacteria *Enterococcus faecalis* after 1h, 5h, 24 h and 48 h.

	MIC % (w/v)			
Sample	1h	5h	24h	48h
Ver	-	-	-	-
VerAg	10	10	3.33	3.33
VerCu	-	10	10	3.33
VerAgCu	10	10	3.33	3.33

The results of antibacterial test in Tab. 3 show that control pure PE exhibited the countless number (CN) of bacterial CFU for 96 h of the tested time. The number of survived bacterial colonies decreased to several hundreds and tens of CFU from initial 1.5×10^8 CFU per ml after 24 h. This represents the decrease about 4-5 orders on surfaces of all samples. Sample PE/VerAgCu inhibited bacterial growth after 24 h and after 96 h no bacteria survived on the surface of this sample. Antibacterial behaviour was result to not only an effect of silver and copper but it was also probably influenced by the surface properties of the plates. Pure PE have smooth surface compared to rougher surface in PE/Ver-Ag,Cu samples. Rougher surface can influence the contact of bacteria with active components in the samples. Also, the charge properties of samples surfaces and bacterial cells could play an important role in antibacterial action, nevertheless, this needs to be better examined.

Tab. 3 Average number of survived colony forming units (CFU) *Enterococcus faecalis* on the three tested composite plates after 24 h, 48 h, 72 h and 96 h. (CN = countless number)

Sample	Average number of CFU *Enterococcus faecalis*			
	24h	48h	72h	96h
PE	CN	CN	CN	CN
PE/VerAg	257	94	49	26
PE/VerCu	71	39	34	13
PE/VerAgCu	40	19	7	0
PE/VerAgCu-b	124	67	44	32

4 CONCLUSIONS

The polyethylene with nanofillers of vermiculite with silver and copper were prepared and characterized. The X-ray diffraction patterns showed that polyethylene was not intercalated into the interlayer space of vermiculite. Further, vermiculite nanofillers were not exfoliated in polyethylene matrix, and only traditional microcomposite structure was formed in the samples. Creep test confirmed increase of rigidity of polyethylene matrix with vermiculite nanofillers compared to the pure polyethylene matrix. The antibacterial test showed good antibacterial effect of powder vermiculite with silver and copper on Gram-positive bacteria *Enterococcus faecalis.* Samples inhibited bacterial growth already after 1 h of bacteria contact with samples. The antibacterial test showed that all prepared samples of polyethylene with nanofillers of vermiculite with silver and copper inhibited the bacterial growth of *Enterococcus faecalis*.

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RESUMÉ

Vzorky polyethylenu s nanoplnivem vermikulitu se stříbrem a mědí byly připraveny a charakterizovány. Rentgenové difrakční záznamy ukázaly, že nedošlo k interkalaci polyethylenu v mezivrství vermikulitu. Difrakční záznamy také potvrdily, že vermikulitové nanoplnivo nebylo exfoliováno v polyethylenové matrici, tudíž došlo pouze ke vzniku mikrokompozitní struktury. Test krípu potvrdil vyztužení matrice polyethylenu s vermikulitovými nanoplnivy oproti čisté matrici polyethylenu. Antibakteriální test ukázal dobrý antibakteriální účinek práškových vzorků vermikulitů se stříbrem a mědí na gram-pozitivní bakterii *Enterococcus faecalis.* Vzorky inhibovaly růst bakterie již po 1 h kontaktu bakterie se vzorky. Dále, antibakteriální test ukázal, že všechny připravené vzorky polyethylenu s obsahem nanoplniva vermikulitu se stříbrem a mědí inhibovaly růst bakterie *Enterococcus faecalis*.