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MINERAL FILLED POROUS COMPOSITES BASED ON POLYVINYLPYRROLIDONE COPOLYMERS WITH BACTERICIDAL PROPERTIES

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Abstract. The polymerization of 2-hydroxyethyl-methacrylate compositions with polyvinylpyrrolidone has been investigated in the presence of mineral fillers (hydroxyapatite, montmorillonite and wollastonite). The influence of the nature and amount of mineral filler on polymerization kinetics and composition of copolymers has been determined. In the composite structure silver particles were obtained *via* silver nitrate reduction by polyvinylpyrrolidone tertiary nitrogen. The synthesized silver-containing composites possess bactericidal properties against *E. coli* and *S. aureus* bacteria, in particular.

Keywords: 2-hydroxyethylmethacrylate, polyvinylpyrrolidone, silver nanoparticles, hydroxyapatite, montmorillonite, wollastonite.

1. Introduction

The search of new and modification of known osteoplastic composites capable of bone tissue regeneration is one of the most topical problems of modern reconstructive medicine [1]. In our previous publications [2-4] we presented the obtaining of osteoplastic composites based on copolymers of glycol methacrylic esters with polyvinylpirrolidone (PVP) filled by hydroxyapatite (HA). HA provides active implantation into bone tissue and enhances its functioning. We investigated the effect of HA amount, kind and amount of pore forming agents, as well as monomer: polymer matrix ratio in the starting material on the composites porosity and mechanical properties. Cyclopentane (10 wt %) was found to be the optimal pore forming agent. Silver nanoparticles in the composites may be obtained *via* silver reduction from argentic salts by PVP. Argentic salts retard the polymerization reaction but its rate is still high [2].

However, other kinds of mineral fillers, their effect on process regularities, structure and properties of the composites were not studied. The experimental results concerning the reaction conditions effect on the silver nanoparticles formation are absent as well.

Therefore, the aim of the present work is to develop new silver-containing composites based on PVP/methacrylic esters copolymers with mineral fillers, to determine the effect of filler kind and amount on the polymerization kinetics, structure and properties of the composites. These composites would be used in the medicine for osteogenesis.

2. Experimental

2-Hydroxyethylmethacrylate (HEMA, Bisomer) was vacuum-distilled before use (130 N/m^2, 351 K). Benzoyl peroxide (BPO, 97 %, SIGMA) was twice recrystallized from ethanol. Polyvinylpyrrolidone (PVP, MW = 28,000, AppliChem CmbH), polyethylene glycol (PEG-1500, Serva), silver nitrate, montmorillonite $(Na,K,Ca)(Al,Fe,Mg,Cr)_2[(Si_4O_{10}](OH)_2 \cdot H_2O$ (Fluka), wollastonite $CaSiO_3$, and cyclopentane (SferaSim) were used as received. Hydroxyapatite $Ca_{10-x}(PO_4)_6(OH)_2$ with the particles size of 0.05–1.25 mm was synthesized at the Department of Silicates Technology at Lviv Polytechnic National University.

Typically, porous copolymers were obtained *via* block copolymerization [5]. Cyclopentane was used as pore forming agent, PEG-1500 polyethylene glycol was used for the foam stabilization.

The average diameter of pores (d_p) and polydispersivity index (*PDI*) were determined by measuring size of at least 200 pores using MBS-9 microscope. The structure of the composites was studied using transmission electron microscope (TEM) JEOL JEM 200 CX. The total porosity and composites density were determined using a Manehold method described in [6]. According to this method, the polymer sample is weighted in two immiscible liquids (*e.g.* heptane and water). Total porosity (*W*, %) is calculated as:

$$W = \frac{V - V_d}{V} \cdot 100 = \frac{V - \dfrac{P_a - P_{H_2O}}{d_{H_2O}}}{V} \cdot 100 \quad (1)$$

$$V = \frac{P_{H_2O} - P_{hept}}{d_{hept} - d_{H_2O}} \quad (2)$$

where V_d and V – volumes of dry substance and sample, impregnated with water in air, respectively, cm^3; P_a, P_{H2O} and P_{hept} – weights of sample in air, in water and in heptane, respectively, g; d_{H2O} and d_{hept} – densities of water and heptane, respectively, g/cm^3.

The compression strength of the materials was determined by the standard method using universal testing machine "Kimura" type RT-601U (Japan). To determine the mechanical properties the samples of polymer composites with a diameter of 15 mm and a height of 10 mm were used. Relative compression strength was identified during 10 % deformation and was calculated according to the following formula:

$$s_{10} = \frac{P_{10}}{S} \quad (3)$$

where P_{10} – loading during 10 % deformation, N; S – sample cross-section area, m^2.

The amount of unreacted PVP was determined using photocolorimetry. The method is based on the formation of PVP/iodine colored complex [7].

UV spectroscopic studies were carried out on the Perkin-Elmer Lambda 20 UV-VIS spectrometer (light source: deuterium and halogen lamp; monochromator: holographic concave grating; detector: photodiodes; spectral bandwidth: 2 nm; software: UV WinLab v. 2.70.01).

Synthesis of silver nanoparticles was performed using the following method: a homogenous solution of 10 g PVP in 40 ml of water and solution of 1 g AgNO$_3$ in 10 ml of water was added into a 250 ml round-bottom flask. The mixture was heated up to 343 K in the dark under vigorous magnetic stirring for 2 h. The resulting mixture was cooled down; the nanoparticles were isolated by centrifugation and then washed three times with deionized Q-water. Ultrapure Q-water filtered on a Milli-Q Gradient A10 system (Millipore, Molsheim, France) was used throughout the work.

Bactericidal and fungicidal properties of the composite samples were investigated in test cultures of the bacteria *Escherichia coli* HB 101 (*E. coli*), *Staphylococcus aureus* (*S. aureus*) and the fungus *Aspergillus niger* (*A. niger*) by the standard method of the active substance diffusion in the agar to solid nutrient medium. Microbial load was $1 \cdot 10^9$ CFU (cells in 1 ml (colony forming unit)) in 1 ml. The bacteria incubation period was 24 h at 308 K, fungus – 72 h at 301 K. The activity degree was evaluated by the size of the growth delay zone diameter, believing that microorganism with a diameter of 11–15 mm is insensitive to the composite, with 16–25 mm – sesitive and > 25 mm – high sensitive.

3. Results and Discussion

Technological parameters of polymer composites obtaining are: composition of the starting material, synthesis time, temperature and others which determine the structure and properties of the composite. Previously we established that polymerization rate decreases in the presence of HA and pores are well formed at HA content of more than 20 wt % [3]. To determine the effect of mineral filler kind on the polymerization rate and copolymer composition we studied the polymerization process in the presence of HA, montmorillonite (MM) and wollastonite (WL).

Comparing polymerization kinetic curves for the composites with different fillers (Fig. 1) we observe higher reactivity for the composites with MM and WL. Moreover, for the same ratio HEMA:PVP = 7:3 (w/w) at 328 K the high "boundary" conversion of the monomer is achieved for 20 min, for the composite with HA – for 80 min (Table 1, Fig. 1). PVP in the composite accelerates polymerization and provides higher "boundary" conversion of the monomer.

To our mind, the high reactivity of the composite with MM is caused by complex structure of its surface with negative and positive charges. Owing to this fact MM may be the catalyst of ionic polymerization, *i.e.* in addition to the initiation of benzoil polymerization by peroxide *via* radical mechanism, the ionic polymerization takes place. The results from Table 1 confirm the above-mentioned assumption, because polymerization rate is high and slightly depends on temperature, which is typical of ionic polymerization.

Table 1

Effect of filler kind on the polymerization rate and "boundary" conversion
of the monomer ([BPO] = 1 wt %, *T* = 328 К)

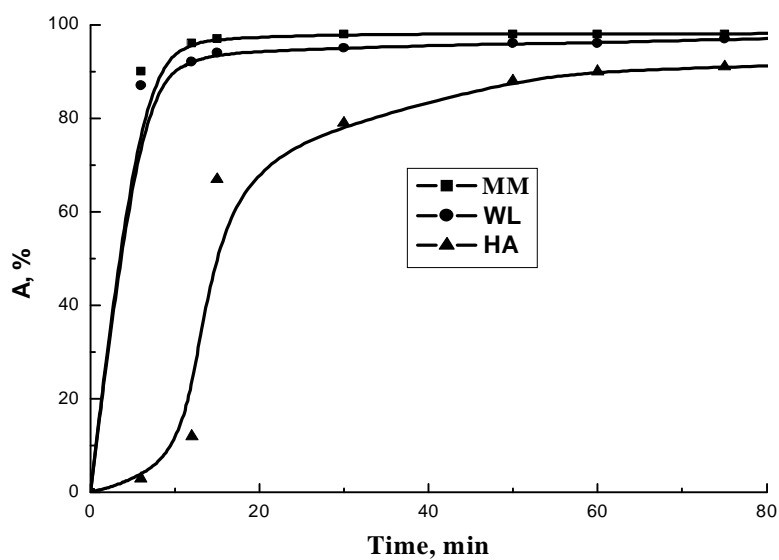| Composition of the polymer-monomer mixture, w/w/w			$V_p \cdot 10^3$, mol/l·s	"boundary" conversion of the monomer for 80 min
HEMA	PVP	filler		
100	0	70 HA	0.74	75
90	10	70 HA	3.36	86
80	20	70 HA	3.71	94
70	30	70 HA	4.2	88
70	30	70 VL	12.5	95
70	30	70 MM	13.6*/12.9	96*/94

Note: * temperature 338 K

Fig. 1. Monomer conversion *vs.*
polymerization time for the composites
with different fillers.
T= 328 K; [BPO]=1 wt %;
HEMA:PVP:filler (w/w/w): 7:3:7

Table 2

Dependence of grafting efficiency *f* and copolymers composition
on the composition of starting monomer-polymer composite ([BPO] = 1 wt %; *T* = 348 К)

Composite No.	Starting composite composition, w/w/w			*f*, %	Copolymer composition, wt %	
	HEMA	PVP	PVP		polyHEMA	PVP
1	90	10	70 HA	99	94.3	5.7
2	80	20	70 HA	59	93.1	6.9
3	80	20	70 WL	75	91.1	8.9
4	80	20	70 MM	98	88.4	11.6
5	70	30	25 HA	50	88.1	11.9
6	70	30	50 HA	37	92.5	7.5
7	70	30	100 HA	32	95.1	4.9

Table 3

**Dependence of final composite physico-mechanical properties on the composition
of starting monomer-polymer composite ([BPO] = 1 wt %; *T* = 348 К)**

Composite No.	Starting composite composition, w/w/w			Compression strength, MPa	Porosity, %	Pore average diameter, mm	*PDI*
	HEMA	PVP	filler				
1	90	10	70 HA	9.2	44	1.26	1.35
2	80	20	70 HA	9.1	53	1.12	1.89
3	80	20	70 WL	9.2	69	1.03	1.43
4	80	20	70 MM	10.6	75	0.79	1.22
5	70	30	25 HA	9.9	90	1.19	1.99
6	70	30	50 HA	10.1	80	1.19	1.98
7	70	30	100 HA	10.3	67	1.40	1.76

It is known that properties of the composite based on PVP/methacrylic esters copolymers are significantly influenced by copolymer composition [8], which is defined by the ratio between methacrylic monomer links and PVP macrochains. It is expected that filler would affect both polymerization rate and copolymer composition. Therefore we investigated the effect of filler kind and starting composite composition on the grafting efficiency *f* and copolymers composition (Table 2).

The increase in PVP amount in the starting composite decreases the grafting efficiency (Table 2, No 1, 2). This fact is in a good agreement with the investigation results of HEMA/PVP block-polymerization and polymerization in solution without fillers [8, 9]. It should be noted that at 348 K for the same composition of the starting composite (Table 2, No. 2-4) the greater amount of PVP (98 %) undergoes a grafting reaction in the case of MM as a filler, compared with WL (75 %) and HA (59 %). It is an indirect confirmation of MM active participation in the polymerization initiation. If HA is used as the filler, grafting efficiency decreases with the increase in HA amount (Table 2, No. 5-7).

The kind and amount of filler also affect the composite porosity (Table 3).

At the same amount of filler the highest porosity and the least diameter of pores are observed for the composite filled with MM (Table 3, No. 4). It should be noted that despite the highest porosity of these composites they have the best mechanical properties, compression strength, in particular.

Photographs of the composites filled with HA are represented in Fig. 2. They confirm the presence of developed microporous structure favoring the effective intergrowth of the composite by bone tissue. Incorporated composites contain more PVP and have higher porosity (Fig. 2a, Table 3).

To obtain the silver nanoparticles and to provide the composites with antibacterial properties the silver reduction reaction between its salts and tertiary nitrogen of PVP was investigated. The results of electron-microscopic investigations show that colloidal solutions are formed from silver nitrate. They consist of nanoparticles of different shapes and sizes, mostly particles of polyhedral (round) shape and polyhedra of various sizes (Fig. 3a). It is important that particles do not form aggregates but remain isolated for a long time. Obviously, PVP is a reducer and a stabilizer of nanoparticles at the same time.

The formation of silver is confirmed by the presence of peak (420–430 nm) at UV spectrum of resulting products (Fig. 4) and by results of chemical analysis, performed by the method described in [10].

The qualitative confirmation of silver nanoparticles obtaining is coloring of the solution from grey to dark-brown depending on the amount of formed nanoparticles, their size and shape, indicating the formation of silver stable colloids.

However, PVP is not a single component capable to reduce silver from its salts. HEMA is also used for this purpose. TEM-photos of silver nanoparticles, which are obtained using this monomer, confirm the fact (Fig. 3b). Nanoparticles have round shape of diameter 20–30 nm (cf. polyhedral shape and polyhedra with the size of 20–70 nm while using PVP).

Reaction of silver reduction by reaction of its salts with PVP tertiary nitrogen was used to provide antibacterial properties of composites during the composite formation. During the synthesis composites with PVP and silver salts change their color from weak-yellow to brown. It is also the indirect confirmation of silver nanoparticles formation at the reaction between silver nitrate and PVP tertiary nitrogen. This method has irrefutable advantages over other known methods, when nanoparticles and hydrogels are prepared separately, or when hydrogel is saturated with silver salts, or when silver nanoparticles are obtained *via* its reduction by amino-containing methacrylic monomer followed by its copolymerization with other monomers [11]. Moreover, there is no necessity in toxic amino-containing reducers.

To confirm the possible practical application of the developed silver-containing composites in biomedical research industry their bactericidal and fungicidal properties were investigated. The research results are present in Table 4.
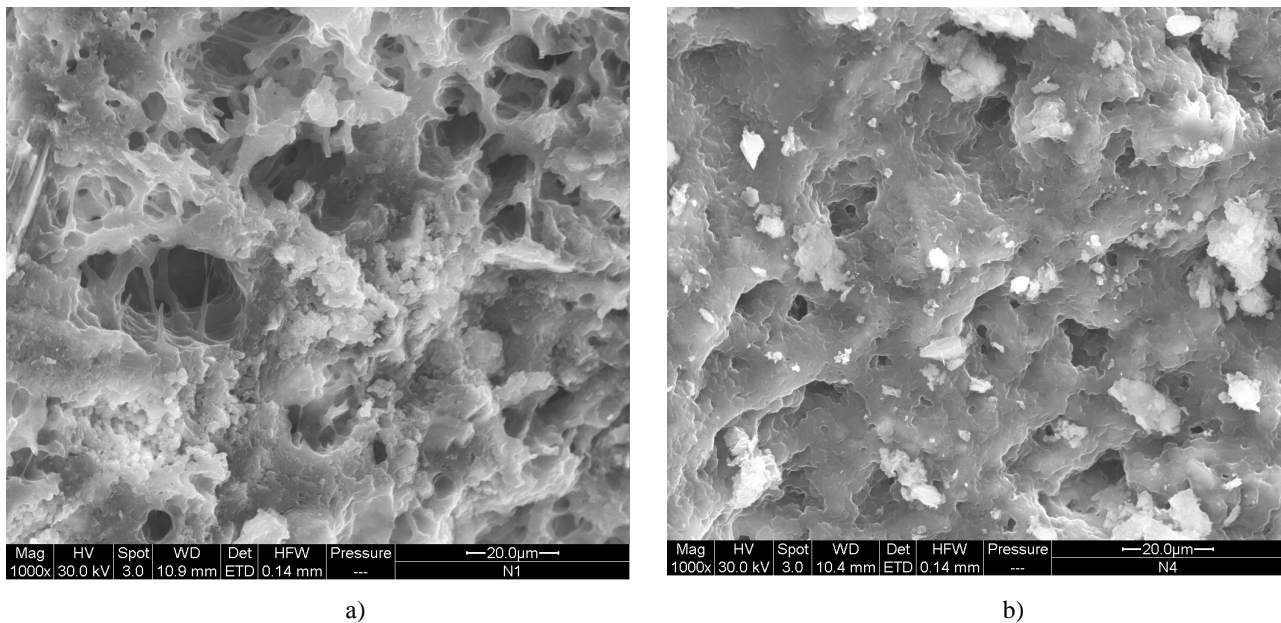
a)

b)

Fig. 2. Photographs of microporous structure of hydroxyapatite filled HEMA/PVP composites with different [HEMA]:[PVP]:[HA] ratio (w/w/w): 6:4:7 (a) and 9:1:7 (b)
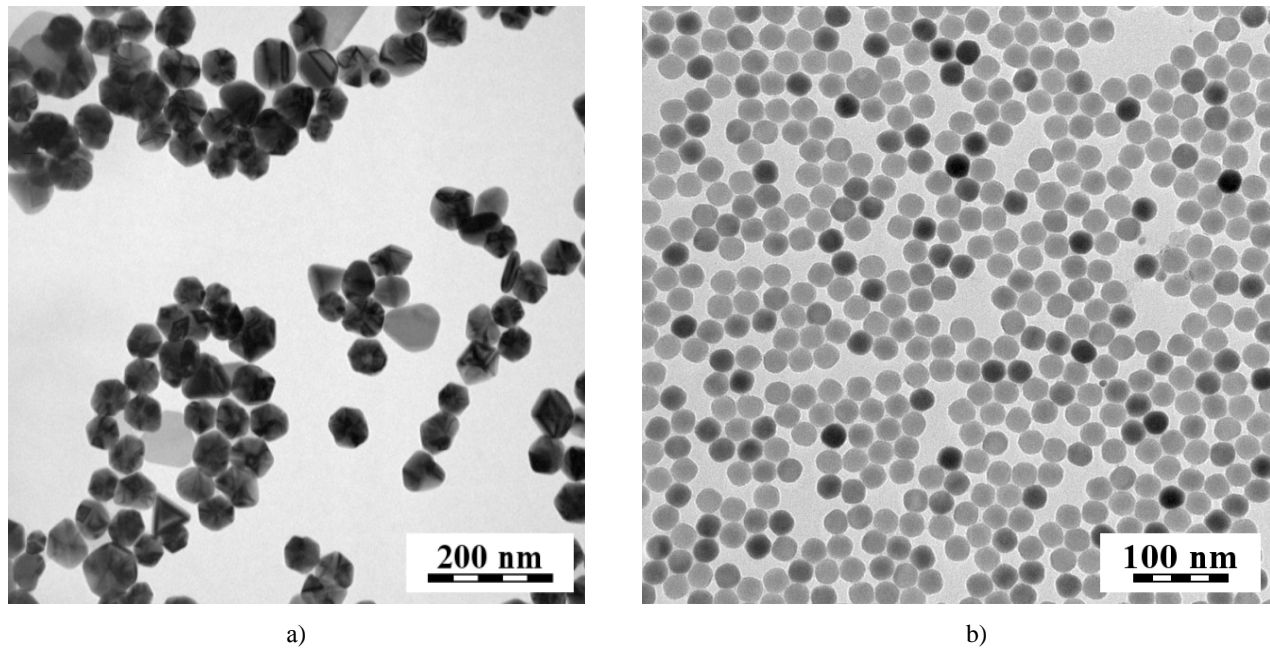

a)

b)

Fig. 3. TEM-photos of silver nanoparticles: $[AgNO_3]:[PVP] = 1:10$ w/w, $M_{PVP} = 10^4$, $T = 348$ K, time is 1 h (a) and $[AgNO_3]:[HEMA] = 1:10$ w/w, $T = 343$ K, time is 2 h

Table 4

Fungibactericidal activity of silver-containing composites

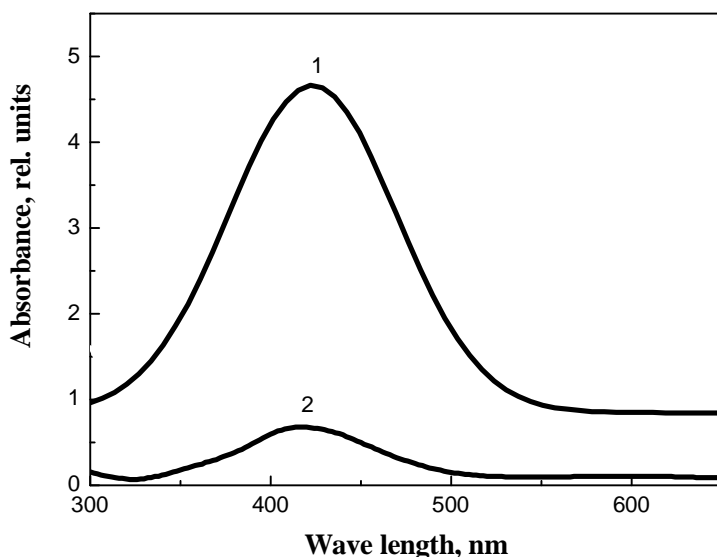| Composition of the starting composite, weight ratio	Diameter of growth delay zone, mm (%)		
	E. coli	*S. aureus*	*A. niger*
HEMA:PVP:HA:AgNO$_3$ = 7:3:7:0.6	24.4 (60)	26.0 (73)	20.0 (33)
HEMA:PVP:HA:CH$_3$COOAg = 7:3:7:0.4	23.0 (44)	18.7 (13)	23.0 (44)

Fig. 4. UV spectra fragment of the solution of AgNO$_3$ products interaction with PVP. M_{PVP}: $3 \cdot 10^4$ (1) and $1 \cdot 10^4$ (2)

Comparing bactericidal and fungicidal properties of resulting HEMA-PVP composites with silver nanoparticles and without them it was established that the composites with silver have fungibactericidal effect.

4. Conclusions

The polymerization of HEMA/PVP composites was studied in the presence of mineral fillers of different kinds. The composites with montmorillonite and wollastonite were found to be the most reactive ones indicating active role of the mentioned fillers in the polymerization process. The effect of filler kind and amount on the grafting process, copolymers composition and composite properties was determined. The formation of porous structure of the composites with mineral fillers was confirmed allowing to use them in osteogenesis. The main starting components (HEMA and PVP) for the polymer-mineral composite synthesis were found to be good reducers in the reaction of silver nanoparticles obtaining from its salts. This fact was used to provide the composites with fungibactericidal properties.

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ПОРИСТІ КОМПОЗИТИ НА ОСНОВІ МІНЕРАЛЬНО НАПОВНЕНИХ КОПОЛІМЕРІВ ПОЛІВІНІЛПІРОЛІДОНУ З БАКТЕРИЦИДНИМИ ВЛАСТИВОСТЯМИ

Анотація. Досліджено полімеризацію композицій 2-гідроксіетилметакрилату з полівінілпіролідоном у присутності мінеральних наповнювачів (гідроксіапатиту, монтморилоніту і воластоніту). Встановлено вплив природи та кількості мінерального наповнювача на кінетику полімеризації і склад кополімерів. Підтверджено можливість одержання в структурі композиту частинок срібла реакцією відновлення нітратів срібла третинним атомом нітрогену полівінілпіролідону. Синтезовані срібловмісні композити проявляють фунгібактерицидні властивості, зокрема, проти Escherichia coli, Staphylococcus aureus i Aspergillus niger.

Ключові слова: 2-гідроксіетилметакрилат, полівінілпіролідон, наночастинки срібла, гідроксіапатит, монтморилоніт, воластоніт.