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Synthesis and mucoadhesive study of thiolated hydrogels based on 2-hydroxyethylacrylate and 2-hydroxyethylmethylacrylate copolymers

Abstract: Hydrogels and copolymers of 2-hydroxyethylmethacrylate and 2-hydroxyethylacrylate structured with 3-mercaptopropyltrimethoxysilane nanoparticles of different composition and nanoparticles concentration have been synthesized and characterized using IR-spectroscopy, SEM, small angle X-ray scattering, compression mechanical test, thermogravimetric analysis, iodometry. Mucoadhesive properties of obtained hydrogels were investigated. Study revealed that increase in concentration of hydrophilic component, nanoparticles content and crosslinking agent concentration reflects an increase of adhesion time of obtained hydrogel materials. Drug loading and drug release studies of HEA-HEMA-MPTS samples with metronidazole were conducted. It was observed that thiolated samples absorb more drug amount than non-thiolated ones. As nanoparticles concentration increases metronidazole content rises respectively. Drug release studies revealed that the higher concentration of 3-MPTS nanoparticles in hydrogels results in prolongation of drug release but concentration of discharged metronidazole decreases.
Key words: organosilane thiol nanoparticles, hydrogels, mucoadhesion, 3-mercaptopropyltrimethoxysilane

Introduction

Thiolated polymers represent new class of mucoadhesive drug delivery systems. The attachment of non-thiolated mucoadhesive polymers to the mucus layer has been achieved only by non-covalent bonds such as hydrogen bonds and ionic interactions. Accordingly, they provide only a weak adhesion, which in many cases insufficient to guarantee the localization of a drug delivery system at a given target site. Thiolated novel polymers are capable of forming covalent bonds with mucus through disulphide bridges formation. Mechanism of thiolated polymers attachment mimics the mechanism occurring in biological systems. Besides, nonionic polymers show weak mucoadhesion comparing to ionic. In this regard, thiolation of nonionic polymers will allow significantly improve their ability to retain on mucous surfaces [1]. Synthesis of such polymers will provide hydrogels with better mucoadhesive characteristics suitable for using them as buccal drug delivery systems. Drug delivery to target organ through classic routes of administration is usually complicated, however buccal drug delivery offers the possibility of circumventing the hepatic 'first-pass' elimination that follows gastrointestinal absorption, degradation in the gastrointestinal tract is also avoided [2]. Therefore, creation of buccal drug forms appears to be a very promising direction of investigation.

Particularly in this work we synthesized homopolymers and copolymers of 2-hydroxyethylmetacrylate (HEMA) and 2-hydroxyethylacrylate (HEA) structured with 3-mercaptopropyltrimethoxysilane (MPTS) nanoparticles of different composition and nanoparticles concentration. The thiolation was confirmed by different physical-chemical methods. Mucoadhesive properties of synthesized hydrogels were investigated. Drug loading and drug release studies of thiolated HEA-HEMA samples with metronidazole were conducted.

Materials and methods

Materials. 3-Mercaptopropyltrimethoxysilane (95%) (supplied by Sigma-Aldrich, Inc., dimethylsulfoxide «Chemical Pure» grade (supplied by AppliChem), 2-Hydroxyethylmetacrylate (96%) (supplied by Acros Organics), N,N'-methylene-bis-acrylamide (supplied by AppliChem), ammonia persulfate (supplied by Sigma-Aldrich, Inc.), 2-Hydroxyethylacrylate (96%) (supplied by Sigma-Aldrich, Inc.), starch were used without preliminary treatment.

$Na_2HPO_4*10H_2O$, $NaHCO_3$, $CaCl_2$ (supplied by Laborfarma) were used as purchased.

HCl, NaOH, C_2H_5OH were used without preliminary treatment.

Dialysis membrane MWCO 12-14 kDa (Medicell International Ltd, UK).

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Synthesis of 3-MPTS nanoparticles and hydrogels based on HEMA, HEA and 3-MPTS nanoparticles. Preparation of 3-MPTS nanoparticles was conducted as described in [3].Synthesis of hydrogels made by free radical polymerization was conducted in the presence of ammonium persulfate as the initiator and N,N'-methylene-bis-acrylamide as crosslinking agent, at a temperature of 40°C. The initial monomer mixture was purged with argon. Hydrogels and copolymers with different concentrations of monomers and nanoparticles in the initial monomer mixture have been synthesized. Also hydrogels without 3-MPTS nanoparticles were synthesized.

Methods of characterization. Chemical structure of obtained copolymers was studied by IR-spectroscopy, on Perkin Elmer FTIR-Spectrum 400 (USA).

Morphology and structure of obtained copolymers were characterized by SEM using FESEM Hitachi SU8220 (Japan).

In order to verify presence of nanoparticles in structure of obtained hydrogels and determination of their size and placement small angle x-ray scattering method was used. It was performed on the combined system of small and wide angle X-ray scattering (Hecus, Austria).

Compression test of obtained copolymers was performed by TA.XT. Texture Analyser (England). Elastic modulus was calculated by the following formula:

$$E=tg\ \alpha$$

Every copolymer composition was tested three times and average values were calculated.

Thermogravimetric analysis was conducted on Netzsch STA 449 F3 Jupiter (Germany) with heating rate 10°C/min in nitrogen atmosphere.

Iodometry. The amount of thiol groups on the hydrogel HEA-HEMA-MPTS was determined using iodometric titration method proposed by Bernkop-Schnürch [4]. First, 30.00 mg of each polymer were hydrated in 9.0 ml of deionized water. The pH-value was then adjusted to 2–3 by adding 1 M HCl. After the addition of 300 µL of aqueous starch solution (1%), samples were titrated with an aqueous iod solution (1.00 mM) until a permanent light blue colour became visible.

Preparation of artificial saliva solution. Artificial saliva solution was prepared by method proposed in [5]. Na_2HPO_4 (0,671 g), $NaHCO_3$ (1,05 g), $CaCl_2$ (0,092 g) were dissolved in distilled water (499,5 ml) with 1M HCl (1,56 ml) addition.

Mucoadhesion studies. In order to evaluate the retention time of hydrogels on mucosa surface the "falling liquid method" was used. It is represented at Figure 1. Preliminarily dried polymer samples were attached to freshly excised buccal porcine mucosa, which has been sticked to a glass pad 2. Glass funnel 1 with artificial saliva solution was placed over the mucosal surface with polymer sample. Flow was regulated by faucet up to 20 drops in a minute (imitating the saliva release). Forementioned glass pad was disposed in a transparent beaker 3 to collect the washings. Received washing were continuously returned back to the funnel. The detachment, disintegration of test hydrogel samples was observed within a time period of 4 h.

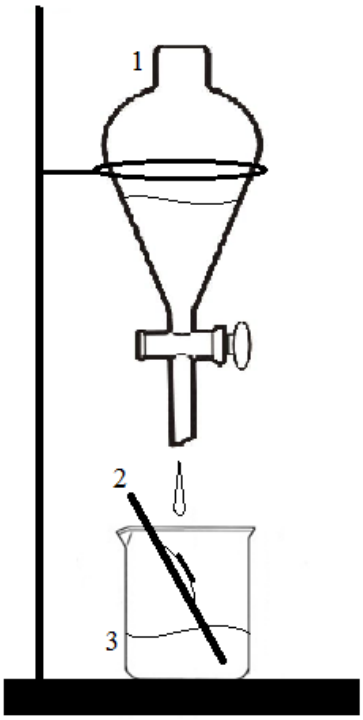


Figure 1 – Schematic illustration of "falling liquid method"

Drug loading. 0,8 % Metronidazole solution was prepared by dissolving metronidazole powder in distilled water. Then preliminarily dried samples were immersed in this solution for 48 hours until equilibrium swelling. Concentration of metronidazole loaded in hydrogels was determined by measuring its residual concentration in solution by UV-spectroscopy (absorbance of metronidazole at 320 nm). For these purposes UV-spectrophotometer Shimadzu UV/VIS-2401 PC was used. Before that, calibration curve of metronidazole in water solutions was plotted.

Drug release studies. The measurement of metronidazole release was conducted using Franz cell

chamber. The cellulose membrane separated the donor compartment containing the sample being analyzed from the receptor compartment filled with collection medium. Artificial saliva solution was used as the collection medium. Diffusion of the drug from polymer sample across the membrane was monitored by taking sample of the receptor medium. At predetermined time points, an aliquot of medium was removed from the receptor compartment and analyzed by UV-spectrophotometer Shimadzu UV/VIS-2401 PC. The aliquot was measured at 320 nm. Then it was returned back to receptor compartment. Determination of drug concentration was carried out using calibration curve of metronidazole in artificial saliva solution.

Results and their discussion

The characterization of synthesized hydrogels was carried out using different physical-chemical methods: FTIR spectroscopy, scanning electron microscopy, thermal analysis, small angle X-ray scattering, mechanical test, "falling liquid method", Franz cell method.

FTIR-spectroscopy. The investigation of obtained composite materials by FTIR-spectroscopy confirmed the presence for C-S (691 cm^{-1}) and S-S at 491 cm^{-1} bonds formation (Figure 2) [6]. However, it's difficult to provide good evidence for –SH groups presence, because that groups give very weak signal in IR-spectra.

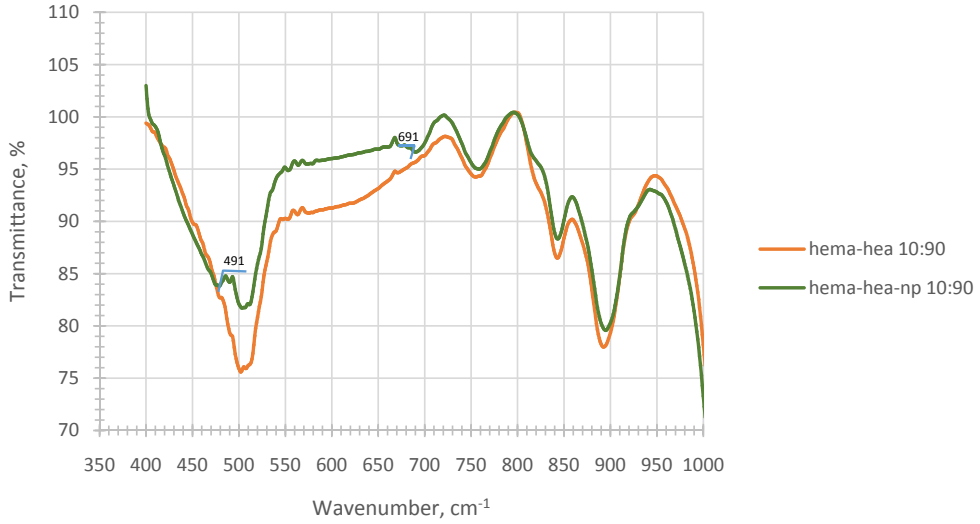


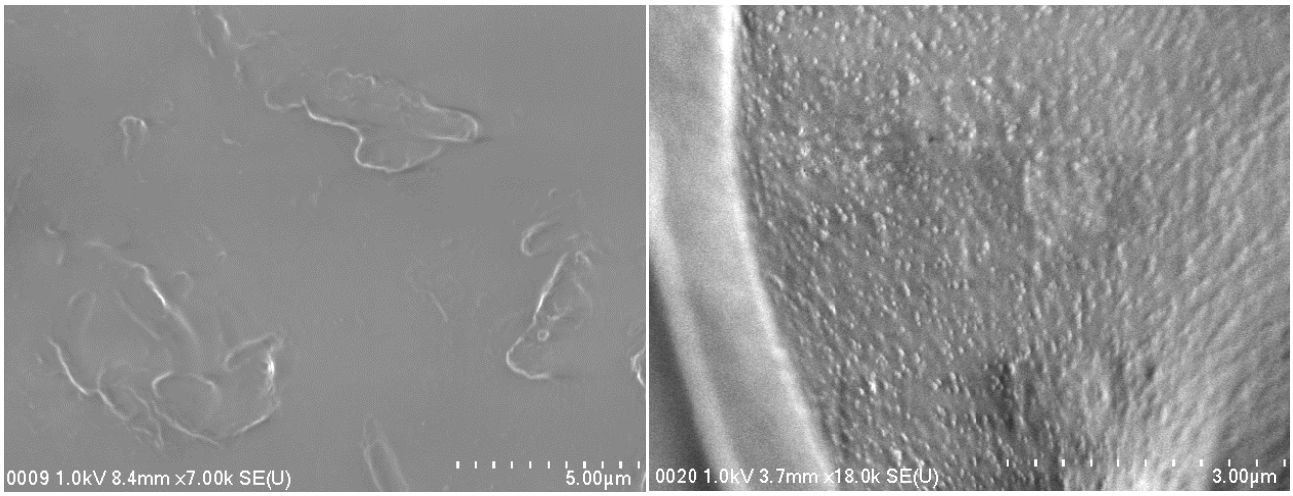
Figure 2 – IR-spectra of thiolated and non-thiolated HEA-HEMA copolymer

Scanning electron microscopy (SEM). In present work SEM was used and obtained microphotographs are presented on Figure 3. Comparing the images of thiolated (b) and non-thiolated (a) hydrogel samples revealed that small nanoparticles are present in gel structure with satisfying homogeneous distribution. Also the size of 3-MPTS nanoparticles can be determined and is around 60 nm.

Small angle X-ray Scattering (SAXS). Nanoparticles presence and their size distribution in polymer matrix were investigated by small angle X-ray scattering spectroscopy (Figure 4). Average size of nanoparticles incorporated in polymer network is about 60-70 nm which is in good correlation with results obtained by SEM.

Thermogravimetric analysis. Thermogravimetric analysis was conducted on Netzsch STA 449 F3 Jupiter (Germany) with heating rate 10°C/min in nitrogen atmosphere. Samples were heated under temperature range of 30-600°C.

From thermogravimetric curves it was possible to determine the final residues % and then calculate the mass of 3-MPTS nanoparticles in hydrogel samples. Obtained data are presented in Table 1. It was observed that with increase of nanoparticles concentration in initial monomer mixture the percentage of final residues of nanoparticles incorporated into HEA-MPTS and HEA-HEMA-MPTS hydrogel samples and their weight also increased.



a *b*

Figure 3 – Microphotographs of HEA-HEMA copolymers without (a) and with 3-MPTS nanoparticles (b)

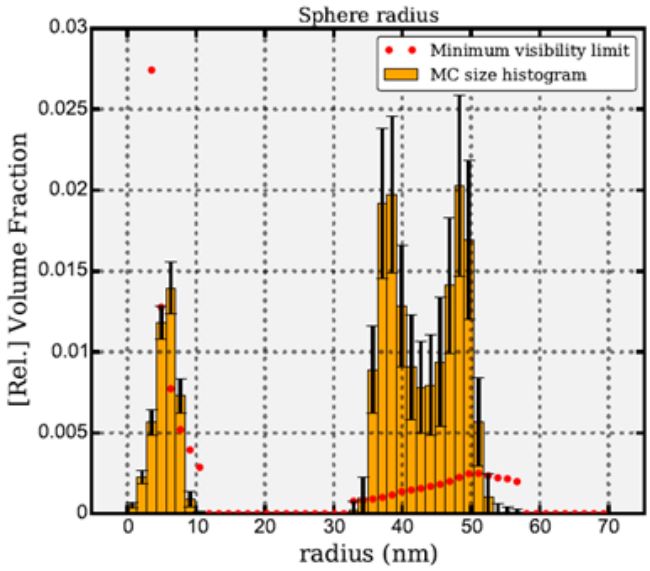


Figure 4 – Small Angle X-ray Scattering of thiolated hydrogel

Table 1 – TGA final residues analysis and calculation of 3-MPTS nanoparticles concentration in hydrogels.

Sample	C_{NP} in IMM, weight %	Final residues of hydrogels, weight %	Weight of sample, mg	C_{NP} in HEA-HEMA hydrogels, weight %	Weight of 3-MPTS nanoparticles in hydrogel sample, mg
HEA	-	10		-	-
HEA-MPTS	0,26	12	18,090	2	0,362
	0,36	13	11,458	3	0,344
	0,40	14	7,490	4	0,300
HEA-HEMA	-	8	22,728	-	-
HEA-HEMA-MPTS	0,26	10	15,402	2	0,308
	0,36	13	13,255	5	0,663

Compression test. Mechanical properties of synthesized materials were investigated by mechanical test in compression mode. Compression test showed that hydrogels based on HEA, HEMA and 3-MPTS nanoparticles are sufficiently elastically strong and flexible. This is due to the fact that glass transition temperature of HEA lies in the negative temperatures, which results in demonstration of highly elastic properties at room temperatures. Thus, while the compression analysis of hydrogel samples containing HEA was performed, their destruction was not observed, and when the load was removed, the hydrogel shape returned to its original state.

The elastic modulus E for analysed hydrogels were calculated from stress-strain curves. Results are shown in the Tables 2 and 3. The elastic modulus was determined as the slope of the initial straight-line section of the deformation curve. The growth of the concentration of the crosslinking agent and nanoparticles in the gel promoted increase of elastic modulus values by 2-3 times for certain samples.

Table 2 – Values of elastic modulus E for HEA-HEMA-MPTS hydrogels of different HEA-HEMA ratios and concentrations of nanoparticles

Composition of initial monomer mixture		Concentration of nanoparticles, mg/ml	Concentration of crosslinking agent, mol %	Elastic modulus E, Pa
[HEMA]		-	0,125	48 700,55
		2,45		121 780,60
[HEA-HEMA]	70:30	-	0,125	7 281,75
		2,45		9 021,91
	80:20	-	0,125	1 685,90
		2,45		8 530,34
		3,43		9 701,46
		3,92		15 720,57
	90:10	-	0,125	1 312,77
		2,45		5 053,85
		3,43		12 492,13
		3,92		17 408,85
[HEA]		-	0,100	5 126,72
		-	0,125	13 194,04
		2,45	0,100	1 768,32
		3,43	0,100	2 713,48
		3,92	0,100	5 925,39
		4,41	0,100	8 406,60

It is clear that the presence of nanoparticles in a gel contributes to a slight decrease in the elasticity of the polymer network, thus increasing its rigidity. This is probably due to the higher number of crosslinks in the network, which reduce flexibility and mobility of macromolecules.

When studying the effect of the monomer ratio in the initial monomer blend on the mechanical properties of copolymers, it was observed that reduction of the HEA content in the copolymer hydrogel the rigidity of the network increased.

When studying the effect of the concentration of crosslinking agent on the physico-mechanical properties, it was found that the capacity for high elastic deformation is the lowest for hydrogel samples with maximum concentration of methylene bis-acrylamide.

Thiol groups content determination. Many methods have been developed for the quantification of thiol groups; electromagnetic resonance spectroscopy, high-performance liquid chromatography with electrochemical detection, capillary electrophoresis, liquid chromatography with mass spectrometry, enzymatic methods and, the traditional analytical approaches based on the thiol derivation procedure, obtaining compounds detectable by UV–visible spectroscopy or fluorimetric. However, all these methods require elaborate equipment and are very expensive. Therefore, in this work thiol content determination was carried out by simple iodometric titration. Although, it's less sensitive and precise, this method is highly appreciated for its easiness, rapidity and no necessity in aggressive procedures.

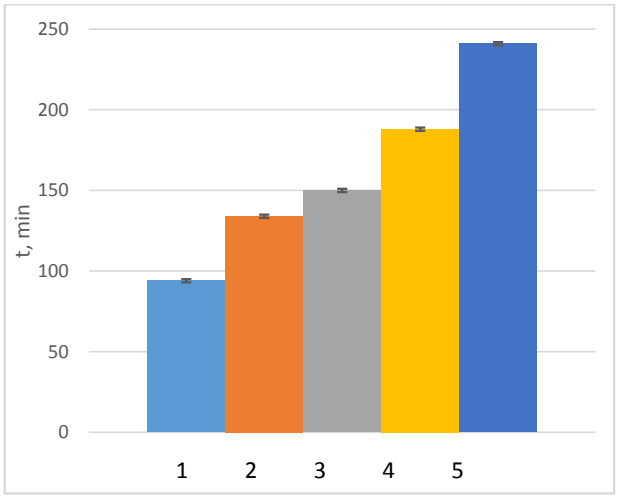
Table 3 – Impact of crosslinking agent concentration on mechanical properties of HEA-HEMA-MPTS hydrogels

Composition of initial monomer mixture		Concentration of nanoparticles, mg/ml	Concentration of crosslinking agent, mol %	Elastic modulus E, Pa
[HEA-HEMA]	80:20	2,45	0,075	6 215,07
		2,45	0,125	8 530,34
		2,45	0,175	15 404,79
		2,45	0,225	34 366,51

Results obtained by iodometric titration are presented at Table 4. First of all, it's vital to say that thiol group content increase with the concentration of nanoparticles in initial blend. For example for thiolated HEA-HEMA copolymers with monomer ratio 80:20 thiol content appears to be 32,9; 36,2 and 45,6 μmol per g polymer for nanoparticle concentration 2,45; 3,43 and 3,92 mg/ml respectively. Another point is that increase in hydrophilicity of copolymer composition results in worse thiolation which is due to hydrophobic nature of nanoparticles.

Table 4 – Dependence of thiol groups content of HEA-HEMA-MPTS hydrogels from nanoparticles concentration

Composition of initial monomer mixture		Concentration of nanoparticles, mg/ml	Concentration of crosslinking agent, mol %	Thiol content, μmol per g polymer
[HEMA]		2,45	0,125	27,8
[HEA-HEMA]	70:30	2,45	0,125	22,7
	80:20	2,45	0,125	32,9
		3,43		36,2
		3,92		45,6
	90:10	2,45	0,125	23,9
		3,43		34,4
		3,92		40,0
[HEA]		2,45	0,100	13,3
		3,43		20
		3,92		23,3
		4,41		26,7



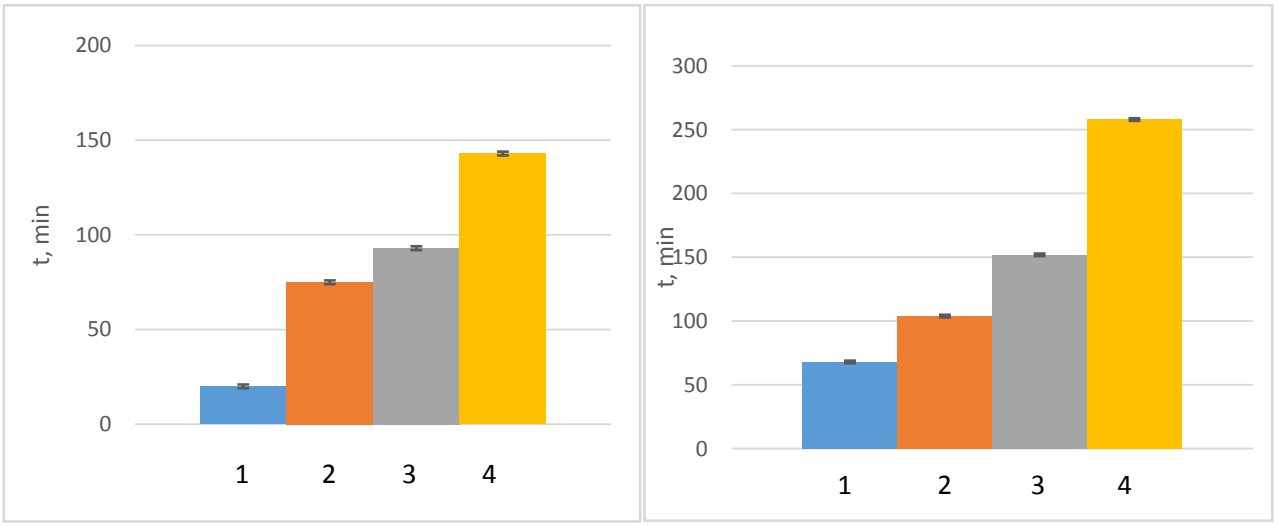
C_{NP} = 0 mg/ml (1); 2,45 mg/ml (2); 3,43 mg/ml (3); 3,92 mg/ml (4); 4,41 mg/ml (5)

Figure 5 – Impact of 3-MPTS nanoparticles concentration on retention time of HEA-MPTS hydrogels

Mucoadhesive studies. Retention time of homopolymer and copolymer hydrogels on mucosal surfaces was evaluated by "falling liquid method".

From Figures 5–6 it's seen that increase of 3-MPTS nanoparticle concentration results in longer retention time for thiolated homopolymer and copolymer hydrogels. Such effect was observed for HEA-HEMA-MPTS hydrogels of different copolymer ratio. Consequently, it should be noted that increase of thiol moieties in hydrogel composition in fact promotes better mucoadhesion.

Influence of IMM ration on mucoadhesive properties was studied. Figure 7 represents dependence of retention time for HEA-HEMA-MPTS hydrogels from copolymer content. The data obtained on this dependence appears to suggest that increase of HEA concentration as hydrophilic component in hydrogel composition results in improvement of mucoadhesion.



[HEA:HEMA], mol % = [80:20] [HEA:HEMA], mol % = [90:10]

C_{NP} = 0 mg/ml (1); 2,45 mg/ml (2); 3,43 mg/ml (3); 3,92 mg/ml (4)

Figure 6 – Impact of 3-MPTS nanoparticles concentration on retention time of HEA-HEMA-MPTS hydrogels

Drug loading. In present work as a model drug for buccal drug delivery forms the metronidazole was chosen. Metronidazole is an antibiotic used to medicate infections of oral cavity [7], so our choice was determined upon this fact. Metronidazole was loaded into obtained thiolated hydrogels by simple absorption method.

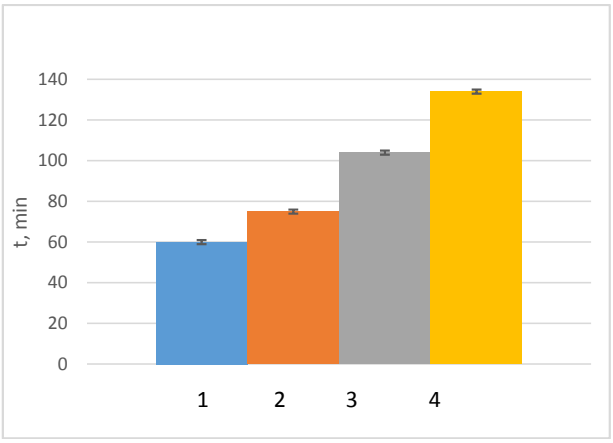
Content of metronidazole loaded into polymer samples is presented in Table 6.

It should be noted that thiolated samples absorb more drug than non-thiolated ones. As nanoparticles concentration increases metronidazole content rises respectively. For example for HEA-HEMA-MPTS sample with copolymer ratio 90:10 and nanoparticles concentration 2,45; 3,43 and 3,92 mg/ml masses of loaded metronidazole were 0,0112; 0,0123 and 0,0132 g respectively.

Another pattern that could be derived is that with increase of HEA content in copolymer samples concentration of loaded drug decreases. For instance, samples with nanoparticles concentration of 2,45 ml/ml and copolymer ratio 70:30, 80:20, 90:10 showed drug concentration of 0,0126; 0,0098. Hypothetically, it's caused by hydrophobic character of metronidazole, so that metronidazole showed affinity to hydrophobic nature of HEMA.

Drug release studies. The measurement of metronidazole release was conducted using Franz cell chamber. The Franz Cell chamber is an in vitro skin permeation test method frequently used in formulation development. In this work permeation of drug was observed through cellulose membrane. Artificial saliva solution was used as collection medium.

From Figure 8 it's seen that increase of nanoparticles concentration promotes more prolonged release of metronidazole. As concentration of nanoparticles becomes higher metronidazole concentrations decrease.



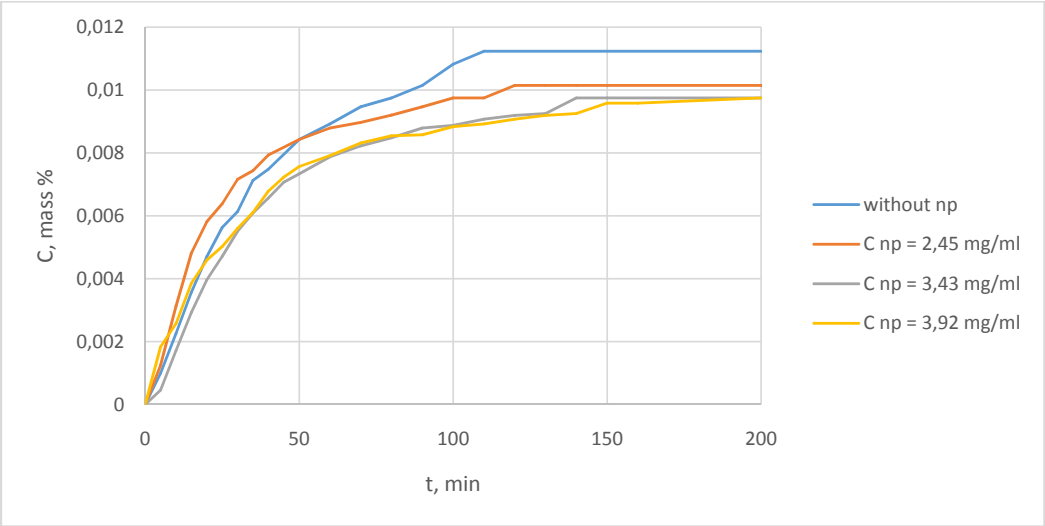
[HEA:HEMA], mol % = 70:30 (1); 80:20 (2); 90:10 (3); [HEA] (4)

C_{NP} = 2,45 mg/ml

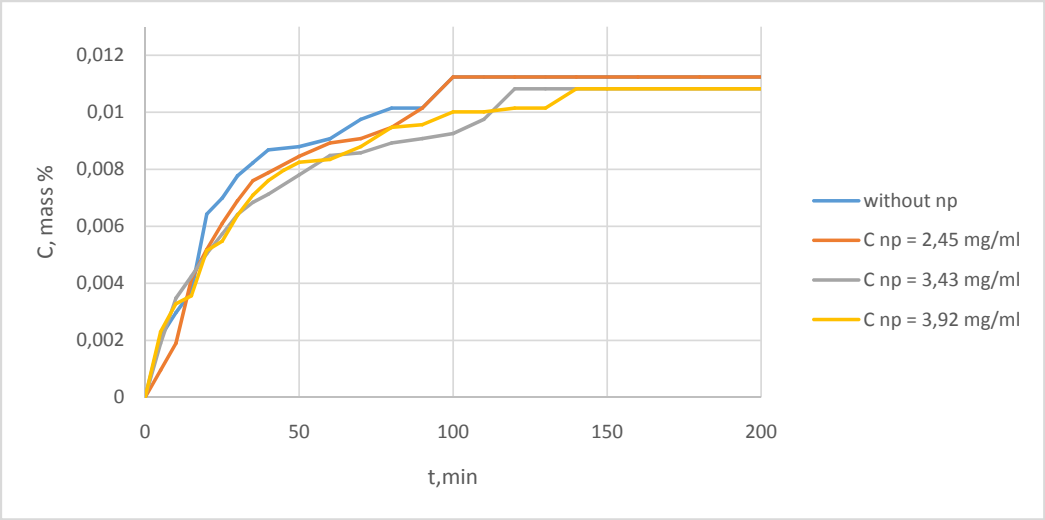
Figure 7 – Impact of copolymer IMM ration on retention time of HEA-HEMA-MPTS hydrogels

Table 6 – Metronidazole concentrations loaded in non-thiolated and thiolated HEA-HEMA hydrogels

Composition of initial monomer mixture		Concentration of nanoparticles, mg/ml	Concentration of crosslinking agent, mol %	Mass of loaded metronidazole, g	Concentration of loaded metronidazole, g/g of polymer
[HEMA]		-	0,125	0,0125	0,2147
		2,45		0,0135	0,1777
[HEA-HEMA]	70:30	-	0,125	0,0120	0,1921
		2,45		0,0126	0,2603
	80:20	-	0,125	0,0084	0,1132
		2,45		0,0098	0,2357
		3,43		0,0108	0,1735
		3,92		0,0192	0,3751
	90:10	-	0,125	0,0075	0,1157
		2,45		0,0112	0,1752
		3,43		0,0123	0,1636
		3,92		0,0132	0,2052
[HEA]		-	0,125	0,0112	0,2463



a. [HEA:HEMA], mol % = [80:20]



b. [HEA:HEMA], mol % = [90:10]

Figure 8 – Impact of nanoparticles concentration on kinetics of metronidazole release.

Figure 9 shows that with increase of hydrophilic component in copolymer ratio the concentration of released metronidazole grows. Also drug release duration become shorter as time of plateau appearance moves to smaller values. Consequently samples with copolymer ratio 90:10 release drug more quickly.

Conclusion

Hydrogels based on HEMA and HEA copolymers structured with 3-MPTS nanoparticles of different concentration have been synthesized and characterized using various physical-chemical methods.

Small angle X-ray scattering and scanning electron microscopy confirmed presence of incorporated nanoparticles. These methods allowed determination of nanoparticles size and their distribution in polymer matrix. Average size of nanoparticles incorporated in polymer network was about 60-70 nm.

Thiolation of copolymers was confirmed by FT-IR-spectroscopy and iodometry as well. Iodometric quantification showed that concentration of thiolated samples lays in concentration range 12-46 μmole per g of hydrogel.

Thermogravimetric analysis displayed that with increase of nanoparticles concentration in initial monomer mixture the mass fraction of residues of HEA-MPTS and HEA-HEMA-MPTS hydrogel samples also increased.

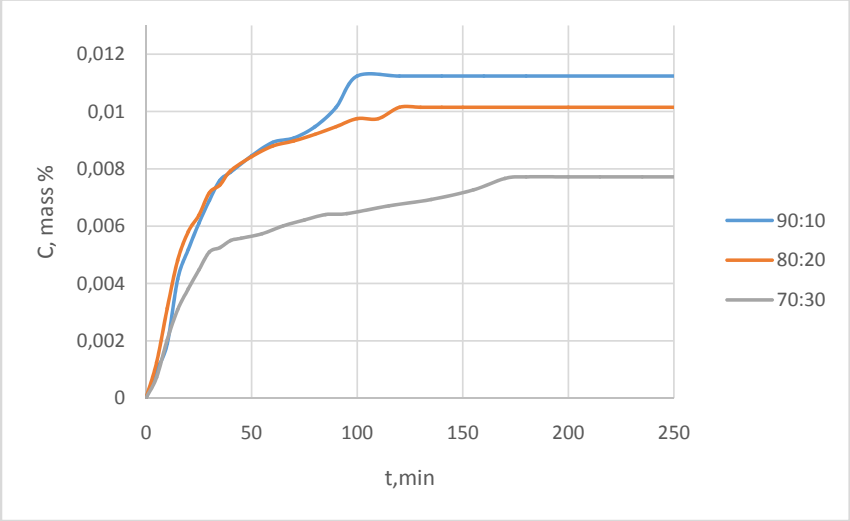


Figure 9 – Impact of HEA-HEMA copolymers ratio on kinetics of metronidazole release.

Compression test showed that the presence of nanoparticles in a gel contributes to a slight decrease in the elasticity of the polymer network increasing its strength. The growth of the concentration of the crosslinking agent and nanoparticles in the gel promoted increase of elastic modulus values by 2-3 times for certain samples.

Mucoadhesion studies showed that increase in concentration of hydrophilic component HEA in hydrogel, nanoparticles content and crosslinking agent concentration reflects an increase of adhesion time of obtained hydrogel materials.

Drug loading and drug release studies of HEA-HEMA-MPTS samples with metronidazole were conducted. It was observed that thiolated samples absorb more drug than non-thiolated ones. As nanoparticles concentration increases metronidazole content rises respectively. With increase of HEA content in copolymer samples concentration of loaded drug decreases.

Drug release studies revealed that the higher concentration of 3-MPTS nanoparticles in hydrogels results in prolongation of drug release but concentration of discharged metronidazole decreases. When hydrophilic component in copolymer content increases the concentration and rate of released metronidazole also growing.

Modifications of hydrogel materials by thiolation lead to broadening of their application possibilities. As a result of this research new thiolated polymers with improved mucoadhesive properties were obtained. And mucoadhesive studies of their application as a buccal dosage forms were performed as well.

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