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IONICALLY AND COVALENTLY CROSSLINKED HYDROGEL PARTICLES BASED ON CHITOSAN AND POLY(ETHYLENE GLYCOL)

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Received: February 19, 2013 / Revised: May 12, 2013 / Accepted: October 29, 2013

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Abstract. Self-assembled pH-sensitive reactive submicron-sized particles have been developed *via* intermolecular electrostatic interactions between chitosan and a carboxylated oligoperoxide having poly(ethylene glycol) fragments. The particles have the structure of an ionically crosslinked hydrogel and their size and surface charge depend on the conditions of their formation (*i.e.*, pH and the functional group ratio). The presence of peroxide groups in the oligoperoxide has enabled to synthesize covalently crosslinked pH-sensitive particles.

Keywords: hydrogel particles; pH-sensitive particles; self-assembly; oligoperoxide; chitosan.

1. Introduction

Various submicron-sized targeted drug delivery systems (*e.g.*, nanocapsules, nanohydrogels, core-shell nanoparticles and so forth) have been recently developed in order to facilitate existing methods of drug administration to a target organ [1]. Moreover, the methods based on intermolecular electrostatic interactions of polyelectrolytes [2-6] have received much attention for the development of self-assembled systems such as microparticles, microcapsules, films, and hydrogels. Among them, electrostatically self-assembled systems based on chitosan are of particular interest. For instance, nanoparticles based on chitosan and poly- γ -glutamic acid can be used as drug delivery vehicles to target cells [7, 8]. A work on the polyelectrolytic interaction between chitosan and DNA has been recently published [9] where

gene transfection has been studied. Particles, which could be used as potential drug delivery carriers for proteins, have been prepared through electrostatic interactions between chitosan and a glucomannan derivative [10].

The use of natural hydrophilic polymers as drug carriers has received considerable attention in the last few years, especially from the viewpoint of safety, cost, and environmental pollution. In particular, polysaccharides such as chitosans and their derivatives are suitable candidates for sustained or controlled drug-release systems. Chitosan [poly-*b*-1,4-(2-amino-2-deoxy-D-glucopyranose) with some proportion of 2-acetyl-amino-2-deoxy-D-glucopyranose units] (Fig. 1) is a hydrophilic polyelectrolyte generally prepared by alkaline deacetylation from chitin, which is the main component of the exoskeleton of crustaceans, such as shrimps [11]. Chitosan is currently receiving a great deal of interest for medical and pharmaceutical applications. The main reasons for this increasing attention are its unique properties. Indeed, chitosan has been reported to be biodegradable, non-toxic, biocompatible and bioadhesive. It is a good hemostatic, hypocholesterolemic, and hipolipidemic agent. Chitosan has antimicrobial, antiviral, and antitumoral activity. Moreover, chitosan is very abundant and its production is of low cost. All these interesting characteristics have led to the development of numerous applications of chitosan in biomedicine. Chitosans bear positively charged groups and, hence, can interact and form three-dimensional networks with molecules of opposite charge. Being swollen in water or biological fluids, these macromolecular networks form hydrogels.

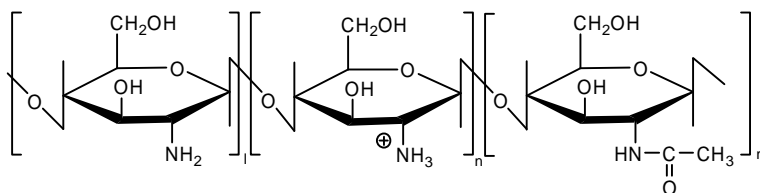


Fig. 1. Scheme of chitosan

On the other hand, poly(ethylene glycol) has been very popular as a synthetic polymer used in medicine and pharmacy. PEG is nontoxic, nonimmunogenic, and favorable to nutrient and oxygen transport. Furthermore, poly(ethylene glycol) is neutral, flexible and very hydrophilic material and, thus, the PEG macromolecules possess “stealth” properties. Poly(ethylene glycol) chains can in fact properly produce surface barrier layers that reduce the adhesion of opsonins present in the blood serum on the surfaces making them “invisible” to phagocytic cells.

This work aims to develop a method for the design of self-assembled submicron-sized hydrogel particles which are ionically crosslinked *via* intermolecular electrostatic interactions between chitosane (Chit) and a PEG-based oligoperoxide (OP) containing pyromellitic acid moieties. A further goal of this study is to use the OP peroxide groups for the formation of covalently crosslinked hydrogel particles.

2. Experimental

Chitosan [poly-*b*-1,4-(2-amino-2-deoxy-*D*-glucopyranose)] with a molecular weight of 32 300 and the degree of deacetylation of 75 % (Aldrich) was used as received. The oligoperoxide (tridecaoxyethylene-bis(mono-*tert*-butylperoxy)pyromellitate) (M_n : calcd. 1206, found 970; acid number: calcd. 186 mg KOH/g, found 202 mg KOH/g; O_{act} : calcd. 2.65 wt %, found 2 wt %) (was synthesized via an interaction between pyromellitic dianhydride, poly(ethylene glycol) (MW=600), and *tert*-butyl hydroperoxide according to the technique described elsewhere [12]. The oligoperoxide was purified by precipitation from acetone in water followed by precipitation from acetone in cyclohexanone. *Glacial acetic acid* (Aldrich) was used without any further purification.

An average molecular weight of Chit (M_h) was calculated according to the Mark-Houwink equation [13]:

$$[\eta] = K \cdot M_h^\alpha,$$

where M_h is an average molecular weight of Chit, $[\eta]$ is intrinsic viscosity of a Chit solution determined with an Ubbelohde viscometer at 298 K.

The Mark-Houwink parameters K and α were calculated according to the following equation [14]:

$$K = 1.64 \cdot 10^{-30} \cdot DD^{14.0}$$

$$\alpha = -1.02 \cdot 10^{-2} \cdot DD + 1.82$$

where DD is the degree of deacetylation of Chit.

The degree of deacetylation of Chit was determined by a potentiometric back-titration of the primary amino groups.

The formation of a dispersion of self-assembled Chit-OP particles was conducted using 0.1 % Chit and OP aqueous solutions. To this end, 0.1 g of Chit was dissolved in 10 g of 2.5 wt % acetic acid and distilled water was added to the total weight of 100 g. Then, the pH of the solution was adjusted to 4.1 using acetic acid. Separately, a 0.1 wt % OP aqueous solution was prepared in distilled water and its pH was adjusted to 4.3 with 0.1 N NaOH. Both Chit and OP solutions were filtered. For the formation of the Chit-OP particles, a calculated amount of the OP solution was added dropwise to the Chit solution under agitation. The reaction mixture was stirred for 30 min at 291 K. The formation of the Chit-OP particles was carried out at different OP:Chit ratios, which were calculated as molar ratios of the OP carboxylic groups to the primary amino groups of Chit in the range given in Table 1.

Thus, the overall concentration of the Chit-OP dispersion was constant for all the samples and was equal to 0.1 %. When required, the pH of the dispersions was adjusted with either 0.1N NaOH or 0.1N HCl.

The formation of covalently crosslinked Chit-OP particles was carried out by heating the dispersion, built up at the COOH:NH₂ ratio as 0.9, at 333 K for 3 h.

The transmittance of the Chit-OP dispersions was measured at pH 4.25 using an UNICO 1201 colorimeter at 450 nm in a 10 mm cuvette.

The light scattering of the Chit-OP dispersions was determined at pH 3.1, 4.25, and 4.9 using an LMF-72 M photometer at 560 nm in a 20 mm cuvette.

The hydrodynamic diameter of the Chit-OP particles was measured by dynamic light scattering using a Nicomp 380 particle size analyzer from Particle Sizing Systems. The hydrodynamic diameter of the particles was calculated as an average value from five successive measurements.

The z-potential of the Chit-OP particles was determined at 298 K using a Malvern Zetasizer Nano-Z instrument equipped with a He-Ne laser. Prior to measurement, the Chit-OP dispersions were diluted with a KCl solution (1.5 ml of 10⁻³ M KCl was added to 100 μl of the Chit-OP dispersion) and the pH was adjusted to a required value with 0.1N HCl).

Scanning electron microscopy (SEM). SEM images of the Chit-OP particles were taken using Leo Zeiss 1530 Gemini scanning electron microscope. For the SEM measurements, the samples were prepared by lyophilic drying of the Chit-OP dispersions followed by the immobilization of the formed powder on a substrate.

FTIR spectra were recorded using a Nicolet 8700 (Thermo Scientific) spectrometer with a resolution of 4 cm⁻¹.

Table 1

Formation of self-assembled Chit-OP particles

Molar ratio of COOH-groups to NH ₂ -groups	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
Weight of 0.1 wt % Chit solution, g	11.3	10.1	9.4	8.7	8.0	7.5	7.1	6.5
Weight of 0.1 wt % OP solution, g	4.7	5.9	6.6	7.3	8.0	8.5	8.9	9.5

3. Results and Discussion

Chitosan is a polyelectrolyte (Fig. 1). In an acidic medium, the Chit macromolecules undergo protonation. An overall charge value and a conformation of the Chit macromolecules essentially depend on the pH of an environment. On the other hand, OP exhibits amphiphilic properties due to the presence of hydrophilic poly(ethylene glycol) fragments and lipophilic aromatic and aliphatic groups in its structure (Fig. 2).

The interaction between the Chit amino groups and the OP carboxylic groups (after mixing together a Chit solution and an OP solution) results in the formation of intermolecular aggregates and their self-assembly into

submicron-sized particles accompanied with the development of interpolymeric structures with a limited solubility in water at certain pH levels.

The ionization degree of the OP carboxylic groups is also determined by pH value. Therefore, the Chit-OP interactions leading to the development of the intermolecular structures depend on pH (Fig. 3). The formation of the Chit-OP particles occurs in an aqueous environment at pH 4.1-4.25 when only the partial dissociation of the OP carboxylic groups and the partial protonation of the Chit amino groups are possible. It results in building up a colloidal system with the Chit-OP particles as a disperse phase.

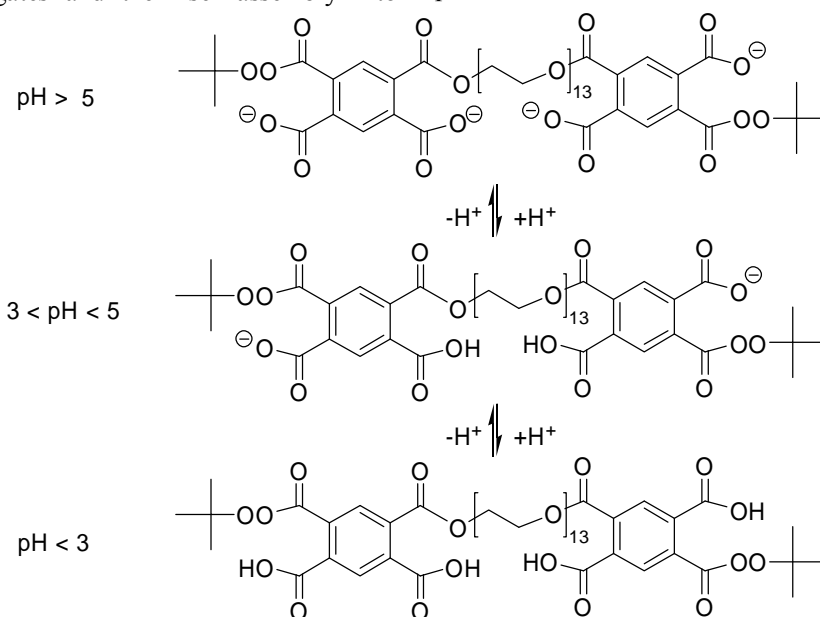


Fig. 2. Scheme of oligoperoxide structures at different pH values

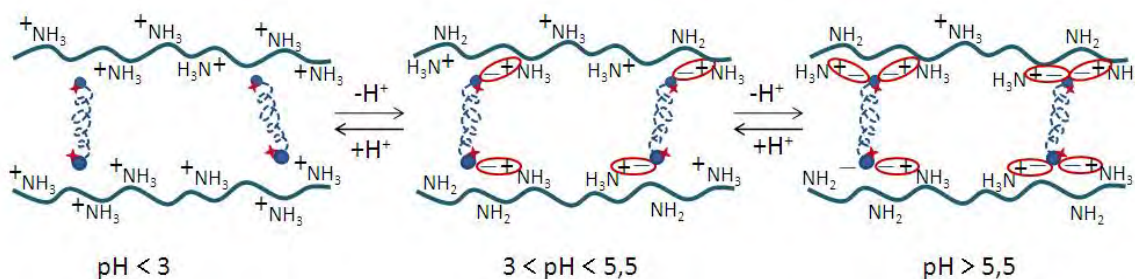


Fig. 3. Scheme of the intermolecular interactions between Chit and OP at different pH values

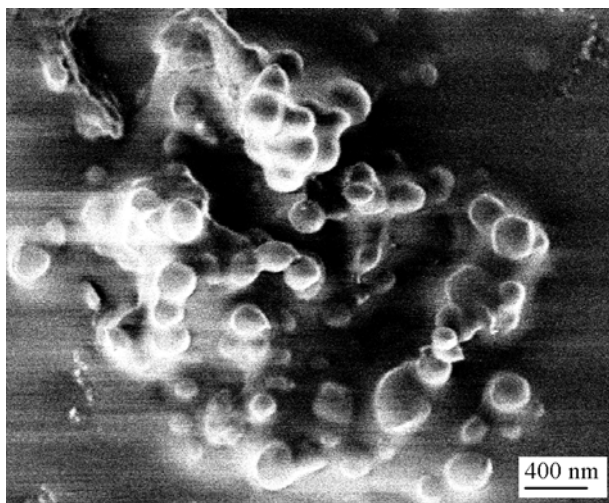


Fig. 4. SEM micrograph of the dispersed Chit-OP particles developed at the COOH:NH₂ ratio as 0.9 at pH adjusted to 5.0

Fig. 4 shows the SEM micrograph of the Chip-OP particles developed at the COOH:NH₂ ratio as 0.9. The picture indicates that the particles are spherical. At pH 5.0, the average diameter of the particles is approximately 300 nm.

In order to determine the type of a chemical bond between the Chit and OP macromolecules, the FTIR spectra of chitosan and the Chip-OP particles have been recorded (Fig. 5). In the spectrum of the Chip-OP particles (*curve 2*), a clear decrease in intensity (as compared to chitosan – *curve 1*) of the absorption band at 1630 cm⁻¹ (NH₂ group) has been observed. With the further increase of a COOH group content in the particles (*curve 3*), this absorption band has completely disappeared. As compared to the chitosan spectrum, the following new characteristic absorption bands, resulting from oscillations of the COO⁻ carboxylate anion, have appeared in the spectrum of the Chit-OP particles: the first band at 1570 cm⁻¹ overlapped with another band at 1600–1575 cm⁻¹ (NH₃⁺ cation) and the second band at 1412 cm⁻¹. Therefore, it could be concluded that the development of the Chip-OP particles is favored by the formation of ionic bonds between the Chit amino groups and the OP carboxylic groups [15].

The particle size has been shown to depend on the molar ratio of the OP carboxylic groups to the Chit amino groups. An increasing content of the COOH-groups leads to an increase in the number of particles and, hence, to a decrease of the particle size as evidenced by the transmittance and light scattering data recorded on the dispersions formed (Fig. 6).

These data have been additionally confirmed by the dynamic light scattering experiments which revealed the

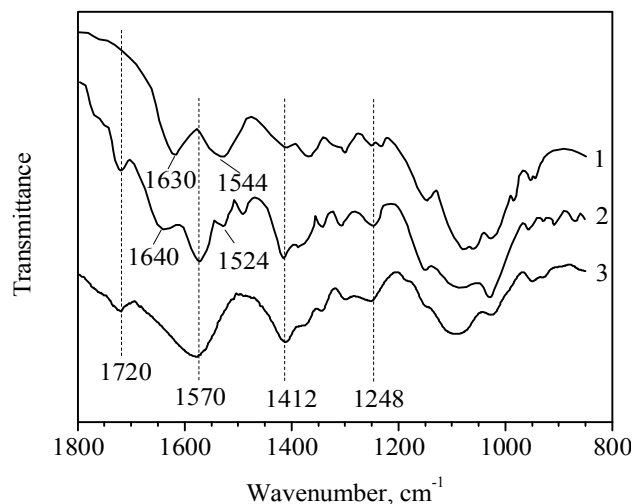


Fig. 5. FTIR spectra of chitosan (1) and the Chit-OP particles developed at pH 4.3 and the COOH:NH₂ ratio as 0.4 (2) or 0.9 (3)

hydrodynamic diameter of the Chip-OP particles decreases from 550 to 334 nm at pH 4.25 with the increasing ratio of the OP carboxylic groups to the Chit amino groups from 0.7 to 1.0 (Fig. 7). An increase in the ratio of the carboxylic functionalities to the amino ones from 0.3 to 1.0 has resulted in a drop of the z-potential of the particles from 35.0 to 17.4 mV (Fig. 8). The increasing content of the carboxylic groups has evidently led to the formation of a higher number of ionic bonds between Chit and OP and, thus, to a smaller number of the free protonated amino groups in each particle. The electrostatic repulsion forces between the positively charged Chit macrochains have weakened, which has resulted in the formation of the denser structure of the Chit-OP particles and, hence, to the smaller particle size (Fig. 7).

The dispersion stability of the self-assembled Chit-OP particles is provided by the presence of the protonated Chit amino groups on the particulate surface, which gain positive charge that hinders aggregation of the particles. The samples with the excessive number of the amino groups have been stable at the pH levels from 3.0 to 5.0. Their particle size has not changed for at least 10–14 days. At the equimolar ratio of the carboxylic functionalities to the amino ones, the dispersions have lost their stability at pH 4.25–5.0 and particle aggregation has been observed. At the above-mentioned ratio of Chit to OP, all the protonated amino groups have been bound with the ionized carboxylic groups, which have resulted in both diminution of the surface charge of the Chip-OP particles (Fig. 8) and decrease in the electrostatic stability of the dispersion. Therefore, an excess of the amino groups should be kept in order to assure dispersion stability.

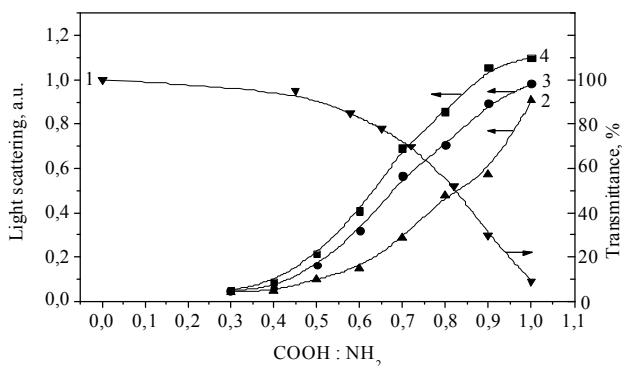


Fig. 6. Transmittance (1) and light scattering (2-4) of the Chit-OP dispersions *versus* the COOH:NH₂ ratio at different pH values: pH 4.25 (1, 3); pH 3.1 (2) and pH 4.9 (4)

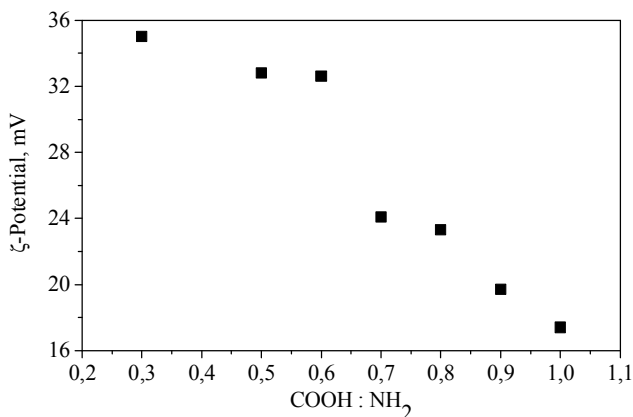


Fig. 8. The z-potential of the Chit-OP particles at pH 5.0 *versus* the COOH:NH₂ ratio

The development of the particles is only possible in a certain pH range (3.0–5.5), which indicates the ionic mechanism of the Chit and OP macromolecules binding. At pH below 3.0, most of the Chit amino groups are protonated whereas the OP carboxylic groups are nondissociated and thus cannot form ionic bonds. At pH above 5.5, the Chit amino groups are deprotonated, they have no charge and, hence, are not capable of forming salts with the OP ionized carboxylic groups. Only in the pH range 3.0–5.5, there are oppositely charged macrochains of Chit and OP able to electrostatically interact with each other and to self-assemble.

The dispersed phase particle size has also been influenced by the pH of the medium. As evidenced by Fig. 9, the hydrodynamic diameter of already formed Chit-OP particles has decreased 2–2.5 times with the pH value increasing from 3.0 to 5.0. This could be explained

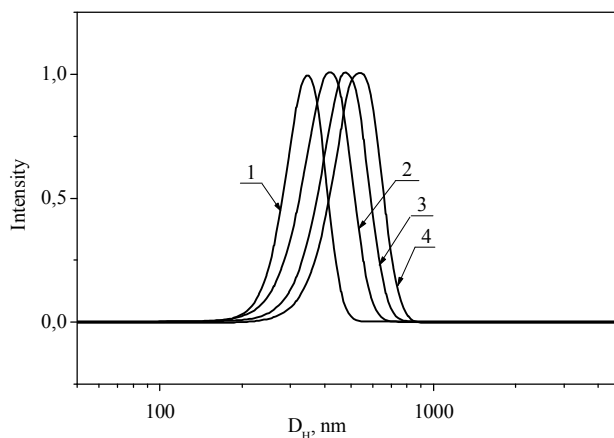


Fig. 7. The hydrodynamic diameter of the Chit-OP particles at pH 4.25 and the different ratios of the COOH and NH₂ groups: 1 (1); 0.9 (2); 0.8 (3) and 0.7 (4)

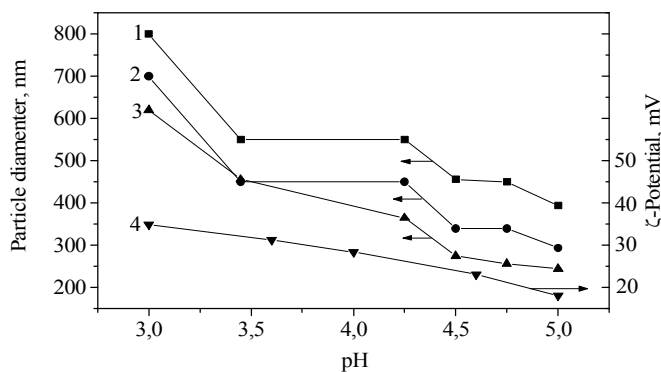


Fig. 9. The hydrodynamic diameter (1-3) and z-potential of the Chit-OP particles at the different COOH:NH₂ ratios *versus* pH: 0.7 (1); 0.8 (2) and 0.9 (3, 4)

by an increase in the dissociation degree of the OP carboxylic groups that has led to stronger electrostatic interactions and denser packing of the Chit and OP macromolecules (Fig. 3). When the pH level has dropped from 5.0 to 3.0, the Chit amino groups have protonated, which has resulted in stronger electrostatic repulsion between the Chit macromolecule units of like charge and their better hydration. Thus, the Chit-OP particle size (Fig. 9, curves 1-3) has increased along with their z-potential (Fig. 9, curve 4).

At pH below 2.8, the OP carboxylic groups do not dissociate and thus cannot form ionic bonds, which renders the electrostatic interactions between Chit and OP impossible and the particles dissolve. Hence, the self-assembly of the Chit and OP into particles is reversible and the particles possess the properties of an ionically crosslinked hydrogel and are pH-sensitive.

A distinctive feature of the developed Chit-OP particles is the presence of reactive peroxide groups in their structure. The peroxide functionalities are capable of generating free radicals at elevated temperatures. Chitosan is known to be able to participate in both chain transfer and macroradical recombination reactions in the presence of radical initiators [16-20]. The course of these free-radical processes leads to the formation of intermolecular cross-links accompanied by the development of the covalently crosslinked Chit-OP particles.

The size of the Chit-OP particles formed at pH 3.5 and the COOH:NH₂ ratio of 0.9 has been observed to decrease from 420 to 240 nm after holding them at 333 K for 3 h because the particles have obviously become denser due to the formation of covalent bonds between the Chit and OP fragments. Moreover, the particles have lost their ability to dissolve in a very acidic medium (pH below 2.8) after heating, which confirms the formation of a covalently crosslinked network within the particles. Unlike the ionically crosslinked particles, which dissolve in an acidic medium, the covalently crosslinked particles just swell and increase in size.

The particulate surface could be modified by the "grafting from" approach in the case if there are residual peroxide groups on the surface [21].

4. Conclusions

The method for preparation of self-assembled reactive submicron-sized Chit-OP hydrogel particles, which are ionically crosslinked *via* intermolecular electrostatic interactions between the oppositely charged macrochains of Chit and oligomeric OP molecules in an aqueous medium has been developed. The particle size depends on both the pH of the medium and the molar ratio of the OP carboxylic groups to the Chit amino groups during the particle formation. The developed particles are pH-sensitive since they are capable of changing their size or even dissolve in response to change in pH of the environment. The presence of the peroxide groups in the particles enables the formation of covalently crosslinked networks within the particles. The covalently crosslinked particles lose their ability to dissolve but retain pH-sensitivity. Both ionically and covalently crosslinked particles are good candidates for the development of polymeric drug delivery systems.

References

- [1] Sinha R., Kim G., Nie S. and Shin D.: Mol. Cancer Ther., 2006, **5**, 1909.
- [2] Kizilay E., Kayitmazer A. and Dubin P.: Adv. Colloid Interface Sci., 2011, **167**, 24.
- [3] Muller M., Reihls T. and Ouyang W.: Langmuir, 2005, **21**, 465.
- [4] Songa W., Hea Q., Mohwald H. et al.: J. Control. Release, 2009, **139**, 160.
- [5] Berger J., Reist M., Mayer J. et al.: Eur. J. Pharm. Biopharm., 2004, **57**, 19.
- [6] Willerich I., Schindler T. and Grohn F.: J. Phys. Chem. B, 2011, **115**, 9710.
- [7] Hajdu I., Bodnar M., Filipcsei G. et al.: Colloid Polym. Sci., 2008, **286**, 343.
- [8] Keresztessy Z., Bodnar M., Ber E. et al.: Colloid Polym. Sci., 2009, **287**, 759.
- [9] Liua W., Suna S., Caoa Z. et al.: Biomaterials, 2005, **26**, 2705.
- [10] Du J., Dai J., Liu J.L. and Dankovich T.: React. Funct. Polym., 2006, **66**, 1055.
- [11] Muzzarelli R.: Chitosan [in:] Muzzarelli R. (Ed.), Natural Chelating Polymers. Pergamon Press, Oxford 1973, 144-176.
- [12] Bratychak M. and Donchak V.: Mat. Res. Innovat., 2002, **5**, 250.
- [13] Toroptseva A., Belgorodskaya K. and Bondarenko V.: Laboratornyy Praktikum po Khimii i Tekhnologii Vysokomolekulyarykh Soedineniy. Khimiya, Leningrad 1976.
- [14] Wang W., Bo S., Li S. and Qin W.: Int. J. Biol. Macromol., 1991, **13**, 281.
- [15] Bellamy L.: The Infra-Red Spectra of Complex Macromolecules. Chapman and Hall Ltd, London 1975.
- [16] Solomko N., Budishevskaya O., Voronov A., Kohut A. et al.: Macromol. Symp., 2010, **298**, 77.
- [17] Sangamesh G., Kumaresh S. and Tejjaz M.: J. Appl. Polym. Sci., 2003, **87**, 1525.
- [18] Jenkins D. and Hudson S.: Chem. Rev., 2001, **101**, 3245.
- [19] Curcio M., Puoci F., Iemma F. et al.: J. Agric. Food Chem., 2009, **57**, 5933.
- [20] Alves N. and Mano J.: Int. J. Biol. Macromol., 2008, **43**, 401.
- [21] Stetsyshyn Y., Donchak V., Harhay K., Voronov S. et al.: Polym. Int., 2009, **58**, 1034.

ЙОННО І КОВАЛЕНТНО ЗШИТІ ГІДРОГЕЛІВІ ЧАСТИНКИ НА ОСНОВІ ХІТОЗАНУ І ПОЛІЕТИЛЕНГЛІКОЛЮ

Анотація. Електростатичною міжмолекулярною взаємодією хітозану та карбоксиповмісного олігопероксиду з фрагментами поліетиленгліколю одержані самовпорядковані рН-чутливі реакційноздатні субмікронні частинки, які мають структуру йонно-зшитого гідрогелю, а їх розмір та поверхневий заряд залежить від умов формування (рН і співвідношення функціональних груп). Наявність пероксидних груп у складі олігопероксиду дала можливість одержати ковалентно зшиті рН-чутливі частинки.

Ключові слова: гідрогеліві частинки, рН-чутливі частинки, самоорганізація, олігопероксид, хітозан.