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MICROCAPSULES BASED ON CHITOSAN-CARBOXYMETHYL CELLULOSE COMPLEXES OBTAINED BY MEMBRANE EMULSIFICATIONVictoryia V. Konovalova¹*, Iryna S. Kolesnyk¹, Ganna A. Pobigay¹, Andriy I. Marynin²¹National University of Kyiv-Mohyla Academy, 2 Skovorody Str., Kyiv, 04070, Ukraine²National University of Food Technologies, 68 Volodymyrska Str., Kyiv, 01601, Ukraine

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Abstract

In this work, thermo- and pH-sensitive microcapsules were obtained based on the polyelectrolyte complex of chitosan-carboxymethyl cellulose by the membrane emulsification. Microcapsules with a size of 200–250 nm were produced using a track PET membrane with a pore size of 0.1 μm . The stability of microcapsules, their size distribution, zeta potential of the surface, and the effect of temperature and pH on particle size were studied by the dynamic light scattering. The formation of the complex was confirmed by IR spectroscopy, and the structure of the complexes at different ratios of polymers was analyzed by viscosimetry. The kinetics of papaverine hydrochloride release from microcapsules based on chitosan : CMC complexes at a ratio of 1 : 1 and 3 : 2 was studied. This method is a simple technique for obtaining microcapsules from interpolymer complexes by alternately introduction of polymer solutions into the dispersion medium, which allows to obtain monodisperse suspensions of a certain size and effective immobilization of a wide range of pharmaceutical ingredients.

Keywords: microcapsules; membrane emulsification; interpolymeric complex; chitosan; carboxymethyl cellulose; drug delivery; papaverine hydrochloride.

ОТРИМАННЯ МІКРОКАПСУЛ НА ОСНОВІ КОМПЛЕКСІВ ХІТОЗАНУ ТА КАРБОКСИМЕТИЛЦЕЛЮЛОЗИ МЕМБРАННОЮ ЕМУЛЬСИФІКАЦІЄЮВікторія В. Коновалова¹, Ірина С. Колесник¹, Ганна А. Побігай¹, Андрій І. Маринін²¹Національний Університет «Києво-Могилянська Академія», вул. Сковороди, 2, Київ, 04070, Україна²Національний Університет харчових технологій, вул. Володимирська, 68, Київ, 01601, Україна**Анотація**

У роботі термо- та рН-чутливі мікрокапсули отримані на основі поліелектролітного комплексу хітозан-карбоксиметилцелюлоза методом мембранної емульсифікації. Мікрокапсули розміром 200–250 нм отримані з використанням трекової ПЕТФ мембрани з розміром пор 0,1 мкм. Стабільність мікрокапсул, їх розподіл за розміром, дзета-потенціал поверхні та вплив температури та рН на розмір частинок досліджено методом динамічного світлорозсіювання. Утворення комплексу підтверджено методом ІЧ спектроскопії та досліджено структуру комплексів при різних співвідношеннях полімерів методом візкозиметрії. Досліджено кінетику вивільнення папаверин гідрохлориду із мікрокапсул на основі комплексів хітозан : КМЦ при співвідношеннях 1:1 та 3:2. Даний метод є простою технікою для отримання мікрокапсул з інтерполімерних комплексів шляхом почергового введення розчинів полімерів в дисперсійне середовище, що дає можливість отримання монодисперсних суспензій визначеного розміру та ефективної іммобілізації широкого спектру фармацевтичних інгредієнтів.

Ключові слова: мікрокапсули; мембранна емульсифікація; інтерполімерний комплекс; хітозан; карбоксиметилцелюлоза; доставка ліків; папаверин гідрохлорид.

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Introduction

Nowadays the development of prolonged-released drug delivery systems has received increased attention [1–3]. Controlled and targeted drug delivery and release play an important role in maintaining a certain drug concentration in blood, thereby reducing the frequency of drug administration, which decreases their toxic effects and increases treatment effectiveness [4]. For development of such drug delivery systems both synthetic [5] (e.g., polylactic acid, polyacrylate) and natural [6] (e.g., gelatin, cellulose, chitosan) polymers are used. Synthetic macromolecular carriers often suffer from lack of biocompatibility, so natural biopolymers are preferred.

Carboxymethyl cellulose (CMC) is a natural anionic water-soluble polysaccharide that is widely used in the drug delivery systems design [7]. Chitosan is one of the most common cationic biopolymers [8]. Owing to its pH-sensitive properties chitosan is widely used in tissue engineering, wound dressing, and drug delivery systems design [9]. Moreover, it can be degraded by enzymes, such as lysozyme, increasing its biocompatibility [10]. However, due to poor mechanical strength and sensitivity to the pH, controlled delivery systems based on pure CMC or chitosan are quite limited [11].

A possible solution to overcome the above shortcomings is to create systems based on chitosan-CMC complexes, which are formed due to the electrostatic interaction of amino groups of chitosan and carboxyl groups of CMC [12]. Such complexes are characterized by improved mechanical properties and stable at different pH values. However, obtaining them in the form of nano- or microparticles is quite complicated. Thus, particles based on chitosan and CMC can be obtained using the layer-by-layer technique [13]. But for practical application, the layer-by-layer technique is quite inconvenient because of the complexity [14; 15].

Microencapsulation is an important technique used for drug delivery systems obtaining [16–19]. However, obtaining microcapsules based on interpolymeric complexes is complicated due to the formation of matrices of large size and uncontrolled dispersion. To solve such problems, emulsification methods are used, when the complex is formed in the organic phase, and the particle size of the emulsion determines the size of the dispersed phase. Thus, Wang et al. obtained water-oil emulsion by sonication of chitosan and anthocyanin solutions with further alginate

introduction on purpose to form alginate-chitosan complex [20]. The obtained microcapsules were separated from the organic phase by centrifugation.

Membrane emulsification is a simple, new, and convenient technology that attracts the attention of researchers [21–23]. It is based on the obtaining of monodisperse emulsions by passing the dispersion phase to the dispersion medium through the pores of the microfiltration membrane of a certain pore size applying pressure [24]. Low shear stress during emulsion production in this method prevents the loss of therapeutic activity of the substance. Moreover, ability of obtaining particles with a narrow size distribution, which increases the system stability, is the main factor determining the usage of membrane emulsification in drug delivery systems design [25].

The present study aimed to obtain microcapsules based on chitosan-carboxymethyl cellulose complex by the membrane emulsification using polyethylene terephthalate track membranes. Papaverine hydrochloride was introduced in microcapsules in the process of their formation. The novelty of this research is determined by obtaining of polyelectrolyte-based microcapsules by alternating the introduction of polymer solutions into the dispersion medium by membrane emulsification, which allows obtaining of monodisperse microemulsions with drops of a certain size.

Experimental

A polyethylene terephthalate (PETF) track membrane with a pore diameter of 0.1 μm (Institute for Nuclear Research) was used to carry out the membrane emulsification process. The membrane is characterized by sufficient strength and rigidity at a thickness of 10 μm and low porosity of 5–10 %.

Microcapsules were obtained based on the polyelectrolyte complex of chitosan-carboxymethyl cellulose. Chitosan with a molecular weight of 400 kDa and a degree of deacetylation of 75 % (Fluka, Japan) was dissolved in 1 % acetic acid. Sodium carboxymethyl cellulose of MW of 450 kDa (Aldrich, USA) was used as the polyanionite. Tween 80, polyoxyethylene sorbitan monooleate, $\text{C}_{64}\text{H}_{124}\text{O}_{26}$ (Tokyo Chemical Industry Co. Ltd., Japan), was used as an emulsion stabilizer. The surfactant was added to the dispersion medium (hexane) at a concentration of 5 %_{wt}. The active substance that was introduced in the microemulsion was papaverine hydrochloride,

(1-[(3,4-dimethoxyphenyl)methyl]-6,7-dimethoxyisoquinoline) (Fig. 1).

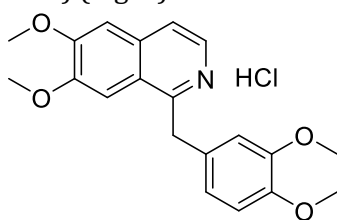


Fig. 1. Chemical structure of papaverine hydrochloride

The membrane cylindrical dead-end cell Amicon 8050, (Milipore, USA) was used for the synthesis of emulsions. The cell was equipped with a stirring bar, which was actuated by a magnetic stirrer IKA® C-MAG HS7.

Complexation in the chitosan-CMC system was studied viscosimetrically, and by IR spectroscopy using an IR spectrometer IRAffinity-1 (Shimadzu, Japan).

Microemulsions obtaining and characterization. Monodisperse emulsions were synthesized using the device shown in Fig. 2. The track membrane soaked in water was sealed in a cell, after which the continuous medium, hexane, containing emulsifier, Tween 80, was poured into the cell. Using a compressed nitrogen cylinder, a pressure of 20 kPa was created above the membrane. Through the feed unit of the dispersion phase 5, solutions of chitosan and CMC were alternately introduced in the specified ratios of the components to avoid the formation of the polyelectrolyte complex in the aqueous phase. The introduction of the components was carried out gradually, at the same speed to avoid air entering the membrane pores. The volume of the dispersed phase was 20 % of the volume of the continuous medium. After the introduction of all components, the emulsion was stirred at a

rotation speed of 240 rpm for 20 minutes. The microcapsules of the chitosan-CMC complex were formed in the organic phase. After that, water in a ratio of 1 : 1 to the organic phase was added to transfer microcapsules to the aqueous phase. The aqueous phase was separated from the organic phase in a separating funnel, and the remains of the organic phase were removed during evaporation. The model drug, papaverine hydrochloride, was administered directly into the chitosan solution. For this purpose, a solution of chitosan with a concentration of 1 %_{wt} was mixed with a solution of papaverine hydrochloride with a concentration of 20 mg/ml in a ratio of 1 : 1.

The size of the microcapsules was determined by dynamic light scattering using Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, UK). Measurement setup and analysis were controlled by the instrument software package. The stability of the obtained microemulsions with papaverine hydrochloride was investigated visually and by the method of dynamic light scattering at temperatures 25 °C and 37 °C.

Investigation of papaverine hydrochloride release kinetics. The kinetics of papaverine hydrochloride release from the chitosan-CMC microcapsules was performed at temperatures of 25 °C and 37 °C. Temperature control was maintained using a water thermostat (DAIHAN Scientific, South Korea). Release was observed at pH values of 2.7, 4.5, and 9.4. The pH of the medium was created by adding a solution of HCl or NaOH. The concentration of papaverine was determined by UV/vis spectrometer UV-1200 (LABInstech, China) at a wavelength of 310 nm.

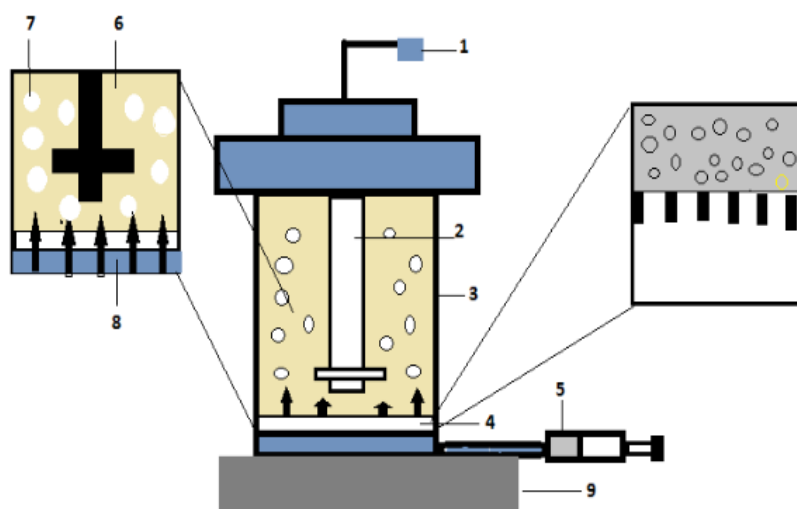


Fig. 2. Scheme of setup for membrane emulsification: 1 - power supply; 2 - stirrer; 3 - working cylinder; 4 - membrane; 5 - unit for the dispersion phase feeding; 6 - continuous medium; 7 - microparticles; 8 - dispersion phase, 9 - magnetic stirrer

Results and discussion

Viscosimetric study of complexation in the chitosan-CMC system. The presence of interaction between the polymer components and structural features of the formed polyelectrolyte complexes were studied by the viscosimetry. As can be seen in Fig. 3, a loose complex is formed at the ratio of

chitosan : CMC = 4 : 1. with increasing the concentration of CMC to 30 % the complex was practically not formed, or a very loose structure was obtained. Dense complexes were formed at ratios of polymers chitosan-CMC 3 : 2 and 1 : 1.

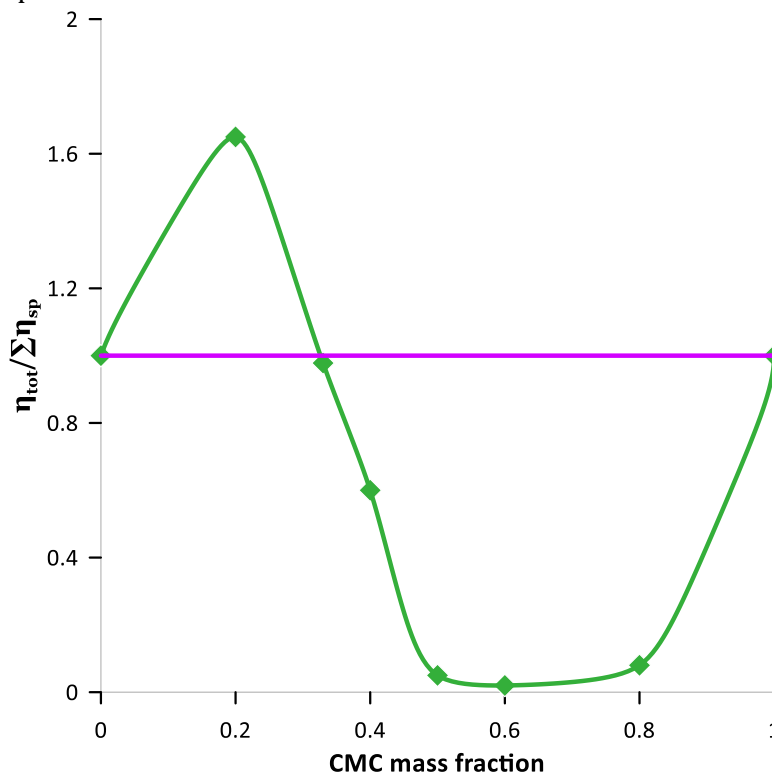


Fig. 3. The influence of the chitosan-CMC ratio on the stability of the complexes

Therefore, complexes with a ratio of chitosan : CMC 1 : 1 and 3 : 2 were selected for the further research.

IR spectroscopic study of complexation in the chitosan-CMC system. of IR spectroscopic studies of polymers and their complexes were performed to confirm the interactions between polyelectrolytes the chitosan-CMC system (Fig. 4).

In the IR spectrum of chitosan, a number of characteristic bands can be observed. The wide absorption band in the region of 3100–3500 cm^{-1} corresponds to the stretch vibrations of the O-H and N-H bonds; bands at 950–1200 cm^{-1} refer to the oscillations of the C-O-C, C-N, and C-C bonds in the polysaccharide structure. The band at 1650 cm^{-1} corresponds to the asymmetric stretch oscillations of C=O bonds of the amide group, and the band $\sim 1600 \text{ cm}^{-1}$ is characteristic for bending vibrations of N-H bonds of amino group. The IR spectrum of CMC is characterized by a wide absorption band at 3100–500 cm^{-1} , which

corresponds to the stretch vibrations of O-H bonds. And the absorption bands at 1590 cm^{-1} and 1400 cm^{-1} correspond to symmetric and asymmetric vibrations of -COO-. As can be seen, in the IR spectra of chitosan-CMC complexes the intense absorption bands at 690 and 750 cm^{-1} appear, which correspond to the fluctuations of the associated -OH groups, while the intensity of the absorption band in the region of 3100–3500 cm^{-1} significantly decreases compared to the original components. This fact can indicate an increase in the fraction of -OH groups involved in intermolecular hydrogen bonds formation. The appearance in the spectra of the complexes of new absorption bands at 1490 and 1450 cm^{-1} , which characterize the bending vibrations of protonated amino groups, as well as the shift of the -COO- and -NH₂ absorption bands from $\sim 1600 \text{ cm}^{-1}$ to $\sim 1550 \text{ cm}^{-1}$, is evidence of electrostatic interaction between polyelectrolytes.

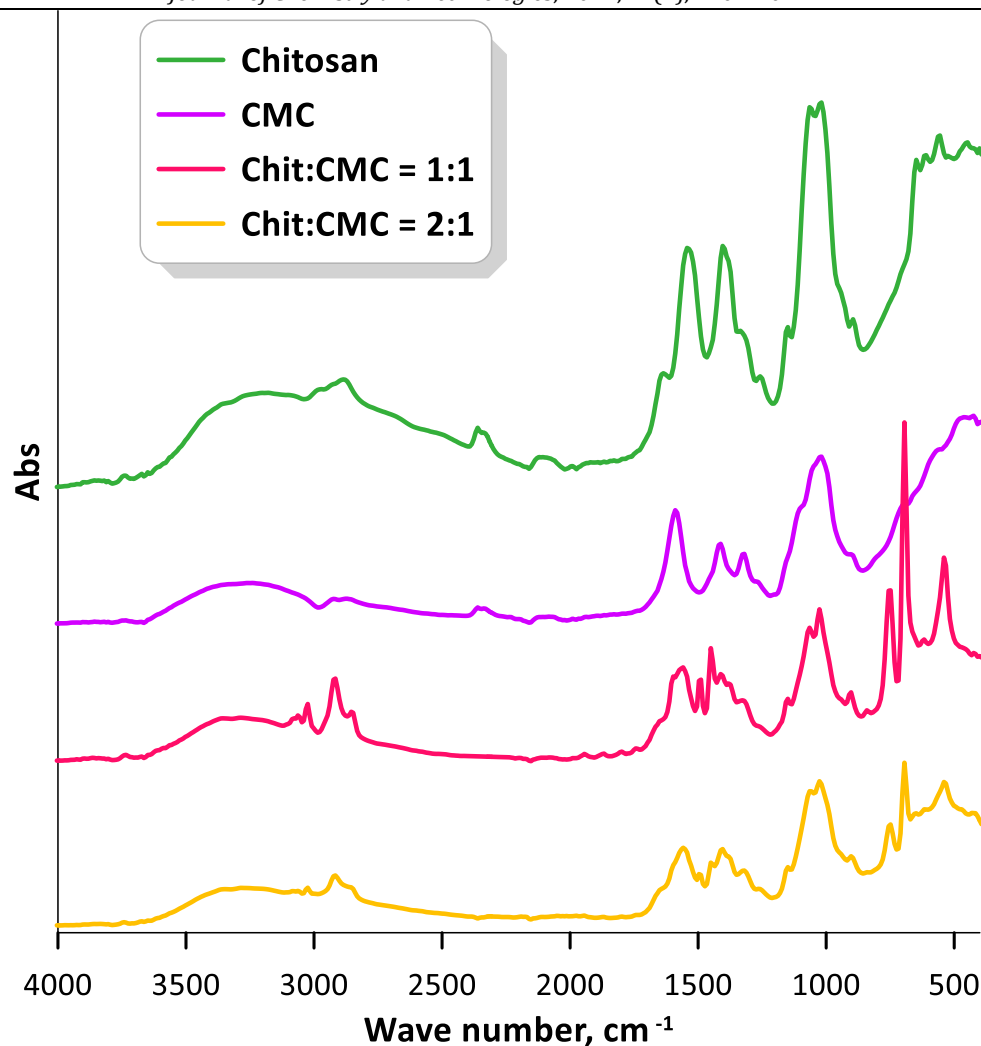


Fig. 4. IR spectra of films based on chitosan, carboxymethyl cellulose, and their complexes

Influence of polymeric composition, temperature, and pH on microcapsules characteristics. The size distribution of the formed microcapsules and their stability were determined using the method of dynamic light scattering, and the influence of temperature and pH was studied.

As can be seen in Fig. 5, the size of the synthesized microparticles varies from 260 nm in a system of Chit:CMC = 3:2 to 200 nm for Chit:CMC = 1:1. Therefore, at the given ratios of components of polymeric system the sizes of the formed microcapsules are close enough and are

defined by the size of membrane pores. Narrower distribution is characterized for polymeric composition Chit:CMC = 1:1, owing to the formation of a complex of denser structure. Moreover, it was found that the resulting emulgels are stable, and the microparticles do not change their size during storage at room temperature for 30 days.

With temperature increasing from 25 °C to 37 °C, the size of microcapsules decreases by almost in 10 times. So, the presented complexes are thermosensitive and with increasing temperature they are compacted (Fig. 6).

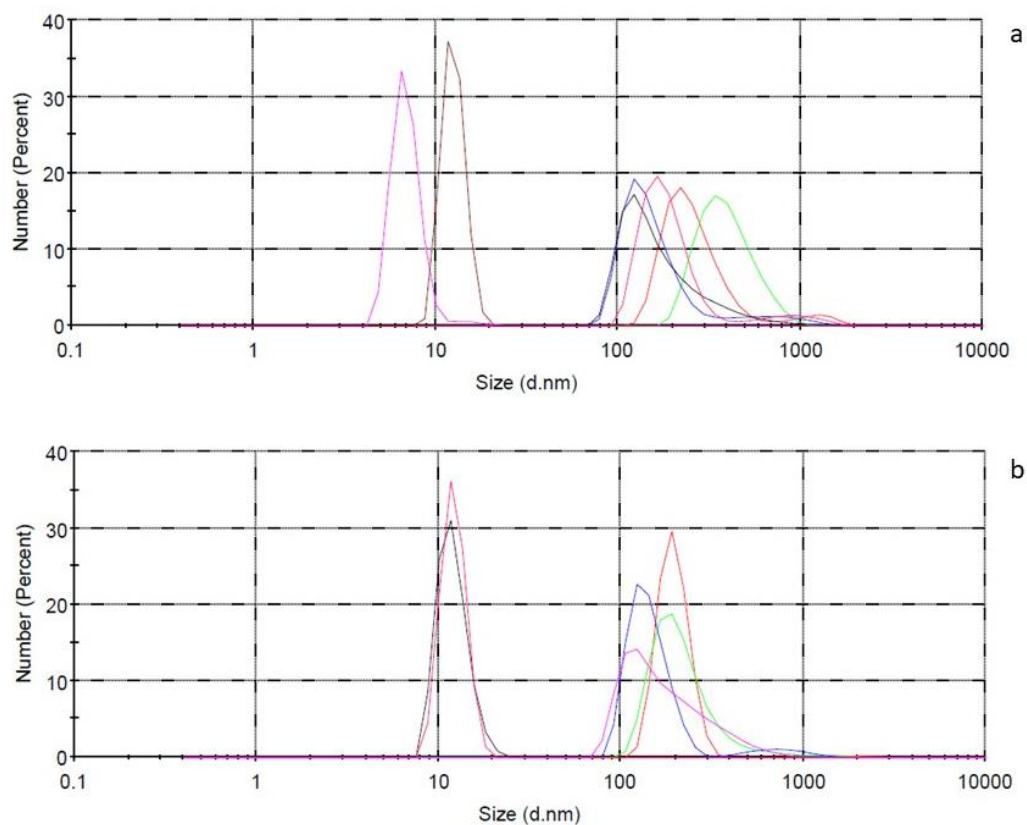


Fig. 5. The size distribution of the microcapsules at a temperature of 25 °C and pH 4.5:
a) chitosan : CMC = 3 : 2; b) chitosan : CMC = 1 : 1

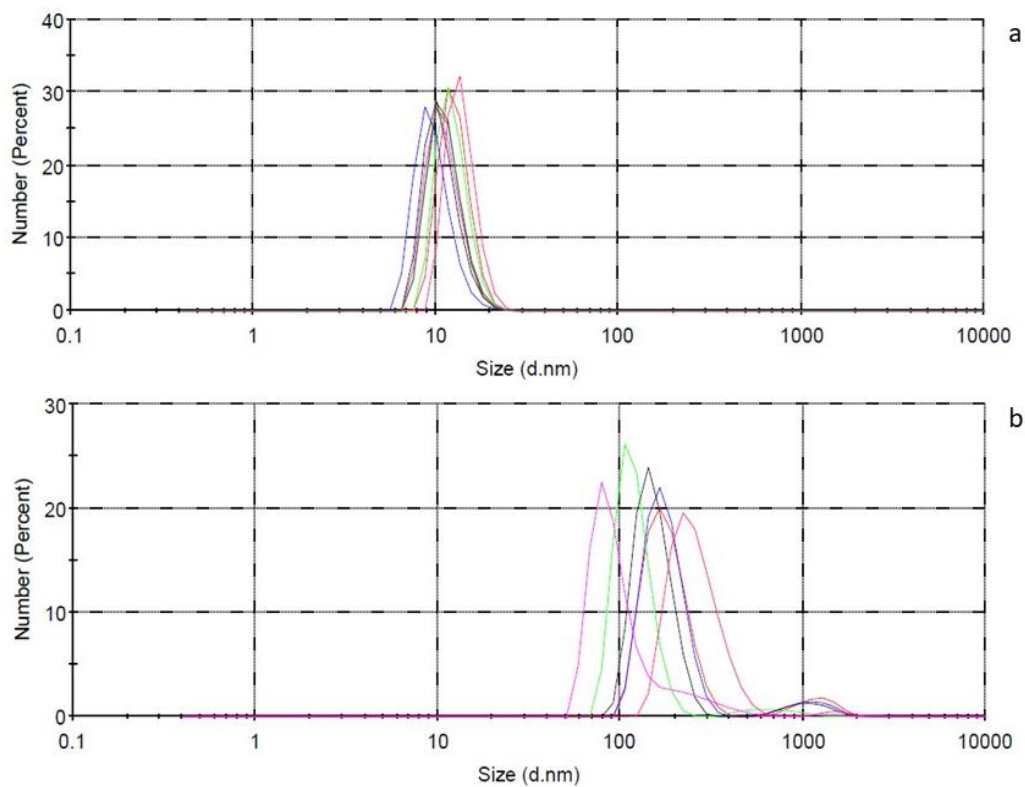


Fig. 6. The size distribution of microcapsules Chit : CMC = 1 : 1
a) and chitosan : CMC = 3 : 2; b) at temperature of 37 °C and pH 4.5

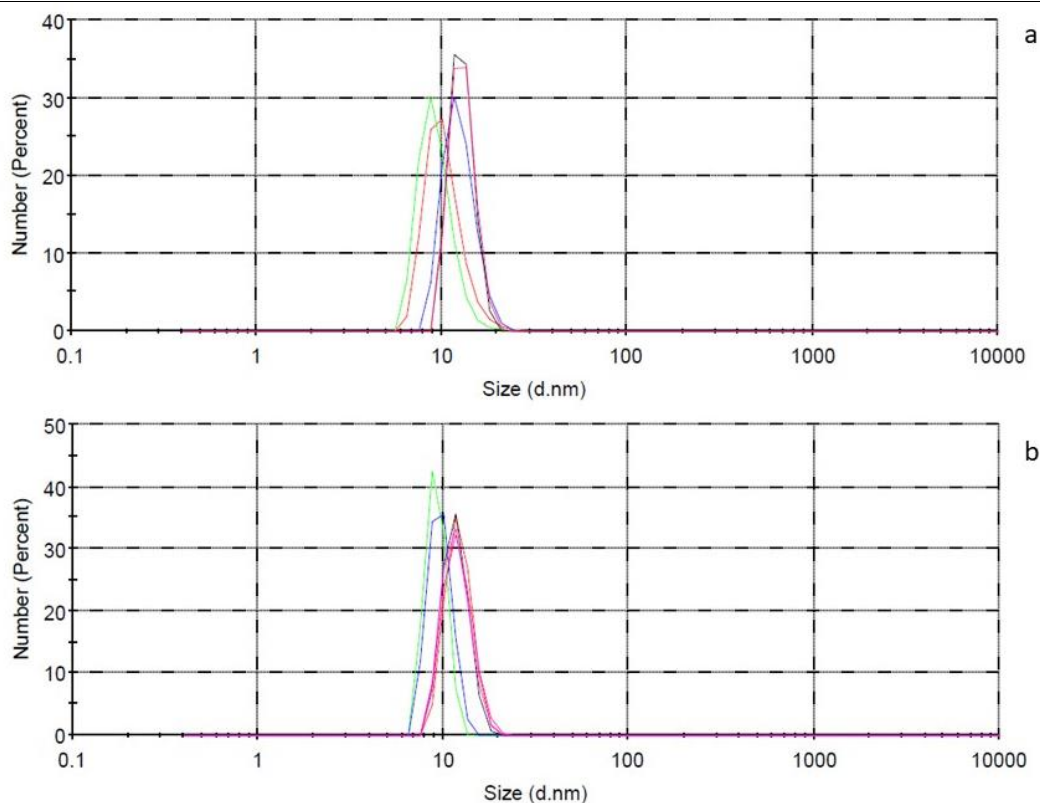


Fig. 7. The size distribution of microcapsules Chit : CMC = 3 : 2 at a temperature of 25 °C at pH 3.0 (a) and 9.0 (b)

Data on the size of microcapsules and ζ -potential of their surface are given in Table 1. As can be seen, microcapsules Chit : CMC = 3 : 2 process more pH sensitivity than Chit : CMC = 1 : 1.

Table 1
Size distribution and ζ -potential of microcapsules depending on the pH value of the medium at 25 °C

Microcapsules	Chit:CMC = 3:2		Chit:CMC = 1:1	
pH	Size, nm	Zeta-potential, mV	Size, nm	Zeta-potential, mV
2.7	13±2	-0.15±2	11±2	3.2±2
4.5	260±10	-0.33±2	200±10	-0.08±2
9.3	12±2	-7.4±2	100±10	-5.2±2

Data on the measurement of the zeta potential of the microcapsule surface show that in an acidic environment zeta-potential becomes more positive owing to the protonation of chitosan amino groups, and it decreases to negative values in alkaline medium owing to dissociation of the carboxyl group of CMC.

Kinetics of papaverine hydrochloride release from microcapsules. Papaverine hydrochloride (PPH) is a drug used in functional disorders of the digestive tract, dilates arteries, increases blood flow. As can be seen from its structural formula (Fig. 1), papaverine has a partial positive charge on the nitrogen atom. During the formation of

microcapsules, papaverine was added to the chitosan solution, as its introduction into the CMC solution caused turbidity of the solution and a change in its viscosity due to chemical interactions of drug with carboxyl groups of CMC.

Fig. 8 shows the kinetics of PPH release from microcapsules Chit-CMC = 1:1 at pH of 4.5 at 25 and 37 °C. As can be seen from the mentioned dependences, at lower temperature the release of the drug has a prolonged effect and reaches the plateau after 100 min. Release occurs according to the first order with a rate constant of 0.004 min⁻¹. At the same time, the degree of release is only 7 %.

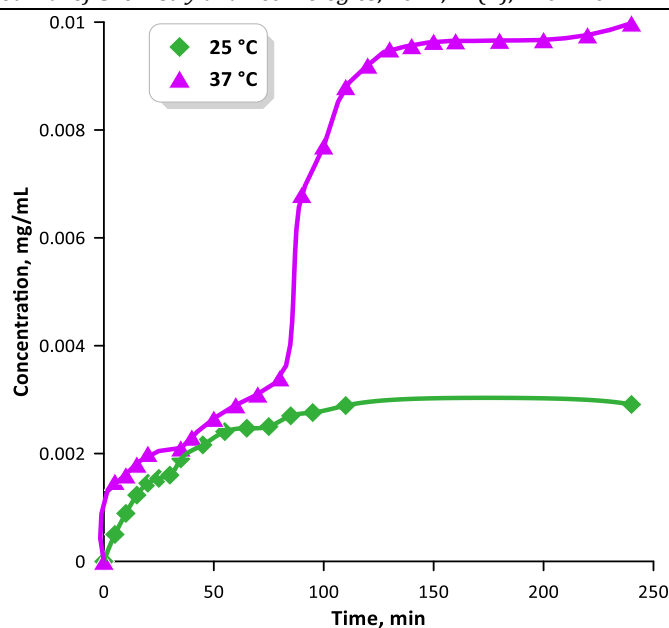


Fig. 8. Influence of temperature on release kinetics of papaverine hydrochloride from microcapsules
Chit : CMC = 1 : 1 at pH 4.5

As the temperature increases, the rate of drug release also increases. At the temperature of 37 °C, a change in the nature of the kinetic curve is observed. The curve has two release zones: in the first interval, which lasts for 100 minutes, the rate constant is 0.008 min^{-1} , followed by an increase in the release rate, while the rate constant increases almost twice and has the value of 0.015 min^{-1} .

Activation energies are calculated for each interval, in the first stage AE is equal to 25 kJ/mol, which indicates the diffusion nature of the drug release. In the second period, the

activation energy is 49 kJ/mol, which indicates that the release occurs in the kinetic region.

During complex formation, electrostatic bonds are formed between the chitosan amino groups and the CMC carboxyl groups. But a significant part of the groups remains free, and chitosan amino groups are protonated in an acidic medium, and dissociation of CMC carboxyl groups in an alkaline medium is occurred. As the dynamic light scattering results show (Table 1), the size of the microcapsules depends on the medium pH. Accordingly, it is very important to investigate the release kinetics at different pH environments.

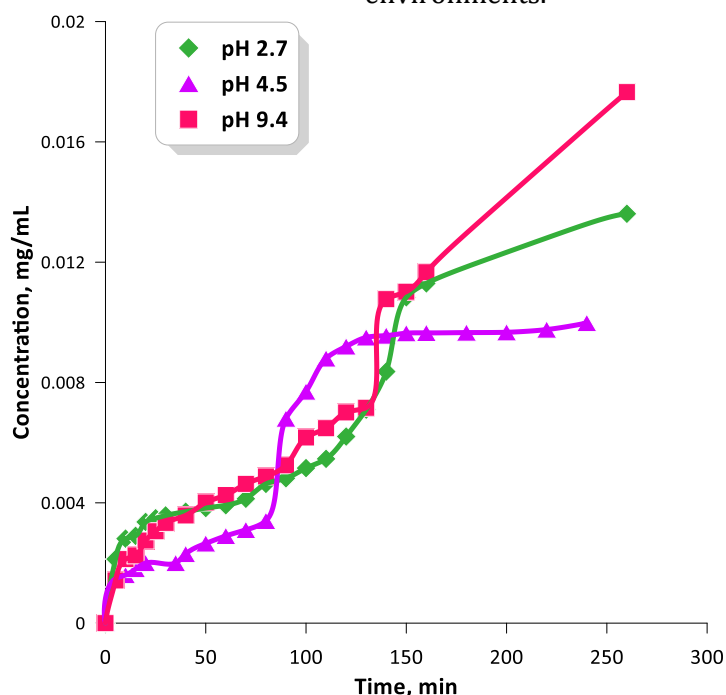


Fig. 9. Influence of pH on release kinetics of papaverine hydrochloride from microcapsules Chit:CMC = 1:1 at temperature of 37 °C

As can be seen in Fig. 9, the nature of the kinetic curves at different pHs is very similar, there are clearly defined two intervals of drug release. At the first stage of release (up to 90 min), the curves are almost indistinguishable. At the second stage, there is difference in the drug release at different pH. The lowest degree of release is observed at pH of 4.5. It is obvious that at this pH the chitosan-CMC complex is in the most compact structural conformation. This is confirmed by measurements of zeta potential, which at pH 4.5 is close to zero. And when the pH changes, both in alkaline and acidic environment, there is an increase in the degree of drug release, which is associated with increased swelling of the complex. Thus, at pH 2.7 the degree of release is 31 %, and at pH 9.4 is 44 %. In general, the kinetics of release from the 1:1 complex are prolonged, but after 240 minutes the degree of drug release does not exceed 50 %, which means that papaverine hydrochloride chemically binds

to the components of the carrier. Therefore, during obtaining of microcapsules based on the chitosan-CMC complex, the drug binds to the complex owing to physical sorption, chemisorption, and electrostatic interactions.

When the ratio of the components of the complex changes, the kinetic curves of PPH release at different pH differ significantly (Fig. 10). At pH 4.3 there are clearly defined two intervals of drug release, but the degree of release increases compared to the previous composition and is 83 %. As for the release curves at pH 2.7 and pH 9.4, no clear separated intervals are observed. With increasing pH, the degree of release decreases. Only 45 % of the drug is released after 240 min. The main part of the carboxyl groups is electrostatically bonded to the amino groups of chitosan with increasing amount of chitosan in the complex, so the change in pH causes compaction of the complex and complicates the release of PPH.

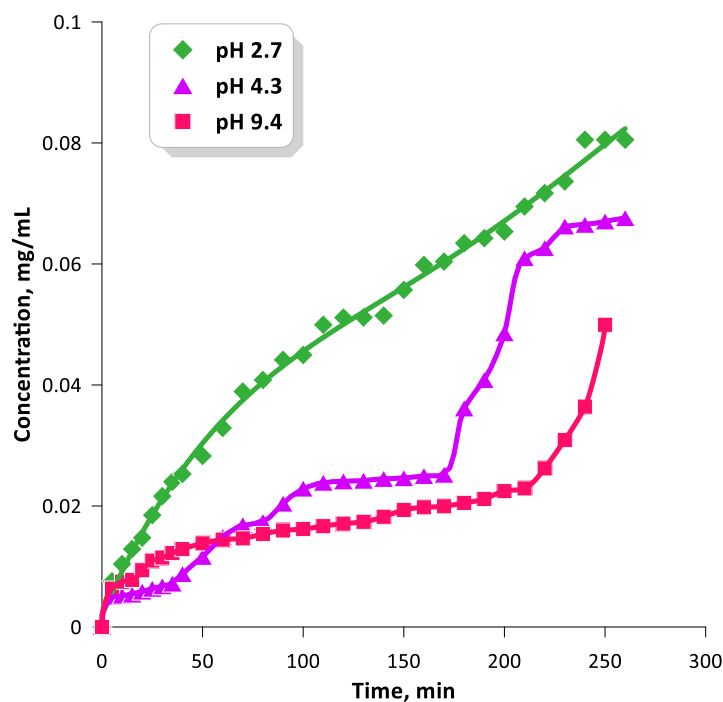


Fig. 10. Influence of pH on release kinetics of papaverine hydrochloride from microcapsules Chit:CMC = 3:2 at temperature of 37 °C

The release is almost linear and occurs according to zero order, 100% degree of release is observed in an acidic environment the release rate is 0.0026 mg/mL·min. In this case, increasing the concentration of chitosan increases the number of protonated amino groups and contributes to the destruction of electrostatic bonds in the complex, which leads to an increase in the rate of release of PPH.

Conclusions

Monodisperse microcapsules based on the polyelectrolyte complex chitosan-carboxymethyl cellulose were obtained by membrane emulsification. Membrane emulsification was performed using track PET membranes with pore size of 0.1 μm . The size distribution and zeta potential of the surface of the obtained microcapsules were determined by the method of dynamic light scattering and the influence of

temperature and pH on the particle size was investigated. The obtained microencapsulates were both thermo- and pH sensitive. The formation of the complex was confirmed by IR spectroscopy and the structure of the complexes at different ratios of polymers was studied by viscosimetry. It is shown that dense complexes were formed at chitosan : CMC ratios of 1 : 1, 2 : 3, and 3:2. It was shown that microcapsules were stable for 30 days at a temperature of 25 °C, and the degree of drug release did not exceed 7 %, which indicates the possibility of their storage under these conditions. The kinetics of papaverine hydrochloride release from microcapsules based on chitosan : CMC complexes at a ratio of 1 : 1 and 3 : 2 was studied. It was shown that at a chitosan : CMC ratio of 1 : 1, pH had little effect on release kinetics. The maximum rate of release was observed in the acidic environment and with increasing concentration of chitosan. In the alkaline environment, the release of the drug was prolonged

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