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HYDROGEL FILMS BASED ON SODIUM ALGINATE MODIFIED WITH OCTANE-1-AMINE: ENHANCED PORE FORMATION AND POTENTIAL APPLICATIONS IN DRUG DELIVERY SYSTEMS

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The use of wound dressings is gaining more and more popularity, especially in the field of tactical and military medicine. Developing wound dressings capable of facilitating wound treatment and reducing healing time is one of the challenges of modern science. So, sodium alginate (Alg) is a good candidate for the development of wound dressings due to its bio- and hemocompatibility and biodegradability. However, Alg has its shortcomings, which can be dispatched by modification.

The purpose of this work was to investigate the effect of Alg modification on the kinetics of ethonium release from crosslinked with Ca^{2+} ions samples. For this purpose, a method of Alg modifying with octane-1-amine was developed without the use of organic solvents and with the use of 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDCl) as an initiator. The optimal parameters of alginate modification process were defined as 60 °C temperature and 24 hours duration. Physicochemical methods confirmed the success of the modification. Films based on the alginate modified with octane-1-amine (AlgM) were obtained using a calcium chloride solution as a crosslinker. The kinetics of swelling was studied and we found that the degree of swelling of the sample based on AlgM after 10 minutes is twice as large (α = 0.71) as for Alg (α = 0.37), which indicates a faster release of drugs.

It has been found that the kinetics of release of ethonium depends not only on the kinetics of swelling but also on the chemical nature of the drug. The ethonium was immobilised in alginate films as a model of bactericidal drug. The kinetics of ethonium release was studied at different pH values corresponding to the pH of healthy skin (5.5), open wounds (7.2) and inflamed wounds (8.2). It was found that the release of ethonium from the sample based on AlgM is more pH-sensitive and prolonged, compared to the sample based on Alg. This effect is explained by the appearance of an additional mechanism of retention of ethonium by AlgM due to hydrophobic-hydrophobic interactions in the films.

The prolonged release properties observed in the drug-loaded samples make them promising candidates for the development of targeted drug delivery systems and wound dressings, which are particularly relevant for the treatment of chronic and burn wounds. Future research will focus on optimizing the crosslinking method and exploring potential applications of modified alginate-based materials in biomedical sciences.

Keywords*: alginate, hydrophobization, hydrogels, ethonium, polysaccharide films, drug delivery system, wound dressing, ionic cross-linking*

INTRODUCTION

Wound dressings have been used in medicine for centuries and previously had the form of natural or synthetic dressings with different abilities to absorb wound exudate. The priority of such dressings was to maintain wounds in a dry and clean form [1, 2]. Many new requirements are put forward to modern dressings, in particular: gas permeability, transparency for the possibility of the wound visual control, the possibility of medical substances delivery, resistance to sterilization, convenience for the patient's usage, easy removal from the skin surface, hypoallergenic properties, protection against contamination by microorganisms in an open wound, promotion of the healing process, and absence of toxic effect [3].

All these new requirements have led to an increase in the number of studies devoted to wound dressings. And today, a variety of hydrogels, especially of natural origin, has great potential in biomedical and pharmaceutical research due to their versatility and capability to form a three-dimensional structure similar to the intracellular matrix by being filled with water [4]. This hydrogel's ability became essential for wound dressings fabrication according to the "wet" theory of wound healing, developed in the 1960s [5]. According to the mentioned theory, maintaining a moist wound environment reduces

healing time, prevents scab formation, and maintain pain-free wound healing by protecting and preventing nerve endings from drying out. Over the last decades the growth of the research and improvement of methods of such hydrogels' preparation and modification was observed [6–10].

Sodium alginate is a common biomaterial for tissue engineering that has an outstanding capability to form hydrogels. It is available, good bio- and hemocompatible, low cytotoxic, low cost, and ease of gelation material, with the capacity to form not only a variety of hydrogels but also microspheres, fibres, and sponges [11, 12]. Modification of alginate is used to enhance its biocompatibility, mechanical properties, and improve the capability to form porous hydrogels capable of gas exchange and providing additional opportunities for drug immobilization for the development of different drug delivery systems [13, 14].

Since partial hydrophobization of hydrophilic molecules induces the self-assembly of amphiphiles into micelles above a certain concentration of amphiphile, known as the critical micelle concentration [15, 16], partial modification of alginate may induce the micelle-formation in aqueous solutions. According to the thermodynamic properties of amphiphiles in aqueous solutions, hydrophobic chains have a strong tendency to avoid direct contact with water. And hydrophilic-hydrophobic interactions can be minimized by self-assembling the molecules into micelles because such structure leads to the isolation of these domains out of hydrophilic solvent [17, 18]. Such partial hydrophobization was successfully used for the development of protein-loaded alginate carriers with prolonged release [15–18].

A large number of wound dressings different in their chemical composition have been developed and implemented; however, they don't meet all nowadays requirements [19–21]. Authors of [22, 23] developed composite wound dressings based on sodium alginate, gelatine, pectin, and the active substance Simvastatin in different ratios. The resulting dressing showed good antibacterial activity, however, at the same time they absorbed too much exudate and showed poor gas permeability. Rojewska *et al.* used hydroxypropyl cellulose and sodium alginate as a basis for obtaining of wound dressings, which demonstrated a controlled release of the drug within 5 days but were gas impermeable [24, 25]. Also, it was investigated dressings based on sodium alginate and polyvinyl alcohol [26]. The obtained samples demonstrated healing properties, the elasticity of the coating and a high degree of swelling, while not possessing the capability for gas permeability and controlled release of drugs [26]. Furthermore, an alginate biopolymer modified with ZnO nanoparticles and poly (ethylene glycol) methacrylate was developed. Such wound dressing was capable of controlled drug release within 3 days, and demonstrated good biocompatibility, but showed low gas permeability coefficients and adhesion to the wound surface [27, 28]. To the best of our knowledge, no patented or published wound dressing capable of controlled drug release with a sufficient gas permeability coefficient, and a moderate degree of swelling has been fabricated so far.

The formation of the porous structure of the wound dressing directly depends on the method of polysaccharide crosslinking [29]. Chemical methods of crosslinking to obtain hydrogels are the best decision for developing a wound dressing characterized by withstands sterilization properties. Among them, methods of crosslinking sodium alginate with calcium ions [12] have such advantages as rapid gelation in aqueous solutions, providing calcium alginate with a highly absorptive and occlusive environment and the possibility of the calcium ions to release from such gels which may promote haemostasis [30, 31].

A method of forming alginate films based on modified alginate by its partial hydrophobization with octane-1-amine was developed during this study. The antibacterial drug ethonium was immobilized in the obtained films. The kinetics of its release was investigated to use these films as modern wound dressings.

As part of this study, the method of crosslinking polysaccharide chains with Ca^{2+} ions (in the form of a $CaCl_2$ solution) was used. To create wound dressings capable of gas exchange and prolonged release of hydrophobic and amphiphilic substances sodium alginate modified with octane-1-amine was employed to discover the feasibility of partial hydrophobization of alginate by amidation in an aqueous solution. The results were compared with samples based on pure alginate. Ethonium was used as a model drug with antibacterial properties [32] for the study of kinetic release.

MATERIALS AND METHODS

Low molecular weight (250 kDa) sodium alginate (Alg) was modified with octane-1-amine with 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDCl) as activator of reaction (all elements supplied by Sigma-Aldrich). As a non-solvent for modified alginate (AlgM), a 96 % ethanol solution was used. A solution of 0.1 M hydrochloric acid was used to control the pH. An ultrafiltration membrane Nadir PM UP020 300783, provided by Microdyn Nadir, Germany, was used for AlgM separation. Anhydrous calcium chloride (Sigma-Aldrich) and 1,2-ethylene-bis- (N,N-dimethyl carbdecyl oxymethyl) ammonium dichloride (ethonium) of pharmacopeial purity (experimental production of the Institute of Organic Chemistry of the National Academy of Sciences of Ukraine) were used to form films.

Alginate modified with octane-1-amine (AlgM) is an alginic acid amide. The modification process was conducted in several stages (Fig. 1). First, a 0.5 % water solution of Alg was prepared. Complete dissolution was ensured by stirring of the mixture using a magnetic stirrer for 30 min at a speed of 600 rpm. Next, sodium alginate was converted into alginic acid by adding 0.1 M hydrochloric acid solution to the resulting mixture until the pH value turned 3.2. After that, an EDCl activator was added to 80 mL of the solution. After 10 min 0.4 mL of octane-1-amine was added to the reaction mixture. The scheme of the reaction between sodium alginate and octane-1-amine was shown in Fig. 2.

The finished reaction mixture in the flask, with an attached air cooler, was thermostated for 24 hours. The modification was performed at different temperatures, in the range of 35–90 °C. After the synthesis was completed, the reaction mixture was brought to room temperature and filtered through a paper filter to remove the activator precipitate. The filtrate was poured into 400 mL of 96 % ethanol to separate modified sodium alginate from the reaction mixture. The reaction product was purified using the ultrafiltration with a PM UP020 membrane. Finally, modified alginate was transferred to a Petri dish to remove solvent residues oven at a temperature of 50 °C for 24 hours.

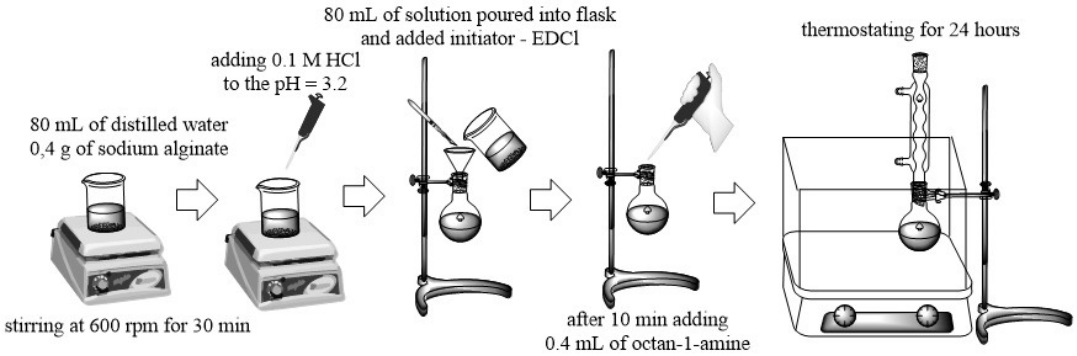

Fig. 1. Scheme of alginate modification process

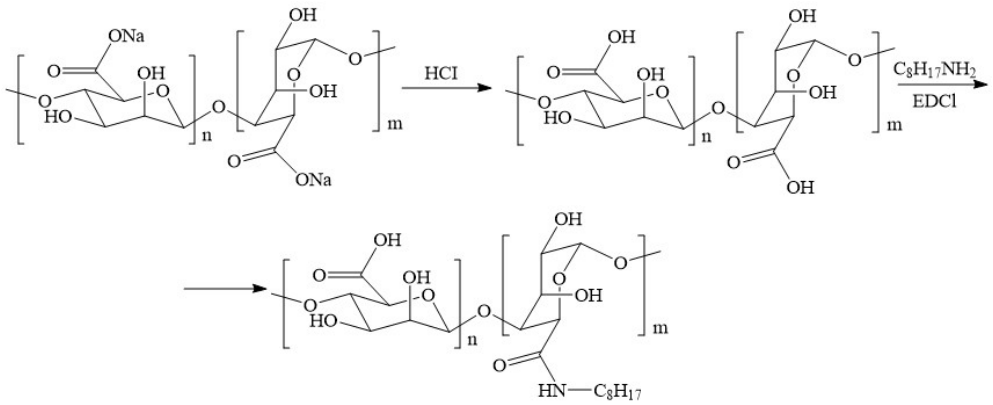

Fig. 2. Scheme of amidation reaction of sodium alginate with octane-1-amine

The IR spectra of Alg and AlgM were obtained using an IR Affinity-1 spectrometer with Fourier transform (Shimadzu, Japan). All spectra were obtained in the range of 4000–400 cm^{-1}.

^1H NMR spectra were recorded using a Bruker Avance III nuclear magnetic resonance spectrometer operating at the frequency of 400 MHz (Bruker, USA).

Thermogravimetric analysis was performed using the Derivatograph Q–1500D system in the temperature range of 20–800 °C in an air atmosphere with the simultaneous removal of gaseous decomposition products. The heating rate was 10 °C/min. The weight of the samples was equal to 50 mg.

Values of enthalpies of decomposition and heat capacities of polysaccharides were evaluated using a differential scanning calorimeter SDT Q600 (TA Instruments, USA). The samples were examined in an argon atmosphere at a heating rate of 10 °C/min.

The micelle-formation ability of modified sodium alginate was determined by the dynamic light scattering (DLS) method for 0.01 % aqueous solution. The study was conducted using a Malvern Zetasizer, Malvern Instruments, UK at 20 °C.

The surface area of the samples, the total pore volume, and the average pore size were determined based on the results of isotherms obtained by the nitrogen adsorption-desorption method at the temperature of 77.4 K, using the automatic gas sorption system Autosorb iQ and AsiQwin (Quantachrome Instruments, USA). Preliminary sample preparation included degassing at 150 °C for 20 h. The weight of the samples was 0.2090±0.0015 g.

To obtain alginate-based films without ethonium, 4 % solutions of polysaccharides were prepared, the process was intensified by stirring at a speed of 700 rpm, 10 mL of the resulting solution was poured into a Petri dish with a diameter of 75 mm. The sample was dried at the temperature of 55 °C for 24 hours. After that, it was placed for 1–1.5 hours in a desiccator to cool to room temperature (20–25 °C). The dried film at room temperature was poured with 20 mL of 0.33 M $CaCl_2$ solution and kept for 30 minutes for ion crosslinking. The resulting film was washed with deionized water and placed in deionized water for 10 min. The washed film was placed in a Petri dish and dried in a thermostat at 55 °C for 24 h.

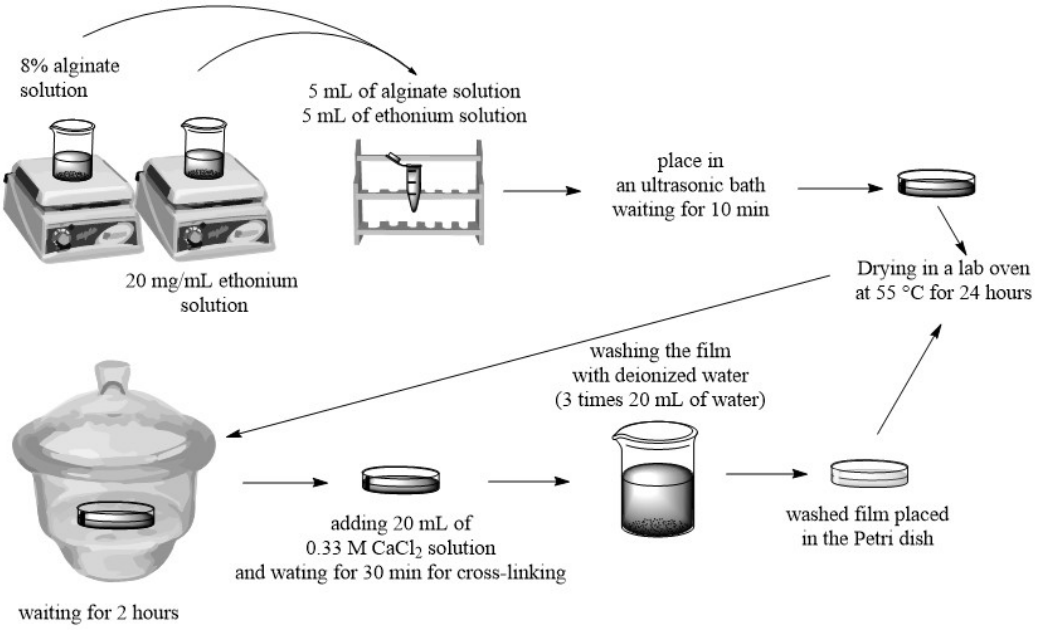

Fig. 3. Scheme of films preparation

Alginate-based films with ethonium were prepared based on 8 % solutions of polysaccharides and 20 mg/mL solution of ethonium. Alginate and ethonium solutions with a total volume of 10 mL were mixed in a 1:1 ratio and placed in an ultrasonic bath for 10 min. The obtained solutions

were poured into separate Petri dishes with a diameter of 75 mm and dried in a thermostat at the temperature of 50 °C for 24 h. Then, they were placed for 2 h in a desiccator at room temperature (20–25 °C). Dried films at room temperature were poured into Petri dishes with 20 mL of 0.33 M calcium chloride solution and left for 30 min to carry out ion crosslinking of the samples. The cross-linked composites were washed 3 times with 20 mL of deionized water and dried in a lab oven for 24 h at 50 °C. The scheme of films preparation is presented in Fig. 3.

Degrees of swelling (α) in deionized water were determined by the weight method and calculated according to the formula:

$$\alpha = \frac{m - m_0}{m_0}$$

where m is the mass of the swollen sample after a certain period of time t, g; m_0 is the initial mass of the sample.

Solutions with pH values of 5.5, 7.2, and 8.2 were used to study the kinetics of ethonium release. 50 mL of a solution with the appropriate pH was poured into a chemical beaker, placed on a magnetic stirrer and half of the sample was added. The system was stirred at a speed of 500 rpm. Every 10 minutes, a sample of the solution was taken from the beaker for spectrophotometric testing at the wavelength of 220 nm, which after the test was returned to the chemical beaker. The study of release kinetics was carried out for 240 min and with constant pH control. After 4 hours, the study was terminated, and the mixture was left undisturbed waiting for 20 h to check the point of complete release of the ethonium from the sample. The sequence of the study of the kinetics from the samples at the temperature of 37 °C was the same as at room temperature, except for constant thermostating of the chemical beaker with the reaction mixture and periodic temperature control using a thermometer.

The release of water-soluble drugs in most cases using porous matrices occurs according to the laws of first-order reactions [33]. Therefore, to determine the rate constants of the release of ethonium from the studied samples, dependences were constructed in the coordinates of the linearized equation of the first-order kinetics of release:

$$\frac{dC}{dt} = -kC$$

where k is the release rate constant; t is release time; C is the concentration.

Based on the results of plotting the graph in linearized coordinates and its approximation line, the ethonium release rate constant is determined as the tangent of the slope angle of the curve, for the value of the approximation coefficient greater than 0.90.

RESULTS AND DISCUSSION

Modification of alginate with octane-1-amine. Amidation of sodium alginate with octane-1-amine in an aqueous solution was carried out at different temperatures. It helped to determine the optimal temperature for carrying out this process (Table 1). The results indicate that the yield of the reaction decreases with an increase over 60 °C in the modification temperature. The use of EDCl as a reaction initiator determines the thermal dependence of the yield over time due to the hydrolysis of EDCl. According to D. Niethera and S. Wiegand [34], the higher the temperature, the faster the hydrolysis of EDCl which leads to the loss of its reactivity. Based on the obtained results, it can be assumed that the optimal ratio of duration and temperature concerning the hydrolysis of EDCl and, accordingly, the optimal conditions for conducting the reaction are maintaining the temperature of 60 °C and a pH of 3.2 with the reaction time of 24 hours.

The ATR-FTIR analyses were used to confirm the success of the modification based on the identification of polysaccharides functional groups. Fig. 4 shows spectra of Alg and AlgM. The IR spectrum of Alg was characterized by two signals of COO^- group divided on symmetric 1589 cm^{-1} and asymmetric 1408 cm^{-1} vibrations of the C=O group, a signal of C–O bond at 1023 cm^{-1}, wide band of –O–H vibrations at 3255 cm^{-1}, and signals of stretching C–H vibrations at 2936 cm^{-1}. The results are consistent with literature data [35].

The spectrum of AlgM was characterized similarly but the signals of C-H bonds were presented by a signal with higher intensity than the corresponding signal for Alg at 2936 cm^{-1}, which indicates the success of the modification. The increase in the intensity of oscillations occurs because of the introduction of a long chain of octane-1-amine, containing C-H bonds. Also, a band of stretching vibration of the -CH_2- group at 2850 cm^{-1} appeared, which confirms the presence of an alkyl chain of octane-1-amine in AlgM. The

presence of these stretching vibrations was confirmed by the bending vibrations of these groups in the regions of 1458 and 1473 cm^{-1}.

The presence of the symmetric signal of C=O bond at 1651 cm^{-1} indicated the formation of an amide I bond (-CONH-) [36]. The weak signal at 1589 cm^{-1} indicates that part of COO^- groups was left unmodified. In addition, the modification by octane-1-amine was evidenced by the appearance of a stretching vibrations band of the –NH– group at 3730 cm^{-1}. The –OH groups signals at the spectrum of AlgM were presented with a broad but less intense absorption band at 3255 cm^{-1}, which indicates a decrease in the amount of bound water, and, therefore, the hydrophobization of the modified sample.

Table 1. Dependence of product yield on temperature

Temperature, °C	Yield, %
35	57 ± 1
45	71 ± 1
60	82 ± 1
70	75 ± 1
90	72 ± 1

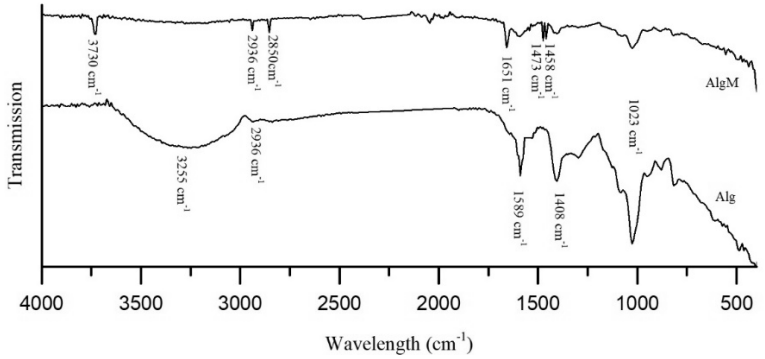

Fig. 4. FTIR spectra of Alg and AlgM

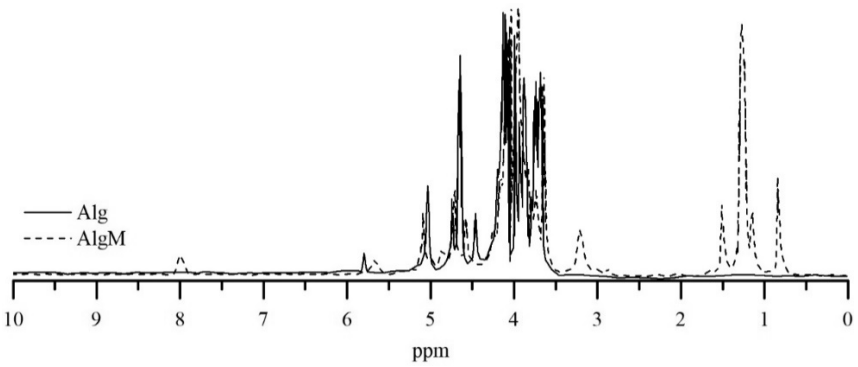

Fig. 5. ^1H NMR spectra of Alg and AlgM

The presence of peaks on the spectrum ^1H NMR of modified alginate (Fig. 5) at 0.8 ppm, 1.2–1.3 ppm, 1.5 ppm, 3.2 ppm, and 8.0 ppm approved modification of alginate. Signals at 3.5 to 5.5 ppm are same for both Alg and AlgM. It confirms the chemical identity of the main carbon chains of pristine and modified polysaccharide [35]. Signals at 0.8 to 1.5 ppm belonged to sp^3-hybridized carbon atoms that appeared in the molecule owing to the introduction of a long carbon chain of octane-1-amine. The presence of several overlapping peaks were located close to each other indicated a different chemical environment and at the same time provided information that there were many such atoms, which confirmed that these signals belong to the

hydrogen atoms of the alkane part of the octane-1-amine chain. The signal at 3.3 ppm belonged to the Carbon atom in the α-position to the amide group formed because of polysaccharide modification. Another signal at 8 ppm was characteristic of the Hydrogen atom in the modified amide group.

The success of the modification was also confirmed by thermogravimetric analysis. Alg lost its mass due to the removal of water from the sample at a temperature range of 16 to 107 °C (Table 2). The TG curve (Fig. 6) allowed to state that two stages of thermal decomposition are typical for Alg: at temperature ranges 107–203 and 204–453 °C. The first stage was caused by the destruction of carboxyl groups and the release of CO_2 at a given temperature [32, 37]. During the second stage, a depolymerization process occured and a carbonized residue was formed [38, 39]. The sample completely decomposed at 453 °C.

Table 2. Thermal destruction of Alg and AlgM

Sample	Stage	ΔT, °C	Loss of mass, %
Alg	Dehydration	16–107	14
	Decarboxylation	107–203	34
	Decomposition	204–453	12
	Dry residue		40
AlgM	Dehydration	16–120	15
	Decarboxylation + octane-1-amine decomposition	121–220	36
	Decomposition·	221–520	15
	Formation and dehydration of $Na_2CO_3 \cdot 10H_2O$	521–620	14
	Dry residue		20

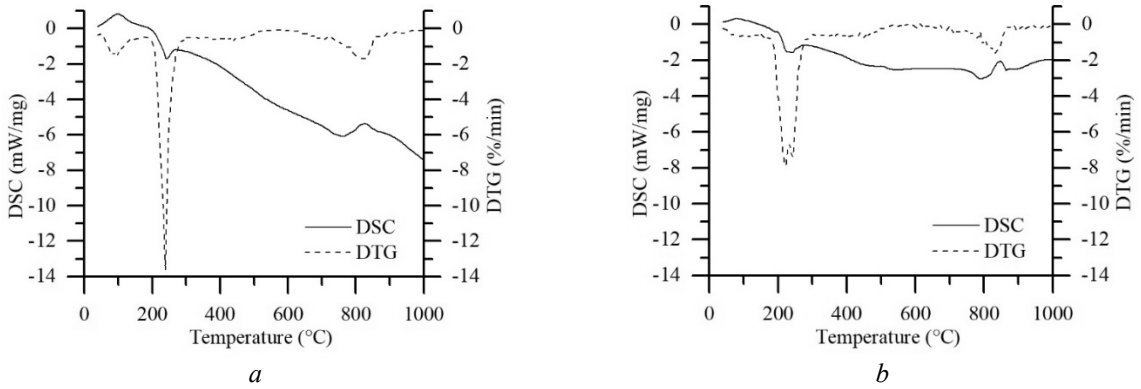

Fig. 6. Thermograms of *a* – Alg, *b* –AlgM

For AlgM, the derivatogram showed a stage of decomposition at a temperature range of 521–620 °C (Table 2), which is not typical for Alg. This corresponds to the stage of formation and decomposition of sodium carbonate. Also, the integral area of peaks corresponding to the stage of thermal destruction of carboxyl groups (peaks at 150–250 °C) in AlgM was larger than the integral area of the corresponding peak in Alg. Such an increase in intensity occured owing to the decomposition of the carbon chain of octane-1-amine in the same temperature range, which confirmed the success of the modification.

The success of the modification was indirectly confirmed by the difference in the mass of the dry residue. The mass of dry residue was twice lower for AlgM, compared with pristine Alg, because the main part of this residue is Na_2CO_3. Thus, there were fewer amount of sodium ions capable of forming carbonate during thermal heating due to the modification of carboxyl groups. Indirectly, this fact was also confirmed that only half of the carboxyl groups of Alg were modified because the content of the dry residue decreased by ~ 50 %.

The values of the enthalpies of the decomposition of polysaccharides and the changes in heat capacities were obtained by differential scanning calorimetry (Table 3). The increase in the enthalpy of decomposition of

AlgM is explained by the presence of the carbon chain of octane-1-amine in the modified sample and the modification of the carboxyl group to an amide group. The decrease in the heat capacity of AlgM also indirectly confirms the presence of new hydrofobic-hydrofobic and hydrofobic-hydrofilic interactions between AlgM moleculaes. The decrease in heat capacity occured owing to the decrease in the number of degrees of freedom in AlgM.

Table 3. Comparison of physicochemical parameters of polysaccharides

Polysaccharide	Alg	AlgM
Decomposition enthalpy, J/g	151±1	223±1
Heat capacity, J/g·K	6.8±0.5	3.3±0.5

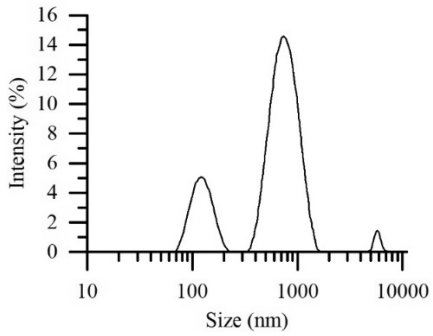

Fig. 7. Particle size distribution by particle number for the 0.01 % aqueous solution of AlgM

Since the modification of alginate leads to its partial hydrophobization, it acquires the capability to self-assemble [15, 16]. The size of the formed polymer micelles was determined by DLS. DLS is a simple method for particles size determination. The distribution of the intensity of scattered light depending on the size of the colloidal particles is presented using a logarithmic scale (Fig. 7).

The result of the dynamic light scattering study confirms that partial hydrophobization of Alg with octane-1-amine leads to micelle formation. AlgM in a 0.01 % aqueous solution forms micelles of various dispersities (polydispersity index 0.813) with average particle sizes 125±20, 650±50 and 6500±250 nm.

The swelling degrees study of samples based on alginate. The results of the films swelling kinetics in deionized water indicated a swelling increase for the modified sample (Fig. 8.). Thus, in the first 10 minutes of the study, the sample based on AlgM swelled twice as much (α = 0.71) as the Alg-based sample (α = 0.37). The stable swollen state of the Ca^{2+}-cross-linked film based on AlgM was reached in 20 min after the sample immersing in water, which showed that the equilibrium condition of swelling was quickly reached. The Alg-based sample swelled more slowly. The equilibrium condition of swelling was reached in 60 min after the start of the study, indicating a three-times slower swelling rate. Such differences in swelling are probably caused by partial hydrophobization of AlgM and the presence of hydrophilic-hydrophobic repulsion between hydrophilic alginate chain and hydrophobic carbon chains of octane-1-amine. Such repulsion contributes to the increase of the free volume for the sample based on AlgM and probably causes the formation of a microporous structure. These results prove that the drug-loaded AlgM-based samples will release physically immobilized drugs from the 3D hydrogel structure faster than the Alg-based samples, opening the prospect of designing controlled AlgM-based drug release systems with defined characteristics.

The nitrogen adsorption-desorption method. According to the results of the samples study using the nitrogen adsorption-desorption method (Fig. 9), it was confirmed that low-porosity samples were formed because of crosslinking alginates with a calcium chloride solution and immobilized ethonium. The results of the study of the surface area, the volume of micropores and the average pore size calculated according to the BET equation are shown in Table 4.

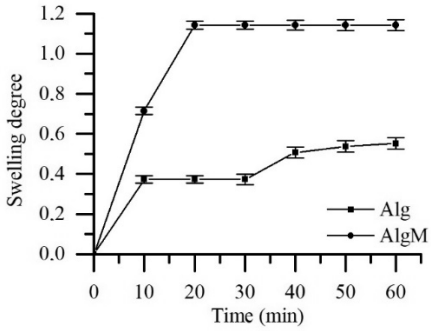

Fig. 8. Kinetics of swelling of samples based on Alg and AlgM

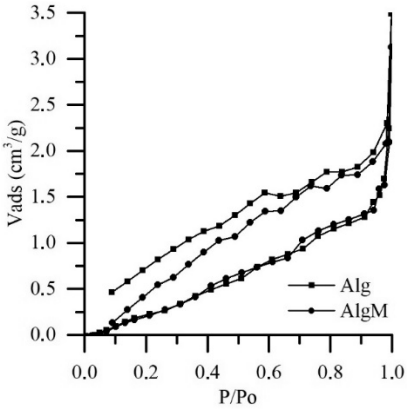

Fig. 9. Adsorption and desorption isotherms

Table 4. Summarized results of sample porosity by nitrogen adsorption-desorption method

Sample	Surface area, m^2/g	Micropores volume, cm^3/g	Average pore size, nm
Alg + ethonium	1.2	$5.7 \cdot 10^{-3}$	4.5±0.5
AlgM + ethonium	2.0	$4.9 \cdot 10^{-3}$	2.8±0.5

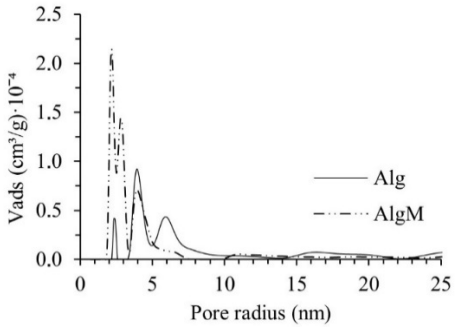

Fig. 10. The size distribution of micropores, obtained by the adsorption-desorption method

For AlgM + ethonium, compared to Alg + ethonium, the surface area of pores increased, while the average volume of micropores and the average radius of pores decreased. Fig. 10 demonstrates the size distribution of micropores. Pores with a size of 2.8±0.5 nm were formed for hydrophobized alginate, and pores with a size of 4.5±0.5 nm were formed for unmodified

hydrophilic alginate with hydrophobic ethonium. A decrease in the volume and pore size in the case of the formation of hydrogels based on modified alginate may indicate the formation of a more compact and orderly hydrogel structure [17, 18].

Determining the kinetics of ethonium release and the release rate constants. The created films can serve as the candidates for the design of wound dressings or transdermal drug delivery systems. Therefore, a study of the kinetics of the release of ethonium as a model bactericidal drug [39] was conducted at three different pH values: the pH of healthy, undamaged skin is 5.5; the pH of inflamed wounds is 7.7; the pH of chronic wounds is 8.2 [40]. The temperature for determining the release kinetics corresponded to the physiological temperature of 37 °C and the room (storage temperature) of 20 °C.

The results of the study of the kinetics of ethonium release are presented in Figs. 11, 12.

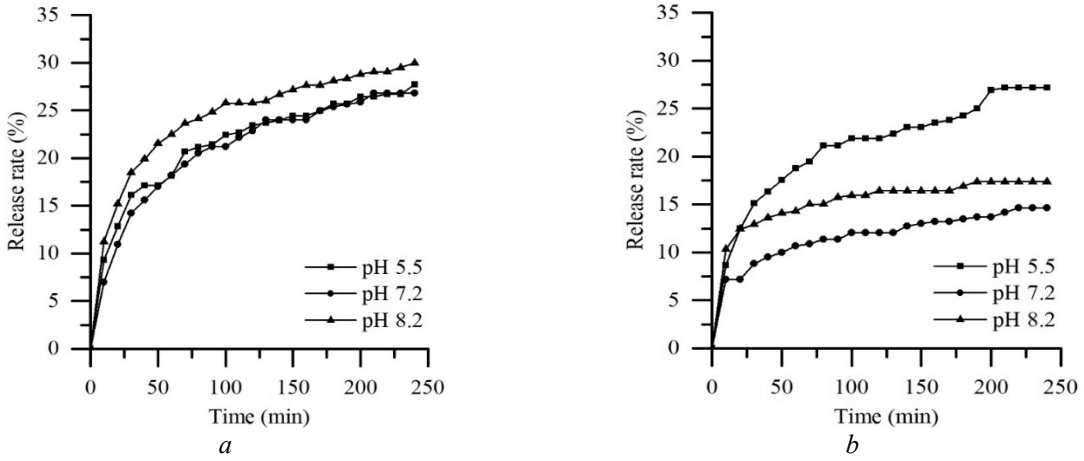

Fig. 11. Kinetics of ethonium release from samples based on *a* – Alg, *b* – AlgM at 20 °C

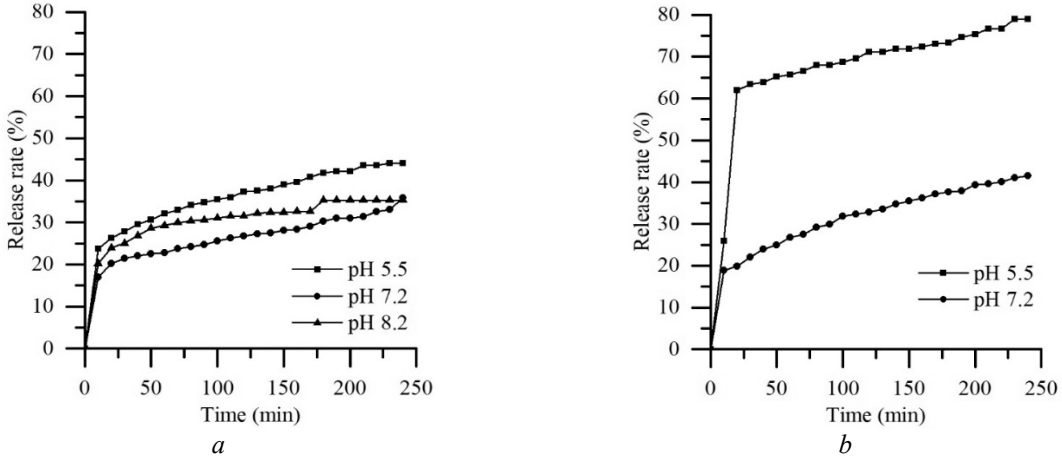

Fig. 12. Kinetics of ethonium release from samples based on *a* – Alg, *b* – AlgM at 37 °C

According to the results of the study, the release of ethonium at 37 °C occurred faster than at 20 °C, but the ability for prolonged release remained. Analysing the obtained data, it can be stated that the rate of ethonium release from the AlgM-based sample was pH-sensitive. This is most likely owing to the mechanism of ethonium immobilization. In the case of ethonium immobilization in AlgM, additional hydrophobic-hydrophobic interactions between ethonium and the carbon chains of octane-1-amine appear, which leads to an increase in the prolongation of the release of the drug. The first way was implemented by the mechanical capture of molecules in the

spatial polymer matrix of the alginate base during the crosslinking process. The second way was implemented by the formation of hydrophobic-hydrophobic interactions between carbon chains of ethonium and chains of octane-1-amine, which explains the increase of release prolongation. The first stage of release from the three-dimensional network of the cross-linked polymer is pH-sensitive since alginate is an anionic polymer and its configuration in solution, depends on the pH of the solution as well as the rate and degree of swelling.

At 20 °C, almost the same rate of drug release was observed for samples based on Alg at pH 5.5 and 7.2 (Table 5). This showed that in a weakly acidic environment, the amount of H^+ ions was insufficient for the protonation of the carboxyl groups of alginate. At pH 8.2, the rate of release increased, which confirmed the destruction of the ionic complex due to the influence of hydroxyl groups. After increasing the temperature to 37 °C for samples based on Alg, the effect of pH on the release rate increased with increasing temperature. The pH dependence of the release constants increased at 37 °C. Thus, at pH 7.2, the lowest release constant is observed for all samples. This fact can be explained by the proximity of this pH value to the dissociation constant of alginate, which complicates the process of hydrogel relaxation and ethonium release.

Table 5. Ethonium release rate constants

Sample	*T*, °C	pH	$k \cdot 10^{-3}$, min^{-1}
Alg + ethonium	20	5.5	2.8
		7.2	2.9
		8.2	3.6
	37	5.5	5.3
		7.2	3.6
		8.2	4.4
AlgM + ethonium	20	5.5	2.7
		7.2	1.2
		8.2	1.7
	37	5.5	9.6
		7.2	4.1

For samples based on modified alginate, the stability of the hydrophobic-hydrophobic complex was observed in the neutral pH range, and the rate of release of immobilized ethonium at the temperature of 20 °C was the lowest one. After shifting the pH of the solution to weakly acidic and weakly alkaline environments, the release rate constantly increased because of the pH impact on the alginate-swelling process.

At 37 °C, the release rate for samples based on AlgM increased compared to the rates at the temperature of 20 °C, as well as for samples based on Alg. The lowest rate of ethonium release was determined for pH 7.2, and the highest one for pH 5.5, which correlated with the results obtained for samples based on Alg.

CONCLUSIONS

Alginate modified with octane-1-amine was obtained using an amidation reaction in an aqueous medium to increase the pore formation capability. It has been found that the highest yield of the product (82±1 %) can be achieved at 60 °C and synthesis duration of 24 hours. The success of the modification was confirmed by the methods of IR spectroscopy, ^{1}H NMR, thermogravimetric method, and differential scanning calorimetry. The ability to form micelles with an average micelle diameter of 470±20 nm was proved by the method of dynamic light scattering and showed that in future investigations alginate modified with octane-1-amine may be used for obtaining micelles and microcapsules. It was shown that modification with octane-1-amine contributed to the hydrophobization of alginate and reduced the average size of the pores formed during ionic cross-linking of hydrogels. This allows us to assume that with further improvement of the crosslinking method, it will be possible to obtain highly porous samples, which will provide the

possibility of forming highly porous hydrogels capable of air exchange. Such hydrogels might be used as a basis for the development of targeted drug delivery systems, in the form of transdermal drug delivery systems. The kinetics of the release of immobilized ethonium confirmed the ability for prolonged and pH-sensitive release for the sample based on modified alginate. These results make these samples interesting for the further development of sustained drug delivery systems based on them and the development of wound dressings that have a therapeutic effect for a long time due to the sustained release of the drug, which is especially relevant for the treatment of chronic and burns wounds.

Гідрогелеві плівки на основі натрій альгінату, модифікованого октан-1-аміном: покращене пороутворення та потенційне застосування в системах доставки ліків

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Використання ранових пов'язок набуває все більшої популярності, особливо в тактичній і військовій медицині. Розробка ранових пов'язок, здатних полегшити лікування ран і скоротити час загоєння, є одним із завдань сучасної науки. Натрій альгінат (Alg) є перспективним кандидатом для розробки ранових покриттів завдяки своїй біо- та гемосумісності та здатності до біодеградації. Однак Alg має свої недоліки, які можна усунути модифікацією.

Метою даної роботи було дослідити вплив модифікації Alg октан-1-аміном на кінетику вивільнення етонію іммобілізованого у зразках, зшитих іонами Ca^{2+}. Для цього розроблено метод модифікації Alg октан-1-аміном без використання органічних розчинників і з використанням 1-етил-3-(3-диметиламінопропіл) карбодііміду гідрохлориду (EDCl) як ініціатора. Оптимальними параметрами для модифікації процесу були встановлені температура 60 °C і тривалість процесу модифікування 24 години. Успіх модифікації підтверджено фізико-хімічними методами. Плівки на основі натрій альгінату, модифікованого октан-1-аміном (AlgM), отримано шляхом зшивання розчином хлориду кальцію. Досліджено кінетику набрякання та встановлено, що ступінь набрякання зразка на основі AlgM через 10 хвилин вдвічі більший (α = 0.71), ніж для Alg (α = 0.37), що свідчить про потенційно більш швидке вивільнення іммобілізованих лікарських речовин із зразків, одержаних на основі AlgM. Однак, кінетика вивільнення залежить не лише від кінетики набрякання, а й від хімічної природи препарату.

Отримано плівки на основі альгінатів та етонію, який використано як модельний бактерицидний препарат. Вивчено кінетику вивільнення етонію за різних значеннь pH, що відповідають pH здорової шкіри (5.5), відкритих ран (7.2) і запалених ран (8.2). Встановлено, що виділення етонію із зразка на основі AlgM є більш pH-чутливим і пролонгованим, порівняно зі зразком на основі Alg. Цей ефект пояснюється появою додаткового механізму утримування етонію AlgM за рахунок гідрофобно-гідрофобної взаємодії в плівках.

Властивості пролонгованого вивільнення, які спостерігаються у зразках, іммобілізованими лікарськими засобами, роблять їх перспективними кандидатами для розробки систем цільової доставки ліків і ранових пов'язок, які особливо актуальні для лікування хронічних та опікових ран. Подальші дослідження будуть зосереджені на оптимізації методу зшивання та встановленні можливого застосування модифікованих матеріалів на основі альгінату в біомедичних цілях.

***Ключові слова**: альгінат, гідрофобізація, гідрогелі, етоній, полісахаридні плівки, система доставки ліків, ранове покриття, йонне зшивання*

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