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PREPARATION AND RESEARCH OF GELATINE HYDROGEL ANTI-BEDSORE MATERIALS PROPERTIES

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The formation of the hydrogel polymer matrix duringthe gelatin cross-linking with dioxirane derivatives of polyoxyethylene glycolswere studied.The optimal conditions for their synthesis were determined. The characteristics of the hydrogel (swelling in different media, mechanical properties at different temperatures) were obtained depending on the type of dioxirane derivative and prepolymer ratio. The possibility of introducing several drugs into hydrogels was established and the release of these drugs was found to be prolonged.

Key words: gelatin; hydrogel; bedsore; gel fraction; swelling degree; chronic wounds.

Introduction

Some specific issues related to the care of wounds of various origins are relevant, modern and important in modern medicine. The number of patients with chronic wounds is constantly growing, due to the population aging, obesity, and accordingly, the increase of type II diabetes and other diseases. Therefore, there is a need for new effective and cost-effective (affordable) dressings [1, 2]. Chronic wounds include wounds that do not heal within 2–3 months (depending on the depth and size of skin damage) [3]. Examples of chronic wounds are bedsores, diabetic wounds, ulcers, deep burns, etc. [4, 5]. All types of wounds require proper and appropriate clinical care to avoid delayed wound healing, which can be caused by microbial infections and other adverse factors.

Dressings play an important role in solving the above-mentioned problems. The assortment of dressing materials is expanding every year, which indicates the relevance of their development. The properties of an ideal wound dressing that make it suitable for creating a suitable environment for the healing process include flexibility, water vapor permeability, no irritation and implantation in the wound, and good mechanical properties [5, 6]. In addition, modern dressings should maintain the water balance of the wound, protect the wound from infections, and at the same time not cause maceration [7]. It is obvious that such universal ideal dressings have not yet been obtained and large groups of researchers around the world are looking for new materials and methods to create them [5, 8, 9].

Among the modern dressings, hydrogel-based medical materials are prominent. Advantages of hydrogels in the field of wound treatment include reduction of wound healing time, high adsorption capacity to exudate and wound fluids, provision of the wound moistening, mechanical protection of wound, the possibility of incorporating drugs into the hydrogel bandage, and convenience for their use by patients [10–12].

Despite a large number of studies on the creation of hydrogel materials for wound care, their universal options do not fully meet the individual objectives. The aim of this study was to create a specialized hydrogel material for the treatment of chronic wounds, including bedsores. We wanted to combine first of all satisfactory mechanical properties, and the possibility of introducing specific drugs into the dressing, that accelerate the healing of this type of wound.

Materials and methods of research

Gelatin (Bloom-180 brand) produced by Aldrich was used without further purification.

Polyethylene glycols (PEG) with molar weights of 200, 400, 600, 1000 g/mol (Aldrich) were used. The correspondence of the molecular weight to the functionality was checked by determining the number of functional groups using the modified method of Ogg and Porter [13].

To evaluate the ability of hydrogels to absorb wound secretions, the liquid simulating the exudate was obtained according to the method described in [14]. This liquid contained 20.0 mmol $CaCl_2$, 400 mmol NaCl, 80 mmol 2-amino-2-(hydroxymethyl)propane-1,3-diol (TRIS) and 2 % of bovine albumin serum in distilled water (pH was maintained at the level of 7.5).

The crosslinking agents – dioxirane derivatives based on polyethylene glycols with different molecular weights (200, 400, 600, and 1200) were synthesized according to the methods described previously [15]. The purity of the substance was determined by the value of the signal integral from 2 protons of the oxirane ring with a shift of 2.65–2.85 ppm in D_2O using ^{1}H NMR spectroscopy on a Bruker Nuclear Magnetic Resonance (NMR) Spectrometer at 200 MHz in an automatic scanning mode. Table 1 shows the characteristics of the synthesized dioxirane derivatives of polyethylene glycols (crosslinking agents).

Table 1

Characteristics of the synthesized dioxirane derivatives of polyethylene glycols with different molecular weights

Sample number	Symbol and molecular weight of PEG	Symbol of crosslinking agent	MW, g/mol	$W_{ep.gr.}$, %	Yield, %
1	PEG-200	DOX-300	300	72–76	80–83
2	PEG-400	DOX-500	500	98–99	85–89
3	PEG-600	DOX-700	700	96–98	84–87
4	PEG-1000	DOX-1100	1100	70–74	81–84

The synthesis of hydrogels based on gelatin and dioxirane derivatives was performed using a 15 % aqueous solution of gelatin, a corresponding crosslinking agent and water, which were mixed at a temperature of 40 °C according to a specified ratio. The polyhexamethylene guanidine was introduced as a bacteriostatic to all samples. Its concentration in the hydrogel was 0.02 %. The resulting mixture of prepolymers was homogenized, sealed in molds and heated at 80 °C for a definite period of time.

The introduction of drugs (chlorhexidine, diclofenac sodium, solcoseril (actovegin)) from aqueous solutions of these drugs by the hydrogel samples was performed according to the method described in [16].

Release of drugs (chlorhexidine, diclofenac sodium, solcoseril (actovegin)) from hydrogels saturated with aqueous solutions of drugs to a given swelling degree and their content (diclofenac sodium in the range from 1 to 2.5 %, chlorhexidine in the range from 0.5 to 1.5 %, actovegin in the range from 1.5 to 4 %) was performed as follows: 1. The hydrogel samples were placed in the eight-fold excess of the appropriate medium for the release of: in water, or in saline, or in exudate. 2. The samples were withdrawn after a certain time and the optical density D was measured. 3. Quantitative evaluation of the drug content in the solution was performed using the appropriate calibration dependence and the percentage of the drug remaining in the hydrogel was calculated. Concentrations of drugs were determined according to the following equations: $C = (D - 0.046)/282.7$ at $\lambda = 275$ nm for diclofenac sodium; $C = (D - 0.02)/342.2$ at $\lambda = 253$ nm for chlorhexidine; $C = (D - 0.002)/7.069$ at $\lambda = 225$ nm for actovegin.

The content of the gel fraction in the samples of hydrogels was determined by the method described in [16].

The swelling degree of hydrogels (the ratio of water/exudate weight in the hydrogel sample to the polymer weight in the hydrogel sample) was

determined by the gravimetric method at 293 K in distilled water/exudate and calculated according to the method described in [16].

The flexibility and appearance of the hydrogels were assessed visually. The thickness of the hydrogel samples was estimated using a digital caliper.

Determination of mechanical properties was performed using a stepwise uniaxial compressive load with a step of 500 μm at a contact area of 0.95 cm^2 with the registration of the force. To determine the mechanical properties, standardized samples of hydrogels with a diameter of 11 mm and a height of ~ 5.5 mm were used. Since the hydrogel samples were not destroyed even by deformation equal to the value of the hydrogel height (5.5 mm) and after the cessation of the applied force gradually returned to its original shape, the maximum value of the force under hydrogel loading was considered as the strength limit.

Research results and their discussion

Gelatin was chosen as the basis of the hydrogel material. The advantages of gelatin as a natural polymer are: good biocompatibility and biodegradability, non-toxicity, non-immunogenicity, and availability. In addition, it has been proven that gelatin accelerates wound healing by promoting the regeneration of skin tissues, it is a component of drugs that stimulate blood clotting [17,18]. However, gelatin forms thermoreversible hydrogels, which lose the mechanical properties of the solid body and turn into a solution at temperatures above 35 °C. To improve the mechanical properties and stability of the obtained hydrogels, it is necessary to crosslink the gelatin macromolecules using active bifunctional agents. However, there is a need to ensure a certain balance between the mechanical and other properties of the hydrogel. Excessive crosslinking of gelatin hydrogels using low molecular weight compounds (formaldehyde, glutaraldehyde) produces inelastic materials with low water and wound secretions absorption. Moreover, it is also necessary to provide purification from unreacted compounds for these materials [19]. Another factor to consider is the high susceptibility of gelatin and gelatin-based hydrogel materials to microbial contamination, which is especially inappropriate in products that involve prolonged exposure to wounds.

These caveats were taken into account in the study. So, as a crosslinking agent for gelatin, we used a bifunctional dioxirane crosslinker based on polyethylene glycols of different molecular weights, which provides high mechanical properties of the hydrogel while maintaining its absorption capacity. This agent has another advantage. Living cells have shown no cytotoxicity when it was used for hydrogels production according to the developed method and this fact allows to omit the purification stage of the finished product.

To eliminate the possibility of microbial contamination polyhexamethylene guanidine was introduced at the stage of hydrogel material synthesis. This bacteriostatic is not released but ensures the absence of bacterial and fungal contamination of the hydrogel during storage. In addition, chlorhexidine was introduced. This compound is prolonged released and provides bactericidal action in the wound areas close to hydrogel material. Chlorhexidine is known to be an antiseptic that is active against vegetative forms of gram-negative and gram-positive bacteria, as well as yeast, dermatophytes and lipophilic viruses, it cleans and disinfects the skin without irritating it.

Actovegin (solcoseril) and diclofenac sodium were used as drugs. Actovegin is a deproteinized hemoderivative from calf blood. The tests in vitro, as well as preclinical and clinical studies, have shown this drug: improves the repair and regeneration of damaged tissues; accelerates collagen synthesis in models in vitro; supports aerobic metabolism and oxidative phosphorylation, and thus promotes the supply of high-energy phosphates to cells, which do not receive adequate nutrition; increases (in vitro) the use of oxygen and glucose supply to tissues and cells; prevents or reduces secondary degeneration and pathological changes in reversibly damaged cellular systems; stimulates cell proliferation and migration in vitro [20]. Diclofenac sodium is a non-steroidal drug with pronounced analgesic/anti-inflammatory properties recommended for the treatment of bedsores [21].

The formation of a hydrogel three-dimensional network occurs during interaction with free amino

groups in gelatin (primarily the free amino group of lysine) as a high-nucleophilic substrate according to the scheme shown in Fig. 1 and this interaction takes place in an aqueous medium at pH 6–6.5 and does not require the introduction of additional catalysts or regulators of the hydrogen index of the environment.

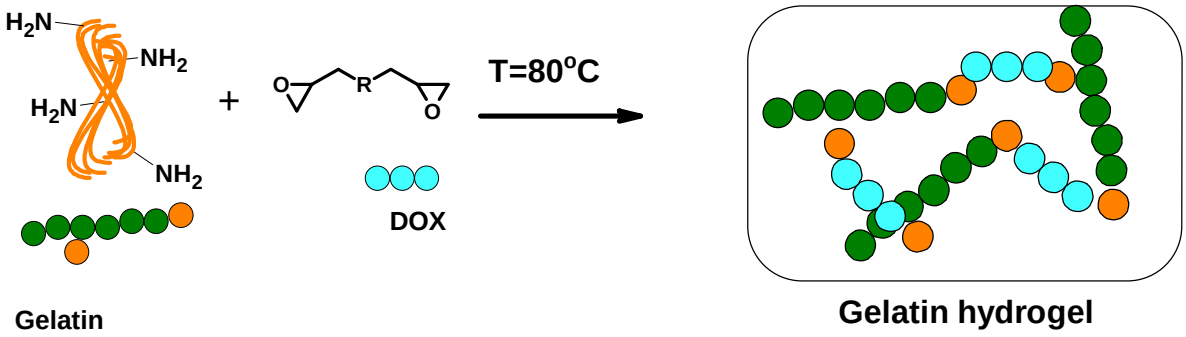

Fig. 1. Scheme of hydrogel three-dimensional network formation (R-polyethylene glycols of molecular weight 200, 400, 600, 1000)

To compare the effectiveness of structuring agents of different molecular weight, model syntheses of gelatin hydrogels were performed at a ratio of structuring agent: gelatin as 1 to 3. It was found that when using structuring agents with DOX-300, DOX-1100 hydrogels with low mechanical properties are formed. This is confirmed by the figures in Fig. 2 results of studies of the content of the gel fraction and data on the swelling of hydrogel materials.

When analyzing the results of studying the hydrogel properties, we observe that the content of gel fraction in the synthesized hydrogels for the samples with DOX-300 and DOX-1100 is low (25–30 % and 5–10 %, respectively). Swelling of the obtained hydrogels shows that these samples are destroyed after 9 hours of swelling in water. It means that DOX-300 and DOX-1100 should not be used for the synthesis of hydrogels at the specified ratio (1 : 3). Therefore, dioxirane derivative DOX-500 (obtained using polyethylene glycol PEG-400) was chosen for further research as a crosslinking agent; it provides a gel fraction content of 81 % in these conditions. Table 2 shows the synthesis parameters and properties of the obtained hydrogels at different ratios DOX-500 : gelatin.

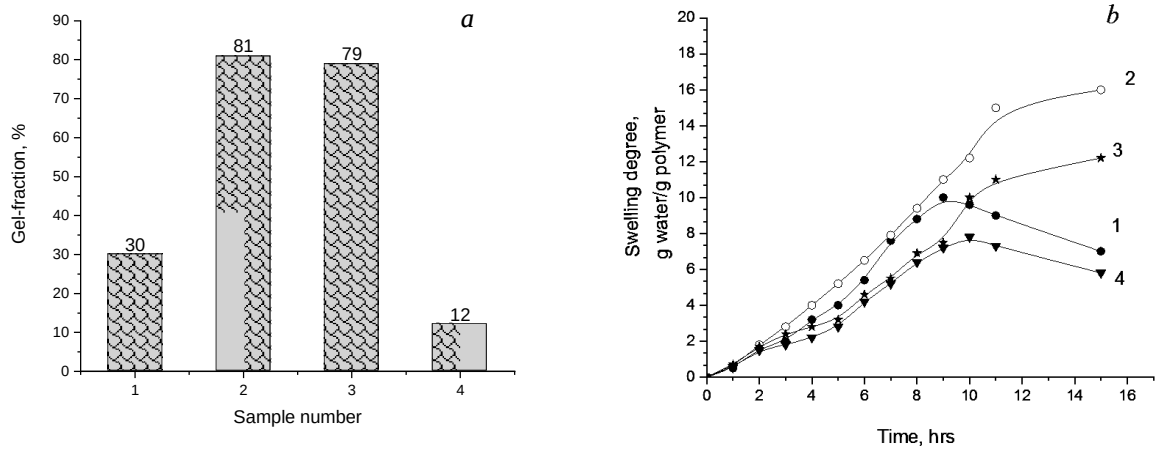

Fig. 2. Gel fraction (a) and swelling degree (b) of hydrogel samples in water synthesized using crosslinking agents with different molecular weights: 1 – DOX-300; 2 – DOX-500; 3 – DOX-700; 4 – DOX-1100*

** Swelling was studied at a temperature of 37 °C, resulting from the conditions of these materials further use*

Table 2

Synthesis parameters and properties of obtained gelatin hydrogels

Sample No.	Reagents ratio		DOX-500 concentration, %	Gelatin concentration, %	Gel-fraction content, %	Maximal swelling, g water/g polymer	Maximal loading*, kPa	
	DOX-500	Gelatin					20 °C	37 °C
1	1	1	9.0	9.0	55.9	59.5	27	1.6
2	1	2	6.0	12.0	64.9	57.2	30	3.2
3	1	3	4.5	13.5	78.2	38.5	42	7.7
4	1	4	3.6	14.4	81.5	35.1	45	8.5
5	1	5	3.0	15.0	79.7	28.0	60	10.9
6	0	1	0.0	18.0	0.0	0.0	11	–

** Hydrogel samples with a thickness of 5.5 mm were not destroyed at maximum deformation (5.5 mm)*

The most promising for further use are samples 3, 4 and 5, for which the high values of gel fraction content, sufficient (relative to the level of bedsores exudation) degree of swelling and high mechanical properties are observed.

With increasing gelatin concentration for hydrogel samples (at a constant concentration of other components) the increase in the gel fraction content to 82 % (samples 3,4,5 Table 2) is observed, which can be explained by the optimal number of bonds between the crosslinking agent and gelatin.

The maximum swelling of the hydrogel samples in water is in the range of 25–60 grams of water per gram of gelling polymer and this value is reached within 5 days while maintaining satisfactory mechanical properties. However, in real applications, the hydrogel material will absorb not water but exudate. The amount of absorbed model exudate for the synthesized samples is in the range from 12 to 15 grams per gram of gelling polymers for 24 hours and reaches a value of 20–23 grams for 72 hours. This value is satisfactory when used for wounds with a medium level of exudation according to the classification given in [22].

It should be noted that studies on the mechanical properties of hydrogels were performed at a temperature of 20 °C and 37 °C. At the temperature of 20 °C the hydrogel samples were not destroyed under high loads and after removal of the load returned to their original form. At a temperature of 37 °C, the destruction also did not occur, but the hydrogels became more plastic and underwent deformation under the action of applied force without losing their integrity.

The defined properties allow to recommend these materials as the basis of anti-bedsore dressings. Therefore, using a sample of hydrogel (No. 4 Table 2) we conducted studies on the introduction/release of drugs, namely chlorhexidine, actovegin, diclofenac sodium, by hydrogel materials.

Table 3

Administration/release factors of drugs*

Sample number	Drug	Concentration in hydrogel plate, %	Administration factor	Release factor (after 24 hrs)	
				exudate	saline
1	Chlorhexidine	1.0	0.92	0.20	0.22
2	Actovegin	1.0	0.87	0.23	0.38
3	Diclofenac sodium	2.0	0.83	0.30	0.45

** The administration factor was calculated as the ratio of the drug amount introduced into the hydrogel plate to the drug amount introduced into the solution for administration.*

The release factor was calculated as the ratio of the drug amount released in different media (saline, model exudate) to the drug amount introduced in the hydrogel plate.

Table 3 shows the results of the study on the administration of drugs in the gelatin hydrogel plate. The administration was performed by absorbing the aqueous solutions of drugs in the formed hydrogel. The drug concentration in the finished hydrogel was created according to the accepted recommendations for their amount in the products for external use. To evaluate the effectiveness of drug administration/release the corresponding factors were used (Table 3). The value of the administration factor for all drugs is in the range of 0.83–0.92.

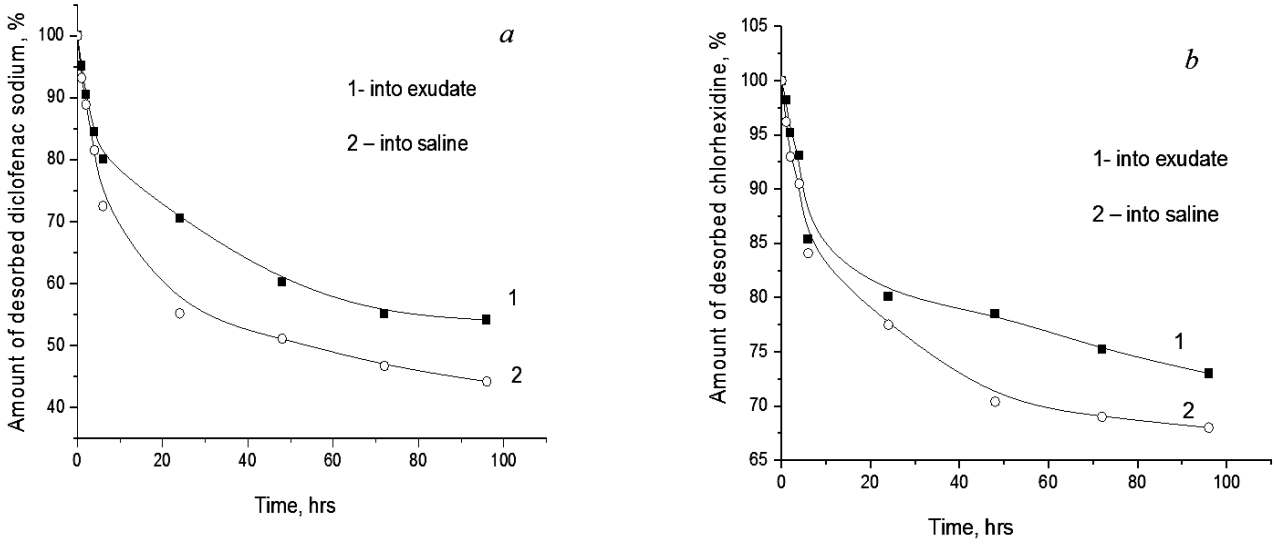

Fig. 3. Drug release curves: a – diclofenac sodium; b – chlorhexidine

Fig. 3 shows, for example, the release curves of diclofenac sodium and chlorhexidine from hydrogel plates into exudate and saline. The release factors determined after 24 hours of experiment are represented in Table 3. It is obvious that the release of drugs from the hydrogel plate occurs at different rates and we can argue about the prolongation of this process in time.

Conclusions

The conditions for gelatin hydrogel materials synthesis during their crosslinking with dioxirane derivatives based on polyethylene glycols of different molecular weights have been studied. The optimal conditions for their production have been determined, which allow to obtain hydrogels with high mechanical properties and satisfactory swelling degree in the exudate. The processes of administration and release of a number of drugs have been examined. The obtained results suggest that the obtained material can be the basis of a specialized hydrogel dressings for the treatment of chronic wounds, in particular, bedsores.

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ОТРИМАННЯ ТА ДОСЛІДЖЕННЯ ВЛАСТИВОСТЕЙ ЖЕЛАТИНОВИХ ГІДРОГЕЛЕВИХ ПРОТИПРОЛЕЖНЕВИХ МАТЕРІАЛІВ

Наведено результати досліджень утворення полімерної матриці гідрогелю у результаті структурування желатину діоксирановими похідними поліоксіетиленгліколів, визначено оптимальні умови їх синтезу та визначено характеристики гідрогелю (ступінь набрякання у різних середовищах, механічні властивості за різних температур) залежно від типу діоксиранового похідного та співвідношення між форполімерами. Встановлено можливість введення низки лікарських препаратів до складу гідрогелів та показано, що вивільнення цих препаратів відбувається пролонговано.

Ключові слова: желатин; гідрогель; пролежні; гель-фракція; ступінь набрякання; хронічні рани.