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Development of a chitosan polymer based composite haemostatic agent and a method for evaluation of performance

Oleksandr Chornyi*

PhD in Technical Sciences
Joint Stock Company "Farmak"
04080, 63 Kyrylivska Str., Kyiv, Ukraine
https://orcid.org/0000-0002-6949-9247

Volodymyr Fedorenko

Postgraduate Student
Kyiv National University of Technologies and Design
01011, 2 Mala Shyianovska Str., Kyiv, Ukraine
Joint Stock Company "Farmak"
04080, 63 Kyrylivska Str., Kyiv, Ukraine
https://orcid.org/0009-0003-5113-6848

Volodymyr Yaremenko

Postgraduate Student
Kyiv National University of Technologies and Design
01011, 2 Mala Shyianovska Str., Kyiv, Ukraine
Joint Stock Company "Farmak"
04080, 63 Kyrylivska Str., Kyiv, Ukraine
https://orcid.org/0009-0001-1757-5500

Olena Ishchenko

Doctor of Technical Sciences, Professor
Kyiv National University of Technologies and Design
01011, 2 Mala Shyianovska Str., Kyiv, Ukraine
https://orcid.org/0000-0002-9510-6005

Svitlana Gureyeva

Doctor of Pharmaceutical Sciences, Professor
Kyiv National University of Technologies and Design
01011, 2 Mala Shyianovska Str., Kyiv, Ukraine
Joint Stock Company "Farmak"
04080, 63 Kyrylivska Str., Kyiv, Ukraine
https://orcid.org/0000-0002-5949-4493

Abstract. The aim of this study was to develop an in vitro method for evaluating the hemostatic efficacy of composites and to investigate the factors influencing the hemostatic efficacy of polymer composite materials based on chitosan using the developed method. Existing contact haemostatic composite materials based on zeolites and chitosan polymer were reviewed. For research and quality control work with haemostatics, it was required to test their haemostatic performance. Conducting in vivo studies required intervention in a living organism and is undesirable for ethical reasons. A generally accepted system for assessing the efficacy of hemostatic agents in vitro does not exist. A methodology for analyses to

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*Corresponding author

determine the haemostatic properties of composites has been developed. The main quality attributes of haemostatics, such as gel formation time and permeability, were determined. The composition and method for producing the finished haemostatic composite material were developed. The composite contains a salt of chitosan and lactic acid, polyethylene glycol 4,000. The effect of carboxylic acids and surfactants on the properties of the composite was studied. Chitosan with a molecular weight of at least 80,000 Dalton and a degree of deacetylation of 85-95% was selected. The "active" and "inactive" forms of chitosan, which affect the haemostatic properties of the composite, were studied. The process for obtaining the "active" form of chitosan from the "inactive" form by redeposition, which consists in the interaction of chitosan with carboxylic acid to obtain a soluble salt and subsequent alkali precipitation, was developed. The influence of the following factors on the properties of the resulting product was studied: solvent in the salt synthesis reaction; molar ratio of lactic acid and chitosan; particle size of the haemostatic powder. The resulting haemostatic is effective in binding water and blood into a fixed gel-like clot. The product has sufficient permeability and haemostatic performance. The clot formed by binding liquids is stable for 24 hours

Keywords: lactic acid; polyethylene glycol; composite polymeric materials; gel formation; permeability; *in vitro*

Introduction

The issue of first aid is extremely important. According to statistics, blood loss is the main cause of death of the wounded soldiers in combat and emergency situations. For example, R.K. Latif *et al.* (2023) state that a large number of casualties die from bleeding, often before they can be treated. N.P. Charlton *et al.* (2021) describe that tactical medicine protocols focus on the effective use of haemostatic agents (haemostatics) such as powders, bandages, and applicators. The most common products include gelatine sponge; collagen plate; oxygenated regenerated cellulose; combined preparation with fibrin glue. However, the Tactical combat casualty care guidelines (2024) state that these topical haemostatic agents cannot stop bleeding from large arteries and veins on their own. They must be used in combination with other methods of stopping bleeding.

Celox Medical (2025) report a dramatic change in mortality statistics with the use of contact haemostatics for massive bleeding, especially in areas where tourniquets are not available (buttocks, armpits, neck). P. Yu & W. Zhong (2021) state that two main groups of contact haemostatics have a great advantage in stopping bleeding – chitosan-based composite materials (e.g. Celox®, ChitoGauze®) and kaolin-based materials (QuikClot®), which can stop almost any bleeding in a matter of seconds. The advantages of this type of haemostat include hypoallergenicity due to the fact that a physical reaction-absorption occurs in the contact zone and induce a local sharp rise in temperature.

Y. Zheng *et al*. (2023) state in their work that typical production technologies for inorganic hemostatics include 3D printing, freeze-drying, electrospinning, and vacuum filtration, which provide materials in the form of three-dimensional scaffolds, porous masses, soft membranes, or hydrogels. Chitosan serves as the foundational material for an alternative class of contact haemostatics. The advantages of chitosan-based composites include their properties: their performance does not decrease at low temperatures; the performance does not depend on impaired blood clotting factors; the presence of the composite in the wound provides the effect of gluing damaged soft tissues and prevents the resumption of bleeding during transport. D. Tripathi *et al.* (2021) report that chitosan-modified gauze achieved a hemostatic effect nearly threefold faster than standard gauze, and facilitated more rapid wound closure.

D. Alemu *et al*. (2023) emphasised the widespread use of chitosan in various industries, including medicine and pharmacy. D. Yan *et al*. (2021) and A. Guarnieri *et al*. (2022) reported that the unique features of chitosan-based composites include antibacterial activity against gram-positive and gram-negative bacteria, which reduces the risk of wound infection and ensures faster healing without the massive use of antibiotics. The haemostatic mechanism of action of chitosan-based composites is that the latter, having a positive charge, binds negatively charged red blood cells and platelets in the blood, thus, forming a dense blood clot. J. Bar *et al*. (2017) reported that chitosan-containing haemostatics work effectively independently of coagulation factors without affecting the natural coagulation cascade.

Chitosan is completely biodegradable under the influence of microbial enzymes such as chitinases and chitobiases. Chitosan is characterised by mucoadhesive properties, i.e. the ability to adhere to mucous membranes, the ability to absorb biological fluids and help regenerate tissues (Liu, 2022). Modified forms of chitosan in the form of composite materials have been used in the form of various haemostatic products (Das *et al.,* 2024; Zeng *et al*., 2025). Chitosan does not exhibit a pronounced gel formation property when in contact with liquids (except for acid solutions) and has weak haemostatic properties (Gheorghiță *et al.,* 2023). Chitosan has free amino groups and is able to form salts with acids that have stronger haemostatic properties, such as salts of lactic, succinic, acetic acids and some other organic acids with a short carbon chain (Pieklarz *et al.,* 2021). Bicarboxylic and tricarboxylic acids can form intermolecular ionic cross-links between the amino groups of adjacent chitosan chains, which allows for a three-dimensional "mesh" of chitosan molecules.

Chitosan derivatives are a promising class of haemostatic agents. Thus, the study and optimisation of methods for the synthesis of haemostatic chitosan-based composite materials is an urgent task that will improve user properties and reduce the level of blood loss in wounds. Research

work with haemostatics requires that they be tested for haemostatic performance (Rezabeigi *et al*., 2022; Zhang *et al*., 2022). At present, there is no generally accepted system for evaluating the efficacy of haemostatics *in vitro*, and *in vivo* studies require intervention in a living organism, which is not desirable for ethical reasons (Kiani *et al.,* 2022). Existing *in vitro* methods for evaluating the efficacy of hemostatic materials are complex to implement and resource-intensive for quality control, being more suitable for the development stage of a hemostatic agent (Nativel *et al.,* 2024). Therefore, the search for simple but effective *in vitro* methods remains relevant for testing haemostatics.

The aim of the work was to develop an *in vitro* method for the evaluation of the haemostatic efficacy of chitosan-containing composites and to study the factors influencing the haemostatic performance of polymeric composite materials based on chitosan using *in vitro* methods.

Materials and Methods

Chitosan is an aminosaccharide, a derivative of a linear polysaccharide, macromolecules consist of randomly linked β-(1-4) D-glucosamine units and N-acetyl-D-glucosamine (Aranaz *et al*., 2021). Chitosan is a solid substance in the form of scales with a particle size of no more than 10 mm or powder of various degrees of grinding. White to off-white, often with yellow or grey tint. Odourless. Insoluble in water, alkalis, solvents. It is soluble in dilute solutions of some organic and inorganic acids. Chitosan is a natural polymer with a molecular weight of several thousand to hundreds of thousands of Dalton (Fig. 1) (PubChem. Chitosan, n.d.). To perform the study, 6 samples of chitosan produced by Zhengzhou Delong Chemical Co., Ltd. (China) with different viscosities and degrees of deacetylation were used (Table 1). Other starting components used to produce haemostatic composites are lactic acid, sodium hydroxide, ethyl alcohol, polyethylene glycol. Lactic acid (α-oxypropionic, 2-hydroxypropanoic) is a monobasic oxycarboxylic acid, a light yellow to dark yellow liquid. It is soluble in water and alcohols. In the study, lactic acid was used from PURAC bioqumica s.a. (Spain). Sodium hydroxide is a white crystalline substance, highly hygroscopic, and highly soluble in water. It has strong alkaline properties. In this study, sodium hydroxide was used from Merck KGaA (Germany). Ethyl alcohol is a single-atom alcohol, a colourless volatile liquid with a characteristic odour and burning taste. It is a good solvent for other organic substances. The study used 96% alcohol produced by Lopatyn Distillery LLC (Ukraine). Polyethylene glycol (PEG) is a water-soluble polymer, a non-ionic surfactant. Depending on the average molecular weight of the polymer, it is a viscous liquid, gel-like or solid substance. PEG 4,000 manufactured by Clariant Product (Deutschland) GmbH (Germany) was used in the study.

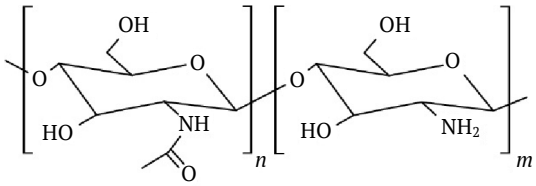

Figure 1. *Chitosan molecule*

Source: *developed by the authors of this study*

Table 1. *Characteristics of the original chitosan*

Parameters from the certificate of quality	Chitosan					
	X1	X2	X3	X4	X5	X6
Viscosity, cPs	7	13	44	54	80	170
Degree of deacetylation, %.	91	90	95	95	85	85

Source: *developed by the authors of this study based on certificates of analysis from the chitosan manufacturer*

Preparation of "active" chitosan. The method of producing "active" chitosan is based on precipitation, i.e., the production of salt from chitosan and lactic acid, followed by alkaline precipitation of chitosan. Water is loaded into the mixer and chitosan is added while stirring. Small portions of lactic acid are added to the suspension in terms of 1 mol/mol of chitosan. The addition of acid significantly increases the viscosity of the reaction mass. A viscous mass is formed with particles of undissolved chitosan. The mass is kept under stirring for 1 hour. During the holding time, a slight decrease in the viscosity of the mass is observed, as well as the dissolution of chitosan particles (Fig. 2, a). After the aging is completed, an aqueous solution of sodium hydroxide in the amount of 0.95 mol per 1 mol of chitosan is added to the reaction mass in small portions. During the reaction, the formation of fibrous chitosan is observed (Fig. 2, b). After the addition of alkali, a suspension is formed consisting of a liquid phase (water and sodium lactate) and fibres of "active" precipitated chitosan. The precipitated active chitosan is filtered and washed first with water and then with ethanol.

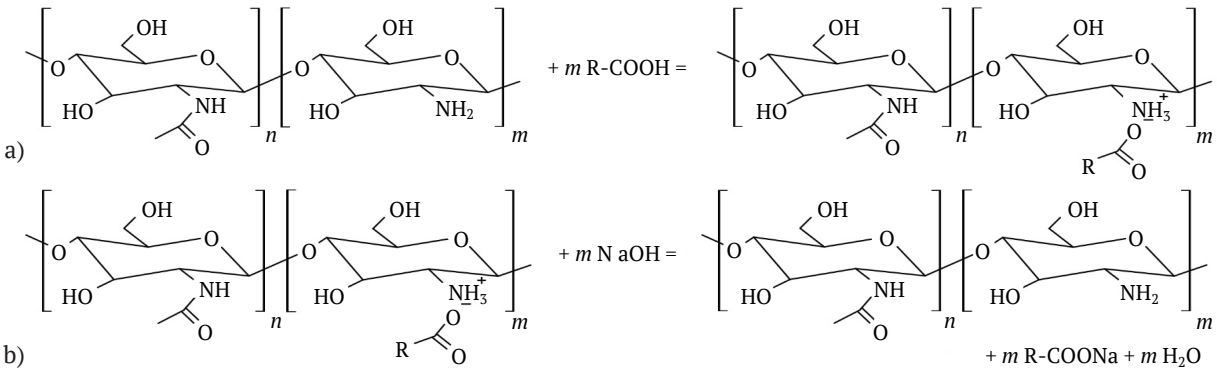

***Figure 2**. Reactions of chitosan redeposition,*
where a – obtaining a carboxylic acid salt; b – precipitation of "active" chitosan
***Source:** developed by the authors of this study*

Preparation of a solid haemostatic composite. The method for obtaining the haemostatic composition is the reaction of "active" chitosan (m.w. 50-200 kDa) with lactic acid at room temperature, followed by mixing with a surfactant. It is planned to use different chitosans for synthesis, in particular, chitosans with different degrees of deacetylation and different molecular weights. The method of producing haemostatic composites consists in the production of a composite material based on a carboxylic acid salt and chitosan (Fig. 3). Ethyl alcohol and water in a ratio of 7:3 wt% are loaded into a mixer. Next, lactic acid is added under vigorous stirring in the amount of 0.5 mol per 1 mol of chitosan. Then, a surfactant, polyethylene glycol 4,000, is added to the reaction mass. The mass is kept under stirring for 1 hour. As a result, a viscous mass is obtained with the inclusion of insoluble chitosan particles. The solvent is removed from the resulting mass under vacuum, then dried with flowing air at a temperature not exceeding 60°C. The composite is dried to a moisture content of no more than 5% by weight. The resulting fibrous films of the haemostatic composite are crushed and calibrated. A fraction of not more than 0.8 mm is used.

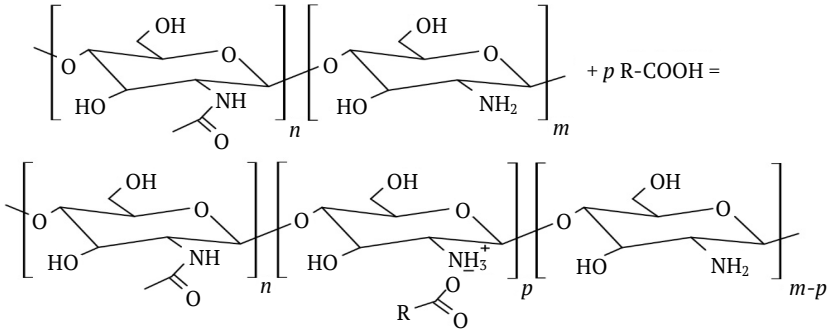

***Figure 3**. Reaction for the preparation of chitosan salt*
***Source:** developed by the authors of this study*

***In vitro* method for the evaluation of haemostatic performance.** The development of *in vitro* method for the evaluation of haemostatic performance was carried out using the ready-made haemostatic composite Celox® as a reference agent. *In vitro* parameters of the method were chosen for the following reasons:

- take *water R* as the Quality Control (QC) medium;
- the process of formation of a fixed gel (hereinafter referred to as gel formation) when the composite is in contact with liquids should be as fast as possible, therefore the time of formation of a fixed gel plug is an important parameter;
- the composite should have permeability limits when in contact with liquids, as there is a risk of: the formation of a gel layer of insufficient depth and, as a result, strength; or in the second case, too high permeability of the liquid through the composite layer and the inability to stop bleeding.

The properties of the haemostatic composite material were studied by evaluating the gel formation time and permeability.

Gel formation time. Added 40 ml of *water R* into a 50 ml chemical beaker with an internal diameter of 38±2 mm, and added 2.0 g of the drug at a time, spreading it evenly over the surface of the liquid in a circular motion from the walls to the centre of the beaker. A gel-like mass should form within no more than 180 seconds, which remains stationary when the beaker is turned 180°. The resulting gel should retain its consistency for at least 24 hours (Fig. 4). At least 2 parallel tests are carried out.

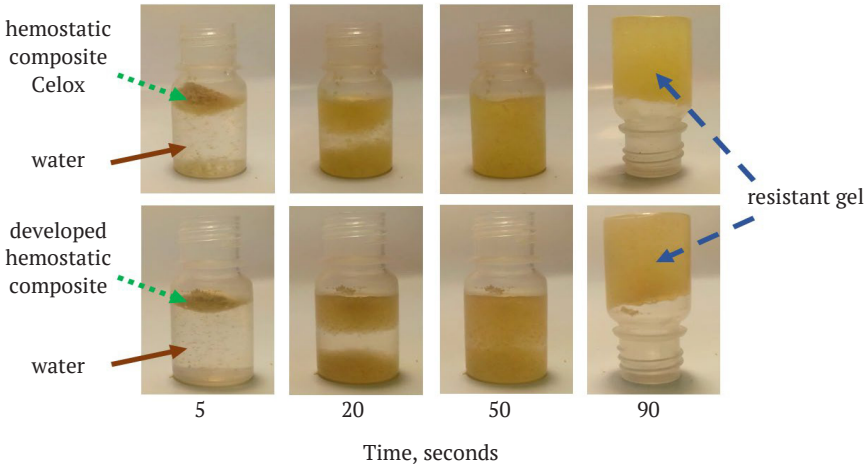

***Figure 4**. Gel formation during testing of Celox and the developed haemostatic composite*
***Source:** developed by the authors of this study based on the findings of experimental research*

Permeability and wetting. Added 5 ml of a 0.25% solution of Ponceau 4R food colourant (E-124) in *water R* in a test tube with a diameter of 13-14 mm, and quickly added 2.00 g (m) of the composite at a time. After 1 minute, performed the following steps:

1) measure the value of the wetted gel-like layer in mm;

2) pour out the remaining dry weight of the drug, weigh it (m_1) and calculate % moisture content using formula (1):

$$\%\ wetting = (m - m_1)/m \times 100, \quad (1)$$

where m – the weight of the drug to be tested, g; m_1 – the residual dry weight of the drug after the test, g.

***Note:** The value of the gel layer should be at least 5 mm. % moisture content should be between 35.0 and 70.0%.*

Results and Discussion

Evaluation of haemostatic performance of the reference product "Celox®". *Water R* was chosen as *in vitro* blood substitute to develop *in vitro* haemostatic performance method. The first stage of the method development was carried out using the reference composite Celox®. The parameters "Gel formation time" and "Permeability and wetting" were studied and developed to test the reference product for haemostatic performance. As a result of the studies, it was found that the gel formation parameters depend on: the quantitative ratio of gestatic composite – water; the amount of haemostatic (and, accordingly, water) (Table 2); the size of the vessel in which the test is carried out (Table 3). As a result of the studies, a threshold ratio of 20:1 was established at which water could still be completely bound by the haemostatic composite to form a stable gel. At higher ratios, no gel clot was observed. Taking into account that water and blood have different nature and properties, the haemostatic composition was tested on blood with a ratio of 20:1 and 25:1. The results were reproduced for water and blood. Thus, the use of *water R* as a QC medium can be considered acceptable for the *in vitro* method, therefore water in the ratio of 20:1 was chosen for further tests. During the gel formation studies, it was observed that in some cases the time of complete water binding differs from the time of formation of a fixed gel. From the results of the studies, it was found that the time indicators depend on the size of the vessel in which the test is performed (Table 2).

***Table 2**. Dependence of the time of water binding and gel formation on the diameter of the vessel, 0.5 g of powder, 10 g of water*

Parameter	Diameter of a cylindrical vessel with a flat bottom, mm							
	12	14	17	20	22	25	30	40
Time of complete water binding, s	-	300	80	70	55	50	35	15
Gel formation time, s	90	70	70	70	70	50	40	40

***Source:** developed by the authors of this study based on the findings of experimental research*

As the vessel diameter increases, the area of contact between the haemostatic and the liquid increases, and the height of the liquid column decreases, which contributes to faster wetting of the haemostatic, water binding and gel formation. At small vessel diameters, the composite forms a gel layer, which allows all the water to penetrate the powder, and for vessels with larger diameters, the powder absorbs water quickly, but the limiting process is gel formation. For further gel formation tests, a 38 mm inner diameter chemical beaker was chosen. For this vessel, the effect of increasing the gel weight was tested (Table 3).

***Table 3**. Dependence of water binding and gel formation time on powder weight, vessel diameter 38 mm*

Parameter	Weight of composite / water, g			
	0.5 / 10.0	**1.0 / 20.0**	**2.0 / 40.0**	**3.0 / 60.0**
Time of complete water binding, s	15	35	45	180
Gel formation time, s	40	70	90	110
Gel retention time, h	> 24	> 24	> 24	2.1

***Source:** developed by the authors of this study based on the findings of experimental research*

As the amount of haemostatic composite and water increases, the height of the liquid column also increases, leading to an increase in the time of water binding and gel formation. After gel formation, the beakers were inverted and the gel mobility was observed. For powder weights of up to 2 g, the resulting gel remained stationary for at least 24 hours, while for a 3 g composite weight, the immobility lasted about 2 hours, after which the gel flowed out of the beaker. This observation can be explained by the too large weight of the gel, which did not allow the adhesive and cohesive bonds to hold it in the glass. Therefore, for *in vitro* method, the amount of the composite was chosen to be 2.0 g. This amount of the composite also correlates well with the commercially available Celox® in the amount of 2 g, which is designed for household use.

Taking into account the studied cases when all the water has not yet been bound by the haemostatic composite during testing in a narrow vessel, and the top layer is already stationary, there is a need to study the permeability of water through the powder. From observations, it was found that the optimal height of the column of the resulting gel should be at least 15-25 mm for wide vessels (25-40 mm in diameter) and at least 5 mm for narrow vessels (12-14 mm in diameter). Gels obtained from composites with lower permeability values have low strength and can collapse under the pressure of the liquid. Based on the data obtained, *in vitro* methods for evaluating the haemostatic performance of chitosan-based composite materials were developed.

Study of the influence of carboxylic acid and solvent type and amount on composite synthesis. To solve this problem, haemostatic composites using chitosan and various organic acids were obtained. The low efficiency of the composites formed with succinic and citric acids was found. Succinates and citrates of chitosan quickly form a gel upon contact with water (20-45 s), but their stability time does not exceed 30-50 minutes, which does not allow obtaining a composite material with effective haemostatic properties. The study of the gel formation properties of chitosan salts and monobasic carboxylic acids showed a slightly longer gel formation time (1-3 min) and significantly better stability of the formed gels – up to 24 hours. It was found that the length of the carbon chain in the range of C_1...C_3 does not significantly affect the properties of the obtained composites. For the further development of a solid haemostatic composite material, lactic acid was chosen among organic carboxylic acids due to its sufficient haemostatic performance and safety for the human body, as it is naturally formed in the body. Lactic acid also acts as a plasticiser in the composite. As a result of the work performed, it was determined that the properties of the composite are affected by the ratio of chitosan to acid in the formed salt. With an increase in the acid content, the gel formation efficiency increases. At acid content of 0.6 mol/mol or more, a gel unstable for 24 hours is formed (Table 4).

In the course of the work, the possibility of using different solvents for the synthesis of haemostatic composite material was studied. It was found that the nature of the solvent significantly affects both the process parameters and the haemostatic performance of the obtained composites. It has been established that salts of organic acids and chitosan exhibit better gel formation properties when water-alcohol solvents are used for their synthesis. The aqueous-alcohol solvent has process advantages: the reaction mass has a lower viscosity, which simplifies the mixing conditions; it evaporates more easily and quickly. It was found that haemostatic performance is affected by the concentration of alcohol in the solution used as a solvent (Table 5).

***Table 4**. Dependence of water binding and gel formation time on the molar ratio of chitosan: acid*

Parameter	Molar ratio acid: chitosan						
	0.25:1	**0.30:1**	**0.40:1**	**0.50:1**	**0.60:1**	**0.75:1**	**1:1**
Water binding time, s	90	65	55	50	50	45	40
Gel formation time, s	-	140	110	90	90	85	80
Gel retention time, h	-	> 24	> 24	> 24	18	8	1.5

***Source:** developed by the authors of this study based on the findings of experimental research*

***Table 5**. Dependence of water binding and gel formation time on the concentration of ethanol in the solvent*

Parameter	Alcohol concentration in the solvent, % wt.					
	40	**50**	**60**	**65**	**70**	**75**
Time of complete water binding, s	55	70	60	70	50	-
Gel formation time, s	125	110	110	95	90	-

***Source:** developed by the authors of this study based on the findings of experimental research*

The best results of haemostatic performance are demonstrated by the samples obtained using 70% (w/w) ethanol. With a decrease in the concentration of alcohol in water, the viscosity of the reaction mass increases during the preparation of the haemostatic composition, which necessitates an increase in the amount of solvent. In addition, the properties of the haemostatic, in particular, the gel formation time, deteriorate. When using a more concentrated alcohol solution than 70%, the formation of a haemostatic composition is impossible, because in this case chitosan does not react with acids.

Study of the effect of surfactant amount and preparation method of "active" chitosan. To improve haemostatic performance, the composite can contain a medically acceptable surfactant. In this study, polyethylene glycol 4,000 was used. It can also serve as a plasticiser and is safe for the human body. The results of the study showed that the presence of 1.0% polyethylene glycol 4,000 in the haemostatic composite reduces the time of water binding and gel formation due to faster water permeability (Table 6). Further increase in the content of PEG-4,000 above 1.0% does not improve the performance of the composite.

***Table 6**. Dependence of water binding and gel formation time on the content of PEG 4,000 in the composite*

Parameter	PEG-4000 content in the composite, % wt.						
	0.00	0.25	0.50	0.75	1.00	1.25	1.50
Water binding time, s	60	60	55	55	50	50	50
Gel formation time, s	105	100	100	95	90	90	90

***Source:** developed by the authors of this study based on the findings of experimental research*

The presence of a surfactant in the haemostatic composite can significantly improve the efficiency of gel formation with blood, since blood has a much higher viscosity and lower permeability compared to water. In the course of the work, it was found that the haemostatic composite material which contains chitosan lactate and PEG 4,000 meets the established requirements and has satisfactory efficiency for both water and blood tests. Studies have shown that the use of chitosans of different grades/viscosities to produce composites has produced inconsistent results (Table 7).

***Table 7**. Performance of haemostatic composites of different brands/manufacturers of chitosans*

Chitosan	Viscosity, cPs	Baseline chitosan "activity"	Tests of chitosan haemostatic samples	
			Permeability	Gel formation efficiency
X1	7	-	-/-	-/-
X2	13	-	-/-	-/-
X3	44	-	-/+	-/-
X4	54	-	-/+	-/-
X5	80	+	+	+
X6	170	-	-/+	-/+

***Note:** test results are for two states of chitosan – "inactive" and "active" except for sample X5.*
***Source:** developed by the authors of this study based on the findings of experimental research*

Thus, haemostatic properties can be influenced by the characteristics of the original chitosan, in particular, molecular weight (viscosity), degree of deacetylation and chitosan "activity". It has been suggested that the phenomenon of unstable results for different chitosans may be due to the presence of chitosan in the "active" form, i.e. when the shape and arrangement of polymer molecules does not facilitate uniform access of acid molecules to them, and the "inactive" form, which does not provide uniform access of acid molecules to amino groups. C. Carrera *et al*. (2023) in their work consider modifications of chitosan with different surface characteristics and the possibility of creating a variety of individual chitosan adapted to different applications. In view of the above, it became necessary to establish the possibility of using "inactive" chitosan for the production of haemostatics. The "active" form of chitosan was obtained by synthesising a chitosan salt followed by precipitating the active chitosan through interaction with an alkali. Thus, the chitosan samples X1, X2, X3, X4 and X5 were redeposited.

The composites obtained from chitosans X1 and X2 showed low permeability and gel formation efficiency both in the initial state and after "activation". Composites based on X3 and X4 showed low permeability and gel formation efficiency in the initial state. After "activation", the permeability values are normal. The gel formation efficiency improved, but beyond the normal range. The composite based on X5 showed good results of permeability and gel formation efficiency in the initial state (without additional "activation"). The composite with X6 showed low permeability and gel formation efficiency in the initial state. After "activation", the sample meets the testing standards. Of the 6 different chitosans, only one is suitable for use in the synthesis of composite haemostatics according to the redeposition-free scheme. The other chitosans do not produce compositions

that meet the requirements without activation. It was found that even in the case of chitosan precipitation, not all of them can be used to prepare a haemostatic composite – chitosan must have a molecular weight of at least 80,000.

Study of the particle size distribution of a haemostatic composite. The dependence of the efficiency of composites on the size of powder particles was studied (Table 8).

Table 8. *Dependence of water binding and gel formation time on the particle size of the composite*

Parameter	Fraction or mixture of fractions, mm									
	0-0.08	0.08-0.25 (A)	0.25-0.5 (B)	0.5-0.63	0.63-0.8	0-0.8	10% A + 90% B	20% A + 80% B	30% A + 70% B	40% A + 60% B
Water binding time, s	-	40	55	80	90	50	50	50	55	60
Gel formation time, s	-	70	80	105	140	90	70	70	85	90

Source: *developed by the authors of this study based on the findings of experimental research*

The results showed that the best gel formation efficiency was achieved with a fraction of up to 0.5 mm. At the same time, it was found that composites with a particle size of less than 0.5 mm have low permeability. Thus, the optimal particle size distribution of the haemostatic was selected – up to 0.8 mm. The IR spectra of the haemostatic composite material based on chitosan were obtained in the course of the study (Fig. 5).

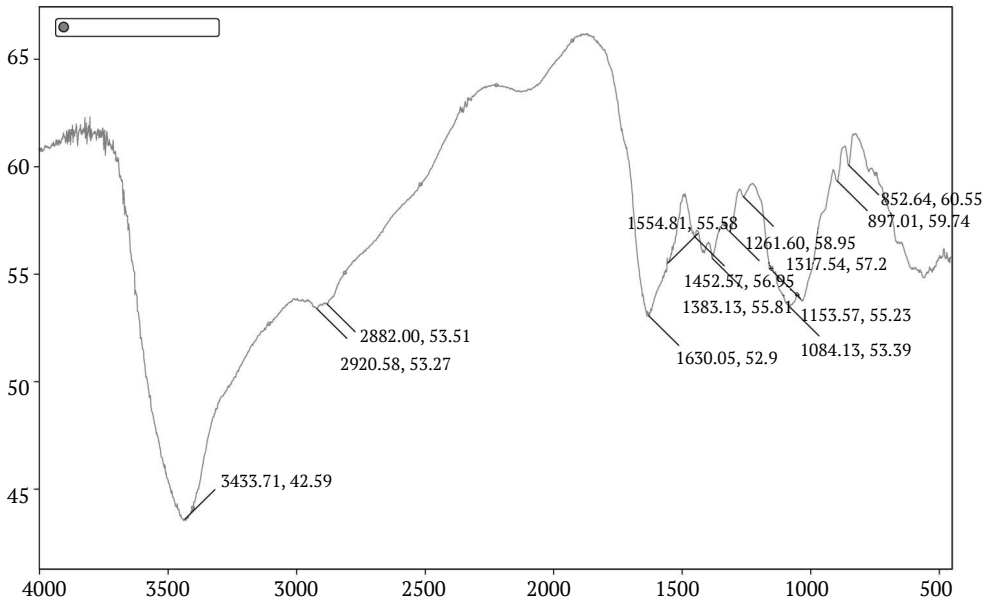

Figure 5. *IR spectrum of haemostatic composite material*
Source: *developed by the authors of this study based on the findings of experimental research*

Thus, during the development of the method, an optimal polymer composition was established, containing chitosan lactate (molar ratio 0.5:1) and polyethylene glycol in an amount of 1% wt. It is proposed to use a 70% wt. water-alcohol solution as a solvent. The solid composition has a particle size of no more than 0.8 mm. The resulting hemostatic composite demonstrates high hemostatic properties. In this work, the authors have for the first time proposed an approach to developing *in vitro* quality control methods for chitosan-based hemostatic composites that do not require *in vivo* intervention. Celox Medical (n.d.) relies on *in vivo* studies for their hemostatic composite, but information on *in vitro* gelation effectiveness studies is absent. The available general information from the manufacturer correlates with the obtained *in vitro* research results. For example, Celox Medical (n.d.) states that the hemostatic composite effectively absorbs blood, forms a gel in no more than 5 minutes, can be used in wounds of any shape, and is simple to apply and remove. The *in vitro* method developed by the authors allows for the quantitative assessment of the gelation effectiveness of the hemostatic composite – how much liquid the composite can absorb, how quickly, and

how long the gel can be retained. The method accurately reproduces results for both the QC environment and blood.

The method developed by the authors allowed for the study of the influence of factors on the gelation properties of composites based on modified chitosan. A. Das *et al*. (2024) demonstrate in their work various methods for obtaining chitosan-based composites. Modified chitosan show significant differences in composite properties compared to the original chitosan. The chitosan lactate obtained by the authors of this work demonstrates a high gelation capacity, unlike regular chitosan. K. Pieklarz *et al*. (2021) in their work describe a method for obtaining chitosan lactate by physically mixing chitosan with acid using crosslinking agents. In contrast to the described synthesis method, the method proposed by the authors has advantages. The use of water-alcohol solvents for synthesis instead of water is proposed. A water-alcohol solvent has technological advantages: the reaction mass has lower viscosity, which simplifies mixing conditions; it evaporates more easily and quickly, and the resulting composite exhibits better hemostatic properties. The method proposed by the authors for obtaining a hemostatic composite based on chitosan lactate does not require the use of additional agents for crosslinking molecules. The authors' work shows the influence of the ratio of chitosan and lactic acid during the synthesis of chitosan lactate salt. The influence of surfactants on the composition is also additionally studied.

During the development of the method for obtaining a hemostatic composite, it was found necessary to pre-treat some chitosan samples that have an "inactive" form. Of the 6 different chitosans studied, only one is suitable for use in the synthesis of a composite hemostatic agent according to the scheme without redeposition. Other chitosans without activation form compositions that do not meet the requirements. C. Carrera *et al*. (2023) in their work consider methods of influencing the surface activity of chitosan at the synthesis stage. This method does not allow changing the properties of existing chitosan after its synthesis stage. To solve this problem, a method for obtaining the active form of chitosan by obtaining a salt of chitosan and acid with subsequent precipitation of active chitosan upon interaction with alkali is proposed. The proposed method made it possible to expand the choice of initial chitosans, but it was found that not all redeposition chitosans can be used to prepare a hemostatic composite – chitosan must have a molecular weight of at least 80,000. This requires careful selection of chitosan depending on its properties, which necessitates further research to develop a method for determining the initial activity.

Conclusions

This paper presents the results of the development of an *in vitro* method for the evaluation of the haemostatic performance of chitosan-based composites using *water R* as a QC medium, including gel formation and permeability tests. The results of studies of the properties of chitosan and chitosan lactate are presented. It was found that to obtain a haemostatic, it is necessary to use chitosan with a molecular weight of at least 80,000 Dalton and a degree of deacetylation of 85-95%. It was found that the "active" and "inactive" forms of chitosan affect the properties of the composite. A method for obtaining the "active" form of chitosan from the "inactive" form was developed, which consists in redeposition: the interaction of chitosan with acid to obtain a soluble salt, followed by alkali precipitation. A method for obtaining the finished haemostatic composite material was developed, in particular, the acid and surfactant were selected. Lactic acid was used as a carboxylic acid because it provides high haemostatic performance. Polyethylene glycol 4,000 in the amount of 1% was used as a surfactant. The influence of various factors on the properties of the resulting composite was determined: the solvent used in the salt synthesis reaction; the molar ratio of lactic acid and chitosan; and the particle size of the haemostatic powder. The research results showed that 70% ethanol is the optimal solvent. The ratio of chitosan to acid should not be more than 0.6 mol/mol of chitosan. The haemostatic powder should have particles not larger than 0.8 mm. The properties of the obtained haemostatic composite were studied. The developed haemostatic composite is effective in binding water and blood into a stationary gel-like clot that is stable for 24 hours. The composite can be used for the production of haemostatic products in the form of applicators, tapes or bandages, which are more convenient for use in the field.

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None.

Conflict of Interest

None.

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Розробка композиційного гемостатичного засобу на основі полімеру хітозану та методу оцінки ефективності

Олександр Чорний

Кандидат технічних наук
Акціонерне товариство «Фармак»
04080, вул. Кирилівська, 63, м. Київ, Україна
https://orcid.org/0000-0002-6949-9247

Володимир Федоренко

Аспірант
Київський національний університет технологій та дизайну
01011, вул. Мала Шияновська, 2, м. Київ, Україна
Акціонерне товариство «Фармак»
04080, вул. Кирилівська, 63, м. Київ, Україна
https://orcid.org/0009-0003-5113-6848

Володимир Яременко

Аспірант
Київський національний університет технологій та дизайну
01011, вул. Мала Шияновська, 2, м. Київ, Україна
Акціонерне товариство «Фармак»
04080, вул. Кирилівська, 63, м. Київ, Україна
https://orcid.org/0009-0001-1757-5500

Олена Іщенко

Доктор технічних наук, професор
Київський національний університет технологій та дизайну
01011, вул. Мала Шияновська, 2, м. Київ, Україна
https://orcid.org/0000-0002-9510-6005

Світлана Гуреєва

Доктор фармацевтичних наук, професор
Київський національний університет технологій та дизайну
01011, вул. Мала Шияновська, 2, м. Київ, Україна
Акціонерне товариство «Фармак»
04080, вул. Кирилівська, 63, м. Київ, Україна
https://orcid.org/0000-0002-5949-4493

Анотація. Метою дослідження було розробити *in vitro* методику оцінки гемостатичної ефективності композитів та вивчити фактори впливу на гемостатичну ефективність полімерних композиційних матеріалів на основі хітозану за допомогою розробленої методики. Розглянуто існуючі контактні гемостатичні композиційні матеріали на основі цеолітів та полімеру хітозану. Для проведення досліджень та контролю якості роботи з гемостатиками необхідно тестувати їх гемостатичну ефективність. Проведення досліджень *in vivo* вимагає втручання в живий організм і є небажаним з етичних міркувань. Загальноприйнятої системи оцінки ефективності гемостатиків *in vitro* не існує. Розроблено методику проведення аналізів для визначення гемостатичних властивостей композитів. Визначено основні показники якості гемостатиків – час гелеутворення та проникність. Розроблено склад та методику отримання готового гемостатичного композиційного матеріалу. Композит містить сіль хітозану та молочної кислоти, поліетиленгліколь 4000. Досліджено вплив карбонових кислот та поверхнево-активних речовин на властивості композиту. Підібрано хітозан з молекулярною масою не менше 80000 Дальтон та ступенем деацетилювання 85-95 %. Досліджено «активну» та «неактивну» форми хітозану, що впливають на гемостатичні властивості композиту. Розроблено технологію отримання «активної» форми хітозану з «неактивної» шляхом переосадження, що полягає у взаємодії хітозану з карбоновою кислотою

з отриманням розчинної солі та наступним висадженням лугом. Досліджено вплив факторів на властивості отриманого продукту: розчинник в реакції синтезу солі; мольне співвідношення молочної кислоти та хітозану; розмір часток порошку гемостатика. Отриманий гемостатик є ефективним в зв'язуванні води та крові в нерухомий гелеподібний згусток. Засіб має достатню проникність та гемостатичну ефективність. Згусток, що утворюється внаслідок зв'язування рідин, є стійким протягом 24 годин

Ключові слова: молочна кислота; поліетиленгліколь; композиційні полімерні матеріали; гелеутворення; проникність; *in vitro*