

Interaction Between Chitosan Solutions, Cellulose Carriers and Some of the Multi-enzyme Complexes

Elina Eldarovna Dosadina¹, Alexey Alexeevich Belov^{1,2,*}

¹Department of Biotechnology, Mendeleev University of Chemical Technology of Russia, Moscow, Russia

²Research Institute of Textile Materials, Moscow, Russia

Email address:

elinadosadina@gmail.com (E. E. Dosadina), abelov2004@yandex.ru (A. A. Belov)

*Corresponding author

To cite this article:

Elina Eldarovna Dosadina, Alexey Alexeevich Belov. Interaction Between Chitosan Solutions, Cellulose Carriers and Some of the Multi-enzyme Complexes. *International Journal of Bioorganic Chemistry*. Vol. 2, No. 2, 2017, pp. 51-60. doi: 10.11648/j.ijbc.20170202.12

Received: January 30, 2017; **Accepted:** February 22, 2017; **Published:** March 9, 2017

Abstract: This research is devoted to create and investigate the wound healing medical materials that contain proteolytic enzymes. The aim of the study is to propose a new wound dressing design “Multifer”TM, that is based on modified cellulose, chitosan gel and proteolytic enzymes, and represent the interaction between all of the drug components for the wound treatment such as: chitosan, modified cellulose (dialdehyde cellulose, cellulose phosphor ester) and different polyenzyme complexes (proteolytic complex from hepatopancreas of crab and bromelain). The research will also propose the scheme of the obtained materials and the components interaction mechanism. This scheme is supposed to create new materials with the special properties such as: minimal loss of biological activity during the process of dressings', obtaining, and using; minimal doze of therapeutic agent; and controlled drug release. The article and the results might be useful for everyone who is interested in cellulose chemistry, properties of proteolytic enzymes and drug development for wound healing.

Keywords: Chitosan, Dialdehyde Cellulose, Proteolytic Enzymes, Wound Healing, Drug Design, Controlled Release

1. Introduction

The problem of creation of the new multipurpose wound healing drug is still relevant for the modern medicine. There are a large number of requirements for the new products as it should be non-allergenicity, non-toxicity, biocompatibility and biodegradability, should have an ability to remove a wound exudate and to prevent a microbial contamination, mechanical injuries, over drying and other. It is also important to note that those drugs should keep its activity during the whole term of storing [1]. Nowadays, none of the existent wound healing materials can respond to all the stated requirements, which makes the creation of a new would care drug one of the most challenging issue of the modern medicine and science [1, 2].

The drugs based on enzymes for the wound treatment have been used for a long time. Proteolytic enzymes could clean the wound of exudate, provide a normal blood circulation in injured tissues and also decrease the number of pathogenic microorganisms on the wound surface, however those factors can lead to a secondary infection [2, 3]. Nowadays, there is a

trend towards multi-enzyme preparations rather than highly pure enzymes. Multi-enzyme drugs contain a complex of different enzymes to hydrolyze a wound exudate by affecting different chemical bonds and destroying them. Moreover the cost of multi-enzyme preparations is far lower than the one of highly pure enzymes since the absence of purification stage in the wound care products production [3-5].

Immobilization of physiologically active compounds promotes a longer preservation of biological properties of the systems. It is also allowing the drug action to spread in the wound to provide a controlled and prolonged drug release. All of these factors result in minimizing the healing term, making the whole process more comfortable for the patients [3, 6]. The immobilization of enzymes on different carriers is commonly used for creation of the wound treatment products. Pharmaceutical market today is full of various forms of wound care drugs: gels, powders, ointments, sponges, films and combinations of different materials [7, 8]. So developing a new product and considering a desirable form of preparation. The developers should always look for an optimal carrier, which is the one that meets all of the listed

criteria, while maintaining all of the necessary properties during the enzymes immobilization process [3, 7, 8]. One of the most common types of wound healing materials is dressing based on cellulose or its derivatives [3, 6, 9]. The cellulose materials are modified in order to impart new chemical properties such as new specific functional groups. Appearance of these groups makes the immobilization process less complicated to perform due to the chemical interaction between groups on modified cellulose matrix and the active compound [3, 10].

One more substance that is also commonly used in modern medicine for wound care is chitosan. There are lots of studies available where chitosan is employed as a carrier for various therapeutic agents [3, 11] or as an active compound with antibacterial properties against *Escherichia coli* and *Staphylococcus aureus* (microorganisms that make up the largest percentage of the pathogenic microflora on the wound surface) [11-13]. Chitosan is a biodegradable and biocompatible polymer; Figure 1 shows the structural forms of chitin and chitosan. Biopolymer is called chitin when the deacetylation degree is less than 50%. Deacetylation degree is a ratio of a glucosamine links (m) to the total amount of monomer units in a polysaccharide molecule (Fig. 1).

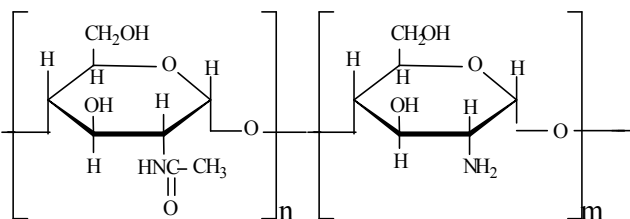


Figure 1. Chitin (n) and chitosan (m) structural forms.

The chitin monomer N-acetylglucosamine is similar to hyaluronic acid by its structure. Hyaluronic acid is known to be a biocompatible compound, which can form a specific bonds between different growth factors cells, receptors and adhesive proteins that facilitates the optimal orientation of the cells in connective tissue of the body [14]. Chitosan is known to be biodegradable: different enzymes in human body such as lysozyme or some of the bacterial enzymes can partly destroy it [15, 16].

The purpose of this research was to propose and to study the structural scheme of the designed systems and to understand the interaction mechanism of all of the components. Although similar systems are already successfully used in medicine, they still need to be improved and modified in order to create smart materials with specific properties for wound treatment.

2. Materials and Methods

2.1. Materials

In this study we used chitosan (Ct) from a crab shell ("Bioprogress", Schelkovo, RF) (humidity 10%, TC 9289-067-00472124-03, deacetylation degree 80,0%, kinematic

viscosity 383,7cSt; MW 500kDa); proteolytic complex from a hepatopancreas of crab (PC) (TC 9281-00411734126-00, proteolytic activity (caseine) 0,9PU/mg, (BAPNA) 43nMol/mg*min, (azocoll) 28U/mg) ("Bioprogress", Schelkovo, RF); bromelain (Brm) (2400GDU/g China) (proteolytic activity (caseine) 0,4PU/mg, (azocoll) 7,7U/mg); 3,5-dinitrosalicylic acid (DNSA); caseine Hammersten (Sigma-Aldrich, USA).

2.2. Methods

2.2.1. Cellulose Activation and Determination of Aldehyde Groups

The activation of the cellulose carrier in form of woven fabric (medical gauze) was carried out by sodium periodate to obtain dialdehyde cellulose (DAC) of the required modification degree of secondary hydroxyl groups [3, 18]. The number of aldehyde groups was determined similarly to [3, 19] or by the oxidation of aldehyde groups with the iodine solution in an alkaline medium (0,1n Na₂B₄O₇) or by reaction with the DNSA and expressed in mM/g (taking into account the carriers humidity) [19].

2.2.2. Study of Kinetics of Interaction Between Chitosan and Cellulose Carriers

The kinetics of interaction between chitosan and cellulose carriers was studied spectrophotometrically. The carriers were treated with the chitosan solution (0,5 mass.%) at a water duty of 10. The study was carried out spectrophotometrically by the subsidence of a chitosan concentration from the impregnation solution with the adjustments on the carriers interfering effect [20-22].

2.2.3. Determination of Enzymatic Activity

The enzymatic activity was determined similarly [3] using caseine [22, 23] as a substrate in 1/15M phosphate buffer (PB) pH 8,0.

For determination of the inactivation effect of different substances we dissolved an inactivating agent in 1/15M phosphate buffer solution with the desirable pH in adjusted ratio. Solution of inactivating agent in PB was added to required volume of the enzyme solution and after 15 minutes at room temperature (or adjusted time and temperature) enzymatic activity was determined. 100% of activity is the activity before the addition of inactivation agent or temperature affection (if temperature is an inactivation factor that is studied).

The study of interaction between chitosan solution and enzymes was carried out in two different ways: interaction between equal volumes of chitosan (1 mass%) and enzyme (solution in 0,1M NaCl) or interaction between chitosan solution (0,5 mass%) and enzyme powder. After the complete enzyme dissolving an enzymatic activity was determined. 100% of enzymatic activity is the enzyme activity without chitosan.

2.3. Measurements

UV-Vis spectra were measured on the recording

spectrophotometer Shimadzu UV-2600 (Japan).

IR spectra were obtained using a spectrometer Nicolet 380 with the FTIR (frustrated total internal reflection) (thermo Scientific, USA).

Zeta potential and the average particles diameter were measured on a Zetasizer Nano ZS, Malvern Instruments, UK.

Elemental analysis (C, N, O and H) was conducted with the CHNS/O analyzer "Thermo Flash 2000" (Thermo Scientific, Italy, UK).

2.4. Statistical Analysis

All the experiments of this study were performed in triplicate when not specifically noted. Analysis of variance (ANOVA) was performed with the Matlab program version 8.4 (USA). The results are expressed as a mean \pm standard deviation (SD) and the least significant differences for

comparison of means were computed at $p > 0,95$.

3. Results and Discussion

3.1. Preparation of the Cellulose Carriers with Active Groups

In this study was used various cellulose materials: medical gauze (cellulose (C)) (RF Standart GOST 9412-93), cellulose phosphor ester (CPE) (RF Standart 42-1726-81) and dialdehyde cellulose (RF Standart 17-09-14-350-91) obtained by the periodate oxidation of cellulose with different modification degrees (shown in Figure 2). All of the used materials were tested for the toxicology in scientific research institutes (Moscow, Russian Federation) and were proved to be safe and available for medical application (Figure 2).

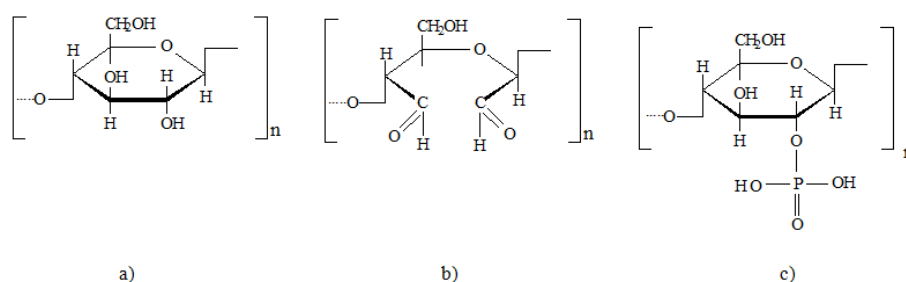


Figure 2. The cellulose materials schemes: a) cellulose; b) dialdehyde cellulose (DAC); c) cellulose phosphor ester (CPE).

Cellulose was activated by periodate oxidation [3] (Figure 3):

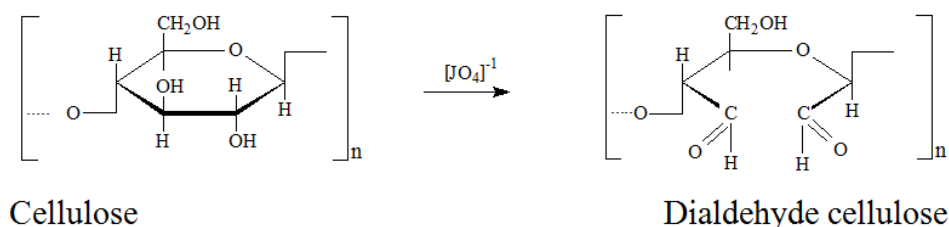


Figure 3. DAC obtaining scheme.

The CPE obtaining scheme is shown in Figure 4.

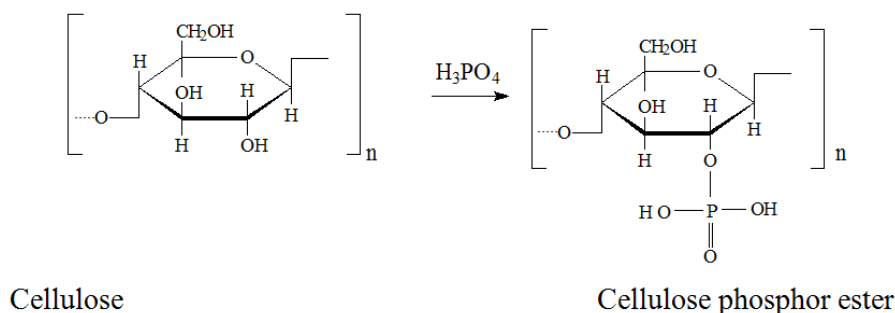


Figure 4. The CPE obtaining scheme.

3.2. Elemental Analysis

For determination of the amount of chitosan and enzymes (PC) that were immobilized it was applied the elemental analysis of the obtained materials: cellulose and oxidized

cellulose (DAC) with the different oxidation degree, cellulose carriers coated with chitosan, materials with immobilized enzymes (DAC-Chitosan-PC). Results are shown in table 1.

Table 1. Elemental composition of obtained systems and materials (CHNS).

Sample	N	C	H	S
Cellulose	0,30±0,02	42,22±0,13	6,24±0,02	-
Chitosan (Ct)	7,75±0,08	40,51±0,14	6,87±0,02	-
Cellulose-Ct	0,33±0,02	42,54±0,14	6,25±0,04	-
DAC(0,23)	0,30±0,04	42,06±0,08	6,22±0,02	-
DAC(0,65)	0,28±0,02	42,24±0,09	6,20±0,03	-
DAC90,23)-Ct	0,34±0,03	41,99±0,12	6,26±0,04	-
DAC(0,65)-Ct	0,36±0,03	42,18±0,13	6,15±0,04	-
PC	10,15±0,10	44,24±0,13	6,57±0,05	1,22±0,08
DAC(0,23)-Ct-PC	0,46±0,02	41,24±0,08	6,15±0,05	-
DAC(0,65)-Ct-PC	0,40±0,04	41,25±0,09	6,09±0,03	-

For aldehyde-containing materials the number of aldehyde groups (mM/g) is given in brackets.

The obtained results are similar to the ones obtained by other researches [24]. The amount of chitosan and PC immobilized on cellulose carriers (calculated according to the amount of nitrogen, recorded in CHNS analysis) is similar to the results obtained using other methods in this research.

3.3. Spectral Characteristics of Obtained Materials

FT-IR spectra of the obtained materials were measured. The results are shown in Figures 5, 7-8.

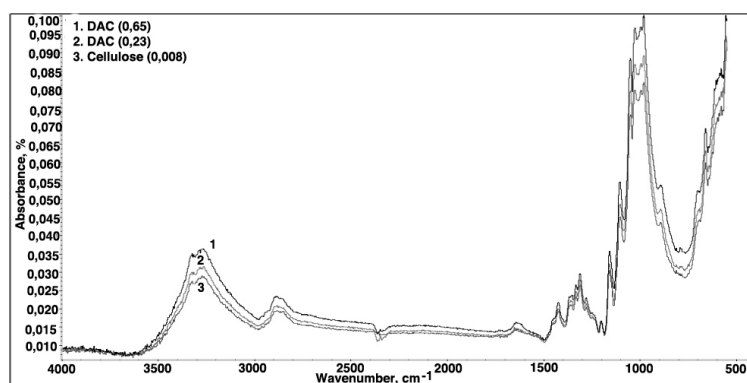


Figure 5. FT-IR spectra of 1 – cellulose(0,008), 2 - dialdehyde cellulose DAC(0,23), 3 – dialdehyde cellulose DAC(0,65).

The comparison of the FT-IR spectra of cellulose and oxidized cellulose (DAC) is shown in Figure 5. The analysis of the results shows the absence of absorbance at 1610 cm^{-1} and 1740 cm^{-1} – characteristic wavelengths of the carbonyl groups, but appearance of the peak at 900 cm^{-1} instead. This could be explained by the formation of the cyclic hemiacetal bonds as a result of an interaction between one of the aldehyde groups with the primary hydroxyl group of the

same elementary unit, while the hydration of the second aldehyde groups also takes place (Figure 6).

The FT-IR spectra of DAC obtained in this work and other researches [10, 18, 25-27] are characterized by the absence of absorbance of double bonds C – O, by less clear absorbance at 1370 , 1360 , 1340 and 1320 cm^{-1} , by disappearing of absorbance at 1200 cm^{-1} and by increasing of absorbance intensity at 900 cm^{-1} (Figure 6).

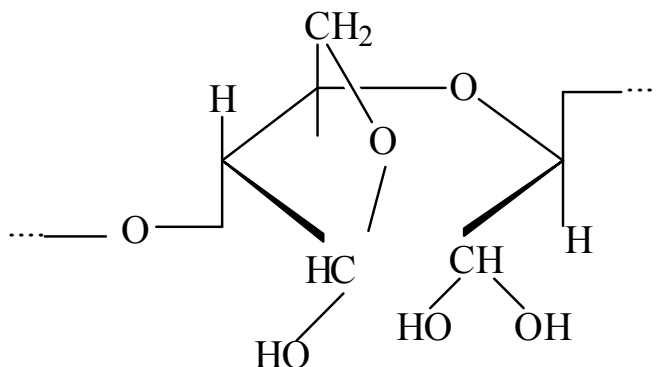


Figure 6. The result of interaction between one of the aldehyde groups with the primary hydroxyl group of the same unit with the hydration of the second aldehyde group at the same time.

Chemical binding, hydration of aldehyde groups, formation of hemiacetal or hemialdal bonds can explain the absence of absorbance at 1740 cm^{-1} . The absorbance that is increasing at 900 cm^{-1} could also be attributed to the processes noted above (Figures 7, 8).

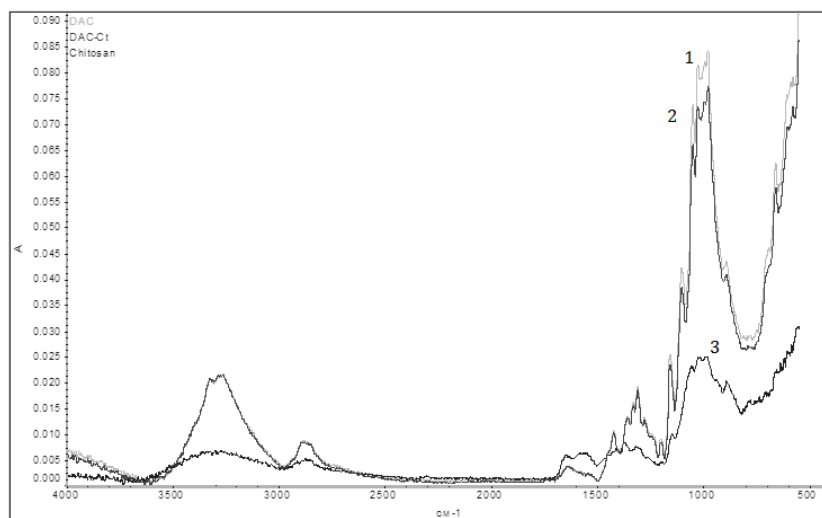


Figure 7. FT-IR spectra of 1 – dialdehyde cellulose DAC(0,23), 2 – dialdehyde cellulose DAC(0,23) coated with chitosan, 3 – chitosan.

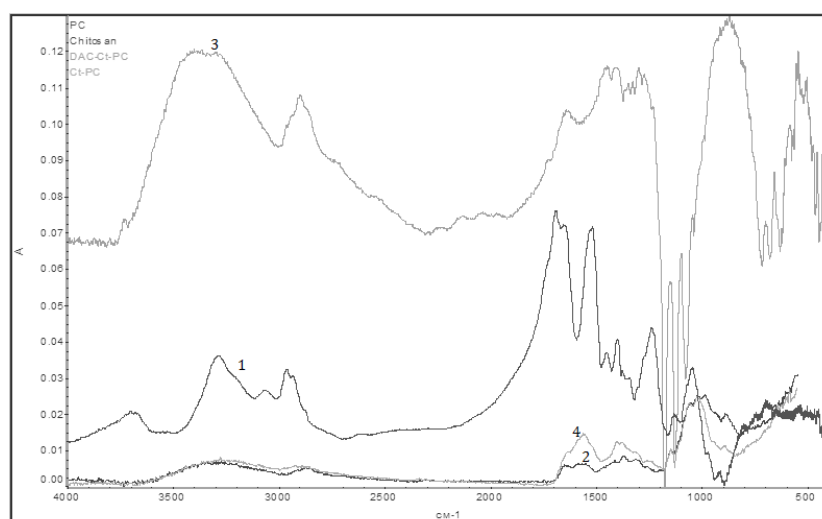


Figure 8. FT-IR spectra of 1 - proteolytic complex from a hepatopancreas of crab (PC), 2 – chitosan-PC, 3 – dialdehyde cellulose DAC(0,23) coated with chitosan and PC, 4 – chitosan.

The differences between the FT-IR spectra of diadehyde cellulose (or cellulose) coated with chitosan and chitosan solution (Figures 7, 8) are negligible. The characteristic analytical signal, free from the spectral superposition was not determined. As well as the C-N bond vibration was not detected on the FT-IR spectra of DAC coated with chitosan (Figures 7, 8). This could be explained by the little concentration of chitosan immobilized on cellulose carriers and on enzymes-containing materials (less than 5mass.%).

3.4. Interaction Between Chitosan and Cellulose Materials

On the first stage of this research it was important to understand how cellulose carriers interact with chitosan and the interaction was studied spectrophotometrically. The results can be seen in Table 2.

Typical spectra of chitosan and cellulose carriers' interaction are shown in Figures 9-10 [20-22].

Table 2. The kinetics of interaction between chitosan and different cellulose carriers. The ratio $C(t)/C_0$ of chitosan is shown in the table.

Sample	Time					
	15 min	30 min	60 min	120 min	4 hours	7 hours
Cellulose(0,008)	0,52±0,01	0,59±0,06	0,53±0,01	0,52±0,02	0,53±0,01	—
Cellulose (glycerin 2%)	0,54±0,06	—	—	0,52±0,04	0,51±0,03	—
Cellulose (glycerin 5%)	0,49±0,04	—	0,47±0,02	0,47±0,02	0,46±0,03	—

Sample	Time					
	15 min	30 min	60 min	120 min	4 hours	7 hours
DAC(0,46)	0,50±0,04	—	0,48±0,02	0,48±0,02	0,46±0,01	—
DAC(0,50)	0,64±0,01	0,65±0,02	0,65±0,02	0,65±0,02	0,64±0,01	—
DAC(0,50) (glycerin 2%)	0,88±0,08	—	—	0,88±0,07	0,91±0,05	—
DAC(0,50) (glycerin 5%)	0,64±0,05	—	0,65±0,04	0,65±0,04	0,66±0,03	—
DAC(2,445)	0,63±0,08	0,63±0,07	0,61±0,05	0,61±0,05	0,60±0,04	—
CPE(H-form)	0,58±0,03	—	0,58±0,02	0,59±0,04	0,57±0,05	0,57±0,04
CPE(Na-form)	0,24±0,11	—	—	0,31±0,04	0,36±0,01	—

For aldehyde-containing materials the number of aldehyde groups (mM/g) is given in brackets. Exchange capacity on Na⁺ is 2mgEq/g of CPE. C(t)- chitosan concentration at the point of time (t); C₀- chitosan initial concentration.

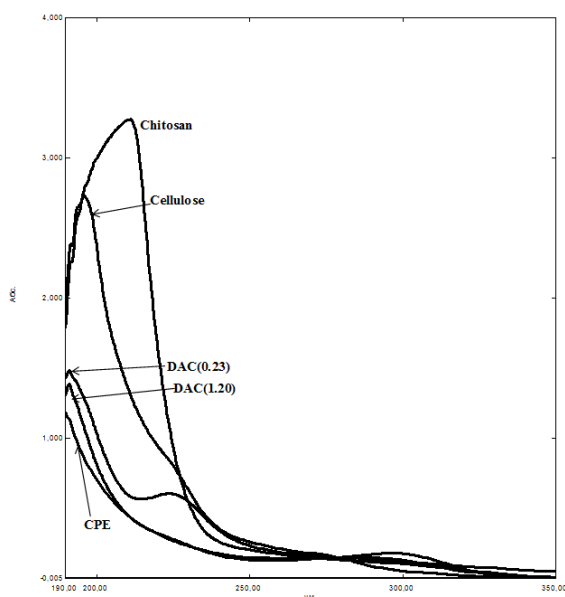


Figure 9. UV-Vis spectra of chitosan solution and cellulose materials matured in distillate water for 1 hour; the number of aldehyde groups (mM/g) is given in brackets.

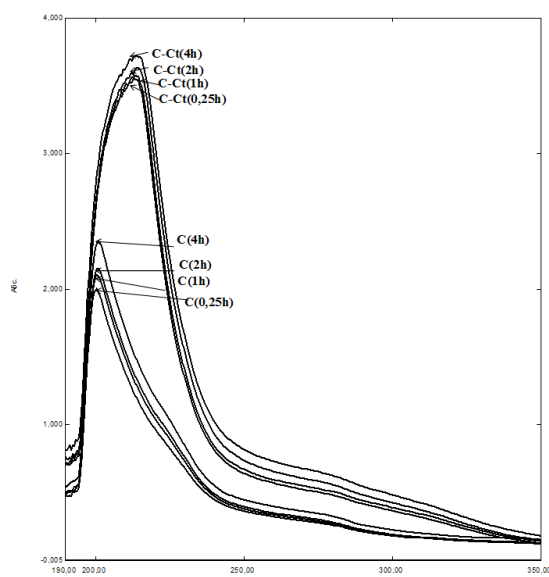


Figure 10. UV-Vis spectra of solutions after chitosan (Ct) and cellulose (C) materials interaction.

The provided results demonstrate that interaction between chitosan and all of the selected cellulose carriers can be completed in 1 hour and can be independent from the number

of aldehyde groups on the carrier's matrix [21-22].

Cellulose contains a large number of pores that can be permeable for chitosan, especially for one with low molecular weight [28]. The researches [18] show that cotton fibrils (100-1400 nm diameter) and micro fibrils (10-25 nm diameter) are full of empty spaces – micropores (10 nm diameter inside of micro fibrils and 10-100 nm diameter inside fibrils). Due to macroporosity cotton fibers exhibit good sorption properties. Swollen cotton has a large inner surface (up to 100 m²/g), what makes it perfect material for the medical purposes. According to several studies [29], proteins (MW up to 100 kD) have a diameter about 2-10 nm, when chitosan particles diameters can vary from 0,515 nm (of a structural unit – glucosamine) to 90 nm (MW chitosan about 500 kD) for single molecules [30] and more than 250 nm for the chitosan associates [31]. Periodate oxidation not only leads to the disruption of glucopyranose circle and break of the cellulose structure [18], but also can appear in extra canals and holes of the cellulose matrix. As a result it could increase the amount of chitosan and proteins (enzymes) that can be immobilized. The researches [28] shown that even 1mass% chitosan solution (MW 150kD) can penetrate into cellulose pores with a diameter of 30nm, when chitosan (MW 400kD) cannot.

The schemes of interaction between proteins' amino groups and cellulose matrix are shown in [32]. The conclusion of this study demonstrates that amino groups of chitosan and cellulose materials interact the same way, according to these schemes (Figure 11).

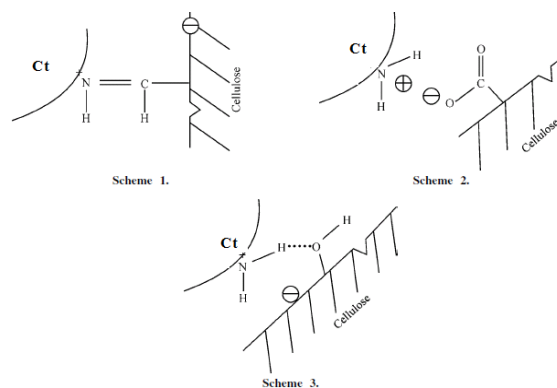


Figure 11. Schemes of possible interaction between amino groups of chitosan (or protein molecule) and aldehyde groups of cellulose (Scheme 1), carboxyl groups of cellulose (Scheme 2) and hydroxyl groups of cellulose (Scheme 3). There can be DAC instead of cellulose

3.5. Interaction Between Chitosan and Enzymes

A lot of the researches are dedicated to the study of interaction between chitosan and different proteins (enzymes) [33, 34]. The studies show that interaction of protein molecule with chitosan can take place both on the polysaccharide surface and inside of the polysaccharide matrix [35].

This research also analysed the interaction between chitosan and enzymes (PC and bromelain) as a system that can be immobilized on the cellulose carriers. On the basis of oxidized cellulose, chitosan and hydrolases from hepatopancreas of crab [3, 17] was created and developed medical healing layer of the polyenzymatic wound dressing "Multifer"™ (Russian Federation) that is available in specialized pharmacies. Bromelain or rather its immobilized modifications can also be used as a therapeutic agent in biomedicine e.g. in wound treatment [36].

Chitosan molecules have a positive charge because of the amino groups. Zeta potential of chitosan solutions (5mass.%) that was measured in this research is +73,9mV. The molecular weights of collagenolytic enzymes of different crustaceans are much smaller than ones of the vertebrate and

microorganisms. Results of electrophoretic analysis demonstrated that MW can vary from 23 to 30 kD, and also showed that in the studied samples carbohydrates are absent. The feature that unites molecular properties of different enzymes is low isoelectric point (lower than 3,5) [37, 38], what leads to a fast interaction between proteins and chitosan molecules. PC is an enzymatic complex and contains a lot of various enzymes that can partly depolymerize chitosan molecules [39].

Bromelain isolated from stem or from a pineapple pulp are known to have different structure and properties [4]. Isoelectric point of stem bromelain is about 9,55 and for bromelain isolated from pineapple pulp $pI = 4,60$ [1].

Bromelain is a high molecular glycoprotein [41]. This enzymatic complex consists of 4 cysteine proteinases with the similar amino acid sequence with a little difference for every protease. This difference results in particular proteolytic specificity and responsiveness to inactivation.

Bromelain used in this research is the one isolated from a pineapple stem.

Results of the study of chitosan and enzymes interaction are shown in Table 3.

Table 3. Chitosan and different enzymes interaction.

a) Proteolytic complex from hepatopancreas of crab (PC).

PC:Ct*	1:00	1:0,008	1:0,1	1:(0,24-2,5)	1:(5-26)	1:38	1:55
Ai/Ao	1,00	0,93±0,11	0,74±0,15	0,79±0,16	0,84±0,11	1,07±0,12	1,62±0,18

b) Bromelain.

Brm:Ct	1:0	1: 0,074	1:0,244	1:0,854	1:2,56	1:2,58	1:4,975
Ai/Ao	1,0	0,86±0,05	0,80±0,03	0,80±0,04	0,99±0,05	1,07±0,08	0,92±0,12

Results show that addition of chitosan does not have dramatic effect on the proteolytic activity of our enzymes.

Appearing of associations of proteins and polysaccharides molecules that are connected by Coulomb and non-Coulomb interactions leads to the formation of polyelectrolyte complex [42]. Proteins can react as polyacids or as polybases depending on the conditions. It is clear that protein should react as an acid to form a complex with chitosan. The interaction between chitosan and other biopolymers can occur by formation of the different bonds: electrostatic interaction, hydrogen bonds and intermolecular hydrophobic interaction.

3.6. Interaction Between Enzymes and the Monomers of Cellulose and Chitosan

In order to explain the chitosan affection on enzymatic activity of PC and bromelain, this research studied how monomers of cellulose (glucose) and chitosan (glucosamine and N-acetylglucosamine as a monomer of chitin) affect the enzymes. During the process of exploitation our materials for wound healing could undergo a hydrolytic destruction, what leads to the formation of monomers. Results are shown in Figures 12-13.

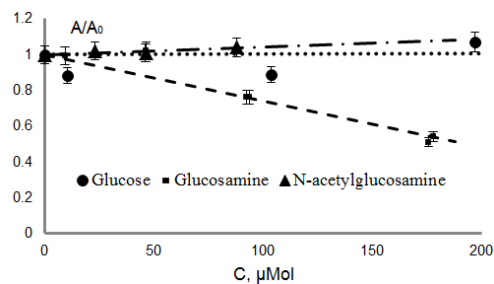


Figure 12. The effect of glucose, glucosamine and N-acetylglucosamine on the enzymatic activity of non-modified PC (caseine was used as a substrate).

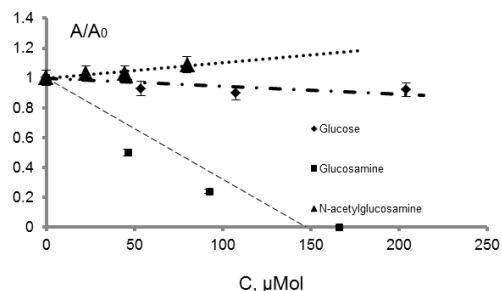


Figure 13. The effect of glucose, glucosamine and N-acetylglucosamine on the enzymatic activity of non-modified bromelain (caseine was used as a substrate).

The results show that glucosamine could decrease enzymatic activity of both PC and bromelain, what can be caused by modification of the enzymes active sites. As we noted earlier, size of glucosamine molecules is about 0,5 nm and the chitosan molecules are about 100nm, so glucosamine could penetrate right into enzymatic complex and bind with the catalytic site. The addition of glucose and N-acetylglucosamine does not affect the enzymatic activity of the systems.

3.7. Wound Healing Materials Structural Scheme

Based on understanding of how all components (cellulose carrier, chitosan and enzymes) interact with each other, we designed a new material for wound healing "Multiferm" and a new way of its industrial production [18].

The first step of this process is activation of cellulose carrier. As we mentioned before (3.1.), cellulose is activated by periodate oxidation with the formation of dialdehyde cellulose.

After we immobilize all necessary therapeutic components such as enzymes, antioxidants, antibiotic etc. on activated material. It should be also noted that different compounds could be immobilized in one stage or in so-called layer-by-layer immobilization process. To choose between those two different ways 3 different materials were designed, changing the way of production:

Multiferm 1 – solutions of chitosan and enzyme (PC) were mixed together, and this composition was immobilized on activated cellulose;

Multiferm 2 - enzyme (PC) powder was dissolved in solution of chitosan, and this composition was immobilized on activated cellulose;

Multiferm 3 – solution of chitosan was immobilized on activated cellulose, than obtained material was air-dried and after that solution of enzyme (PC) was immobilized on dried material (Table 4).

Table 4. The effective constants of inactivation rate of obtained immobilized materials.

Samples	Effective constants of inactivation rate, month ⁻¹	
	k ₁	k ₂
Multiferm 1	0,54±0,15	0,10±0,08
Multiferm 2	0,32±0,11	0,11±0,08
Multiferm 3	0,44±0,18	0,06±0,04

The analysis of effective constants of inactivation rate shows that the production way does not affect inactivation rate significantly, so immobilized material can be obtained in one stage without any loss of biological activity.

The obtained materials are air-dried at room temperature to avoid denaturation of immobilized enzymes.

Then they are packed in special polyethylene package.

All materials for the medical purposes should be carefully sterilized. Our products are sterilized by radiation with a standard doze of 25kGr.

We designed and elaborated all necessary technical specifications for industrial production of immobilized materials for wound healing [1].

We believe that it is essential not only to know how to product our materials but also to completely understand their structure, to have a scientifically based possibility to improve these materials in future and design some new ones. So the study and analysis of all of the interactions between single compounds and complexes lead us to understanding of the possible structural scheme of obtained materials immobilized on modified cellulose (Figure 14).

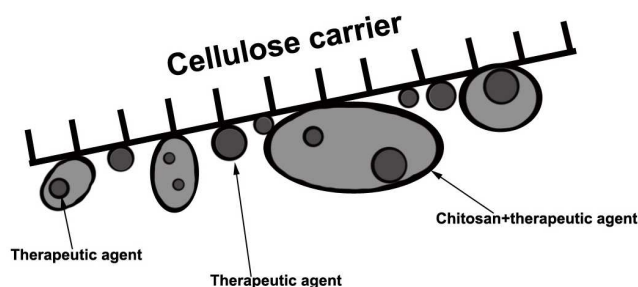


Figure 14. Structural scheme of obtained immobilized materials.

4. Conclusions

In this research we offered a structural scheme of immobilized materials for a wound healing, studied the mechanism of interaction of all of the components of the system, created the optimal way of producing medical materials based on oxidized cellulose, chitosan and enzymes for wound healing. Obtained material is a perfect base for any drug that can be immobilized on cellulose coated with chitosan.

We studied an interaction between all of the components of designed wound dressing: cellulose carriers, chitosan and enzymes. It was stated that interaction between chitosan and all of the selected cellulose derivatives can be completed in 1 hour and does not depend on the number of aldehyde groups on the carrier's matrix. This research also provides mechanisms of 3 possible chemical interactions between active groups of chitosan and modified cellulose. The analysis of chitosan effect on different enzymes (PC and bromelain) revealed the fact that chitosan does not cause a decrease of proteolytic activity of both studied enzyme complexes. Considering the degradation of designed material during exploitation, we explored the effect of monomers units of cellulose (glucose) and chitosan (glucosamine and N-acetylglucosamine). It appeared that only glucosamine significantly reduce proteolytic activity of enzymes possibly due to chemical interactions with active site.

In this paper we also elaborated technological specifications for industrial production of designed wound dressing material. It was proved that essential properties (biological activity) do not depend on chosen production method.

The scheme that was proposed and studied in this paper has a lot of advantages such as: possibility of co-immobilization of the compounds that are incompatible because of the "co-annihilation" (proteins and enzymes); controlled release of therapeutic agent because of the

irregular destruction of a complex carrier (cellulose and chitosan gel); prolonged action of therapeutic agent because of the constant drug flow in wound.

The scheme meets all of the requirements for a wound dressing material and can be successfully used in medicine in future.

References

- [1] A. D. Sezer, E. Cevher, Biopolymers as Wound Healing Materials: Challenges and New Strategies, *Biomater. Appl. Nanomedicine*. (1992) 383–414. doi:10.5772/25177.
- [2] R. D. Sinclair, T. J. Ryan, Proteolytic enzymes in wound healing: the role of enzymatic debridement., *Australas. J. Dermatol.* 35 (1994) 35–41.
- [3] A. A. Belov, Textile materials containing immobilized hydrolases for medical and cosmetic purposes. Production. Properties. Application., LAP LAMBERT Acad. Pub. GmbH & Co, Germany, 2012.
- [4] A. D. Rowan, D. J. Buttle, A. J. Barrett, The cysteine proteinases of the pineapple plant., *Biochem. J.* 266 (1990) 869–875.
- [5] S. Yoshioka, K. Izutsu, Y. Aso, Y. Takeda, Inactivation kinetics of enzyme pharmaceuticals in aqueous solution., *Pharm. Res.* 8 (1991) 480–4. doi:10.1023/a:1015899011324.
- [6] B. V. Vernikovskii, E. F. Stepanova, Immobilized proteases for wound cleaning, *Russ. J. Gen. Chem.* 82 (2012) 572–578. doi:10.1134/S107036321203036X.
- [7] P. Zhuang, D. a Butterfield, Spin labeling and kinetic studies of a membrane-immobilized proteolytic enzyme., *Biotechnol. Prog.* 8 (1992) 204–210.
- [8] K. C. Broussard, J. G. Powers, Wound dressings: Selecting the most appropriate type, *Am. J. Clin. Dermatol.* 14 (2013) 449–459. doi:10.1007/s40257-013-0046-4.
- [9] M. C. Costache, H. Qu, P. Ducheyne, D. I. Devore, Polymer-xerogel composites for controlled release wound dressings., *Biomaterials.* 31 (2010) 6336–43. doi:10.1016/j.biomaterials.2010.04.065.
- [10] G. S. K. Singh M., Ray A. R., Vasudevan P., Verma K., Potential biosiluble carriers, *Biomaterials.* 7 (1982) 495–512.
- [11] S. P. M. Castro, E. G. L. Paulín, Is Chitosan a New Panacea? Areas of Application, in: *The Complex World of Polysaccharides*, 2012: pp. 3–46. doi:10.5772/51200.
- [12] T. Dai, M. Tanaka, Y.-Y. Huang, M. R. Hamblin, Chitosan preparations for wounds and burns: antimicrobial and wound-healing effects., *Expert Rev. Anti. Infect. Ther.* 9 (2011) 857–879. doi:10.1586/eri.11.59.
- [13] M. Ganan, A. V Carrascosa, A. J. Martinez-Rodriguez, Antimicrobial activity of chitosan against *Campylobacter* spp. and other microorganisms and its mechanism of action, *J. Food Prot.* 72 (2009) 1735–1738.
- [14] L. C. Keong, A. S. Halim, In Vitro Models in Biocompatibility Assessment for Biomedical- Grade Chitosan Derivatives in Wound Management, *Int. J. Mol. Sci.* 10 (2009) 1300–1313. doi:10.3390/ijms10031300.
- [15] J. K. Suh, H. W. Matthew, Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering: a review., *Biomaterials.* 21 (2000) 2589–2598. doi:10.1016/S0142-9612(00)00126-5.
- [16] T. Kean, M. Thanou, Biodegradation, biodistribution and toxicity of chitosan, *Adv. Drug Deliv. Rev.* 62 (2010) 3–11. doi:10.1016/j.addr.2009.09.004.
- [17] A. A. Belov, V. N. Filatov, E. N. Belova, Medical dressing containing enzymatic complex from hepatopancreas of crab and the way of producing, 2 323 748, 2006.
- [18] Z. A. Rogovin, Chemistry of cellulose, *Chimia*, Moscow, 1972.
- [19] G. L. Miller, Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar, *Anal. Chem.* 31 (1959) 426–428. doi:10.1021/ac60147a030.
- [20] E. E. Dosadina, A. A. Belov, Glycerine influence on the kinetic of chitosan containing cellulose matrix destruction, in: «Biochemical Physics» XIV Annu. Int. Youth Conf. IBCP RAS-Universities, 2014: pp. 50–55.
- [21] E. E. Dosadina, A. A. Belov, The obtaining of chitosan containing materials based on modified cellulose for medical use, in: *Advances Chem. Chem. Technol.*, Moscow, 2014: pp. 15–17.
- [22] A. A. Belov, A. I. Korotaeva, E. A. Raspopova, Influence solutions of glycerol on the enzymatic activity of proteolytic complex of hepatopancreas crab stabilized polysaccharide compounds, in: *Chem. Technol. Key Dev. Appl. Chem. Mater. Sci.*, Apple Academic Press, 2014: p. 380.
- [23] A. A. Belov, T. E. Ignatuk, V. V. Riltsev, Methods of determination of proteolytic activity in industrial samples of immobilized proteinases preparations, *Pharm. Chem. J.* 26 (1992) 101–103.
- [24] N.-A. Rangel-Vázquez, E. Guilbert-Garcia, R. Salgado-Delgado, E. Rubio-Rosas, E. G. Hernandez, Z. Vargas-Galarza, et al., Synthesis and Characterization of Chitosan Coated Dialdehyde Cellulose with Potential Antimicrobial Behavior, *J. Mater. Sci. Eng.* 4 (2010) 62–67.
- [25] V. I. Gumnikova, Synthesis of dialdehydedextrane and dealdehydecaboxymethylcellulose and its chemical modification, *Mendelev University of Chemical Technology of Russia*, 2014.
- [26] C. J. Knill, J. F. Kennedy, Degradation of cellulose under alkaline conditions., *Carbohydr. Polym.* 51 (2002) 281–300. doi:10.1016/S0144-8617(02)00183-2.
- [27] R. G. Zhibankov, R. M. Marunov, N. V. Ivanov, A. M. Shishko, Spectroscopy of cotton, *Nauka*, Moscow, 1976.
- [28] L. F. Zemljic, S. Strnad, O. Sauperl, K. Stana-Kleinschek, Characterization of Amino Groups for Cotton Fibers Coated with Chitosan, *Text. Res. J.* 79 (2009) 219–226.
- [29] M. Ettenauer, F. Loth, K. Thümmel, S. Fischer, V. Weber, D. Falkenhagen, Characterization and functionalization of cellulose microbeads for extracorporeal blood purification, *Cellul. (Dordrecht, Netherlands)*. 18 (2011) 1257–1263. doi:10.1007/s10570-011-9567-2.
- [30] M. Weinhold, Characterization of chitosan using triple detection size-exclusion chromatography and ¹³C-NMR spectroscopy, *University Bremen*, 2010.

- [31] E. V. Korchagina, O. E. Philippova, Multichain aggregates in dilute solutions of associating polyelectrolyte keeping a constant size at the increase in the chain length of individual macromolecules., *Biomacromolecules*. 11 (2010) 3457–66. doi:10.1021/bm100990u.
- [32] N. E. Kotelnikova, S. A. Mikhailova, E. N. Vlasova, Immobilization of proteolytic enzymes trypsin and α -chymotrypsin to cellulose matrix, *Russ. J. Appl. Chem.* 80 (2007) 322–329.
- [33] B. Krajewska, Application of chitin- and chitosan-based materials for enzyme immobilizations: A review, *Enzyme Microb. Technol.* 35 (2004) 126–139. doi:10.1016/j.enzmictec.2003.12.013.
- [34] Z.-X. Tang, J.-Q. Qian, L.-E. Shi, Preparation of chitosan nanoparticles as carrier for immobilized enzyme., *Appl. Biochem. Biotechnol.* 136 (2007) 77–96. doi:10.1007/BF02685940.
- [35] E. Biró, A. S. Németh, C. Sisak, T. Feczko, J. Gyenis, Preparation of chitosan particles suitable for enzyme immobilization., *J. Biochem. Biophys. Methods*. 70 (2008) 1240–1246. doi:10.1016/j.jprot.2007.11.005.
- [36] R. Pavan, S. Jain, Shraddha, A. Kumar, Properties and therapeutic application of bromelain: a review., *Biotechnol. Res. Int.* 2012 (2012) 976203. doi:10.1155/2012/976203.
- [37] G. N. Rudenskaia, [Brachyurins--serine collagenolytic enzymes from crabs], *Bioorg. Khim.* 29 (2003) 117–28. <http://www.ncbi.nlm.nih.gov/pubmed/12708311>.
- [38] G. N. Rudenskaya, V. A. Isaev, A. M. Shmoylov, S. V. Shvets, M. A. Karabasova, A. I. Miroshnikov, et al., Preparation of proteolytic enzymes from Kamchatka crab *Paralithodes camchatica* hepatopancreas and their application, *Appl. Biochem. Biotechnol.* 88 (2000) 175–183.
- [39] E. I. Kulish, V. P. Volodina, V. V. Chernova, Enzymatic chitosan destruction (in Russian), *Plast. Masses*. 5 (2008) 32–38.
- [40] Z. I. M. Arshad, A. Amid, F. Yusof, I. Jaswir, K. Ahmad, S. P. Loke, Bromelain: an overview of industrial application and purification strategies, *Appl. Microbiol. Biotechnol.* (2014) 7283–7297. doi:10.1007/s00253-014-5889-y.
- [41] L. P. Hale, P. K. Greer, C. T. Trinh, C. L. James, Proteinase activity and stability of natural bromelain preparations., *Int. Immunopharmacol.* 5 (2005) 783–93. doi:10.1016/j.intimp.2004.12.007.
- [42] M. A. Krayukhin, N. A. Samoilova, I. A. Yamskov, Polyelectrolyte complexes of chitosan: formation, properties and applications, *Russ. Chem. Rev.* 77 (2008) 799–813. doi:10.1070/RC2008v077n09ABEH003750.