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Modified Cellulose Products for Application in Hygiene and Dressing Materials (Part I)

Abstract
Modification of commercial cellulose products commonly used in homes and hospitals was the main purpose of the research herein presented. Enhanced moisture sorption and antimicrobial properties were conferred upon regular commercial gauze. Nanoparticles of microcrystalline chitosan and complex chitosan/alginateNa/Ca were used in the modification. A method was elaborated for the preparation of polymeric materials with a particle size below 1 μm by means of an ultrasonic reactor (Hielscher UP 200S). Modified commercial dressing materials were obtained charecterised by a largely increased absorption capacity, thus easing the transportation of moisture to the outside of the dressing and providing an environment optimal for wound healing. Thanks to the internal surface developed and adequately selected composition, the modified cellulosic materials exhibit antibacterial and antifungal properties.

Key words: *cellulosic products, nanoparticles, chitosan, chitosan/alginate Na/Ca complex.*

Introduction

The number of diseases caused by microbiological, especially specific hospital infections has increased in recent years, triggering extensive research into new materials and procedures which would bring permanent bioactive effects and high safety for humans. Fungi and Gram positive and Gram negative bacteria are commonly met in cellulosic textile and paper materials. Textiles used for medical and hygiene purposes usually come into contact with highly bacteria- contaminated media, leading to the decay of the materials and the secretion of odour. This is the reason why so many antimicrobial hygiene and medical products have emerged on the medical market. The antibacterial properties of materials are conferred by processing with nanosilver or the salts of silver [1 - 17]. Recently these substances have come under criticism for their negative impact upon the environment and humans [18 - 20].

A number of medical products are available in which antimicrobial properties are achieved by admixing some antiseptic substances of a broad activity spectrum, for example dressing called Kerlix® (Tyco Healthcare Kendall), made of low density gauze impregnated with polyhexamethylene biguanide, which reveals a wide bactericidal range. The dressing is devised for use in inflammatory states and in the purification of infected wounds [21]. The dressing Bioguard, prepared from gauze with a content of poly (dimethyldiallylammonium chloride) – pDADMAC, is extremely effective in the healing of wounds infected with S*taphylococus aureus,* being resistant to metacycline (MRSA) and other microorganisms: *S. aureus and P. aeruginosa* [21].

Increasing interest can recently be seen in the development of textile dressing materials modified by the addition of natural polymers of the polysaccharide group, like chitosan and alginates. Biodegradability, lack of toxicity and adequate antimicrobial properties characterise the polymers. A chitosan-modified double-layer nonwoven dressing made of Tencel fibres and cotton as well as a chitosan-modified dressing of polypropylene or cellulose fleece [23] count among the latter products. A dressing material composed of a bio-absorbable medium tinted with an oxidation-inhibiting dye has been proposed for the healing of regular and difficult wounds both infected and uninfected. The dressing base may comprise collagen, chitosan, and regenerated cellulose with the admixture of silver salt. It is offered in the form of film, fabric, knitwear, nonwoven, sponge, foam or a combination thereof [24]. Known are commercial dressing materials like plasters for the disinfection of wounds, where a cotton gauze activated with chitosan, chitin or its antibacterial and antifungal oligosaccharides [25] make the active layer. A nonwoven and sponge with a content of chitosan and cellulose are one other variant of the active layer [26]. A micro-porous elastic band with a content of chitosan, glycerol or dehydrate of calcium chloride was also prepared, which exhibits a high tenacity and absorption capacity [27].

Known from the literature survey is a double-layer medical dressing containing chitosan and a complex of an anionic polymer and textile reinforcement which readily absorbs exudates from burn wounds. Moreover it does not stick to the wound, and exhibits adequate strength and excellent antibacterial properties [28].

Other materials of this kind also include:
1) a dressing of chitosan nanofibres characterised by high antimicrobial activity as well as a high value of water retention and absorption indices [29],
2) a dressing made of a blend of synthetic and polysaccharide fibres [31], and
3) a textile dressing material based on chitosan-modified oxidised cellulose [31].

Textile dressing materials are also available in the form of gauze, cotton wool and sponge modified with alginate salts containing an admixture of penicillin or its derivatives designed to prevent bleeding from wounds [32].

The main goal of the research presented here was to modify a commercial cellulosic dressing gauze for home and hospital use by conferring enhanced moisture absorption and antimicrobial properties. Microcrystalline chitosan and chitosan/alginate complex with a particle size below 1 μm were used for the purpose. The particles size of the two polymeric materials was reduced to a nano-range by means of an ultra-sonic reactor - Hielscher UP 200S

Experimental

Materials

1. Virgin chitosan Chito Clear HQG Primex Co, Iceland, with the following quality parameters: average molecular mass - $\bar{M}_v$ = 372 kD, dea-

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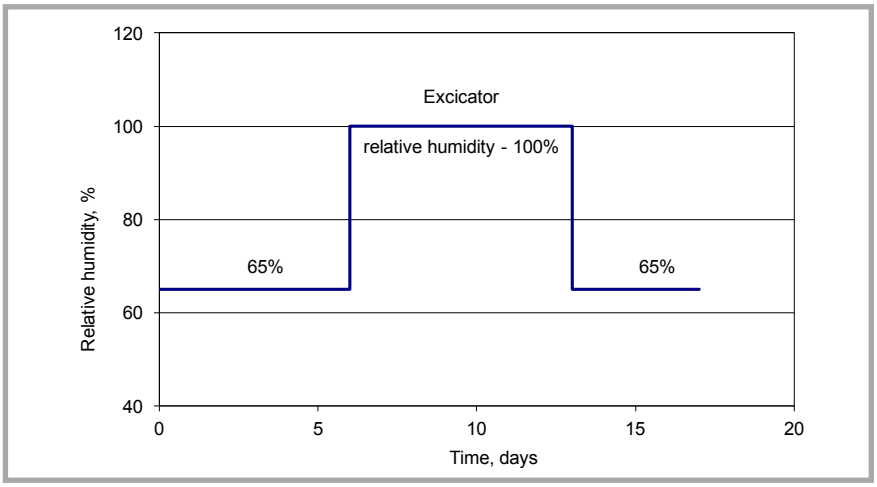


Figure 1. *Scheme of the theoretical run of sorption and desorption at RH 65% and 100% of air.*

cetylation degree (SD) = 81%, ash content = 0.22%, and content of heavy metals: As < 0.1, Cd < 0.1, Pb = 0.27, Zn = 0.80, Hg < 0.05 = 0.0%.

2. Sodium alginate Protanal 10/60FT, FMC Biopolymer Engineering, Inc., USA
3. Cellulosic textile materials
   - non-sterile gauze (100% cotton), surface density - 23.5 g/m^2, Merkator Medical Co, Poland (symbol: gauze),
   - non-sterile gauze, 8-layers (100% cotton), surface density - 200 g/m^2, Matocomp TZMO Co. Torun, Poland (symbol: Scel).

Analytical methods

Estimation of average molecular mass of chitosan (M_v)- Viscometric method

The viscometric average molecular mass was calculated based on the limiting viscose number [η]. The viscosity was measured by means of a dilution viscometer with capillary No1, K ≈ 0.01 according to own procedure [38].

Estimation of deacetylation degree in chitosan (SD) – method of first derivative of UV spectrum

The deacetylation degree was estimated by the spectrophotometric method, which consists in the determination of the maximum of the first derivative curve of the UV spectrum and mathematical computation of SD of the material according to own procedure prepared on basis of articles [39, 40].

Estimation of ash content

Ash content was estimated at 800 °C according to a procedure prepared on the basis of standard [35].

Estimation of heavy metal content

The content of heavy metals was estimated by the use of Atomic Absorption Spectrometry according to test procedure NL-13/2008 ed. IV prepared on the basis of standards [36, 37].

Estimation of the size of nanoparticles of MCCh and complex chitosan/ alginate (chit/alg/)

The size of the nanoparticles was estimated in suspension by the use of the Dynamic Light Scattering (DLS) technique and apparatus Nicomp 380; Santa Barbara, California, USA.

Methods

Modification of cellulosic products

The cellulosic materials underwent surface modification with nano-particles of MCCh and chit/algNa/Ca. An aqueous suspension of the nanoparticles with a concentration of ca. 0.11 - 0.8 wt % of the polymer was used in the coating of the dressing materials. The modified textile materials were classically dried at 35 °C and by sublimation. The latter was carried out at a temperature in the range of (-20) to 10 °C and in a vacuum from 10 to 70 Pa for 20 to 24 hours, with the use of a lyophiliser type ALFA 2-4 LD Plus, made by Martin Christ GmbH Germany.

Assessment of chitosan content in the modified cellulosic product

The ninhydrin method was applied for the assessment of the chitosan content by means of the spectroscopic technique in the range of visible light. The colour reaction of ninydrin with primary amino groups is exploited in the method. Measurements were made at a wavelength of 570 nm with apparatus UV/VIS, UNICAM Co., England.

Examination of sorption and desorption of moisture in cellulosic materials

The sorption and desorption of moisture in the cellulosic materials prior to and after modification with MCCh and complex chit/alg were examined according to own method [33, 34]. The sorption and desorption was accomplished at a relative humidity of 65 ± 4% and 100% at a temperature of 20 ± 2 °C according to the scheme in ***Figure 1***.

Assessment of antibacterial activity of modified cellulose textile materials

The assessment of antibacterial activity against *E. coli and S. aureus* was made according to Standard JIS L 1902:2002, "Examination of Antibacterial Activity of Textile Products" Quantitative Test.

Assessment of antifungal activity of modified cellulose textile materials

The assessment of antifungal activity against *C. albicans* and *A. niger* was made according to Standard ASTM: E2149-01 "Standard Test Method for Determining the Antimicrobial Activity of an Immobilised Antimicrobial Agent under Dynamic Contact Conditions" – Shaking Flask Method.

Analysis of morphology structure by SEM method

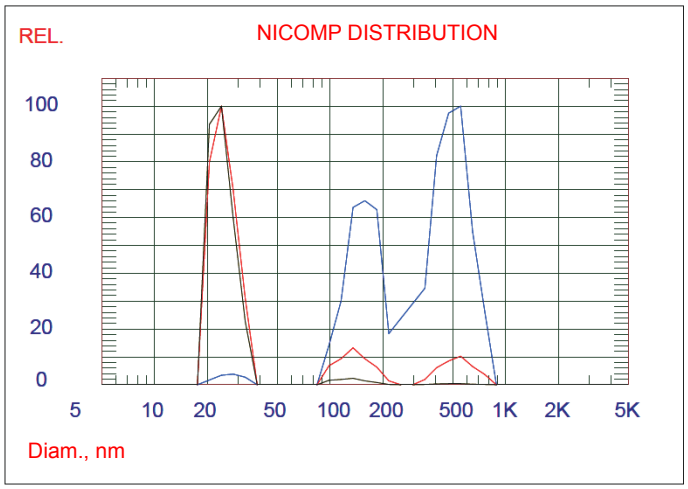
The surface and cross-section of the starting and modified cellulose materials were assessed at the Department of Scanning Electron Microscopy of IBWCh with the use of an electron scanning microscope - SEM Quanta 200, FEI Co., USA)

Resarch results and discussion

Method to prepare nanoparticles of microcrystalline chitosan (MCCh) and complex chitosan/alginate (chit/alg)

MCCh

An ultrasonic reactor - Hielscher UP 200S equipped with a S14L2D sonotrode was used to prepare MCCh charectised by a particle size below 1 μm. Chitosan lactate was prepared by dissolving virgin chitosan in 0.08 wt% lactic acid. A peristaltic pump continuously fed 0.1 wt% aqueous sodium hydroxide to a solution of 0.2 wt% chitosan lactate which was subjected to ultrasounds at 52.5 W/cm^2 acoustic density and at a maximal immersion of the sonotrode - 40 mm. The coagulation of chitosan lactate was con-



Intensity weighting:

	Peak 1	Peak 2	Peak 3
Mean diam., nm	26.9	158.2	528.7
Percent, %	2.2	38.9	58.9

Volume weighting:

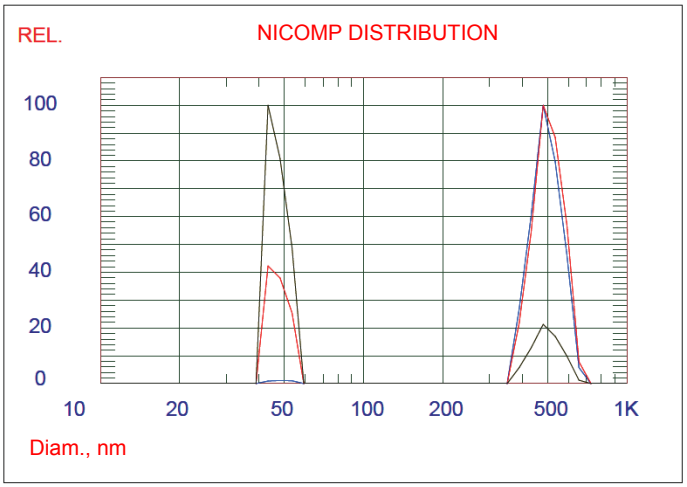
	Peak 1	Peak 2	Peak 3
Mean diam., nm	25.0	137.3	548.7
Percent, %	81.0	10.7	8.3

Number weighting:

	Peak 1	Peak 2	Peak 3
Mean diam., nm	24.5	132.1	528.7
Percent, %	97.3	2.3	0.4

Intensity wt: —— ***Volume wt:*** —— ***Number wt:*** ——

Figure 2. *Assessment of MCCh particle size in suspension.*



Intensity weighting:

	Peak 1	Peak 2	Peak 3
Mean diam., nm	48.4	493.6	-
Percent, %	1.1	98.9	-

Volume weighting:

	Peak 1	Peak 2	Peak 3
Mean diam., nm	47.4	500.8	-
Percent, %	29.7	70.3	-

Number weighting:

	Peak 1	Peak 2	Peak 3
Mean diam., nm	47.1	493.6	-
Percent, %	82.4	17.6	-

Intensity wt: —— ***Volume wt:*** —— ***Number wt:*** ——

Figure 3. *Assessment of complex chit/alg/Na/Ca's particle size in suspension.*

tinued at 20 - 30 °C directly in the stream of ultrasounds until the pH of the suspension reached the level of 6.7 - 6.8. After the coagulation was complete, the suspension of the polymer prepared was exposed to the further action of ultrasounds at an acoustic density of 30 - 52.5 W/cm^2 for 20 minutes. A suspension of MCCh nanoparticles at a concentration of 0.14% was obtained.

Complex chit/alg

A 0.13 wt% aqueous solution of chitosan lactate with the addition of calcium ions in the amount of 5.2 mg on 100 g of the chitosan solution, and a 0.045 wt% alkaline solution of sodium alginate were used to prepare a suspension of complex chit/alg/. A coagulation process of the solution of chitosan lactate with the addition of calcium ions by means of an alkaline solution of sodium alginate was conducted in the same way as in the process of preparing chitosan nanoparticles. Measured by the ninhydrin method, the content of chitosan in the complex was 90%.

Solid particle distribution in the biopolymers prepared was analysed by use of the Dynamic light scattering (DLS) technique. The results obtained are shown in ***Figures 2 - 3***.

The analysis has shown that the particles of both MCCh and complex chit/alg are of a size below 1 micrometer. 3 fractions were determined in MCCh, varied in relation to the particle size and percentage content; in the chit/alg complex 2 fractions were considered. In MCCh, particles of size ca. 25 nm had the highest percentage, reaching 97%, while in the complex chit/alg, 47 nm particles made up a majority of 82%.

The results obtained confirm the suitability of the method with the use of an ultrasonic reactor for the preparation of

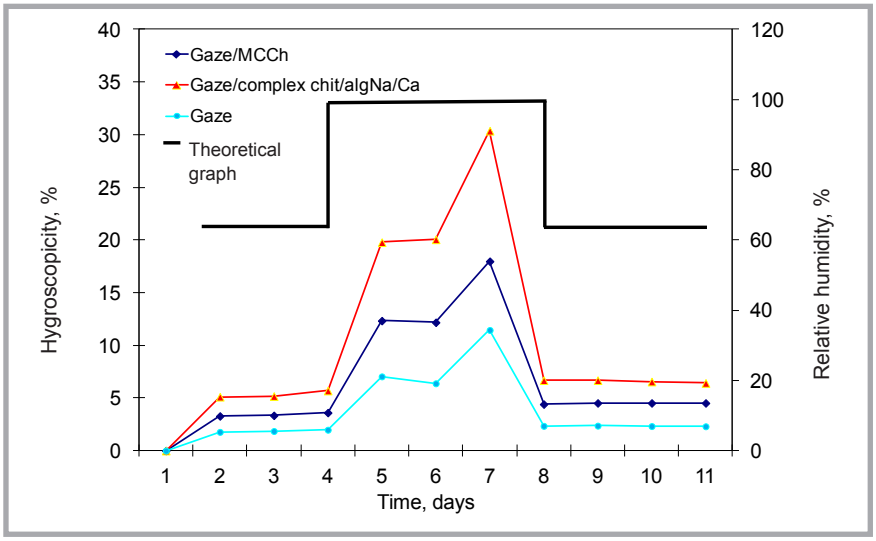


Figure 4. *Sorption and desorption of moisture by the gauze (surface density of 23.5 g/m²) before and after modification with nanoparticles of MCCh and complex chit/algNa/Ca at 65% and 100% relative humidity of air.*

MCCh and complex chit/alg with a particle size in the nano-range.

Assessment of the impact of the modification of cellulose products with bioactive polysaccharide nanoparticles upon the moisture absorption ability

Commercial cellulosic material - gauze with a 23.5 g/m² surface density was used in the testing. It was modified with an aqueous suspension of MCCh at a concentration of about 0.15 wt%, and with complex chi/alg/ at a concentration of 0.11 wt% with the addition of a plasticiser (glycerol) at a weight proportion of 1 part per 0.5 parts of the polymer (dry).

The suspension of polymer nanoparticles was sprayed onto the cellulosic material with the use of an areograph. After coating with either MCCh or complex chit/alg/, the material was dried at 35 °C. The coating intensity was about 25 wt% (coat/basic material). The materials prepared were examined in respect of the sorption and desorption of moisture in proportion to the starting material. An examination was made in accordance with IBWCh's own method at a relative humidity of 65 ± 4% and 100% and temperature of 20 ± 2 °C. The results obtained are presented by the graph in ***Figure 4***, giving the relationship between the water content in the product and the relative humidity of the environment.

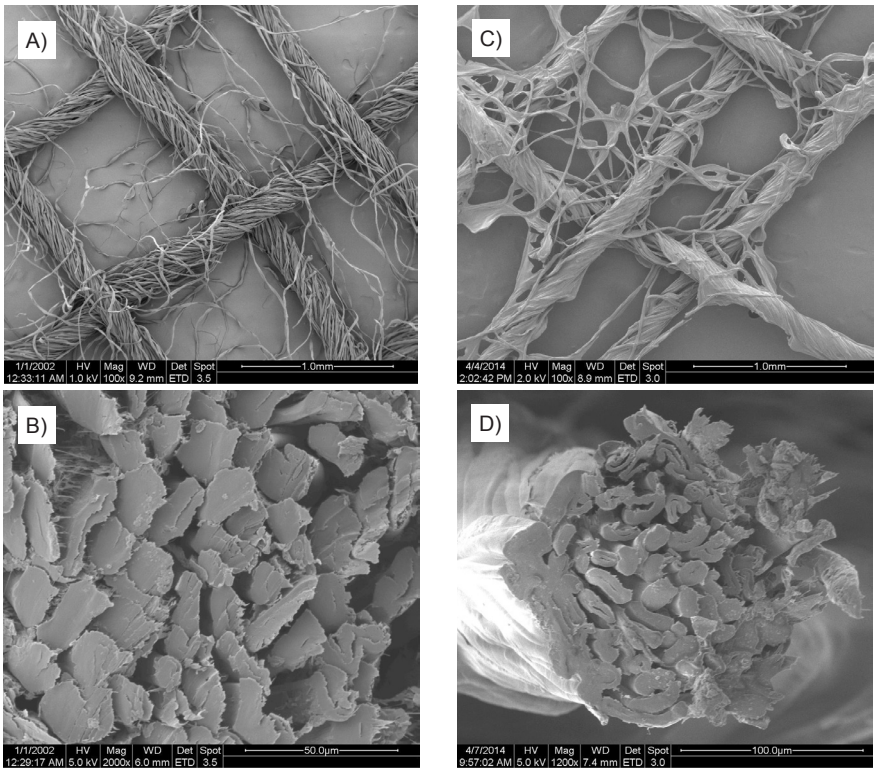


Figure 5. *Structure of the surface and cross section of the gauze before and after modification with nanoparticles of MCCh employing a classical drying method: A) structure of surface of unmodified gauze, mag. 100×, B) structure of cross-section of unmodified gauze, mag. 2000×, C) structure of surface of modified gauze (nano-MCCh), mag. 100×, D) structure of cross-section of modified gauze (nano-MCCh), mag. 1200×.*

The moisture sorption amounts to about 28% and 29% for the material modified with MCCh and complex Chit/alg, respectively, which makes an increase of merely 10% in comparison to the unmodified material. The low concentration of the polymer nano-particle-containing suspension used in the modification, and selection of the coating and drying methods are perceived as the implicit reasons for the low increase in sorption.

Structural examination of the dressing by SEM revealed that the coating by aerograph spraying combined with classical drying produced on the fibre surface an active layer of dense structure with a poorly developed internal surface (***Figure 5.C - 5.D***), which did not permit to effectively improve the water imbibition.

It was therefore decided to increase the polymer concentration in the coating liquid, to adopt padding instead of spraying and freeze-drying to replace conventional drying. Investigations were made with a cellulosic gauze (Scel) with a surface density of 200 g/m². It was modified with a aqueous suspension of MCCh and of complex chit/alg/ at a concentration of 0.5 to 0.8 wt% with the addition of a plasticiser (glycerol) in the amount of 1wt. part per 0.5 wt. parts of the polymer (dry). A coating with the suspension of polymer nanoparticles was accomplished by padding, followed by freeze-drying by means of a lyophiliser - ALFA 2-4 LD Plus by Martin Christ GmbH, Germany. Conditions of the lyophilisation: temperature range from (-20) to 10 °C,vacuum from 10 to 70 Pa, time - 24 hours. The coating intensity was, as in the preceding trials, about 25 wt% (coat/basic material). The modified cellulose materials were examined in respect of moisture sorption-desorption in comparison to the

starting one. Results of the investigation are presented in ***Figure 6***.

The results of the investigation indicate a six times higher humidity sorption in the cellulosic material modified according to the latter method than in the starting commercial material. The adoption of freeze-drying resulted in a development of the internal surface of the polymer layer (***Figure 7,C - 7.F***) and, in turn, in a much enhanced sorption of the dressing. It is a positive quality from the patient's point of view since the higher imbibition of wound exudates prevents the retention of liquid under the dressing material, thus protecting the skin from serious damage.

The method elaborated for the modification of cellulosic materials with nanoparticles of selected polymers opens a way for the preparation of dressing material with a soft, elastic, quasi-fibrous structure (***Figures 7.C & 7.E***) characterised by the increased sorption and desorption of humidity. Moreover the product modified with complex chit/alg permits a faster control of bleeding. The form of polymer selected, which, under the influence of moisture, reveals an ability to transform into a gel form, prevents to-wound-sticking, thus permitting the painless change of the dressing

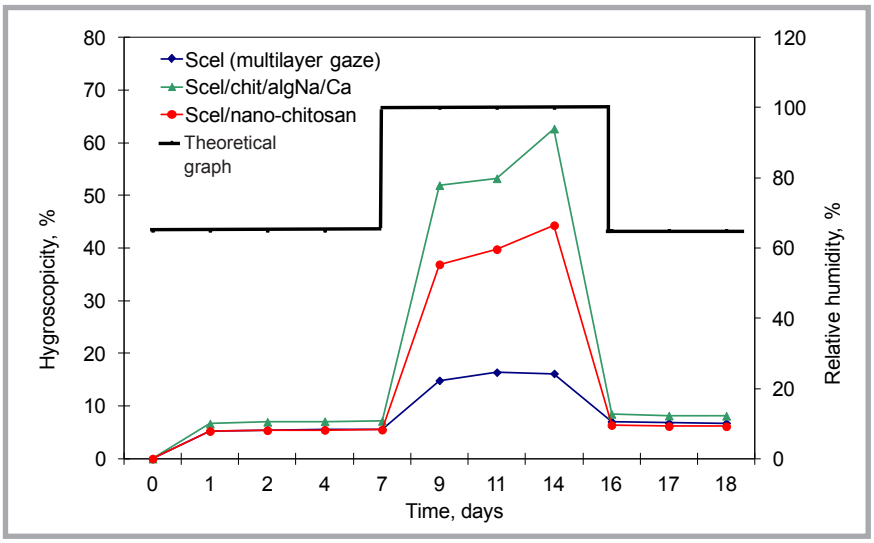


Figure 6. *Sorption and desorption of moisture in the multilayer gauze (surface density of 200 g/m^2) before and after modification with nanoparticles of MCCh and complex chit/algNa/Ca at 65% and 100% relative humidity of air.*

Assesment of the antibacterial and antifungal activity of modified cellulose textile products

Gauze with a 200 g/m^2 surface density modified with MCCh- and complex chit/alg nanoparticles was examined in respect of antibacterial properties. Gram negative and gram positive bacteria, as well as fungi were included in the testing. Antibacterial properties were tested *in vitro* in accordance with Japanese Standard JIS L 1902:2002, while the quality method Standard SN 195921:1994 constituted the basis of antifungal activity

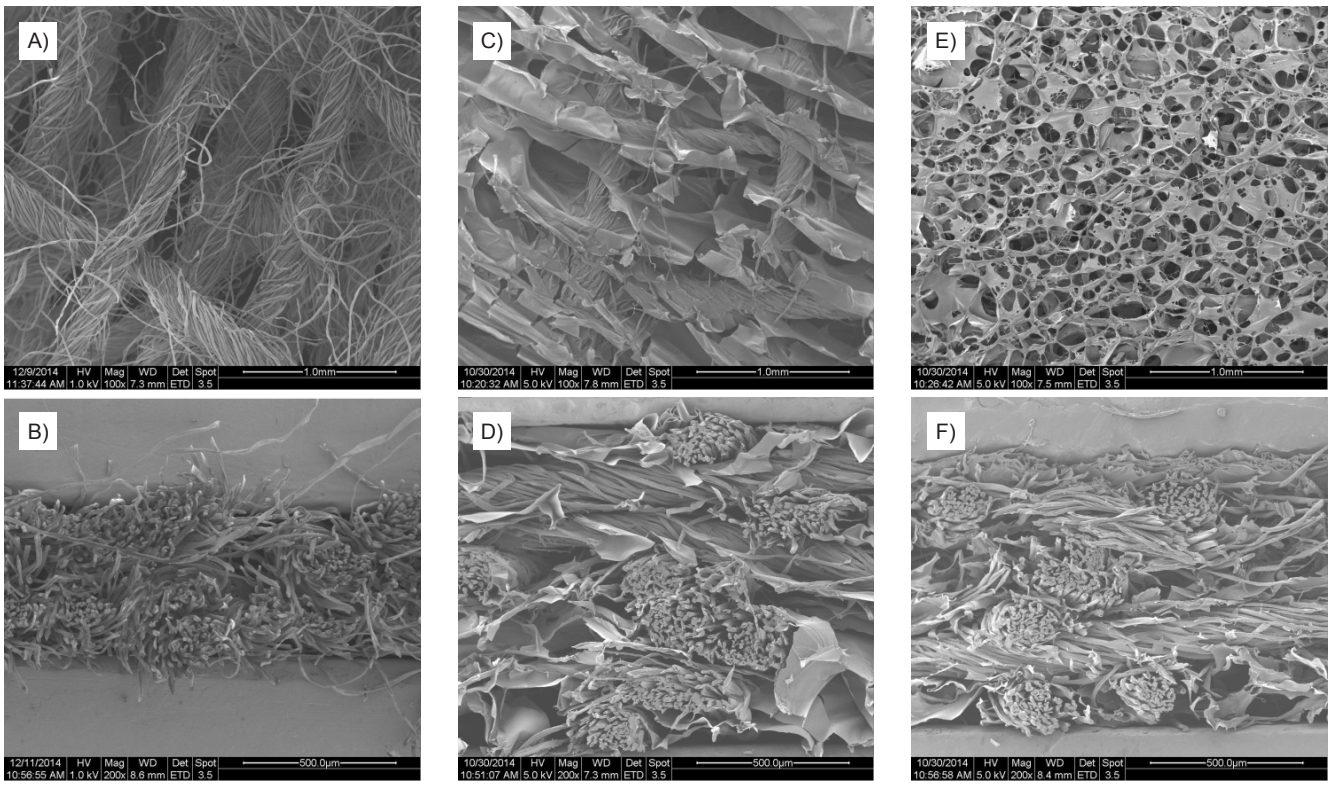


Figure 7. *Structure of surface and cross-section of cellulosic gauze modified with nano-polymers prepared with the application of freeze-drying: A) structure of surface of unmodified Scel, mag. 100×, B) structure of cross-section of unmodified Scel, mag 200×, C) structure of surface of Scel modified with nano-MCCh, mag. 100×, D) structure of cross-section of Scel modified with nano-MCCh, mag. 200×, E) Structure of surface of Scel modified with nano-complex chit/algNa/Ca, mag. 100×, F) structure of cross-section of Scel modified with nano-complex chit/algNa/Ca, mag. 200×.*

Table 1. *Estimation of antibacterial activity of modified cellulose textile products against Escherichia coli.*

Sample	Time, h	No. of bacteria, CFU/sample	Bacteriostatic activity against	Bactericidal activity against	Value of increase
Reference	0	3.1×10^4	-	-	-
Reference	24	1.4×10^8	-	-	3.7
SCel/chit	24	1.9×10^4	3.9	0.2	-
Reference	0	6.6×10^4	-	-	-
Reference	24	1.4×10^8	-	-	3.3
Scel/chit/alg Na/Ca	24	8.9×10^4	3.2	-0.1	-

Table 2. *Estimation of antibacterial activity of modified cellulose textile products against Staphylococcus aureus.*

Sample	Time, h	No. of bacteria, CFU/sample	Bacteriostatic activity against	Bactericidal activity against	Value of increase
Reference	0	3.7×10^4	-	-	-
Reference	24	7.3×10^6	-	-	2.3
Scel/chit	24	6.0×10^1	5.1	2.8	-
Reference	0	6.8×10^4	-	-	-
Reference	24	6.1×10^6	-	-	2.0
Scel/chit/alg Na/Ca	24	5.7×10^2	4.0	2.0	-

Table 3. *Estimation of antibfungal activity of modified cellulose textile products against Aspergillus niger.*

Sample	Repetition	Measured after 24 h of incubation		
		CFU/ml	Decrease, %	Average decrease
Reference	I	4.4×10^4	-	-
	II	7.3×10^4	-	
Scel/chit	I	6.0×10^3	98.7	98.0
	II	6.0×10^3	97.3	
Reference	I	8.0×10^3	-	-
	II	5.5×10^3	-	
Scel/chit/alg Na/Ca	I	3.1×10^2	95.5	92.6
	II	7.1×10^2	89.6	

Table 4. *Estimation of antibfungal activity of modified cellulose textile products against Candida albicans.*

Sample	Repetition	Measured after 24 h of incubation		
		CFU/ml	Decrease, %	Average decrease
Reference	I	$1.4x10^5$	-	-
	II	$1.1x10^5$	-	
Scel/chit	I	<1	100	100
	II	<1	100	
Reference	I	$6.3x10^4$	-	-
	II	$2.9x10^4$	-	
Scel/chit/alg Na/Ca	I	$8.5x10^1$	99.8	99.9
	II	$1.4x10^1$	100	

examination. The results obtained are presented in ***Tables 1 - 4.***

The investigations have shown that commercial dressing gauze when modified with nano-particles of MCCh and complex chit/alg reveal bactericidal activity against bacteria gram (+) *S. aureus.* Both modifications show excellent bacteriostatic action against both bacteria gram (-) *E. coli* and gram (+) *S. aureus.*

The modified cellulosic textile products also present very good antifungal properties against *Aspergillus niger* and *Candida albicans.* Selected pathogens were reduced by both modified products to the extent of 93 to 100%.

Investigations aimed at explaining the interaction that proceeds between the nanoparticles of polysaccharides and cellulose fibres, and the release of functional nanoparticles from the modified fibres were also carried out within the scope of the research. Furthermore the durability of the composite materials prepared was also examined by the method of accelerated ageing. Results of these investigations will be presented in the next article.

Conclusions

1. The method for producing microcrystalline chitosan and complex chit/alg in a stream of ultrasounds with a density of the acoustic strength amounting to 52.5 W/m^2 permits to prepare biopolymers in a nano-range of dimensions.
2. The size of nano-particles of microcrystalline chitosan falls in the range of 24.5 – 550 nm, with about 90% of the total amount of nanoparticles being about 25 nm in size.
3. The size of nano-particles of complex chit/alg fall in the range of 47 - 500 nm; particles with a size of about 47 nm make up a majority of about 82%.
4. Modification of cellulose dressing gauze with nanoparticles of microcrystalline chitosan and complex chit/alg/ with the application of freeze-drying permitted the preparation of a dressing material with water imbibition 6 times higher than in the starting commercial material.
5. Harnessing freeze-drying to the process instead of conventional drying led to a dramatic improvement in the modified material's absorption capacity. That advantage of freeze-drying outweighs the disadvantage of a longer process time and increased costs. However, in case of commercialization of manufacturing the wound dressings, the transfer from laboratory scale to technical production will significantly reduce the cost of the production process.
6. The modified cellulose textile products prepared exhibit excellent bacteriostatic action against bacteria gram (-) *Escherichia coli* and gram (+) *Staphylococcus aureus,* as well as bactericidal action against bacteria *Staphylococcus aureus.*
7. The modified cellulose textile products exhibit excellent antifungal activity against fungi *Candida albicans* and *Aspergillus niger*, which were reduced by nearly 100%.

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