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Chitosan-Modified Cellulosic Nonwoven for Application in Gynecology. Impact of the Modification Upon Chemical Purity, Structure and Antibacterial Properties

DOI: 10.5604/12303666.1226224

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Abstract
Physical-chemical, morphological and physical-mechanical characterization was made for cellulose nonwoven modified with chitosan nanoparticles with a view to their possible use in medicine as gynecological tampons. It was an aim of the work to assess the impact of the addition of chitosan nanoparticles upon the biological activity and toxicity of the materials prepared. Methodology was prepared for the examination of the gynecological devices in the range of their useful properties, notably the mechanical strength, surface density and absorption. Aqueous extracts were examined after an extraction process that simulated standard use of the medical device, and after a surplus extraction. The content of water-soluble-, surfactant- and reductive substances was estimated as well as the contents of heavy metals like cadmium, lead, zinc and mercury by the ASA method. Morphology examination permitted to assess the impact of the extraction processes on the fibre structure. Antibacterial activity against Escherichia coli and Staphylococcus aureus, and antifungal activity against Candida albicans was measured. Altogether examinations were made to assess whether the cellulosic nonwoven modified with chitosan nanoparticles meets the demands of medical devices and lends itself to the manufacture of tampons.

Key words: *biomedical polymers, medical tampons, chitosan, cellulose, chemical purity, medical devices.*

Introduction

Natural polymers like cellulose, starch and chitosan find use in pharmacy and medicine due to their desired properties like biocompatibility, lack of toxicity and allergenic action [1, 2]. Prepared from natural polymers are materials that mimic the extracellular matrix; they reveal a soft and strong but also elastic structure which provides mechanical stability to tissue and organs [3, 4]. The availability and low price is are the economic assets of natural polymers. Environment protection issues, now strongly pronounced by the European Union, also speak for the use of natural polymers, which are seen as environmentally-friendly. In general, natural polymers enjoy a growing interest. They are primarily used in modern medical devices contributing to advanced healing procedures.

Chitosan counts as a polymer and is in abundance in nature, revealing beneficial biomedical properties like antibacterial activity against *Escherichia coli* and *Staphylococcus aureus,* which is primarily responsible for septic shock [5 - 7]. It also displays activity against the fungi *Candidia albicans,* which often produces vaginal candidiasis [8, 9], and antiviral action, for example against the human papilloma virus (HPV), which is the cause of cervical carcinoma [9 - 11]. Thanks to its ability of controlled slow-release, chitosan is often used as carrier of active substances. Various sterilization methods can be employed for chitosan without upsetting its structure and physical-chemical properties[12]. Its beneficial properties ensure chitosan is widely used in pharmacy and medicine as a safe, non-toxic polymer originating from nature [12, 13]. Both natural and synthetic polymers like PLA, poly(DL-lactide-co-glycolide) and PP are often modified with chitosan [14 - 17]. The process is intended to prepare new materials with beneficial biological, physical-chemical and mechanical properties [17 - 19]. The modified composite materials obtained open wide avenues of application and contribute to the introduction of new healing techniques. The incorporation of chitosan into the cellulose matrix yields devices with high biological activity and good mechanical strength [12].

The medical devices have potential use in gynecology as medical materials with beneficial biological and mechanical properties. Basic cellulose material with built-in chitosan nanoparticles provides optimal and controlled diffusion of chitosan to the mucous membrane of the vagina and ovarium. Tampons holding antimicrobial chitosan particles may also find use as post-operation dressings and in the healing of diseases and infections due to high antimicrobial activity; alternatively they may be employed for carrying active substances. This is crucial because infections and gynecological ailments are problems women are mostly plagued with. More than 40 microorganisms are the reason for infections in the region of female sexual organs. The copolymer system proposed also permits to minimalise irritation due to the adjustment of chitosan concentration as the active agent. It is also hoped that the more developed surface of the biopolymer material shall provide better contact with the vagina and neck of the uterus, thus enhancing the antimicrobial effect. The use of modern biopolymer medical devices opens new ways in medicine.

Assessment of the impact of chitosan nanoparticles added to a cellulose matrix upon biological activity and toxicity was the aim of the research conducted. Structural examinations and estimation of chemical purity, as well as antibacterial and useful properties were made. Prepared was a system to control the medical device in respect of microorganism growth and fulfilment of quality requirements of medical devices [20, 21].

This article presents preliminary studies on the assessment of the effect of modification of cellulose nonwoven with nanoparticles of chitosan upon biological activity, toxicology and mechanical properties. The addition of nanochitosan was supposed to influence antimicrobial properties, however it was necessary to verify the correlation between the concentration of chitosan and the activity. A very important purpose was to examine how the addition of chitosan would influence the mechanical properties and chemical purity. It is necessary to guarantee optimum activities and the safety of human life and health simultaneously. Therefore according to the standards and scientific literature available, research methods were selected and used to assess cellulose nonwovens for their possible use in medicine as gynecological tampons.

Materials and methods

This article is only a part of research conducted within the framework of an international project realised by a Polish consortium member, the Institute of Biopolymers and Chemical Fibres. The aim of the article is to present how to interpret the results obtained. The technological development of cellulose nonwoven modified with chitosan nanoparticles was achieved by a Slovenian consortium member, which was the main coordinator at the University of Maribor, Slovenia, Department of Mechanics.

Cellulosic nonwovens of about 2.4 - 2.8 mm thickness and 40 × 300 mm dimensions with built-in chitosan nanoparticles (sized about 100 nm) were used in the investigations

The nonwovens were prepared at the University of Maribor, Slovenia, Department of Mechanics.

Aqueous chitosan solution buffered with acetic acid was sprayed on cellulose fibres, from which the nonwovens were prepared. Nano-chitosan was added to the cellulosic matrix in three concentrations: 0.25, 0.5 and 1.4%.

Chitosan used in the modification was supplied by Mahtani Chitosan PVT.LTD CO, India, characterized by an average molecular mass of 780 kD aand deacetylation degree amounting to 92%. Cellulosic fibres to prepare the nonwoven were characterized by a fibre diameter of about 20 μm, fibre length of about 30 mm, linear mass of 3 dtex, and tenacity of about 20 cN/tex.

The non-sterile textiles prepared underwent structural investigation, and assessment of the chemical purity, physical-mechanical properties, physical–chemical parameters and antimicrobial activity. A physical-chemical investigation was also made for the initial materials (chitosan and cellulosic fibres) and commercial hygiene materials used as reference.

The reference materials selected were commercially available cellulose hygiene products - tampons, unmodified with active substances, and therefore it was used only in research of chemical purity. Because of the absence the antimicrobial agent in the reference materials, microbiological tests were unnecessary. Commercially available medical materials are probiotic tampons containing lactic bacteria, but because of the content the microorganisms their use in the microbiological testing would be wrong. Also the form of reference materials differed from that of cellulose nonwovens modified with chitosan nanoparticles, and comparing them in terms of mechanical tests would be wrong.

Assessment of antimicrobial acivity[1]

Antibacterial activity of the medical devices prepared was estimated according to internal Examination Procedure No.1*"Examination of the antimicrobial activity of textiles. Quantitative test"* prepared on the basis of Standard JIS L 1902: 2002. The procedure concerns activity against *Escherichia coli* (ATCC 11229) and *Staphylococcus aureus* (ATCC 6538) [22]. The method of assessing antibacterial activity consists in the inoculation of the sterilized test samples prepared and cotton without antibacterial additives as reference samples The samples are incubated at 37 °C for 24 h. After the incubation, bacteria were washed out with normal saline with the addition of the Tween 80 agent. Adequate solutions were then prepared from the bacteria suspension. Culture plunge was then made on agar plates. After the incubation, colonies were counted, and the number of bacteria and antibacterial activity were calculated on each of the plates.

Antifungal activity was also tested by the Shaking method according to Standard ASTM: E2149-01 [23]. Fungi *Candida albicans* (ATCC 10259) cultured by the reductive method on SDA culture (30 °C, 48 h) were used in the tests. The samples tested were shaken at 37 ± 1 °C for 24 hours in a buffer containing 9.15×10^4 CFU/ml of the *C. albicans* strain. The amount of cells was estimated by the method of decimal dilutions and culture plunge on SDA agar.

Physical-mechanical examination[2]

Physical-mechanical testing was accomplished at standard climatic conditions: temperature of 20 ± 2 °C and relative humidity of 65 ± 4% in accordance with Standard PN-EN ISO 139:2006 [24]. The following parameters were examined:

- Linear mass of fibre (PN-EN ISO 1973:2011p. 9.1) [25]
- Tenacity of fibre (PN-EN ISO 5079:1999) [26]
- Surface density of nonwoven (PN-EN 29073-1:1994) [27]
- Thickness of nonwoven (PN-EN ISO 9073-2:2002) by a mechanical thickness gauge - Tilmet-64 (Lodz University of Technology, Poland)[28]
- Force and elongation at break (PN-EN 29073-3:1994) by the use of a testing machine - Model 5544 (Instron, UK) [29].

Morphology investigation

The composite materials were SEM-tested by means of a microscope - Quanta 200 (FEI Co, USA) Samples powdered with a 20 nm layer of gold were tested in a high vacuum at an electron-beam-accelerating voltage of 5 KV.

Physical-chemical examination

Water absorption of the samples tested was determined according to PN-P -04781/12:1989 [30]. The method was based on the determined amount of water absorbed through the medical materials in conditions defined according to the standard. The average molecular mass of chitosan was measured by the viscometric method based on the terminal (limited) number of viscosity [η] [31, 32]. The deacetylation degree was determined by the first differential of UV-spectrum method [33]. The content of chitosan in cellulose was estimated by the ninidrine method with the use of a Unicam 5625 UV/VIS Spectrophotometer (ATI Unicam, UK) [34].

Chemical purity

Extraction of chemical substances

Simulating delivery in standard application, the dynamic extraction method was employed according to PN-EN ISO 10993-12:2009 [35], and to requirements of USP 38 NF 33 [20]. Water for injection was used as an extractant. The process was conducted in a shaker with a thermostat (Water Bath SW 23 by Julabo Co, Germany) at a temperature of 37 ± 0.1 °C for 72 ± 2 h. The samples tested also underwent surplus extraction to increase the delivery of chemical components as a comparison to the amount delivered at simulated conditions according to PN-EN ISO 10993-12:2009 [35]. The process was carried out in an autoclave (SMS, Poland) at 121 ± 1 °C for 1 ± 0.1 h.

The method of extraction and conditions of this process: temperature and time, were rigidly described in Standard PN-EN ISO 10993-12, section 10.3. They were selected in order to guarantee the measurement of possibilities risks of use in medicine as gynecological tampons.

The following parameters of chemical purity were estimated in three parallel tests: pH, turbidity, content of foaming agents, content of water-soluble substance, content of heavy metals, and permanganate oxidation.

The pH was measured by means of a pH-meter (Schott Instruments, Germany) and Blue Line 14 pH electrodes at ambient temperature (Schott Instruments, Germany) according to Standard PN-EN ISO 3071:2007 [36].

Turbidity was estimated by the turbidimetric method according to European Pharmacopoeia 8.0 by measuring scattered light with the use of a Unicam 5625 UV/VIS Spectrophotometer (ATI Unicam, UK). A suspension of formazine was used as the basic reference standard equal to 4000 NTU; it is a blend of hydrazine sulfate and hexamethylenetetramine (urotropine) [21].

Estimation of the content of foaming agents was based on Standard PN-P-04781/14:1989 [37]. The method of detecting foaming agents in aqueous extracts consists in measuring the height of the froth after shaking of the sample. The appearance of, at least, one single full circle of air bubbles around the wall of the test tube is evidence of the presence of foaming agents.

The content of water-soluble substance was determined according to PN-P-0478/06:1988 [38]. The amount of dry residue in the water extract is estimated in this method. The residue is the sum of water-soluble and insoluble matter (turbid extracts) after water evaporated in determined conditions according to the standard.

The content of heavy metal ions was determined by Atomic Absorption Spectrometry using a SCAN-1 spectrometer (Thermo Jarrell ASH Co., USA)[3]. Cd, Cr, Pb and Zn were directly determined in aqueous extracts by the flame method ASA (FAAS) at the following parameters:

- Cd: wave length λ = 228.8 nm, flame – acetylene-air, limit of detection - 0.02 mg/dm^3
- Cr: wave length λ = 357.9 nm, flame – acetylene N_2O, limit of detection - 0.2 mg/dm^3
- Pb: wave length λ = 217.0 nm, flame – acetylene-air, limit of detection - 0.2 mg/dm^3
- Zn wave length λ = 213.9 nm, flame – acetylene-air, limit of detection - 0.01 mg/dm^3

Mercury was determined by the method of cold vapour atomic spectroscopy ASA (CVAAS) using an Atomic Vapor Accessory 440 (Thermo Jawell ASH, USA) at the following parameters: wave length- λ = 253.7 nm, reductive solution - 5% $SnCl_2$ in 20% HCl, carrier gas-Ar, limit of detection - 0.01 mg/dm^3 [39].

Determination of permanganate oxidation was based on Standard PN-P-04896:1984. The method consists in determining the amount of potassium permanganate solution per milligram of oxygen spent to oxidize organic matter and certain inorganic compounds. These compounds were eluted from the medical device during extraction [40].

Results and discussion

Assessment of antimicrobial activity

Cellulosic fibres and the chitosan-nanoparticles–modified nonwovens prepared thereof were examined in respect of their ability to slow down the growth of microorganisms, both Gram(+) and Gram(-), and their antifungal activity against *Candida albicans.* Results of the investigation are presented in ***Table 1.***

It was found that the starting cellulosic fibres are more active against *S. aureus* than against *E. coli.* The addition of chitosan with a content of 0.25% and 0.5% has limited the growth of *E. coli*, while the effect did not occur for *S. aureus.* Nonwoven with a 1.4% content of chitosan entirely stopped the growth of *E. coli* and *S. aureus,* meaning an activity of 100%.

After 24 hours of incubation, the virgin cellulose fibres revealed antifungal action against *C. albicans* on at the level of 91.7%; the addition of 0.5% of chitosan caused a complete reduction on microorganism growth. Bacteriostatic action at a level of 99.6% was observed with a 0.25% addition of chitosan.

Assessment of physical-mechanical parameters

Physical-mechanical parameters of the cellulose nonwovens modified with nanoparticles of chitosan were tested to assess their useful properties. Results are compiled in ***Table 2***.

Nonwoven with a 0.25% content of chitosan shows the lowest thickness and tenacity, while its elongation is the highest. In spite of its thickness bing comparable with the other versions, the material with 0.5% of chitozan reveals the highest

Table 1. *Antimicrobial activity against Escherichia coli and Staphylococcus aureus of the initial cellulosic fibres and chitosan-modified nonwovens .*

Symbol of sample	Bacteriostatic effect, % activity		Antifungal effect -reduction, %
	E. coli	*S. aureus*	*C. albicans*
Cellulose fibres	51.5	91.7	69.6
Nonwoven 1.4% chitosan	100	**100**	100
Nonwoven 0.5% chitosan	73.2	**69.8**	100
Nonwoven 0.25% chitosan	65.9	**67.9**	99.6

mass per unit area, witnessing high compactness of fibres in the fabric, which is advantageous in respect of useful properties since an increase in sorption/imbibition is expected along with increased surface density. The material also shows the highest strength both along and across the fabric. The testing disclosed remarkable differences in tenacity along (138, 262, 30.8 N) and across the fabrics (35.2, 56.4, 1.28 N), the alleged reason for which are differences in calendering in the course of preparing the nonwovens. Diameters of the fibres in the nonwovens are comparable. Based on the results of the physical–mechanical examinations it may be concluded that the nonwoven with a 0.5% content of chitosan presents the most favourable and useful mechanical properties.

Table 2. Physical-mechanical properties of the chitosan-modified nonwoven.

Parameter	Chitosan-modified nonwoven, %		
	1.4	0.5	0.25
Thickness,mm	**2.87** ± 0.11	**2.54** ± 0.19	**2.39** ± 0.32
Mass per unit area,g/m^2	**233** ± 11	**252** ± 6	**243** ± 14
Breaking load in longitudinal direction, N	**138** ± 79	**262** ± 32	**30.8** ± 10.4
Elongation at maximum force in longitudinal direction, %	**7.75** ± 1.14	**9.84** ± 2.52	**7.75** ± 1.10
Strength in longitudinal direction, MPa	**0.96** ± 0.55	**2.06** ± 0.25	**0.26** ± 0.09
Breaking load in cross direction, N	**35.2** ± 9.2	**56.4** ± 11.4	**1.28** ± 0.83
Elongationat max force, %	**5.79** ± 1.99	**5.77** ± 0.76	**29.2** ± 26.2
Strength in cross direction, MPa	**0.25** ± 0.06	**0.44** ± 0.09	**0.01** ± 0.01

Table 3. Physical properties of the chitosan-modified nonwovens.

Parameter	Cellulose fibres	Chitosan-modified nonwoven, %		
		1.4	0.5	0.25
Water absorption, g	20	11	15	18
Sinking speed,s	2.0	104.3	44.3	7.2
Water content,%	7.10	7.90	9.07	8.80

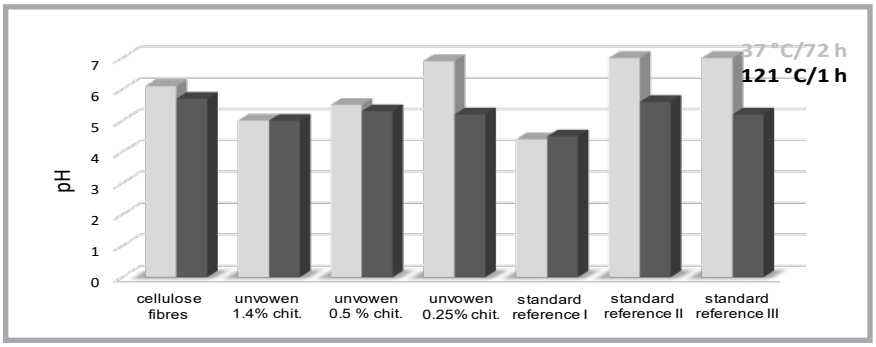

Figure 1. pH of aqueous extracts.

Physical examination

Samples of the nonwoven were put to physical testing to assess their useful properties, namely imbibition and absorption. Results are shown in ***Table 3***.

Nonwovens with a chitosan content of 0.25 & 0.5% show similar water imbibition: 18 and 15 g, respectively, resulting from the similar surface density of the two materials: 243 and 252 g/m^2 respectively. Much lower is the value (11) in the nonwoven with a chitosan content of 1.4%, resulting from the lower surface density (233 g/m^2), which means a lower compactness of fibres in the fabric.

Due to the loose structure, the initial cellulose fibres sink fastest (sinking time-2 seconds), while nonwoven with a 1.4% content of chitosan showed the longest sinking time of 104.3 seconds, the supposed result being higher thickness of the material and a higher content of chitosan. Very short (7.2) was the sinking time for the thinnest material with a 0.25% content of chitosan. The material with 0.5% chitosan sunk in about 44.3 seconds.

It is worth noting that the amount of chitosan added to the nonwoven distinctly influences the sinking time. The moisture content measured in the nonwovens with 0.25, 0.5 and 1.4% of chitosan amounts to 8.80, 9.07 and 7.90%, respectively.

Examination of chemical purity

Cellulose nonwoven modified with chitosan nanoparticles, the initial cellulose fibres and reference samples were examined in respect of chemical purity. The samples tested underwent extraction for 72 hours at 37 °C, which simulates the delivery of the substance in normal use of the device, and 1 hour surplus extraction at 121 °C in an aqueous medium according to Standard PN-EN ISO 10993-12:2009 [35]. The aqueous extracts obtained were then analysed to estimate the impurities which were leached in the process (4.1 - 4.6). Results of the chemical purity analysis are presented in ***Figures 1 - 6***.

pH of aqueous extracts

Values of pH fall into a permissible limit which was estimated by measuring the pH of the reference materials (***Figure 1***), the requirement of medical devices is thereby met in that respect. The measurement results obtained are in response to the parameters of bacteriostatic water for injection according to USP 38 NF 33, which was used in the preparation of the aqueous extracts [20]. Therefore it may be inferred that the use of such a product shall not cause irritation at the contact point and provide safe use.

Turbidity of aqueous extracts

Aqueous extracts prepared from the nonwoven with a chitosan content of 0.25 and 0.5% show turbidity at the level of that of the reference samples, thereby meeting the requirements of medical devices. On the other hand, the value is far exceeded in case of the nonwoven with a 1.4% content of chitosan (18.9 NTU), caused by leaching of the chitosan nanoparticles in the course of simulating extraction or it may be the result of cellulose delivery since the nonwoven is characterised by the highest thickness (about 2.9 mm) and loose compactness of fibres (surface density is at the level of 233 g/m^2). The solution prepared from cellulosic fibres also shows a high value of the parameter, which probably stems from the loose fibre structure in the material tested (***Figure 2***).

After the surplus extraction (at 121°C), turbidity increases for cellulose fi-

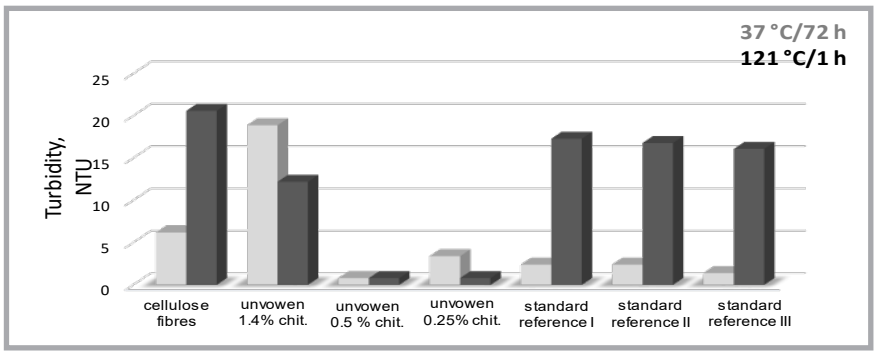

Figure 2. *Turbidity of aqueous extracts.*

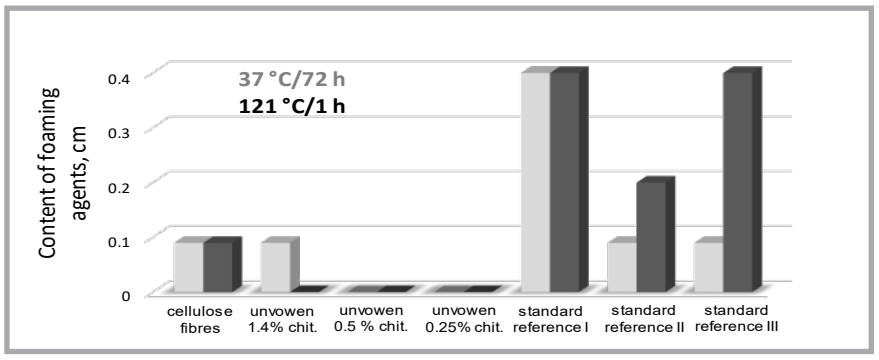

Figure 3. *Content of foaming agents in aqueous extracts.*

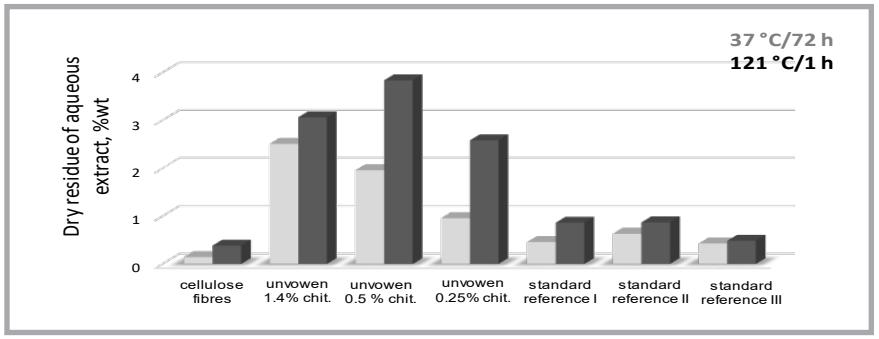

Figure 4. *Content of substance dissolved in aqueous extracts.*

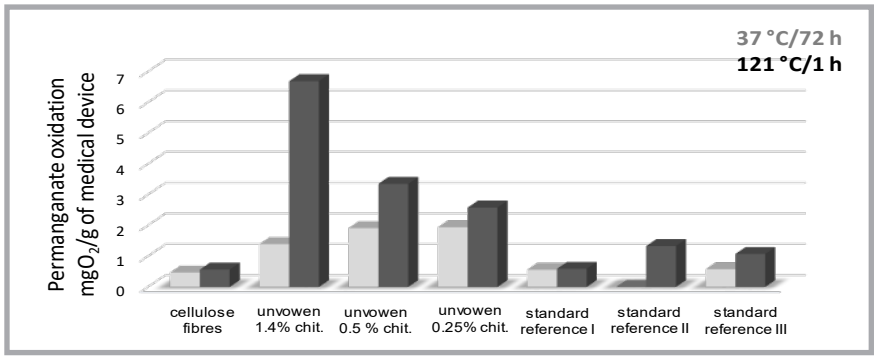

Figure 5. *Content of reductive substances in aqueous extracts.*

bres and reference materials, probably caused by the degradation of the material and delivery of both chitosan nanoparticles and cellulose to the extraction solution.

Content of foaming agents in aqueous extracts

The content of foaming agents in the nonwovens with a content of chitosan nanoparticles is much lower than in the reference materials (***Figure 3***).

No foaming agents were found in aqueous extracts prepared from the nonwovens with 0.5 and 0.25% of chitosan content. In the simulating extraction, the 1.4% chitosan-containing nonwoven delivers to the solution an amount of the foaming agents close to that of the reference material. Cellulosic fibres show a concentration of foaming agents similar to that of the reference materials; the surplus extraction does not generate any further amount of the agents. The low content or absence of the surfactants contributes to the positive assessment of chemical purity.

Content of substance dissolved in aqueous extracts

The amount of substance dissolved in water goes up along with increasing chitosan concentration (***Figure 4***) as a result of the delivery of chitosan nanoparticles to the aqueous medium in the course of extraction. It is advantageous since the chitosan delivered is expected to reveal many beneficial properties like antimicrobial- and anti-inflammatory action. In all materials tested the surplus extraction generates increased amounts of water-dissolved substance.

Content of reductive substances in aqueous extracts

Oxidizability is at a similar level for the initial fibres and reference samples. Under conditions that simulate normal use, the amount of substance which undergoes oxidation is higher in the chitosan-containing nonwoven in comparison to the reference samples (***Figure 5***). The delivery of chitosan from the samples tested in the course of extraction is the reason. The correlation between oxidizibility and the content of chitosan was not found in the nonwovens tested under conditions that simulate normal use. On the other hand, an increase could be observed in the oxidized substance with an increasing content of chitosan in the nonwovens tested.

The cellulose fibres tested show a content of reductive substance similar to that of the reference samples, the indicating suitability of the material for medical application.

Content of heavy metals

Toxicological assessment of the chitosan-modified textile materials examined was made by estimating the content of heavy metals in the aqueous extracts

Table 5. *Content of heavy metals in aqueous extracts from cellulose fibres, chitosan-modified nonwovens and reference samples.*

Temperature, °C	37	121	37	121	37	121	37	121	37	121
Symbol of sample	mg/100 cm³ of the aqueous extract									
	Cd		Cr		Pb		Zn		Hg	
Cellulose fibres	<0.002	<0.002	<0.02	<0.02	<0.02	<0.02	<0.002	0.007	<0.0001	<0.0001
Nonwoven 0.25% chitosan							<0.002	0.008		
Nonwoven 0.5% chitosan							0.002	0.018		
Nonwoven 1.4% chitosan							0.006	0.038		
Standard reference I	<0.002	<0.002	<0.02	<0.02	<0.02	<0.02	0.018	0.045	<0.0001	<0.0001
Standard reference II							<0.002	0.015		
Standard reference III							0,004	0,009		

by the method of Atomic Absorption Spectroscopy. Results are compiled in ***Table 5***.

The contents of Cd, Cr, Pb and Hg in the aqueous extracts from the chitosan–modified nonwovens fall below the limit of determination.

The zinc content in the nonwovens with 0.5 and 1.4% of chitosan is in the permissible limit found in the reference material. The content of heavy metals which could exert a hazardous impact upon organisms was not found in the materials tested, hence the medical devices tested may be regarded as safe.

It was demonstrated that the results of physical-chemical parameters attained: pH, turbidity, content of foaming agents, measured in the nonwoven with a content of chitosan nanoparticles fall into the permissible limits determined in the samples of reference materials.

On the other hand, much higher than in the reference is the content of water-soluble substance in the chitosan-containing nonwovens because the polymer is soluble in an aqueous medium. The increased chitosan content also causes an increase in the amount of oxidizable substance. The parameters related to the chemical purity of the nonwovens examined, and the limiting values of the reference materials are compiled in ***Table 6***.

Examination of morphology

The morphology of the chitosan-modified nonwovens and the initial cellulose fibres was examined by means of a scanning electron microscope - Quanta 200 (FEI. Co., USA). The fibre diameter was measured by the use of the software AnalySISDocuby Soft Imaging System (***Table 7***).

The diameter of the fibre goes up along with an increasing content of chitosan in the cellulose matrix, SEM analysis was made for the nonwovens after the simulation and surplus extractions to learn the impact of the extraction upon the fibre structure (***Figure 7***).

SEM investigation shows that the fibre structure in the nonwoven was not upset; an insignificant deformation can be seen in the form of twist. It may be inferred that the product will remain safe while in use (***Figure 7.B, 7.E*** & ***7.H***). On the other hand, surplus extraction performed in an autoclave (T = 121 °C) disturbs the fibre structure, causing micro-cracks and substantial deformation (***Figure 7.C, 7.F*** & ***7.I***); however, Standard PN-EN ISO 10993-12:2009 permits changes in the material tested [35].

Conclusions

Cellulose fibres used in the preparation of the nonwovens have good properties, both physical-mechanical and physical-chemical, including chemical purity.

The addition of chitosan in the amount of 0.25 and 0.5% to the nonwoven caused an inhibition of the growth of *E. coli,*

Table 6. *Results of determination of chemical purity in chitozan-modified nonwovens, and in the reference.*

Parameters		Temperature, °C	Symbol of sample			
			Chitosan-modified nonwoven, %			Reference
			0.25	0.5	1.4	
pH		37	6.9	5.5	5.0	**4.4 - 7.0**
		121	5.2	5.3	5.0	**4.5 - 5.6**
Turbidity, NTU		37	3.4	0.8	18.9	**2.4**
		121	0.8	0.8	12.2	**17.3**
Content of foaming agents, cm		37	0	0	0.09	**0.4**
		121	0	0	0	**0.4**
Content of dissolved substance, %		37	0.96	1.96	2.51	**0.63**
		121	2.58	3.83	3.6	**0.87**
Content of reductive substances,mgO_2/g		37	1.94	1.91	1.39	**0.57**
		121	2.58	3.35	6.69	**1.33**
Cd	mg/100 cm³ of aqueous extract	37	<0.002			**<0.002**
		121				
Cr		37	<0.02			**<0.02**
		121				
Pb		37	<0.02			**<0.02**
		121				
Zn		37	<0.002	0.002	0.006	**0.018**
		121	0.008	0.018	0.038	**0.045**
Hg		37	<0.0001			**<0.0001**
		121				

Table 7. *Thickness of initial cellulose fibres and fibres contained in the nonwoven.*

Parameter	Symbol of sample			
	Cellulose fibres	Fibres in nonwoven		
		1.4% chitosan	0.5% chitosan	0.25% chitosan
Thickness of fibre, μm	19.65	21.94	21.89	20.27

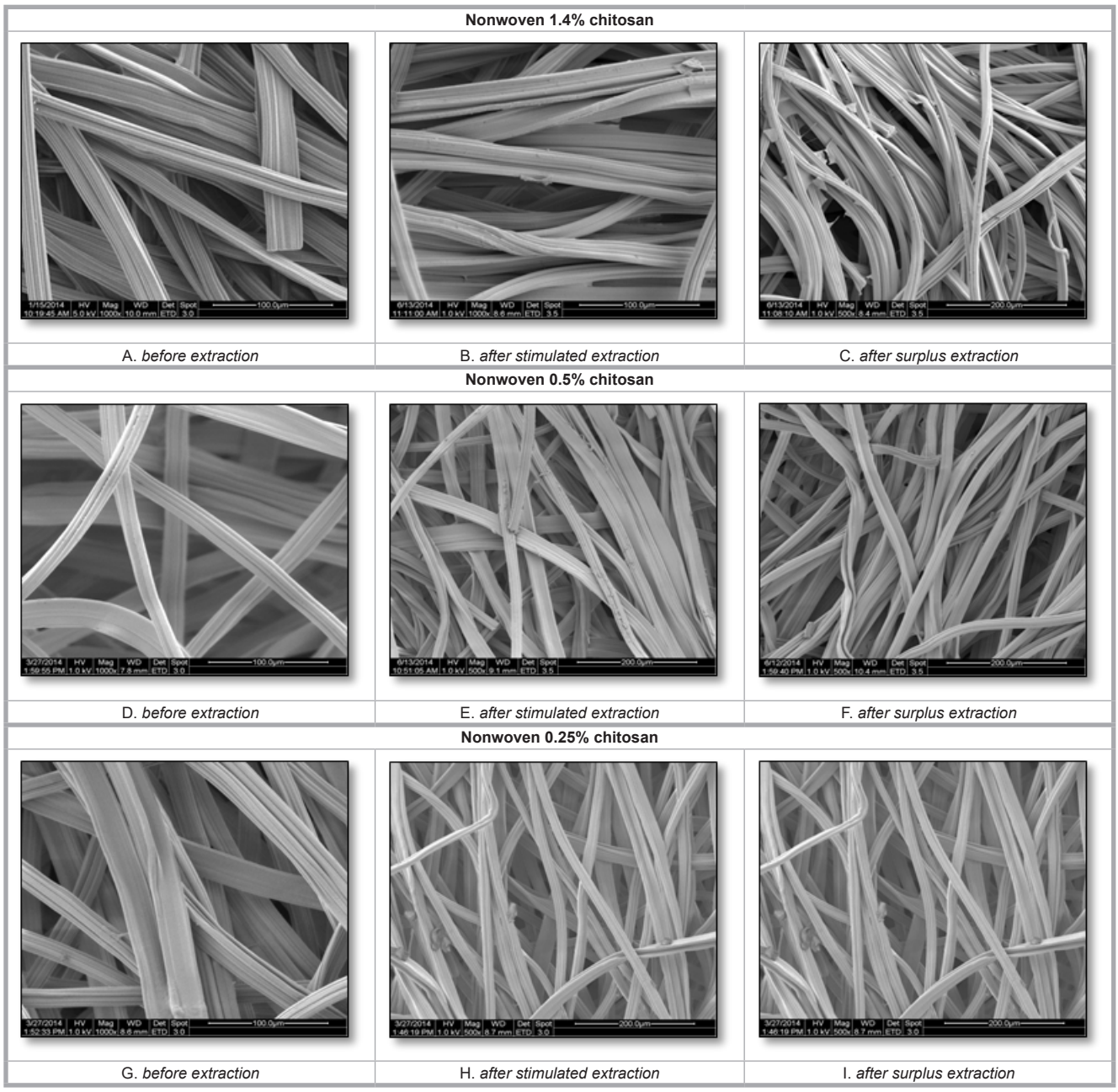

Figure 7. *Nonwovens before and after stimulated and surplus extraction; magnification 1000×.*

while such an effect was not observed with *S. aureus*. Nonwoven with a 1.4% content of chitosan completely stopped the growth of bacteria *E. coli* and *S. aureus*.

The initial material revealed antifungal activity against *C. albicans* on at the level of 91.7%; the addition of 0.5% of chitosan entirely inhibited the growth of microorganisms. Antifungal activity of the nonwoven with 0.25% of chitosan against *C. albicans* was slightly below 100%. Based on the results of physical-mechanical testing, it was found that the nonwoven with 0.5% of chitosan has the best mechanical and useful properties.

Assessment of the chemical purity of the materials tested points, at best, to useful properties in the case of nonwoven with 0.5% of chitosan.

The structure of the fibres examined after extraction simulating normal use was not disturbed, which may evidence safe use of the hygiene material manufactured.

Summarizing the results of the examinations, it may be concluded that the initial cellulose fibres are a good raw material for use in the preparation of innovative medical devices.

Acknowledgments

This work was completed at the Institute of Biopolymers and Chemical Fibres within the project "Nano-functional tampons for gynecological applications" Eureka E!5852NANOFUNTAMPONS/2013 under Era-Net, co-financed by The National Centre for Research and Development.

Editorial Note

1 *Examination was made in the accredited Microbiological Laboratory of IBWCh (Certificate of accreditation AB 388)*

2 *Examination was made in the accredi-*

ted Metrological Laboratory of IBWCh (Certificate of accreditation AB 388).

[3] *Examination was made in the accredited Laboratory of Paper Quality of IBWCh (Certificate of accreditation AB 065).*

References

1. Amidi M, Mastrobattista E, Jiskoot W and Hennink WE. Chitosan-based delivery systems for protein therapeutics and antigens. *Adv. Drug Deliv. Rev*. 2010; 62(1), 59-82.
2. Friess W. Collagen - biomaterial for drug delivery. *Eur. J. Pharm. Biopharm* 1998; 45, 2: 113-136.
3. Oledzka E, Sobczak M, Kołodziejski W. L.: Polymers in medicine - review of recent studies. Polimery 2007; 52,11-12: 793-916.
4. Stojadinovic A, Carlson J W, Schultz G S, Davis T A and El-ster E A. Topical advances in wound care. *Gynecol. Oncol*. 2008; 111, 2: 70-80.
5. Niekraszewicz A, Lebioda J, Kucharska M and Wesołowska E. Research into Developing Antibacterial. *Fibres and Textiles in Eastern Europe* 2007; 15, 1(60):101-105.
6. Brzoza-Malczewska K, Kucharska M, Wiśniewska-Wrona M, Guzińska K and Ulacha-Bacciarelli A. Modified Cellulose Products for Application in Hygiene and Dressing Materials Part I. *Fibres and Textiles in Eastern Europe* 2015; 23, 3(111): 126-132.
7. Brzoza-Malczewska K, Kucharska M, Wiśniewska-Wrona M, Kaźmierczak D, Jóźwicka J, Pałys B. Modified Cellulosic Products for Application in Hygiene and Dressing Materials - Part II. *Fibres and Textiles in Eastern Europe* 2015; 23, 5(113): 124-128. DOI: 10.5604/12303666.1161768
8. Seyfarth F, Schliemann S, Elsner P and Hipler UC. Antifungal effect of high- and low-molecular-weight chitosan hydrochloride, carboxymethyl chitosan, chitosan oligosaccharide and N-acetyl-d-glucosamine against Candida albicans, Candida krusei and Candida glabrata. *International Journal of Pharmaceutics* 2008; 353: 139-148.
9. Aksungur P, Sungur A, Ünal S, Iskit AB, Squier CA and Şenel S. Chitosan delivery systems for the treatment of oral mucositis: in vitro and in vivo studies. *Journal of Controlled Release* 2004; 98, 269-279.
10. Tahamtan A., Ghaemi A., Gorji A., Kalhor H.R., Sajadian A., Tabarraei A., Moradi A., Atyabi F., Kelishadi M.: Antitumor effect of therapeutic HPV DNA vaccines with chitosan-based nanodelivery systems. *Biomed Sci*. 2014, 21(1): 69.
11. Tahamtan A, Tabarraei A, Moradi A, Dinarvand M, Kelishadi M, Ghaemi A and Atyabi F. Chitosan nanoparticles as a potential nonviral gene delivery for HPV-16 E7 into mammalian cells. *Artif Cells Nanomed Biotechnol.* 2015, 43(6), 366-72.
12. Mucha M. *Chitosan-versatile polymer from renewable resources.* Wydawnictwa Naukowo-Techniczne, Warszawa, 2010, 7, 118-127.
13. Niekraszewicz A, Kucharska M, Wawro D, Struszczyk M H, Kopias K. Development of a Manufacturing Method for Surgical Meshes Modified by Chitosan. *Fibres and Textiles in Eastern Europe* 2007; 15, 3(62), 105-109.
14. Struszczyk MH, Ratajska M and Brzoza-Malczewska K. Films and Non-wovens Coated by Chitosan for Special Applications: Biological Decomposition Aspect. *Fibres andTextiles in Eastern Europe* 2007; 15, 2(61): 105-109.
15. Niekraszewicz A, Kucharska M, Wiśniewska-Wrona M and Kardas I. Biological and Physicochemical Study of the Implantation of a Modified Polyester Vascular Prosthesis. *Fibres and Textiles in Eastern Europe* 2010; 18, 6(83): 1 00-105.
16. Kucharska M, Niekraszewicz A, Kardas I, Marcol W, Właszczuk A, Larysz-Brysz M and Lewin-Kowalik J. Developing a Model of Peripheral Nerve Graft Based on Natural Polymers. *Fibres and Textiles in Eastern Europe* 2012; 20, 6B(96): 115-120.
17. Niekraszewicz A, Kucharska M, Kardas I and Szadkowski M. Resorbable Tightening of Blood Vessel Protheses Prepared from Synthetic Polymers. *Fibres and Textiles in Eastern Europe* 2009; 17, 6(77): 93-98.
18. Urreaga J M and de la Orden M U. Chemical interactions and yellowing in chitosan-treated cellulose. *European Polymer Journal* 2006; 42: 2606-2616.
19. Niekraszewicz A, Kucharska M, Struszczyk M H, Rogaczewska A and Struszczyk K. Investigation into Biological Composite Surgical Meshes. *Fibres and Textiles in Eastern Europe* 2008; 16, 6(71): 117-121.
20. The United States Pharmacopeia USP 38 NF 33 2015.
21. European Pharmacopoeia-8th Edition volume I 01/2014 2.2 Psychical and physicochemical methods.
22. JIS L 1902: 2002. Testing Antibacterial Activity and Efficacy on Textile Products.
23. ASTM: E2149-01. Standard Test Method for Determining the Antimicrobial Activity of Immobilized. Antimicrobial Agents Under Dynamic Contact Conditions - Shaking Flask C. albicans.
24. PN-EN ISO 139:2006. Textiles-Standard atmospheres for conditioning and testing (in Polish).
25. PN-EN ISO 1973:2011. Textile fibres-Determination of linear density-Gravimetric method and vibroscope method (in Polish).
26. PN-EN ISO 5079:1999. Textiles – Fibres-Determination of breaking force and elongation at break of individual fibre (in Polish).
27. PN-EN 29073-1:1994. Textiles-Test methods for nonwovens-Part 1: Determination of mass per unit area (in Polish).
28. PN-EN ISO 9073-2:2002. Textiles-Test methods for nonwovens-Part 2: Determination of thickness (in Polish).
29. PN-EN 29073-3:1994. Textiles-Test methods for nonwovens-Part 3: Determination of tensile strength and elongation (in Polish).
30. PN-P -04781/12:1989. Textile medical materials. Determination of water absorptivity (in Polish).
31. GLP-SPR/BPB/5 -The own-procedure, which was developed according to standards and research literature.
32. Niekraszewicz A, Kucharska M, Kardas I, Wiśniewska-Wrona M, Kustosz R, Jarosz A. Chitosan Coatings to Seal Cardiovascular Prostheses. Fibres andTextiles in Eastern Europe 2011; 19, 3(86): 106-111.
33. GLP-SPR/BLF/21 The own-procedure, which was developed according to standards and research literature.
34. GLP-SPR/BPB/11 The own-procedure, which was developed according to standards and research literature.
35. PN-EN ISO 10993-12:2009. Biological assessment of medical devices - Part 12: Preparation of the sample and reference materials (in Polish).
36. PN-EN ISO 3071:2007. Textiles. Determination of pH of aqueous extract (in Polish).
37. PN-P-04781/14:1989. Textile medical materials. Determination of frothing agent (in Polish).
38. PN-P-04781/06:1988 Textile medical materials. Determination of water soluble matter (in Polish).
39. Jóźwicka J, Gzyra-Jagieła K, Struszczyk MH, Gutowska A, Ciechańska D and Krucińska I. The aspects of chemical characterization of leachables profile from ultra-light knitting textiles for uses as medical implants in urogynecology and general surgery. *Fibres and Textiles in Eastern Europe* 2012; 6A, 128-134.
40. PN-P-04896:1984. Methods of chemical tests. Knitted medical articles. Determination of permanganate oxidation (in Polish).

Received 30.11.2016 Reviewed 06.12.2016