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Modified Cellulosic Products for Application in Hygiene and Dressing Materials - Part II

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Abstract
The results presented herein relate to a work published earlier which is now complemented with the outcome of additional analyses and examinations. Cellulosic dressing materials modified with nanoparticles of microcrystalline chitosan or complex chitosan/alginateNa/Ca were characterized by the following quality parameters: absorption capacity, dynamics of the release of active substances from the dressing, structure by the FTIR method, physical-mechanical properties, accelerated ageing

Key words: *cellulosic products, nano-particles, chitosan, complex chitosan/alginateNa/Ca, accelerated ageing.*

Introduction

The article is a continuation of an earlier publication [1]. Investigations were made to clarify the interaction between the nano-particles of polysaccharides and cellulosic fibres, and to analyse the release of chitosan oligomers from the active layer of the cellulosic material. In designing dressing materials it is important to assess the allowable storage time and to learn the impact of storing conditions upon the critical properties of the medical device. In the accelerated ageing procedure assessed there were changes in quality parameters like the-absorption capacity as. well as physical-mechanical and biological properties of the materials modified.

Experimental

Materials

1. Chitosan Chito Clear HQG Primex Co, Iceland, with quality parameters; average molecular mass (M_V) = 372 kD, deacetylation degree (DD) = 81%, ash content = 0.22%, content of heavy metals: As 0.1; Cd 0.1; Pb = 0.27; Zn = 0.80; Hg 0.05%.
2. Sodium alginate Protanal 10/60FT, FMC Biopolymer Engineering, Inc., USA
3. Non-sterile cellulose gaze (100% cotton), 8-layers, surface density 200 g/m^2, from Matocomp TZMO Toruń, Poland, (symbol: Scel)

Assessment of absorption capacity of the modified cellulosic materials

The absorption capacity was assessed by estimating the water retention value (WRV). The value of WRV was calculated from the equation:

$$WRV = [(m_1 - m_o)/m_o] \times 100 \text{ in } \%$$

where, m_1 – mass of the sample after centrifugation in g, m_o – mass of the sample after drying up in g,

Ca. 5 g of a sample was immersed with 50 cm^3 of distilled water, left for 20 h, filtered and centrifuged at 4000 r.p.m. for 10 minutes. The sample was then weighted with an accuracy of 0.0001 g and dried up to constant mass at 105 °C and again weighted.

Methods

Examination of the release of chitosan oligomer fractions from the active layer of the dressings

The release of the chitosan oligomers fractions from the dressing active layer was followed by employing extraction with water, which was conducted in a thermal chamber under static conditions at 37 °C for 15 minutes and 1, 3, 6, 24 h. The amount of chitosan oligomers released was measured by the ninhydrin spectroscopy method in the range of visible light. The colour reaction of ninhydrin with primary amino groups is exploited in the method. The measurements were made at a wave length of 570 nm with apparatus UV/VIS , UNICAM Co.

Examination of the structure of the dressing materials by FTIR spectrophotometric method

Spectrophotometric examinations were carried out by Fourier transformation infrared transmission (FTIR). Spectrophotometric IR spectra were prepared by use of apparatus Genesis Series FTIR^{TM}, Unicam Co. The device is equipped with specialized software WinFIRST of ATI Mattson Co USA. Operational parameters of the device:

- wave number range: 4000 - 500 cm^{-1}
- resolution: 4.0 cm^{-1}
- number of scans collected from the background and spectrum: 16.

The material was prepared for testing in the form of tablets in KBr (1 mg of sample, 300 g of KBr).

Estimation of physical-mechanical parameters of the cellulosic textile products

The analysis was carried out at the Laboratory of Metrology of IBWCh (certificate of accreditation AB 388). The thickness of the gauze, wood-wool and hygiene tissues was measured according to Standard PN-EN ISO 9073-2:2002. The surface density, tenacity elongation and sinking time were estimated in accordance with Standard PN-EN 14079:2004.

Accelerated ageing

Accelerated ageing that simulates a one-year ageing proceeding under natural conditions was accomplished according to Standard ASTM F1980-07 (2011). Conditions of the examination: 4 weeks at 60 °C.

Sterilization of the modified textile materials

The dressings were sterilized at the Institute of Applied Radiation Chemistry. The irradiation dose with fast electrons was 25 kGy.

Results of the investigations and discussion

Investigation of the release of chitosan oligomer fractions from the dressing active layer

Estimated was the amount of chitosan oligomers that is being released from the active layer of dressing modified with nano-particles of MCCh and complex chit/algNa/Ca. Results are compiled in ***Tables 1 - 2***.

The release of chitosan oligomers in an amount of more than 1% from the active layer of the dressing proceeds after barely 15 minutes of water extraction under static conditions at 37 °C. It is an indication of the antimicrobial activity of the dressing. [2]. The release of oligomers is more intensive from the gauze Scel/chit/algNa/Ca modified with complex chit/algNa/Ca in comparison to Scel/MCCh, which is probably related to the stronger electrostatic interaction between chitosan and cellulose than in the case of chitosan-alginate nanoparticles.

Analysis of the chemical structure by the spectrophotometric method FTIR

The FTIR method was employed to clarify the interaction between the polysaccharides nano-particles and cellulosic fibres, and to follow the release of chitosan oligomers from the active layer. The fast detection of the kind of functional groups that appear in the test material is a great advantage of the method. Infrared spectroscopy offers the chance of analysing the structure of the nanoparticles and their interaction with cellulosic fibres.

In the FTIR spectrum of the modified dressing Scel/MCCh (***Figure 1***) one can discern more distinct and intensive bands relating to the extension oscillation of groups –OH, –NH in the range of wave numbers 3411 - 3432 cm^{-1} in comparison to the same for nano-MCCh [3].

Based on the analysis of the spectrum of modified dressing Scel/chit/algNa/Ca (***Figure 2***) one can infer that the interaction between the nanoparticles of complex chit/algNa/Ca and cellulosic fibres is slightly weaker than in the dressing modified with MCCh. It is observed with a broader band of the extension oscillation of groups –OH, –NH in the range of wave numbers 3411 - 3432 cm^{-1} along with its reduced intensity [4]. The analysis also explains the bigger amount of chitosan oligomers released from the dressing modified with complex chit/algNa/Ca compared to that modified with MCCh (***Tables 1*** and ***2***).

Analysis of the FTIR spectrum of the complex chit/algNa/Ca (***Figure 2***) leads to the conclusion that in the intensity, width and asymmetry of the extension oscillations band of groups –OH and –NH at wave numbers 3390, 3399 & 3296 sees the presence of hydrogen-bond-associated hydrophilic groups: –OH, $–NH_2$, –NHAc chitosan and –OH alginate. Compared to the spectrum of

Table 1. *Amount of chitosan oligomers delivered from gauze modified with nano-particles of MCCh (12% chitosan content) after water extraction.*

Test	Unit	Time, h				
		0.25	1	3	6	24
Amount of chitosan oligomers delivered from Scel/MCCh	%	1.43	2.00	2.51	2.94	3.40

Table 2. *Amount of chitosan oligomers delivered from gauze modified with nano-particles of complex chit/algNa/Ca (12% chitosan content) after water extraction*

Test	Unit	Time, h				
		0.25	1	3	6	24
Amount of chitosan oligomers delivered from Scel/chit/algNa/Ca	%	3.27	3.72	4.18	4.31	6.31

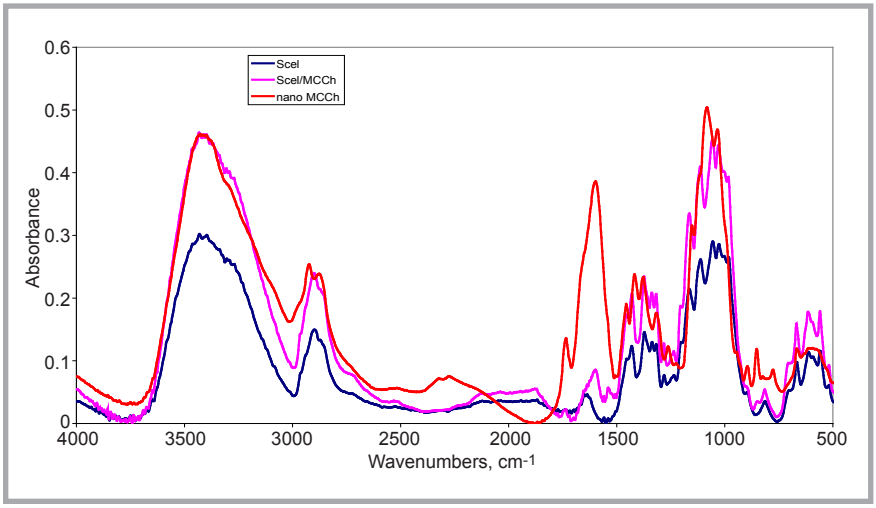

Figure 1. *FTIR spectrum of the cellulosic material modified with MCCh.*

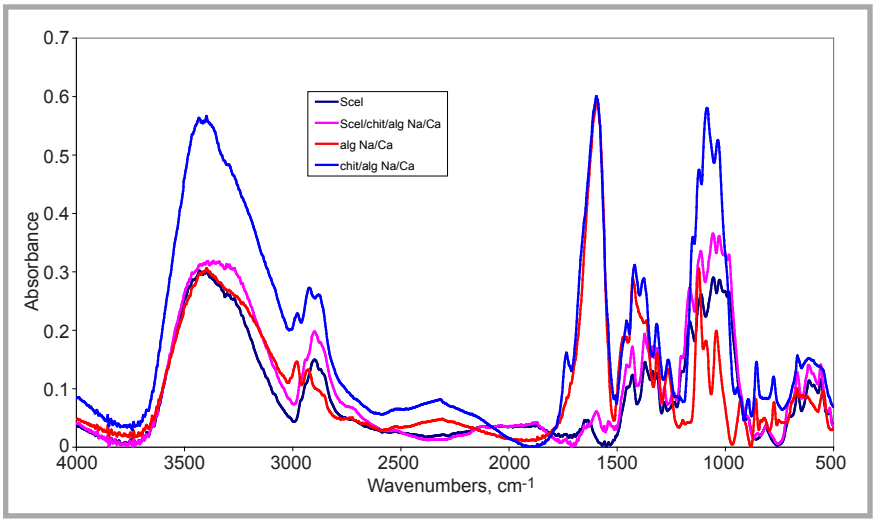

Figure 2. *FTIR spectrum of the cellulosic material modified with nano-particles of complex chit/algNa/Ca.*

Table 3. *Impact of accelerated ageing on the WRV of the dressing materials; Scel – multilayer gazue, unmodified, Scel/MCCh – gauze modified with nano-particles of MCCh, Scel/chit/algNa/Ca - gauze modified with nano-particles of complex chit/algNa/Ca.*

Symbol	Coating degree , [%]	WRV, [%]	
		Before ageing	After ageing
Scel	0.00	21	22
Scel/MCCh	25.5	42	24
Scel/chit/algNa/Ca	25.6	77	46

Table 4. *Antibacterial activity of the dressings modified against Escherichia coli.*

Symbol	Time, h	Amount of bacteria, cfu/sample	Bacteriostatic activity *Escherichia coli*		Bactericidal activity *Escherichia coli*		Growth value
			Log	%	Log	%	
Reference	0	3.1×10^4	-	-	-	-	-
Reference	24	1.4×10^8	-	-	-	-	3.7
Scel/MCCh	24	1.9×10^4	3.9	56.3	0.2	6.3	-
Reference	0	2.3×10^4	-	-	-	-	-
Reference	24	1.6×10^8	-	-	-	-	3.8
Scel/MCCh after ageing	24	2.1×10^4	3.9	56.5	0.1	3.2	-
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	47.1	-0.1	0.0	-
Reference	0	2.3×10^4	-	-	-	-	-
Reference	24	1.6×10^8	-	-	-	-	3.8
Scel/chit/alg Na/Ca after ageing	24	8.9×10^4	3.3	47.8	-0.5	0.0	-

Table 5. *Antibacterial activity of the dressings modified against Staphylococcus aureus.*

Symbol	Time, h	Amount of bacteria, cfu/sample	Bacteriostatic activity Staphylococcus aureus		Bactericidal activity Staphylococcus aureus		Growth value
			Log	%	Log	%	
Reference	0	3.7×10^4	-		-		-
Reference	24	7.3×10^6	-		-		2.3
Scel/MCCh	24	6.0×10^1	5.1	91.1	2.8	84.8	-
Reference	0	3.7×10^4	-	-	-	-	-
Reference	24	6.7×10^6	-	-	-	-	2.2
Scel/MCCh after ageing	24	1.1×10^3	3.7	67.3	1.5	45.5	-
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	72.7	2.0	57.1	-
Reference	0	3.7×10^4	-	-	-	-	-
Reference	24	6.7×10^6	-	-	-	-	2.2
Scel/chit/alg Na/Ca after ageing	24	1.3×10^3	3.7	67.3	1.5	45.5	-

Table 6. *Antifungal activity of the dressings modified against Candida albicans.*

Symbol	Antifungal activity against Candida albicans			
	Time, h	cfu/ml	Reduction, %	Reduction, Log
Reference	24	1.2×10^5	-	-
Scel/MCCh	24	< 1	100	5.1
Reference	24	1.2×10^5	-	-
Scel/MCCh after ageing	24	< 1	100	5.1
Reference	24	4.6×10^4	-	-
Scel/chit/algNa/Ca	24	4.9×10^1	99.9	3.3
Reference	24	1.2×10^5	-	-
Scel/chit/algNa/Ca after ageing	24	< 1	100	5.1

nano-MCCh (***Figure 1***), a bigger amount of the groups appearing in the range of 3300 - 3500 cm^{-1} may be seen by a broadening of the band, being a confirmation of the higher absorption capacity of complex chit/algNa/Ca nano-particles in comparison with MCCh nano-particles.

Absorption bands in the spectrum of Scel/chit/algNa/Ca (***Figure 2***) in the range of wave numbers 1625 - 1637 cm^{-1} indicate the forming of ion bonds between functional groups of complex nano-chitosan and and alginateNaCa, which exerts an impact upon several properties of the modified dressing, notably moisture imbibition [1].

The range of wave numbers 2880 – 3000 cm^{-1} responds to stretching vibrations of the C-H bonds of Scel, nano-MCCh and the dressing modified with MCCh nanoparticles. Bending vibrations of groups C-H and CH_3 for Scel, nano-MCCh and the dressing modified with MCCh nanoparticles appear in the 1422 – 1373 cm^{-1} range of wave numbers (***Figure 1***). The stretching vibrations band of the C-H bond for complex Chit/AlgNaCa appears for wave numbers 2880 – 2977 cm^{-1}, while in the dressing modified with complex Chit/Alg NaCa, the C-H vibrations are in the 2900 – 2967 cm^{-1} range of wave numbers. The bands of bending vibrations of the C-H and CH_3 groups in complex Chit/AlgNaCa and dressing modified with complex Chit/AlgNaCa appear in the range of 1423 – 1380 cm^{-1}. (***Figure 2***) In the range of wave numbers 890 – 1165 cm^{-1}, a change can be seen in the intensity and shape of peaks characteristic of ether bonds.

The band of anti-symmetrical bridge stretching vibration of C-O-C in Scel, dressing modified with MCCh nanoparticles and dressing modified with complex Chit/AlgNaCa appears for wave number 1165 cm^{-1}. The same band in MCCh nanoparticles and complex Chit/AlgNaCa can be seen for the wave number 1152 cm^{-1} (***Figures 1, 2***).

For the dressing modified with both MCCh nanoparticles and complex Chit/AlgNaCa in the band of wave numbers 1650 - 1636 cm^{-1} there appear stretching vibrations C=O (Amid I) as a doublet representing two kinds of intermolecular hydrogen bonds of the carbonyl group. seeing an interaction between the groups of cellulose and MCCh nanoparticles, and between cellulose and complex Chit/AlgNaCa (***Figures 1***, ***2***).

An intensive peak can be seen in the range of wave numbers 1600 - 1800 cm^{-1} relating to bending vibrations of the N-H bond (Amid II), characteristic of nano-MCCh, and a peak relating to vibrations of the ester group (***Figure 1***). Another peak appears in the same range with respect to AlgNaCa and complex Chit/AlgNaCa for wave number 1600 cm^{-1} derived from symmetric valence vibrations C-O in the carboxylate ion (***Figure 2***).

Impact of ageing on absorption properties of the modified dressings

The water retention value (WRV) was the basic parameter in the examination. After a simulated ageing equivalent to 12 month's real time, WRV was down by 18 to 30%. It must, however, be noted that the accelerated ageing was performed at 60 °C for 4 weeks in accordance with the standard. In consequence of the long lasting high temperature, the porous structure of the dressing active layer was probably closed up, which consequently caused a decrease in the material's absorption capacity (***Table 3***).

Impact of the accelerated ageing on antibacterial and antifungal activity

After simulated ageing equivalent to one year's storage, the dressings modified with nano-particles of MCCh and complex Chit/alg Na/Ca were analysed in relation to antimicrobial properties against Gram (-) and Gram (+) bacteria and fungi. Results are shown in ***Tables 4 - 6***.

As can be seen, the simulated ageing equivalent to one year of real time did not actually impede the antibacterial activity of the modified dressings. Both biopolymers retain their good antibacterial and antifungal activity after the ageing. The ability to reduce bacteria *Escherichia coli* remained unchanged in both materials prepared (***Table 4***) and thus antifungal activity against *Candida albicans* remained (***Table 6***). A decrease of 20 - 30% in the activity against the bacteria *Staphylococcus aureus* (***Table 5***) was measured in the dressings.

Impact of ageing on physical-mechanical properties

Also the impact of accelerated ageing on mechanical properties of the modified dressings was investigated. Results are compiled in ***Tables 7 - 8***.

Table 7. *Mechanical parameters of the modified dressings before and after accelerated ageing - along direction.*

Symbol of sample	Surface mass, g/m²	Breaking force, N	Elongation at break, %	Tenacity, MPa	Thickness, mm	Sinking time, s
Scel	2014	86848	11 1	9.870.55	1.760.05	1
Scel after ageing	2003	80340	11 0	9.400.46	1.720.04	2
Scel/MCCh	2402	100628	11 1	6.620.18	3.040.05	3
Scel/MCCh after ageing	2412	92750	11 1	6.620.36	2.800.08	13
Scel/chit/algNa/Ca	2512	87641	11 1	6.770.32	2.590.06	3
Scel/chit/algNa/Ca after ageing	2512	89624	11 1	7.220.20	2.480.05	13

Table 8. *Mechanical parameters of the modified dressings before and after accelerated ageing - across direction.*

Symbol of sample	Surface mass, g/m²	Breaking force, N	Elongation at break, %	Tenacity, MPa	Thickness, mm	Sinking time, s
Scel	2014	25718	15 1	2.920.20	1.760.05	1
Scel after ageing	2003	23311	13 1	2.720.13	1.720.04	2
Scel/MCCh	2402	33012	15 0	2.170.08	3.040.05	3
Scel/MCCh after ageing	2412	31314	15 1	2.230.10	2.800.08	13
Scel/chit/algNa/Ca	2512	29117	15 1	2.250.12	2.590.06	3
Scel/chit/algNa/Ca after ageing	2512	28613	17 0	2.310.10	2.480.05	13

The examination has shown that accelerated ageing does not affect the tenacity of the materials tested.

The rate of fluid absorption by the materials prepared was also measured. The sinking time is informative; it indicates how fast the material imbibes water. The requirement of the standard quotes 10 seconds as the upper limit allowable for the sinking time. Both test materials modified with nano-particles of MCCh and complex chit/algNa/Ca showed a very short sinking time (3 sec.) prior to accelerated ageing, while after the ageing test the time was prolonged to 13 seconds. Such results together with the measurements of the WRV raise a serious concern as to whether the accelerated ageing procedure as laid down in the standard is suitable for materials modified with natural polymers with a developed internal surface. The long-lasting influence of high temperature causes a closure of the porous structure in the active layer of the dressing, thereby substantially decreasing the ability of the material to absorb moisture.

Conclusions

1. Cellulosic material modified with nano-particles of MCCH and complex chit/algNa/Ca reveals a release of chitosan oligomers in the amount of more than 1% immediately after 15 minutes at a temperature of 37 °C under static conditions.
2. FTIR analysis of the dressing modified with MCCh indicates a more distinct and intensive band relating to the extension oscillation of groups –OH, –NH in the range of wave numbers 3411 - 3432 cm^{-1}. It sees a strengthening of hydrogen bonds and a strong interaction between the cellulose fibres and MCCh nanoparticles.
3. Analysis of the spectrum of the dressing modified with nano-particles of complex chit/algNa/Ca shows a broader band of extension oscillation of –OH, –NH in the range of wave numbers 3411 - 3432 cm^{-1} and a decrease in its intensity. It implies that the interaction between the complex and cellulose fibres is not as strong as in the MCCh modification.
4. The intensity, width and asymmetry of the extension oscillation band of the–OH i –NH groups for wave numbers 3390, 3399, 3296 show the presence of the hydrophylic–OH, –NH_2, –NHAc groups of chitosan and –OH group of alginate associated with hydrogen bonds. Compared to the FTIR spectrum of nano-MCCh, a larger amount of the groups placed in the range of 3300 - 3500 cm^{-1} is marked by abroadening of the band, which confirms the higher absorption capacity of complex chit/algNa/Ca nanoparticles than those of MCCh.
5. For the dressing modified with both MCCh nanoparticles and complex Chit/AlgNaCa in the band of wave

numbers 1650-1636cm^{-1}, there appear stretching vibrations C=O (Amid I) as a doublet representing two kinds of intermolecular hydrogen bonds of the carbonyl group, with an interaction existing between the groups of cellulose and MCCh nanoparticles, and between cellulose and complex Chit/AlgNaCa.

6. An investigation into accelerated ageing documented that the equivalent of a 12 month period did not affect the bactericidal activity and tenacity of the biomaterials prepared, with the absorption capacity being reduced. Therefore we can conclude that the specific process of accelerated ageing according to the requirements of the standard i.e. the storing of the material at 60 °C for 4 weeks, is probably inadequate for material modified with natural polymers with the internal surface developed. The long lasting elevated temperature causes the closure of the porous structure of the active dressing layer, and in consequence the moisture absorption of the material is reduced.

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