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A Nonwovens Coated By Chitosan with Potential Anti-microbial Behaviour – Preliminary Results

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Abstract
The aim of the research was to elaborate the manufacturing method of modified non-woven materials for medical use as absorbent material in consumers' plasters. The modified non-woven materials obtained from polypropylene or viscose fibres, using buffered chitosan salt as binding agent. The introduction of chitosan in the form of salt gave the non-woven fabric anti-microbe and bacteriostatic properties. The absorptiveness, physical and chemical properties of modified non-woven fabrics were evaluated, moreover their cytotoxicity and irritating properties were evaluated in accordance with harmonized norms.

Key words: *nonwoven, chitosan forms, medical application, medical devices.*

Introduction

Chitosan, as well as its derivatives, is often used for the preparation of biodegradable films. Use of chitosan in the form of salt causes the solubility of films formed in a solution having pH < 7. However, the application of the microcrystalline form of this biopolymer due to the relatively high crystallinity of formed films results in resistance to dissolution in neutral pH, as well as a prolongation of biodegradation.

The deposition of special forms of chitosan with documented antimicrobial properties onto nonwovens is helpful to produce effective, simple and inexpensive wound dressings [8 - 11]. Moreover, chitosan is well-known as a substance which indicates the absence of risk of transferring animal pathogen onto humans [6].

The aim of this study was to determine the effect of application of chitosan lactate to create useable properties of modified nonwovens for medical applications, taking into account its antimicrobial properties as well as preliminary estimation of bioactivity in the range of cytotoxicity and irritation made acc. Standards harmonized with EC Directives 42/93/EWG. Biological decomposition aspect were published in previous article [13].

Materials and methods

Materials

Chitosan
The chitosan was made by Vanson Ltd., USA. The biopolymer properties used are presented in Table 1.

Table 2 show the properties of chitosan lactate in the form of film.

Oriented fiber webs
For the preparation of nonwovens by the sticking technique, two types of raw material of synthetic and natural origin were used, namely polypropylene (PP) - nonwoven and viscose: (VS) - nonwoven.

The oriented polypropylene and viscose fiber web were used for the manufacture of nonwovens with surface weights of 20 g/m^2 and 50 g/m^2.

Analytical methods

Determination of chitosan properties
The degree of deacetylation (DD) of chitosan was determined by potentiometric titration [1], average molecular weight ($\bar{M}_V$) by viscometry [2, 3] and by gel chromatography (GPC) [12], water retention value (WRV) by gravimetrical method [2]. The $\bar{M}_V$ and DD of microcrystalline chitosan (MCCh) were determined by the modified method described in [2, 3].

Determination of chitosan lactate
Estimation of molecular weigh distribution, average molecular weight and polydispersity were determined by the gel chromatography method (GPC) acc. [12].

Determination of modified nonwovens properties
The WRV of modified nonwovens in distilled water and artificial blood was determined as described in [4]. Artificial blood was prepared based on the DIN 53923 Standard.

Table 1. *Properties of chitosan used; $\bar{M}_V$ – average molecular weight; $\bar{M}_n$ – number-average molecular weight; $\bar{M}_w$ – weight-average molecular weight; $\bar{M}_n$ / $\bar{M}_w$ - polydispersity; DD – degree of deacetylation; WRV – water retention value, 1) – number-average molecular weight and weight-average molecular weight determined by M. Rinaudo [12], 2) – heavy metal content determined by PN-82/C-84002.15 Standard, 3) – ash content determined by ISO 3451-1:1997 Standard.*

Symbol of samples	V3
Form	Powder
$\bar{M}_V$, kDa	166.3
$\bar{M}_n$ 1), Da	14329
$\bar{M}_w$ 1), Da	74742
$\bar{M}_n$ / $\bar{M}_w$	5.2
DD, %	76.2
WRV,%	121.0
Solubility in 1% acetic acid, wt.%	100
Moisture content, wt.%	7.8
Heavy metal content 2), wt.%	0.005
Ash content 3), wt.%	0.3

Table 2. *Properties of chitosan lactate.*

Symbol of samples	V3/Lac
Form	Film
$\bar{M}_n$, Da	14690
$\bar{M}_w$, Da	92903
$\bar{M}_n$ / $\bar{M}_w$	6.3
WRV (in distilled water), %	5620
WRV (in artificial blood), %	1800

The surface weight of nonwovens was determined in accordance with PN-EN 29073-1:1994 Standard, the thickness of nonwovens was determined in accordance with PN-EN ISO 9073-2:2002 Standard, the breaking strength and elongation of nonwovens were determined using PN-EN 29073-3:1994 Standard. The Biocompatibility of modified nonwovens was tested on the basis of PN-EN ISO 10993-1:2004 Standard in the range of:

- cytotoxicity (PN-EN ISO 10993-5 Standard);
- irritation properties (PN-EN ISO 10993-10 Standard, section 5.2., using polar solvent due to the determination of chitosan effect on the irritation).

Antimicrobial properties of modified nonwovens in vitro were carried out on the basis of JIS L.1902: 2002 and PN ISO 7218:1998 Standards using *Escherichia coli* and *Staphylococcus aureus* strains. The non-modified nonwovens were taken as a control. All samples before tests were steam-sterilized for 21 min. at 121 °C acc. requirements of PN-EN 554 Standard for removing of microbiological contamination.

Chitosan lactate content on modified nonwovens was determined by the gravimetrical method. The modified nonwovens were immersed in 1% (v/v) acetic acid solution for 12 h with continuous shaking. The nonwovens were separated and washed with 500 ml of distilled water. The procedure was repeated twice. Then the nonwovens were dried at 105 °C till the weight is constant. The percentage of bonding agent content was calculated as a ratio of the initial weight of modified nonwoven to the nonwoven weight obtained after removing the chitosan lactate.

Methods

Preparation of chitosan lactate solution

2.4 g of chitosan was dispersed in distilled water (150 ml) for 24 h. Then a suitable amount of lactic acid was added to obtain a final concentration of 0.8% (v/v). The Chitosan solution was filtered using a cheese-cloth and filled to 200 ml with distilled water. Then the suitable amount of 1% (w/v) sodium hydroxide was dropped till the pH ranged 5.4. The buffered solution was filled with distilled water to 240 ml.

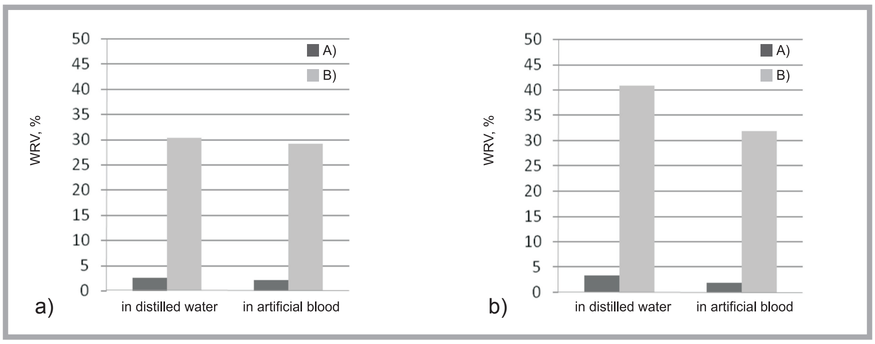

Figure 1. *WRV in distilled water and artificial blood of polypropylene nonwoven (A) nonwoven modified by buffered chitosan lactate (B): (a) - surface weight of 20 g/m^2, (b) - surface weight of 50 g/m^2.*

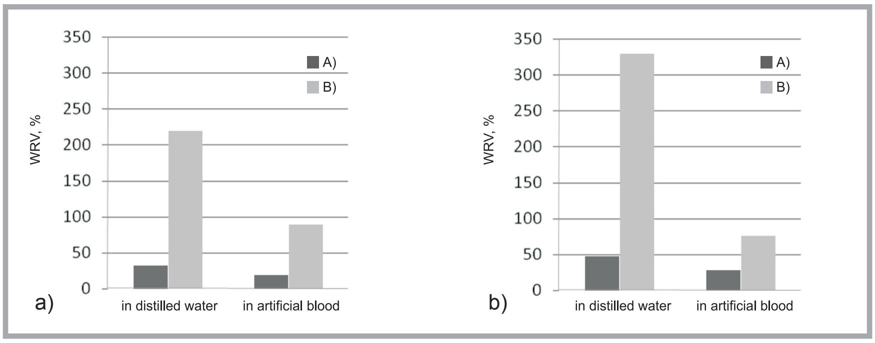

Figure 2. *WRV in distilled water and artificial blood of viscose nonwoven (A) nonwoven modified by chitosan lactate (B): (a) - surface weight of 20 g/m^2, (b) - surface weight of 50 g/m^2.*

Buffered chitosan lactate films were formed by air-drying on a PTFE plate (ca. 6 × 4 × 0.3 cm) for ca. 24 h [4, 7].

Preparation of modified nonwovens in aqueous chitosan lactate solution

Oriented fiber web (made of polypropylene and viscose) were submerged in the aqueous chitosan lactate solution buffered to pH = 5.4. The excess of binding agent was impressed using FULARD apparatus with gab 0.25 mm. Then the formed nonwoven was dried in an oven for 24 h at 40 °C [5].

Effect of modification by chitosan lactate on the physical properties of nonwovens

The content of chitosan lactate onto modified nonwovens differed in relation to the type of source used to manufacture the nonwoven. The application of viscose as a source yielded in chitosan lactate content of 10.7 and 18.8% (w/w) for the initial surface weight of 20 and 50 g/m^2, respectively. Moreover, the application of polypropylene as a source caused a reduction in chitosan lactate in the final, modified PP nonwoven obtained, to the level of 5.7 and 11.1% (w/w) for the initial surface weight of 20 and 50 g/m^2, respectively.

Results and discussion

Effect of impregnation in chitosan lactate on swelling of nonwovens in distilled water and artificial blood

Figure 1 shows the effect of modification of PP nonwovens differing in initial surface weight, using buffered chitosan lactate solution on their WRV coefficient in distilled water and artificial blood.

The application of chitosan lactate solution as a binding agent of PP oriented fibers pulp caused significant increase in WRV of modified PP nonwovens in distilled water and artificial blood, both for initial surface weight of 20 g/m^2 and 50 g/m^2. WRV coefficient in distilled water and artificial blood increased 10-times after modification by buffered solution of chitosan lactate.

Figure 2 shows the effect of modification of VS nonwovens having initial surface weight of 20 g/m^2 and 50 g/m^2 using buffered chitosan lactate solution on WRV coefficient in distilled water and artificial blood.

WRV coefficients of modified VS nonwovens significantly increased in relation to the WRV of modified PP nonwovens.

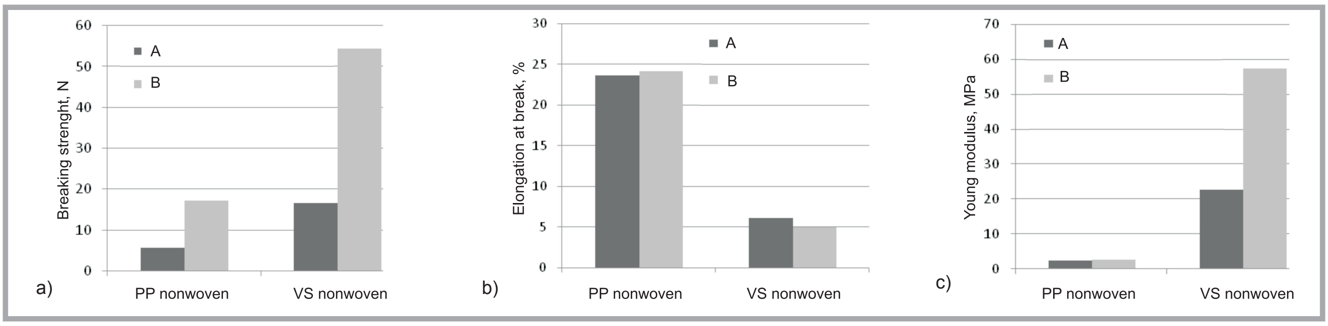

Figure 3. *Mechanical properties of PP and VS nonwoven modified by chitosan lactate: a) – breaking strength in N; b) – elongation at break in %; c) – Young modulus in MPa; initial surface weight: A - 20 g/m², B - 50 g/m².*

The application of oriented VS fiber web with initial surface weight of 20 g/m² and 50 g/m² yielded an increase in WRV of seven times in distilled water. WRV in artificial blood increased only three or five times, depending on initial surface weight as compared with the WRV of initial oriented VS fiber web.

The above phenomena may prefer to be modified by chitosan lactate VS nonwovens for the application to become a source for an absorbing wound dressing design.

Mechanical properties of modified nonwovens

The comparison of mechanical properties of VS and PP nonwovens modified by a buffered solution of chitosan lactate is shown in Figure 3.

An increase in the surface weight of the modified nonwovens caused an increase in breaking strength in both PP and VS nonwovens. The PP nonwoven modified by chitosan lactate showed around a 3.5-times lower breaking strength than the VS nonwoven impregnated in the same conditions. Moreover, the modified PP nonwoven indicated around a 5-times higher elongation at break as compared with modified VS nonwovens. The Young modulus of modified viscose nonwovens resulted in a much higher value as obtained for PP nonwovens prepared in similar conditions.

Determination of biological properties of modified nonwovens

Novel wound dressing materials, as well as sources for their manufacture, should be characterised by high purity, should not to be cytotoxic and not have any irritation properties. Additionally, antimicrobial properties increase the benefit of use, especially if the antimicrobial effect is not connected with a drug introduced.

Estimation of antibacterial properties of modified nonwovens

Tables 3 and 4 shows the results of antimicrobial tests of modified nonwovens made of polypropylene and viscose in the presence of *E. coli*. Tables 5 and 6 shows the results of antimicrobial tests of modified nonwovens made of polypropylene and viscose in the presence of *S. aureus*.

Table 3. *Antimicrobial activity of VS nonwoven modified by chitosan lactate in the presence of E. coli.*

Sample	Time, h	Bacteria number, cfu/sample	Confidence interval, cfu/sample	Bacteriostatic activity	Antimicrobial activity	Growth
oriented viscose fiber web	0	7.6 x 10^4	(5.4 – 10.0) x 10^4	-	-	-
oriented viscose fiber web	24	6.7 x 10^7	(3.2 – 11.0) x 10^7	-	-	2.9
VS nonwoven modified by chitosan lactate	24	< 20	-	6.5	3.6	-

Table 4. *Antimicrobial activity of PP nonwoven modified by chitosan lactate in the presence of E. coli.*

Sample	Time, h	Bacteria number, cfu/sample	Confidence interval, cfu/sample	Bacteriostatic activity	Antimicrobial activity	Growth
oriented polypropylene fiber web	0	7.9 x 10^4	(6.0 – 10.0) x 10^4	-	-	-
oriented polypropylene fiber web	24	7.0 x 10^7	(4.5 – 9.6) x 10^7	-	-	2.9
PP nonwoven modified by chitosan lactate	24	< 20	-	6.5	3.6	-

Table 5. *Antimicrobial activity of VS nonwoven modified by chitosan lactate in the presence of S. aureus.*

Sample	Time, h	Bacteria number, cfu/sample	Confidence interval, cfu/sample	Bacteriostatic activity	Antimicrobial activity	Growth
oriented viscose fiber web	0	5.03 x 10^4	(4.03 – 6.12) x 10^4	-	-	-
oriented viscose fiber web	24	2.22 x 10^7	(6.42 – 40.2) x 10^7	-	-	1.6
VS nonwoven modified by chitosan lactate	24	< 20	-	5.0	3.4	-

Table 6. *Antimicrobial activity of PP nonwoven modified by chitosan lactate in the presence of S. aureus.*

Sample	Time, h	Bacteria number, cfu/sample	Confidence interval, cfu/sample	Bacteriostatic activity	Antimicrobial activity	Growth
oriented polypropylene fiber web	0	6.37 x 10^4	(4.41 – 8.84) x 10^4	-	-	-
oriented polypropylene fiber web	24	2.54 x 10^7	(1.67 – 3.52) x 10^7	-	-	2.6
PP nonwoven modified by chitosan lactate	24	< 20	-	6.1	3.5	-

Both PP and VS nonwovens modified by buffered chitosan lactate showed bacteriostatic and antimicrobial properties. The application of a polypropylene and viscose carrier did not change the antimicrobial properties. Additionally, for the determination of the antimicrobial effect of the binding agent – chitosan lactate, the test was repeated for chitosan lactate films only with reference to cotton, due to the requirements of JIS L.1902: 2002 and PN ISO 7218:1998 Standards (Tables 7 and 8).

The results above-mentioned confirmed significant up the antimicrobial and bacteriostatic behaviour of chitosan lactate applied as a binding agent for the modification of nonwovens. The modified nonwovens are able to be used effectively in the design of wound dressing having bacteriostatic bahaviour.

Cytotoxicity

A cytotoxicity test was carried out at the National Institute of Public Health,Poland for two types of nonwovens made of oriented PP and VS fibre webs modified by a buffered solution of chitosan lactate. A quality test was prepared by the direct contact method, using a L929 fibroblast cell. The cytotoxic effect was estimated based on a 5-grade scale of toxicity (ranged from 0 to 4) described in PN-EN ISO 10993-5 Standard.

Both evaluated modified nonwovens did not show any toxicity and were classified as 0 grade cytotoxicity – meaning they are non-cytotoxic.

Irritation

An irritation test was carried out at the National Institute of Public Health, Poland for two types of nonwovens made of PP and VS fibres modified by a buffered solution of chitosan lactate.

6 albinotic rabbits weighing from 2.5 to 3.5 kg were used for the determination of irritation effect. The sheet of modified nonwoven (2.5 cm × 2.5 cm) was placed onto mechanically depilated skin. Then the samples of nonwovens were wetted by distilled water to make better contact with the skin, covered with aluminum film and occlusion dressing. The samples were removed after 6 h and the degree of inflammatory reaction was estimated acc. 0 - 4 grade scale described in PN-EN ISO 10993-10 Standard. The condition of the rabbit skin in contact with the tested sample was estimated after 1 h, 24 h, 48 h or 72h.

Both evaluated types of modified nonwovens did not indicate any changes in skin tissue that may be connected with the irritation caused by the tested samples. The coefficient of irritation amounted to 0 – grade for both tested samples (no skin irritation). The rabbit skin looked correctly in place of contact with the tested samples.

Conclusions

The study presented, being the basis for the application of novel, modified nonwovens for possible medical application, concludes the following:

- the modification of both nonwovens showed a significant increase in their WRV, both in distilled water and artificial blood;
- the mechanical properties of modified nonwovens seems to be enough for medical application as simply wound dressing;
- the modification of both nonwovens by chitosan lactate introduced antimicrobial behaviour;
- nonwovens modified by chitosan lactate showed non-cytotoxicity and did not have any irritation behaviour.

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Table 7. *Antimicrobial activity of chitosan lactate films in the presence of E. coli.*

Sample	Time, h	Bacteria number, cfu/sample	Confidence interval, cfu/sample	Bacteriostatic activity	Antimicrobial activity	Growth
control - cotton	0	5.0 x 10^4	(4.0 – 5.8) x 10^4	-	-	-
control - cotton	24	1.8 x 10^7	(1.4 – 2.3) x 10^7	-	-	2.6
chitosan lactate film	24	< 20	-	6.0	3.4	-

Table 3. *Antimicrobial activity of chitosan lactate films in the presence of S. aureus.*

Sample	Time, h	Bacteria number, cfu/sample	Confidence interval, cfu/sample	Bacteriostatic activity	Antimicrobial activity	Growth
control - cotton	0	3.72 x 10^4	(3.04 – 4.48) x 10^4	-	-	-
control - cotton	24	1.01 x 10^7	(6.18 – 14.7) x 10^6	-	-	2.4
chitosan lactate film	24	< 20	-	5.7	3.3	-