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PRELIMINARY STUDIES ON THE PREPARATION AND PROPERTIES OF CHITOSAN NONWOVENS MODIFIED WITH ACID VAPORS

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ABSTRACT

The aim of the study was to develop methods for modifying chitosan nonwovens in the gas phase (application of acid vapors). Organic and inorganic acids were used in the research. The time of treating chitosan nonwovens with acid vapors ranged from 10 to 120 min. The conducted research has shown that it is possible to modify chitosan nonwovens with the use of acid vapors (organic and inorganic). It was found that the action of acid vapors does not have a destructive effect on chitosan fibers (SEM tests), which means that the developed method can be applied to various forms of chitosan materials, while the modification is carried out on the finished form.

Microbiological tests were used to investigate the activity and growth of the microorganisms, which were related to the acids in the modified nonwovens. Only materials modified with acetic acid and hydrochloric acid were found to have bacteriostatic properties against *S. aureus* and *E. coli* (gram-negative and gram-positive bacteria). It was also found that chitosan formate significantly reduced the number of colonies of *S. aureus*.

KEYWORDS

Chitosan; surface modification; bacteriostatic activity; protonation of the amino group; ammonium salt.

INTRODUCTION

Chitin, is a polysaccharide most commonly found in the natural environment. It can be found in the structure of sponges, corals, the shells of marine invertebrates, insects, and fungi cell walls [1–4]. Chitosan is a derivative of chitin and is obtained by chemical or enzymatic deacetylation of chitin. The difference between chitin and chitosan is in the degree of deacetylation. Chitosan is considered as a biofunctional polymer used in the health care sector due to its antimicrobial properties, film formation, and bio-adhesive characteristics. Additionally, the special properties such as non-toxicity, anti-bacterial activity, biodegradability, and excellent biocompatibility render widely use of chitosan in the biomedical applications as a drug carrier antimicrobial, antioxidant, antitumor, and a wound dressing agent [5].

The mechanisms of chitosan's action on bacteria and fungi have been studied and described in many articles. The main antimicrobial activity of chitosan is electrostatic interactions between this cationic molecule and the negatively charged cell walls [6,7]. The antimicrobial properties of chitosan can be improved by chemical modification of chitosan structure. The two reactive sites -NH₂ and -OH present



in chitosan open enormous opportunities for its chemical modification. These groups allow to carry out the sulfonation, amination, and carboxymethylation [8–10] reactions.

The aim of the study was to develop methods for modifying chitosan nonwovens in the gas phase (application of acid vapors). It was planned to use various acids for the research: acetic, propionic, butyric, and valeric organic acids, as well as hydrochloric acid. It was expected that this would allow testing the applicability of this type of modification. As part of the research, it was also planned to perform pilot tests of the antibacterial activity of modified chitosan materials in order to select the most optimal chitosan ammonium salts for further research on the use of various forms (nonwovens, films, spheres, and others) of modified chitosan. The antibacterial activity was planned to be determined against *E. coli* and *S. aureus*.

MATERIALS AND METHODS

Material

The chitosan fibers were a commercial product of Hismer Bio-technology Co., Ltd, China. Fibers of 2.02 dtex with a strength of 12 cN/tex and a relative elongation of 1.5% were used. The chitosan-based nonwoven was obtained according to standard nonwoven fabric manufacturing procedure, by using a carder machine and needling process. After the needling process, the final product from elementary fleeces has obtained a surface weight of 120 g/m² per sheet.

Modification of chitosan nonwovens using vapors of various acids (gas-phase modification process)

For gas-phase modification hydrochloric, acetic, butyric, valeric, propionic, and formic acids were used. All chemicals were from Avantor Performance Materials Poland POCH Polish Chemicals Reagents. The procedure of modification of chitosan nonwovens was multi-stage. In the first step, Petri dish containing 20 mL of acid solution was placed in a vacuum to desiccator with a 3 dm³ capacity. The closed desiccator with acid solutions was left for 24 h in order to slowly fill the desiccator with acid vapors (saturation). After this time, 10 g of nonwoven sample was placed in a desiccator filled with acid vapors. The samples were exposed to acid vapors for 10 to 120 min. In the next stage, chitosan fibrils treated with acid vapors were placed in a vacuum desiccator with potassium hydroxide granules to remove excess acids for 24 h and then degassed under reduced pressure. Additionally, the nonwoven was dried in an oven at 40°C for 2 h.

SEM analysis

The effect of acid gas modification on the chitosan fiber morphology was examined by using the SEM method. Nova NanoSEM 230 scanning electron microscope (SEM) from FEI Company (Eindhoven, The Netherlands) was used in the study. For the SEM measurement, the nonwoven samples were prepared by fixing the parts of the samples to an SEM holder using conducting carbon adhesive tape. The studies were carried out using a low-vacuum mode and beam energy of 10 keV, which eliminated the requirement to cover the sample with a conductive material such as gold.

Antimicrobial activity

Modified chitosan samples were cut into 1 cm² piece. Two microbiological assays were conducted to evaluate the antimicrobial properties of the tested materials.

Susceptibility to microbial colonization

Samples of the modified chitosan-based nonwovens were placed in 24-well flat-bottomed plates containing Mueller Hinton II Broth. The chitosan fragments were incubated for 24 h at 37 °C to observe the growth of bacteria. After incubation, 10 µL of medium from each well was seeded on the agar plates. After 24 h of incubation, the appearance of colonies was evaluated.

Bactericidal activity

The second assay was performed similarly to that described above, with the difference that this time bacterial inoculums were used. Modified chitosan nonwoven samples were soaked in 0.5 mL of bacterial suspensions with a density of 0.5 McFarland. Two reference strains were used: *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922. The samples were incubated for 24 h at 37°C. After incubation, 10 µL of medium from each well was seeded on the agar plates and the number of colonies was observed.

RESULTS AND DISCUSSION

In the first stage of the research, chitosan nonwovens modified with acid vapor (gas phase modification) were obtained. The following C1-C4 carboxylic acids were used in the study: formic, acetic, propionic, butyric and valeric acid. Additionally, hydrochloric acid was used. During the modification, the amine groups of chitosan are converted to the corresponding ammonium salts. It is generally believed that the antibacterial activity of chitosan derivatives is due to the presence of positively charged ammonium groups. It was expected that the use of this pool of acids would provide a response regarding the influence of the anion structure in the ammonium salt on the antimicrobial activity of the final materials. In addition, the use of carboxylic acids with a different number of carbon atoms should affect the nature of the hydrophilic-hydrophobic ammonium salts of chitosan, which in turn should also affect the ability of materials to interact with bacteria. The time of treating chitosan nonwovens with acid vapors ranged from 10 to 120 min. The nonwovens treated with acid vapors were analyzed by SEM to assess the effect of the acid on the morphology of the fibers. Figure 1a shows sample SEM pictures of chitosan nonwoven before acid treatment, and Figure 1b shows an SEM picture of a nonwoven exposed to acetic acid vapor for 60 min.

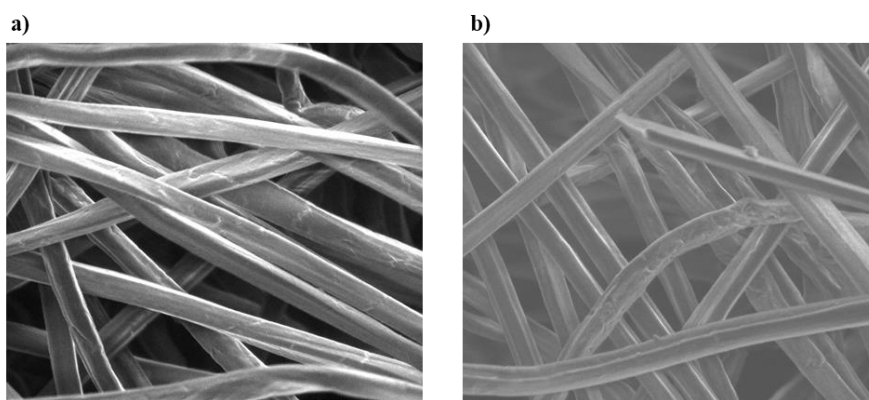


Figure 1. SEM images, a) unmodified chitosan, b) chitosan nonwoven treated with acetic acid vapor for 60 min.

In any case, the negative influence of the acid vapors on the fibers, and thus their destruction, resulting in the loss of the mechanical properties required for the fibrous materials, was not found.

Initial attempts were made to assess the antibacterial effect of chitosan nonwovens modified with acid vapor (gas phase modification).

Table 1. Effects of modification on microbial growth for nonwovens modified by acid vapors.

Sample	<i>S. aureus</i> ATCC 25923	<i>E. coli</i> ATCC 25922
Valeric acid	Growth	Growth
Propionic acid	Growth	Growth
Formic acid	Growth – 5 colonies	Growth
Butyric acid	Growth	Growth
Hydrochloric acid	No growth	No growth
Acetic acid	No growth	No growth

Table 1 shows the effects on microbial growth of modifying with acid vapors of chitosan nonwovens. Antimicrobial activity was observed for materials treated with hydrochloric acid and formic acetic acid against *S. aureus* (decreasing numbers of bacterial colonies or no growth). For *E. coli*, antibacterial activity was found for chitosan hydrochloride and chitosan acetate.

CONCLUSION

The conducted research has shown that it is possible to modify chitosan nonwovens with the use of acid vapors (organic and inorganic). It was found that the action of acid vapors does not have a destructive effect on chitosan fibers (SEM tests), which means that the developed method can be applied to various forms of chitosan materials, while the modification is carried out on the finished form.

Microbiological tests were used to investigate the activity and growth of the microorganisms, which were related to the acids in the modified nonwovens. Only materials modified with acetic acid and hydrochloric acid were found to have bacteriostatic properties against *S. aureus* and *E. coli* (gram-negative and gram-positive bacteria).

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