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Viscose Functionalisation with a Combination of Chitosan/BTCA Using Microwaves

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Abstract
Improved hygiene and health care standards have a great impact on the development of hygiene and health care products. For this purpose, viscose is a very popular substrate. One of the most promising anti-microbial compounds of modern times is chitosan. The anti-microbial action of this polysaccharide depends on the amino group amount, which is crucial for ensuring the effectiveness of anti-microbial treated material. In textile finishing, 1,2,3,4–buthanetetracarboxylic acid (BTCA) is usually used as a non-formaldehyde crease-resistant reagent. But, on the other hand, the negatively charged carboxyl groups of BTCA can be explored as additional binding sites for positively-charged protonated amino groups of chitosan. When using microwaves, polar materials (e.g. chitosan) orient and reorient themselves according to the direction of the electro-magnetic field, which means that chitosan chain-bending may have taken place during the drying with microwaves. This could result in a higher specific surface of the chitosan and, consequently, in a higher proportion of available amino groups. It is concluded that the combination chitosan/BTCA supported by microwaves drying represents an ideal combination to increase the proportion of available amino groups.

Key words: *viscose, chitosan/BTCA, microwaves, Acid Orange VII, methylene blue, antimicrobial activity.*

Introduction

Compared to cotton, no matter if mercerised or not, viscose is more reactive and is therefore a particularly interesting material for functionalisation in medical product manufacturing [1, 2]. In order to protect textile materials against micro-organisms, there are a sufficient number of relatively cheap and effective anti-microbial compounds [3]; however, many of these do not provide the necessary security required by modern legislation. One of the most promising anti-microbial compounds of modern times is chitosan. The anti-microbial action of this polysaccharide depends on the amino group amount, which is crucial for ensuring the effectiveness of anti-microbial treated material [4 - 6]. An undesirable property of cellulose fibres is their wrinkling; for this reason cellulosic fabrics are finished in order to improve dimensional stability. In textile manufacturing, 1,2,3,4-buthanetetracarboxylic acid (BTCA, ***Figure 1***) can be used as an alternative non-formaldehyde crease-resistant reagent [7 - 10]. But in this research we primarily wanted to explore negatively charged carboxyl groups of BTCA as binding sites for negatively-charged protonated amino groups of chitosan.

An interesting approach to increase the proportion of amino groups is, therefore, the chitosan/BTCA combination, since carboxyl groups of BTCA may represent an additional reactive site for chitosan binding onto the material. Such a material could be particularly interesting to create different products of hygienic materials, where a reduction in various pathogenic microorganisms is desirable. In addition, it is assumed that drying using microwaves may cause a specific-orientation of chitosan onto the surfaces of fibres. When microwave heating, polar materials (e.g. chitosan) orient and reorient themselves according to the direction of the electro-magnetic field. This could result in a higher specific surface of the chitosan bound onto the viscose i.e. in the proportion of available amino groups [11 - 14]. Viscose functionalisation is reflected in changes of mechanical properties [15], hence the wrinkle-recovery angle and breaking-strength were measured. Since we anticipate that the carboxyl groups of BTCA represent an additional binding-site for subsequent chitosan binding onto the substrate, it is also important to evaluate the proportion of available carboxyl groups, which can be calculated on the basis of the ion-exchange capacity of the fibre. The classical indirect method of methylene blue was used for this purpose. It is known that the amount of the amino group is the main driving force for chitosan's antimicrobial effectiveness, because these groups within an acidic media form ammonium salts, which have an effect on the permeability of the cell membranes. In such a way, the normal metabolism regarding micro-organisms is disturbed and leads to the deaths of cells [16]. The proportion of available amino groups was evaluated by the spectrophotometric method CI Acid Orange VII, subsequently supported by microbiological-testing. As the main criterion for assessing the efficiency of viscose functionalisation with the combination chitosan/BTCA, a proportion of the available amino groups that reflects the anti-microbial efficien-

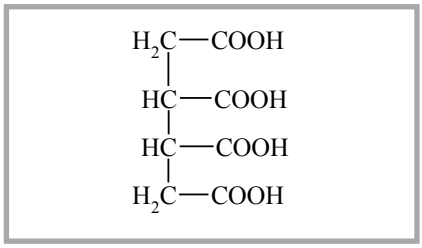

Figure 1. *BTCA molecule.*

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FIBRES & TEXTILES in Eastern Europe 2013; 21, 5(101): 24-29.

cy was chosen. Namely for hygienic or medical products, resistance to wrinkling is not the most important parameter. For this reason, resistance to wrinkling was chosen as a secondary plan for assessing the effectiveness of the viscose functionalisation with a system of BTCA/chitosan. The results obtained by using micro waves were compared with those found on the basis of the conventional pad-dry process.

It was concluded that the combination chitosan/BTCA is ideal to increase the proportion of available amino groups, and not one that could increase the tendency to wrinkle. This is especially pronounced in the case of microwave drying. Microbiological-testing based on the so-called dynamic-vibrating test confirmed the excellent reduction of microorganisms *S. Aureus* and *Candida albicans*, respectively.

Experimental

Fabric specifications

Investigations were carried out on 100% viscose fabric (Lenzing, Austria) with:

- a surface mass of 140 g/m^2,
- warp density 32 threads/cm,
- filling density 27 threads/cm and
- a weave of linen.

Viscose functionalisation

Low molecular weight chitosan (Aldrich, Mr ~ 150.000, 85% degree of deacetylation) of 1% mass fractions was diluted in 3% BTCA. The dissolution of the chitosan was made with BTCA, where the pH was first set at 2.8, then continued over 24 hours by stirring at room temperature using a magnetic stirrer. The complete dissolution of the chitosan was achieved in this way. Impregnation with chitosan/BTCA solution was also performed: The samples were impregnated with the help of a foulard (W. Mathis AG, Switzerland, p = 0.3 MPa, the rollers' speed rotation = 2 m/min). 80% wet pick-up was achieved under these conditions. After completing the process of impregnation, the samples were dried i) in a microwave oven (Moulinex optiquick CK3) and ii) in a laboratory dryer, as shown in ***Table 1***. Then the samples were rinsed at room temperature until reaching the conductivity of the distilled water - 0.4/(μS/cm).

Wrinkle recovery angle

The standard test method for the wrinkle recovery angle measurement of woven textile fabrics was used (SIST EN 22313) in the experiment.

Determination of mechanical properties

Measurements of breaking strength (SIST EN ISO 13937-1) were carried out by the strip method, within a standard atmosphere (T = 20 °C and RH = 65 %), using a Textechno Statigraph M-dynamometer.

Spectrophotometric method with Methylene blue [17]

A weighted oven-dried cellulose sample (app. 0.5 g) of known water content up to 0.5 g was suspended in 25 ml of aqueous methyhlene blue chloride solution (300 mg/l) and 25 ml of borate buffer of pH = 8.5 for 1 h at 20 °C in an 100 ml Erlenmeyer flask and then filtered through a sintered-glass disk. 5 ml of the filtrate were transferred to a 100 ml calibrated flask. Then 10 ml of 0.1 N HCl and subsequently water up to 100 ml were added and the methylene blue content of the liquid was determined spectrophotometrically, employing a calibration plot. The total amount of non-adsorbed methylene blue dye was calculated in this way (parameter A in ***Equation 1***), later inserted into ***Equation 1***.

The amount of carboxyl groups relative to the mass of the absolutely dry cellulose sample can be calculated as follows (***Equation 1***):

$$carboxyl\ group's\ amount = \frac{(7.5 - A)x0.00313}{m}\ \text{mmol/g} \qquad (1)$$

where: A is the part of non-bound methylene blue dye in g, and m the mass of the absolutely dry sample of cellulose in g.

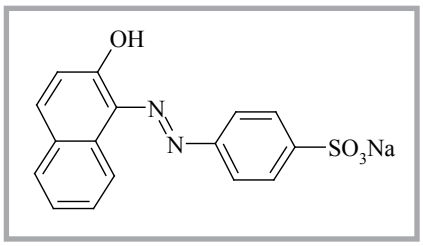

Figure 2. *Chemical structure of Acid Orange VII dye.*

The concentration of the dye solution was determined using a Perkin Elmer Lambda 2 UV/VIS spectrophotometer. Three parallel measurements were performed for each individual sample under identical experimental conditions. The results were statistically evaluated using standard deviation [18 - 20].

The Acid Orange VII spectrophotometric method

The accessible amino groups of the functionalised fibres were determined using the CI Acid Orange VII spectrophotometric method. This method is based on the absorption of the CI Acid Orange VII dye (***Figure 2***) by the principle of reducing the dye concentration in the dye bath by following the Lambert-Beer Law.

Sulphonic groups (SO_3^-) of the dye form within the acidic medium's ionic bonds in the ratio of 1 : 1 with the positively-charged amino groups of chitosan (NH_3^+), thus the amount of dye bound to the fibres corresponds to that of accessible amino groups [21]. The initial absorbance at a wavelength of 484 nm, which corresponds to a maximum absorption of the Acid Orange VII dye, was measured after adding 2 ml of CI Acid Orange VII dye (c = 0.005 mol/l) into 250 ml of water with a pH of 2.8. Then 0.25 g of the fibres was immersed in the dye solution and stirred for three hours at a constant speed, using a magnetic mixer.

Table 1. *Contents of the impregnation bath and conditions for functionalisation with a combination of chitosan/BTCA; *Manufacturer - Fluka.*

w (BTCA)*, %	3
Chitosan *w* (low molecular weight), %	1
pH	2.8
Wet pick-up, %	80
Microwave drying, W	850
Time of microwave drying, min.	3
Temperature of drying _ classical pad-dry procedure, °C	100
Time of drying _ classical pad-dry procedure, °min.	10
Temperature of condensation _ classical pad-dry procedure, °C	170
Time of condensation _ classical pad-dry procedure, °min.	3

After a three hour period, the balance between the concentration of the dye in the dye-bath and that of the dye bound on the fibres was established; and the final absorbance was measured using a Cary 50 spectrophotometer, Varian (USA). Using measurements of the initial and final absorbances, the concentration of the dye on the fibres was calculated as ***Equation 2***:

$$A_i = (A_0 - A_f) \cdot V/(k \cdot l \cdot m_f) \qquad (2)$$

Where: A_i – is the amount of amino groups of chitosan bound to the fibres in mmol/kg); A_0 – initial absorbance; A_f – final absorbance; V – volume of the dye bath in l; k – correction factor in l/mol cm; l – length of the optical field in cm; m_f – mass of absolutely dry fibres in g.

The parameter k was determined following the calibration curve and Lambert Beer Law ($A = k \cdot l \cdot c$), respectively .Based on the known concentration (c from the calibration curve), the known absorbance (A - spectrophotometric measurement) and known length of the optical field (1 cm), the correction factor k was determined afterwards and used in ***Equation 2***.

Antimicrobial test

Antimicrobial properties of the samples treated were evaluated at the Institute of Public Health, Maribor, according to ASTM E2149-01, which is a quantitative antimicrobial test method performed under dynamic contact conditions. *Escherichia coli* and *Candida albicans* were used as test organisms. An incubated test culture in a nutrient broth was diluted using a sterilised 0.3 mM phosphate buffer (KH_2PO_4; pH = 6.8), to give a final concentration of 1.5 - 3.0 × 10^5 colony forming units (CFU)/ml. This solution was used as a working bacterial dilution. Each sample (0.5 to 2 g) was cut into small pieces (1 ×1 cm) and transferred into a 250 ml Erlenmeyer flask containing 50 ml of the working bacterial dilution. All the flasks were loosely capped, placed in the incubator, and shaken for 1 h at 37 °C and 120 r.p.m, using a Wrist-Action incubator shaker. After a series of dilutions using the buffer solutions, 1 ml of the diluted solution was placed in nutrient agar. The inoculated plates were incubated at 37 °C for 24 h and surviving cells were counted. Average values of the duplicates were converted to CFU/ml in the flasks by multiplying by the dilution factor. The antimicrobial activity was expressed as an R = % (***Equation 3***) reduction of the organism after contact with the test specimen, compared to the number of bacterial cells surviving after contact with the control [16].

$$R = \frac{A-B}{A} x\ 100/\% \qquad (3)$$

Where: R – the reduction of microbes in %; A – the number of bacterial colonies after 1 min. (time "0"), and B - the number of bacterial colonies after 1 hour

Results and discussions

For a successful application of chitosan onto the cellulose substrate, it is important that the chitosan within the medium chosen is highly-soluble [11]. In our previous studies [16, 21], it was found that good anti-bacterial effectiveness of cellulose can be achieved by 1% mass fraction of chitosan with an 85% degree of de-acetylation, which also ensures the appropriate viscosity of the chitosan solution for its subsequent application onto cellulose. For this reason a 1% concentration of chitosan dissolved in 3% BTCA was used during the experiment for the functionalisation of the viscose. Preliminary tests confirmed that chitosan is impossible to dissolve in an aqueous solution below 3% of BTCA. On the other hand, concentrations above 3% of BTCA demonstrated poor results with respect to mechanical properties (breaking strength reduction) if compared to cross-linking with BTCA of between 1% and 3%, respectively [22].

In this experiment, two different ways of substrate drying were used after the samples had been impregnated by a combination of chitosan/BTCA:

A) Drying using microwaves

In this case, after impregnation with chitosan/BTCA, the samples were transferred to a microwave oven, where they were dried at a microwave power of 850 W within a processing time of 3 minutes. The drying-time was defined on the basis of preliminary tests, which confirms that the impregnated samples showed no visual changes (e.g. colour) and were completely dry to the touch.

B) Drying followed by the classical pad-dry process

A laboratory drying machine represents the conventional method for textile materials' pad-dry treatment, in which the procedure of dry cross-linking is performed over a two-stage heat treatment in sequences of drying-condensation. By drying at 100 °C the impregnated material is dried at a condensation of about 170 °C; however, a reaction between the substrate and cross-linking compound takes place.

Breaking strength

The results for the breaking-strength measured in the warp and weft directions of the viscose samples dried using microwaves and by the pad-dry procedure are shown in ***Figure 3.a***. The results are compared with those of the non-functionalised sample.

The results show a breaking strength reduction in the warp and weft directions, irrespective of the drying procedure, and was significantly higher in the case of drying by the conventional pad-dry procedure (warp - 190.3 N, weft - 183.3 N) when compared to non-functionalised viscose. The reduction in breaking-strength was significantly lower after drying by microwaves (warp - 57.2 N, weft - 85.5 N) compared with the non-functionalised sample, which was also much less than in the case of drying with the use of the conventional pad-dry process. The reduction in breaking-strength is the inevitable result of cross-linking, which is generally more pronounced in the case of cotton, rather than viscose. In general, the increase in breaking-strength of the viscose after cross-linking confirms optimal cross-linking efficiency [22]. Viscose has shorter molecules than cotton fibres, but after (optimal) cross-linking, these relatively short molecules are connected together and lengthened, resulting in higher mechanical properties e.g. higher breaking-strength [22].In this case, the loss of a fabric's mechanical strength can be attributed to two main factors: acid-catalysed depolymerisation and the crosslinking of cellulose molecules. The magnitude of fabric strength-loss is affected by the temperature and time of exposure, the concentration of acid applied to the fabric, the dissociation constant of the acid, and the pH of the acid solution applied to the fabric. The reason for the relatively high reduction in the breaking-strength with the pad-dry procedure resulted from the acid-catalysed depolymerisation of viscose where BTCA was included in the system. During the pad-dry process, the material is exposed to the conditions of the treatment for a relatively-long period in compari-

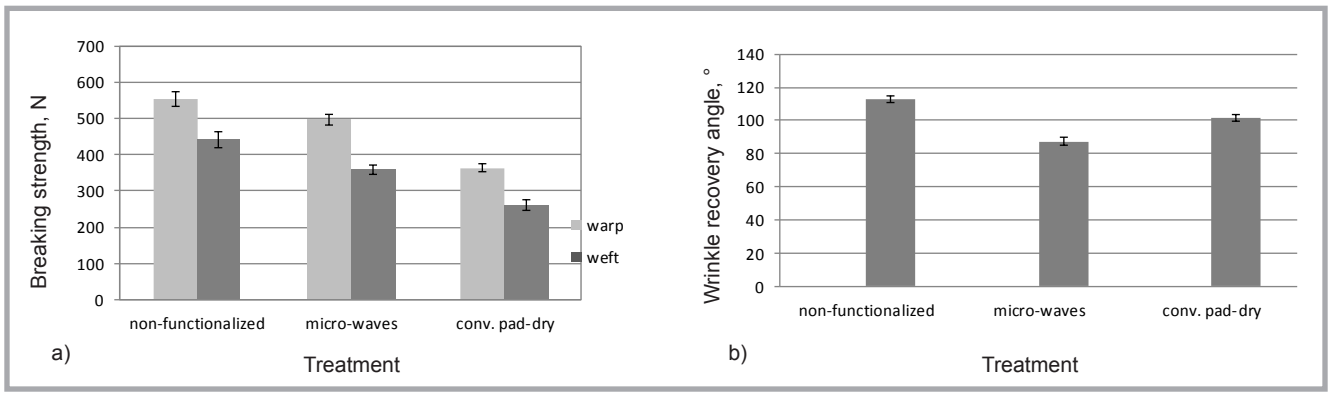

Figure 3. *Breaking strength - a) and wrinkle recovery angles - b) of non-functionalised viscose and viscose functionalised with a combination of chitosan/BTCA, after drying by microwaves and by the conventional pad-dry procedure.*

son to the microwaves, where the whole process of treatment expires within a few minutes. Previous research has shown that the optimal condition of viscose in pad-dry cross-linking is achieved with a concentration of 1% BTCA [22] within the impregnation bath which in this case was excluded in the system due to the poor solubility of chitosan in 1% BTCA. In the case of viscose functionalisation with the system, 1% chitosan diluted in 3% BTCA and drying using microwaves is, therefore, regarding the maintenance of mechanical properties, a better choice.

Wrinkle recovery angle

Results for the measured wrinkle recovery-angle of samples dried using microwaves and dried by the pad-dry procedure are shown in ***Figure 3.b***. The results are compared with those for the non-functionalised sample. The diagram in ***Figure 3.b*** shows that in comparison with the non-functionalised viscose, there was a reduction in the wrinkle recovery-angle for samples dried by microwaves (25.2°), as well as for those dried by the conventional pad-dry procedure (11°). As can be seen from the results, the wrinkle recovery angle in the case of functionalised viscose was lower than even that of the untreated viscose. In praxis this means that viscose treatment as a combination of 1% chitosan/3% BTCA within the impregnation bath causes higher wrinkling of the viscose, as in the case of non-functionalised viscose.

Such a trend is possible in a case where the hydrophylicity of the treated material is increased using functionalization. Hydrophilic polar groups, such as -COOH and $-NH_2$ introduced into the material with a combination of chitosan/BTCA, resulting in higher hydrophyilicity in comparison with the non-functionalised samples. Another reason for the wrinkle-recovery angle reduction of the treated samples may also result from the fact that functionalisation was performed within a range of pH of 2.8; however, it is known that the cross-linking of cellulose with BTCA is satisfactory when it is performed at a pH of around 2.2. A pH closer to 3 obviously does not provide such effective cross-linking as is the case of pH values close to 2.2 [11]. This relatively low pH was not used in the experiment due to the poor solubility of chitosan, which at relatively low pH values is not optimal. The optimal dissolving of chitosan can be expected within a pH range of around pH 3.6 because at this pH protonation of the amino groups is optimal and thus is the solubility of chitosan. During the experiment, the pH of the impregnation bath was set at pH 2.8, hence the boundary conditions were met. On the one hand, for crosslinking with BTCA and for satisfactory dissolving of chitosan in polycarboxylic acid a catalyst is usually also added during the process of cross-linking, which takes place over the stages of impregnation-drying-condensation. The catalyst during the (last) phase of condensation increases the concentration of hydrogen ions, and thus accelerates the rate of reaction [23,24]. In the case of cross-linking with BTCA, the most effective catalyst is sodium di-hydrogen phosphate (I) (NaH_2PO_2). Its drawback is its adverse impact on the environment, as the thermal decomposition of these salts leads to the formation of toxic phosphorus oxides and, in the worst case, highly toxic phosphine (PH_3) [23].This was the reason why a catalyst was not added to the impregnation bath. The results indicate that after functionalisation with the system chitosan/BTCA, higher wrinkle recovery angles in the case of the non-functionalized sample could not be achieved, not even with the use of microwaves nor by using the conventional pad-dry process. A comparison between the results of the two processes used in this research shows that the reduction in the wrinkle recovery-angle was smaller in the case where cross-linking was performed using the conventional method of pad-dry cross-linking.

Carboxyl group amount determination by the Methylene blue method

Results regarding the proportion of available carboxyl groups of non-functionalised viscose, viscose functionalised with microwaves, and those functionalised by the conventional pad-dry process are shown in ***Figure 4.a*** (see page 28).

The Methylene blue method was used to determine the available carboxyl groups which, after functionalisation, remain available in the material treated. As can be seen in ***Figure 4.a***, with the functionalisation, a certain proportion of available carboxyl groups entered into the viscose. Results showed that samples treated using the conventional pad-dry procedure (38 mmol/kg) exceeded the values of those treated using microwaves (31 mmol/kg) for 7 mmol/kg of available carboxyl groups. Based on the results of carboxyl group determination, it is assumed that two different reaction mechanisms exist between chitosan and BTCA in dependence on the drying techniques (microwaves, pad-dry) used in this research. Obviously in the case of drying with microwaves, electrostatic interactions between chitosan and BTCA primarily resulted in a lower carboxyl group amount (but in a higher amino group amount-see below) in comparison with the pad-dry process, where an opposite trend is seen. With the latter, according to Yang, first a cyclical anhydride is

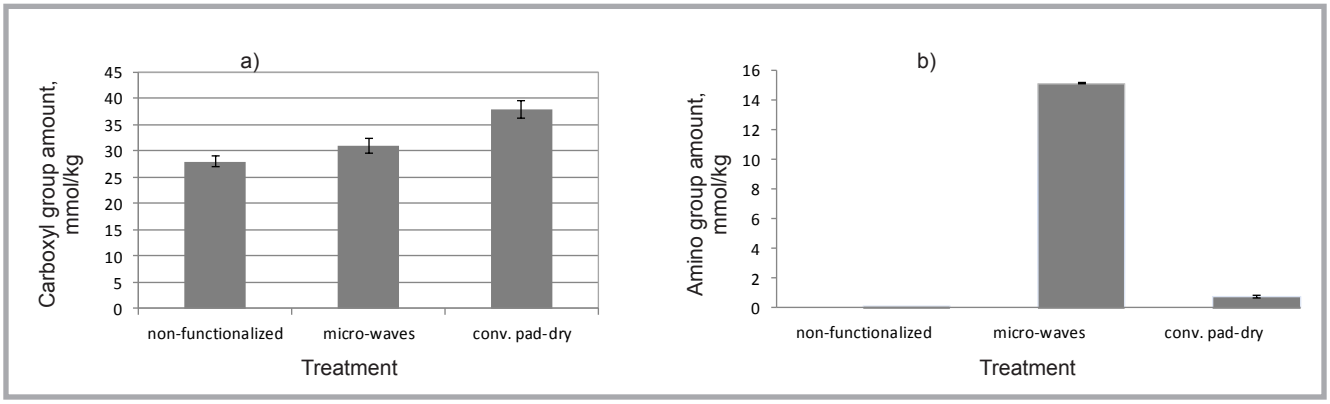

Figure 4. *Carboxyl - a) and amino - b) group amount of non-functionalised viscose and viscose functionalized with the combination chitosan/BTCA after drying by microwaves and by the conventional pad-dry procedure.*

formed, which in the next phase forms an ester with the hydroxyl group of cellulose fibres (***Figure 5***) [25]. In this way (under ideal circumstances) two carboxyl groups remain free, which could explain the situation in the case of pad-dry, where the amount of available carboxyl groups is higher in comparison to drying with microwaves.

It can also be seen from the diagram in ***Figure 4.a*** that even the non-functionalised samples contained a certain proportion of available carboxyl groups (28 mmol/kg), which can be explained on the basis of various chemical pre-treatments of the cellulosic fibres that can cause quantitative changes in the carboxyl group amounts. The ion-exchange capacity of the fibre is increased according to various pre-treatment processes, which is due to an increase in those carboxyl groups of cellulose that are ion-exchangers of cellulose macromolecules [18, 24].

Determination of amino group amount by the Acid Orange VII method

In this research, the proportion of available amino groups was chosen as the most important parameter because the anti-microbial action of the material depends on the amount and availability of amino groups, which are crucial to ensure efficiency regarding micro-organism reduction. For this reason, the functionalisation of viscose using the system of chitosan/BTCA was used primarily to increase the proportion of amino groups that could potentially reduce a wide variety of pathogenic microorganisms. The proportion of amino groups determined by the Acid Orange VI method, depending on the treatment, is shown in ***Figure 4.b***. Here a significantly higher proportion of available amino groups can be seen in the case of treatment using microwaves than in that of samples which, after impregnation with the system chitosan/BTCA, were treated according to the conventional pad-dry process.

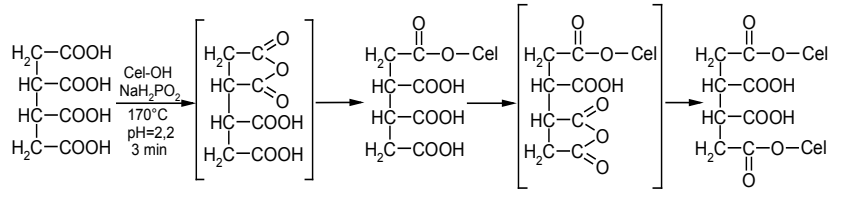

Figure 5. *Mechanism for cross-linking hydroxyl groups of cellulose with BTCA, forming cyclical anhydride [25].*

Table 2. *Reductions (R in %) of microorganisms regarding non-functionalised viscose, and viscose functionalised with a combination of chitosan/BTCA, after drying by microwaves and the conventional pad-dry procedure.*

Sample	Reduction R, %	
	Staphylococcus aureus	*Candida albicans*
Reference	57	57
Micro-waves	100	100
Conventional pad-dry	92	94

As can be seen from the diagram in ***Figure 4.b***, there was a significantly higher difference (14.41) regarding the amino group amount for those samples treated using microwaves (15.4) in comparison with those samples treated by the conventional pad-dry procedure (0.73). The results clearly show that drying using microwaves was more effective with respect to achieving a relatively high proportion of amino groups. In this case, where the molecules are in a constant motion due to the dipole moment, certain changes occurred in the conformation of the chitosan polymer chains. It is assumed that in such an example the oriented/bended chitosan molecules generated a greater extent of available amino groups in comparison with drying performed using the pad-dry procedure. On the other hand, as is explained based on results of carboxyl group determination, the reaction mechanism contributes to the difference in the amino group amount between samples treated using microwaves and those treated using the pad-dry process, respectively.

Anti-microbial testing

Anti-microbial testing was used to examine the impact of the amino group amount on the antimicrobial properties of functionalised viscose [26]. From the results shown in ***Table 2***, a reduction can be seen regarding pathogenic *Staphylococcus aureus* and *Candida albicans*, respectively. These results also demonstrate that the reference, which means the non-functionalised sample, showed a positive reduction in the pathogenic microorganisms selected, which was 57% in both cases. The most likely cause of the inhibition of the pathogenic microorganisms of the non-functionalised sample is the adsorption of the microorgan-

isms onto the textiles. It must be pointed out that the reduction of microorganisms under 60% using the ASTM E2149-01 test method [16], which is a quantitative antimicrobial test method, means a lack of antibacterial effectiveness. Notwithstanding this, the results showed that that the adsorption of chitosan onto viscose, irrespective of the type of treatment chosen, contributed to reductions in *Staphylococcus aureus* and *Candida albicans*, which is, in the example of microwave treatment, 100% against both microorganisms used in this research. On the other hand, in the case of the conventional pad-dry procedure, reductions regarding *Staphylococcus aureus* were 92% and for *Candida albicans* 94 %, respectively.

From the results it is obviously that the antimicrobial property is dependent on the amino group content, which is the highest in the case of microwave drying. These samples show the most intensive reduction in pathogens.

Conclusions

Based on the results, it can be concluded that the functionalisation of viscose using the system of chitosan/BTCA indicated various manifestations and properties regarding functionalised viscose, which depends on the processing method.

Thus treatment with the conventional pad-dry procedure proved that in this case relatively good efficiency can be achieved regarding breaking strength and wrinkle-recovery angles. For this type of treatment, the proportion of amino groups that would be comparable to the proportion of amino groups present in the viscose treated by microwaves is impossible to achieve.

By contrast, in the case of treatment using microwaves, satisfactory mechanical properties cannot be expected through the functionalisation of viscose using a system of chitosan/BTCA when compared to the samples treated according to the conventional pad-dry procedure.

It was seen that the treatment of samples using microwaves can create conditions for the contribution of a relatively high proportion of affordable anti-microbial-acting amino groups of viscose, functionalised with the system of chitosan/BTCA, which was the main plan of this research.

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Received 05.01.2012 Reviewed 31.01.2013