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Functionalization of Non-woven Viscose with Formulation of Chitosan and Honey for Medical Applications

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Abstract

The aim of this research was to develop the formulation of chitosan in combination with honey in different mass proportions of each of the components within the separate mixture. Such a formulation could serve as a functional coating suitable for wound healing. From the perspective of different formulations used within research presented, it is assumed that the different mass fraction of components will affect antimicrobial and antioxidant activity of the functionalised substrate differently. To apply the separate formulation onto a non-woven viscose substrate, the conventional pad-drying process was selected. Moreover a study of the effectiveness of the individual treatment was performed systematically, which is also reflected in the systematics of the experimental techniques selected. Considering antioxidant and antimicrobial action, honey-functionalised non-woven viscose shows higher effectiveness if compared to non-woven viscose functionalized with the chitosan:honey combination.

Key words: chitosan, honey, viscose, non-woven, antimicrobial, antioxidantivity.

Introduction

A modern concept of textile functionalisation introduces natural compounds in the field of textile finishing due to the fact that they have no adverse impact on the potential user or the environment [1-4]. Many natural compounds of plant and animal origin are interesting, but chitosan is still considered as one of the most notable functionalisation compounds [5], whose antimicrobial character is associated with its amino groups [1, 6]. Despite the excellent property of antimicrobial activity of chitosan, it should be taken into account that it is not an antioxidant, since antioxidantivity is a desirable and necessary property regarding the creation of medical sanitary products as antioxidants improve wound healing [7]. For this purpose, honey is very interesting, as the physical properties of which, in terms of acidity (pH 3.2 to 4.5), stimulate the release of oxygen from hemoglobin, thus slowing down the action of proteases, which otherwise inhibits wound healing. Besides this, honey, due to the high content of sugars, has high osmolarity, which means that it, as such, promotes the secretion of fluids, resulting in faster wound healing [7]. Wound healing is a biological process taking place through

a variety of related phases: hemostasis, inflammation, cell division or proliferation, as well as tissue remodeling, or maturation, which should take place within an appropriate time sequence, when talking about correct and complete wound healing [8]. In relation to this, a combination of natural compounds applied onto a cellulosic textile substrate is extremely promising to create sanitary and medical products. Therefore the primary purpose of the research work presented was to develop a formulation of chitosan in combination with honey in different mass proportions of one and the other of the natural compounds. Such a formulation could serve as a functional coating suitable for wound healing. It was predicted that the ratio difference between chitosan and honey would be seen as the difference in antimicrobial and antioxidant activity of the differently functionalised substrate. For application of the functionalization formulations selected, non-woven viscose was used, which is a common carrier substrate in the field of medicine and medical products, respectively [2, 3].

Experimental

Materials

Viscose: As a basis for the application of functionalised formulations, a non-woven commercial product from non-woven viscose in the form of a strap (producer Tosama d.o.o) obtained by the process of carding, laying and needling was used (surface mass: 165 g/m², width: 45 mm).
Honey: 100% Slovenian chestnut honey

(producer: Alojz Jakopic, Slovenia), the sum of glucose and fructose: 45g/100 g.
Preparation of chitosan solution: 0.5% solution of chitosan (Kitozyme, Belgium, molar mass- ~ 82.000, 22.4% acetylated) was prepared by suspending chitosan in ultra-pure Mili-Q water and afterwards setting it to a pH of 3.6 with the use of hydrochloric acid (HCl). The resulting suspension was then stirred for 24 hours at room temperature using a laboratory magnetic stirrer until achieving complete dissolution of the chitosan.
Preparation of the chitosan: honey mixture: A mixture of 0.5% chitosan solution and honey in mass ratios of 1:1, 1:2 and 1:3 was prepared and then stirred for 30 min. using a laboratory magnetic stirrer until achieving an homogeneous mixture.
Viscose functionalisation: Non-woven viscose samples were treated under constant conditions using the procedure of impregnation (30 min, room temperature, wet pickup 80-100% (W. Mathis, Switzerland) and drying in a laboratory dryer (Kambič d.o.o, Slovenia), T_{drying} = 30 °C, t_{drying} = 16 min).

Abbreviations of the non-functionalized (reference), and functionalised non-woven viscose samples can be seen in *Table 1*.

Preparation of desorption bath

Deionized water was used in the experiment. In order to obtain acidic pH of laboratory soft water, 0.1 M HCl was used to set pH to 3.7. Such pH is required to protonate amino groups of chitosan (NH₃⁺).

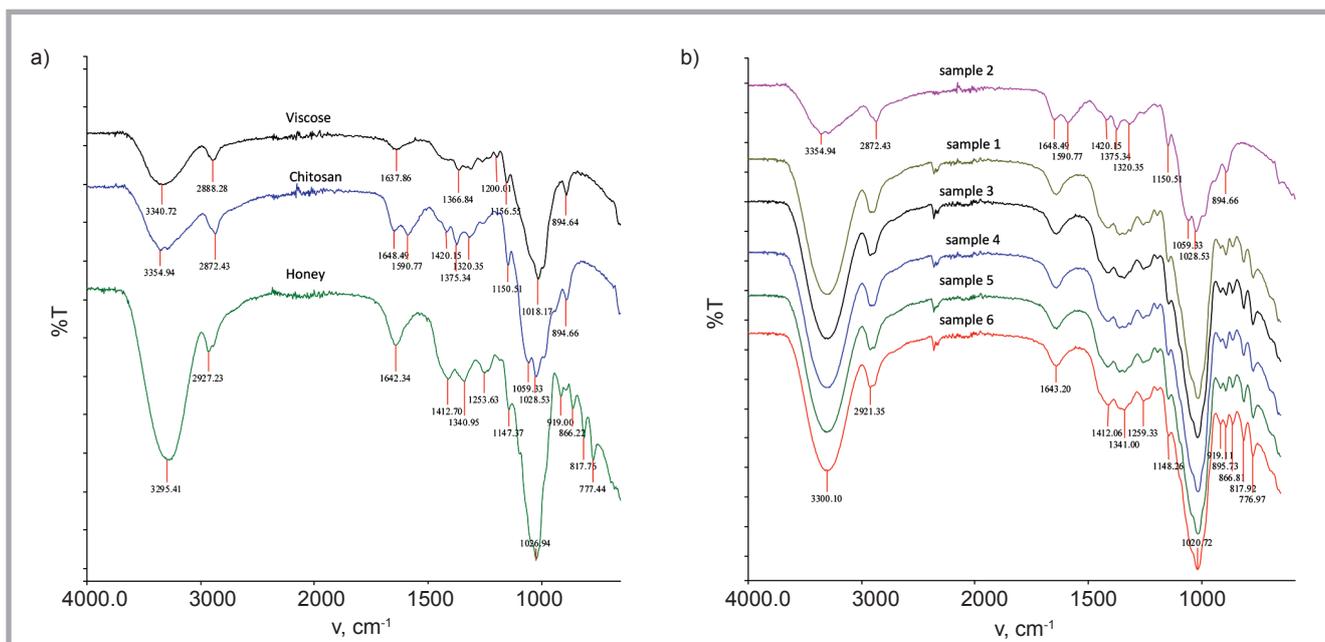


Figure 1. ATR FTIR spectra of non-woven viscose, hitosan and honey a), and of viscose functionalised with a separate functionalisation formulation (sample 1-sample 6) b).

Analytical methods

Functionalisation efficiency

ATR FTIR spectroscopy

Samples were examined by an Attenuated Total Reflection Fourier transform infrared (ATR FTIR) Perkin Elmer Spectrum GX spectrometer. A total of 16 scans were taken for each sample at a resolution of 4 cm⁻¹.

Spectrophotometric method:

Acid Orange VII

The method is based on the use of the acid dye Acid Orange VII. Acidic dyes possess anionic sulfonic groups with the tendency to be attracted electrostatically by cations of chitosan at a stoichiometry ratio of 1:1 [1, 12].

The proportion of available amino groups can be calculated using **Equation (1)**:

$$A_i = (A_0 - A_f) V 10^6 / k \cdot l \cdot mf \quad (1)$$

Where: A_i is the amount of amino groups of chitosan bound to the fibres/(mmol/kg),

A_0 – initial absorbance, A_f – final absorbance, V – volume of the dye bath/l, k – correction factor/(l/mol cm), l – length of optical field/cm, mf – mass of absolutely dry fibres/g.

Antimicrobial activity

Antimicrobial testing was carried out at the company Tosama d.o.o. using the method of the zone of inhibition determination in accordance with the modified standard AATCC 147.

Antioxidant activity

Antioxidant activity of the functionalized non-woven viscose was evaluated using the ABTS^{•+} method (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid, Sigma Aldrich). Radical ABTS^{•+} occurs during the oxidation of ABTS, as well as potassium persulfate absorbance in the visible region at a wavelength of 734 nm. This is determined spectrophotometrically. Antioxidants are hydrogen donors, therefore a free radical is reduced in contact with potassium persulfate, which is

visible as discoloration i.e. in the change of solution absorbance **Equation (2)**.

$$\text{Inhibition} = \frac{A_{\text{starting}} - A_{\text{sample}}}{A_{\text{sample}}} \cdot 100, \% \quad (2)$$

Where: A_{starting} – absorbance measured at the initial concentration of ABTS^{•+}, A_{sample} – absorbance measured at the rest concentration of ABTS^{•+} [9, 10].

Desorption

Polyelectrolyte titration

The specific charge of the polymer is dependent on the ionisation of the polymer's functional groups. In the case of conventional polyelectrolyte titration, the end point of reaction i.e. changes in colour, is usually estimated visually, or by using a phototrode [11], as in this research. **Sample preparation:** To 40 ml of distilled water, 500 ml of indicator Toluidinblau O/Basic Blue 17 and 1 g of a previously prepared sample of non-woven viscose were added. Polyvinyl sulfonic sodium salt was used as a polyelectrolyte titration medium for each of the samples. For measurement, a phototrode – DP660 (Mettler Toledo) was used to record the end-point for each of the samples.

Acid Orange VII method

To determine the amount of accessible amino groups of chitosan released from the surface of the functionalized non-woven

Table 1. Abbreviation of samples.

Abbreviation	Sample
1	Reference (non-functionalized sample)
2	Non-woven viscose treated with chitosan
3	Non-woven viscose treated with honey
4	Non-woven viscose treated with chitosan: honey, ratio 1:1
5	Non-woven viscose treated with chitosan: honey, ratio 1:2
6	Non-woven viscose treated with chitosan: honey, ratio 1:3

ven viscose, the Acid Orange VII method was used. The procedure was the same as in the case of the functionalisation efficiency evaluation.

Results and discussion

Functionalisation efficiency

ATR FTIR spectroscopy

ATR FTIR spectroscopy results are shown in **Figure 1**. **Figure 1.a** shows FTIR spectra of viscose, chitosan and honey. The FTIR spectrum of viscose shows characteristic absorption bands in the spectral regions of 2950-2750 cm^{-1} and 3600-3000 cm^{-1} , attributed to aliphatic C–H stretching vibrations and O–H stretching vibrations. The FTIR spectrum of chitosan shows typical peaks at 1648 and 1590 cm^{-1} . These two wavenumbers are assigned to the carbonyl stretching vibration (amide I) and N-H bending vibration (amide II) of a primary amino group, respectively. The FTIR spectrum of honey shows peaks at the following wavenumbers: 3295 cm^{-1} , 1642 cm^{-1} , 1412 cm^{-1} and 1340 cm^{-1} .

In **Figure 1.b**, sample 2 (non-woven viscose treated with 0.5% solution of chitosan) indicates typical signals for chitosan within the area between 1660 and 1590 cm^{-1} , which are assigned to the carbonyl stretching vibration (amide I) and N-H bending vibration (amide II) of a primary amino group. Sample 1 represents FTIR spectra of non-functionalised non-woven viscose. FTIR spectra of sample 3, non-woven viscose functionalised with honey, show peaks at the following wavenumbers: in the range 3290-3300 cm^{-1} , 1642 cm^{-1} , 1412 cm^{-1} and 1340 cm^{-1} , which are typical signals for honey. The FTIR spectrum of samples 4, 5 and 6 indicates typical signals for honey. In those cases, typical chitosan signals at 1660 cm^{-1} and 1540 cm^{-1} overlap with the broad honey signal at 1650 cm^{-1} . In contrast, with all functionalised non-woven viscose there are detected signals attributed to honey (**Figure 1.a**).

Acid Orange VII method

From **Figure 2** it can be seen that untreated non-woven viscose and non-woven viscose functionalized with a mixture of chitosan and honey at the ratio of 1:3 have no accessible amino groups. This trend arises from the fact that amino groups are not part of the chemical constitution of non-woven viscose, and hence they are not possible to be detected. The same situation applies in the exam-

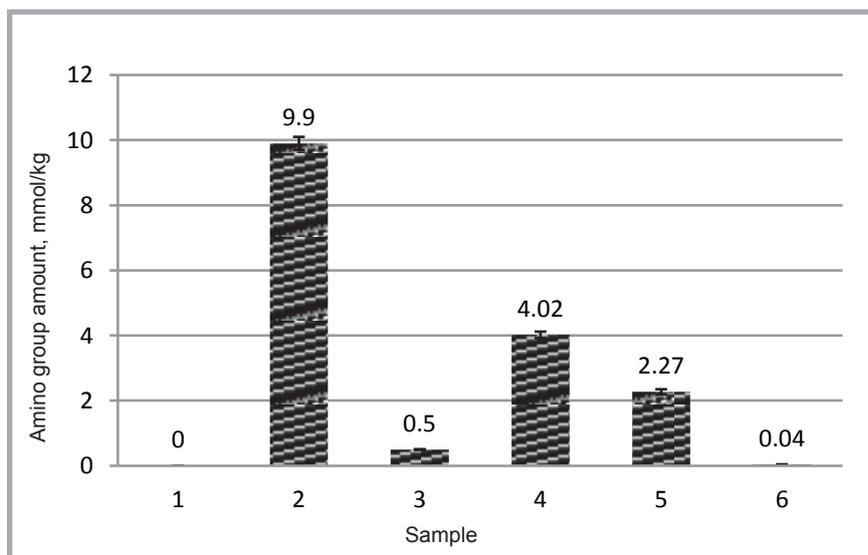


Figure 2. Proportion of available amino groups of functionalised non-woven viscose depending on functionalisation formulation.

ple where the major proportion of the functionalisation formulation (sample 6) is represented by honey. By covering chitosan present in functionalized non-woven viscose, this compound prevents the access of Acid Orange VII dye to the chitosan, consequently amino groups cannot be detected in such a situation. When compared to sample 6, a gradual increase in the proportion of accessible amino groups is seen with non-woven viscose functionalisation based on formulations that are a combination of chitosan and honey. The trend is as follows: sample 5 (2.07 mmol/kg), sample 4 (4.02 mmol/kg). Such a phenomenon is attributed to the lower amount of honey in the mixture with chitosan; hence chitosan is covered with honey to a lesser extent, as in the case of sample 6.

With respect to amino group presence (0.5 mmol/kg), this is not the case in non-woven viscose functionalised with honey (sample 3), no matter if a relatively small proportion of amino groups is detected; thus confirming the situation

that Acid Orange VII dye is only slightly adsorbed onto the surface of the honey-treated non-woven viscose. Obviously the high osmolarity of honey caused the attraction of the dye to the surface of the substrate. In general with this technique, a relatively small number of amino groups are detected in the case of chitosan:honey treated non-woven viscose, which is probably due to the chemical or physical interaction of these groups with specific groups of honey. Contrary to that, non-woven viscose functionalised with chitosan (sample 2) shows the highest amount of accessible amino groups (9.9 mmol/kg), since amino groups are part of its chemical constitution.

Antimicrobial activity

Results of the zones of inhibition are shown in **Table 2**.

The reduction in *S. aureus* is proved to be relatively successful in the case of honey-treated non-woven viscose as well as with non-woven viscose treated with honey in combination with chitosan.

Table 2. Zone of inhibition for functionalised non-woven viscose together with the reference. Note: * Results are the average of four measurements.

Vzorec	*Average zone of inhibition – reduction in growth in mm		
	Test organisms		
	<i>Staphylococcus aureus</i> (Gram positive)	<i>Escherichia coli</i> (Gram negative)	<i>Pseudomonas aeruginosa</i> (Gram negative)
1	0	0	0
2	0	0	0
3	3.34	7.60	10.62
4	5.89	0	0
5	6.40	3.45	0
6	7.51	5.15	0

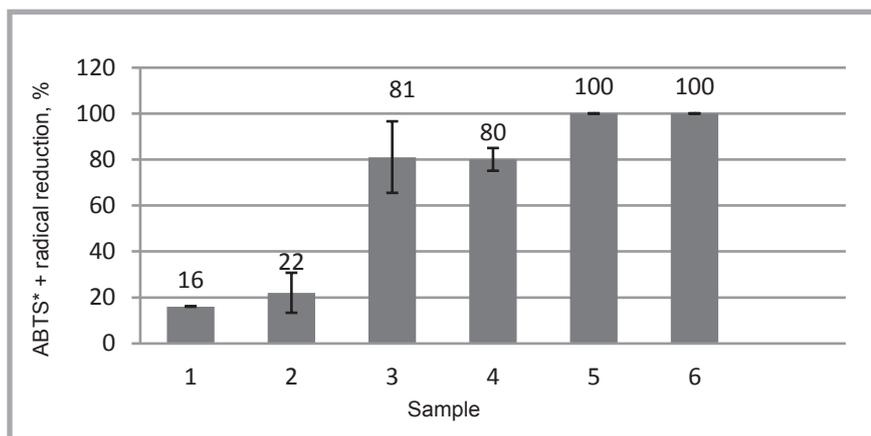


Figure 3. Antioxidativity of the reference and functionalized non-woven viscose depending on the functionalization formulation.

The reduction in this type of microbe rises by increasing the proportion of honey in combination with chitosan, where the average zone of inhibition for sample 4 is 5.89 mm, 6.40 mm for sample 5 and 7.51 mm for sample 6. On the other hand, the functionalisation of non-woven viscose with honey (sample 3) shows a relatively low zone of inhibition of 3.34 mm, exhibiting a relatively low inhibitory activity of honey in relation to this bacterial strain. Non-woven viscose functionalised with 0.5% chitosan solution (sample 2) gives no zone of inhibition for *S. aureus*, thus in this case there is no antimicrobial efficiency seen with this sample. In any case of untreated non-woven viscose, this cannot contribute to antimicrobial activity at all, and hence there is no zone of inhibition with the reference (sample 1). *E. coli* as a representative of Gram-negative bacterial strain is insufficiently reduced with sample 2, reflecting the fact that the functionalisation of non-woven viscose with 0.5% chitosan solution gives no zone of inhibition. In contrast, a relatively high inhibition of *E. coli* is seen with the honey-functionalized viscose (sample 3), since in this case the average zone of inhibition is 7.60 mm. Taking into account the different proportion of honey in combination with chitosan, results of the average zone of inhibition are as follows: sample 4-no zone of inhibition, sample 5-3.45 mm, and sample 6-5.15 mm. With exception of sample 4, where the zone of inhibition is not seen with this sample, in other cases of non-woven viscose chitosan:honey functionalisation it can be seen, but with the same reduction-trend as with *S. aureus*, i.e. with an increasing proportion of honey in combination with chitosan the zone of inhibition increases as well (sample 5 and sample 6). The re-

duction in *P. aeruginosa* is, in general, not successful, as there is no zone of inhibition for samples 2, 4, 5 & 6. Only honey-functionalised non-woven viscose shows 10.62 mm of the zone of inhibition in relation to the reduction in *P. aeruginosa*. Again the reference shows no zone of inhibition, since it is a non-functionalized sample and, therefore, cannot contribute to antimicrobial efficacy at all. In general, the best result regarding antimicrobial efficiency is shown by honey-functionalized non-woven viscose. Also the addition of honey to chitosan contributed to a more effective microbial inhibition of *S. aureus*. Together with this, it is somewhat surprising that the non-woven viscose treated with 0.5% solution of chitosan seems to be less appropriate regarding a reduction in selected pathogenic microbes due to the fact that chitosan is considered as a compound with good antimicrobial efficiency. In any case, such a study may be extended by increasing the time of exposure to the microbial functionalisation formulation using a higher concentration of chitosan (e.g. 1%) or some other antimicrobial testing procedure (e.g. dynamically-shaking test) to see if under these conditions chitosan is more pronounced as an antimicrobial agent. It should be mentioned that 0.5% of chitosan was used within the present research to avoid relatively high viscosity of functionalization formulations, especially those combined with honey. In the context of this research two types of gram-negative microorganisms (*E. coli* and *P. aeruginosa*) were chosen as test organisms, but it is seen that they respond differently to treatment with the same compound. All this emphasised the fact that various bacterial strains react differently to individual compounds,

and hence results cannot be generalised. Results highlight the fact that the most successful seems to be non-woven viscose functionalisation with honey, although in practice it is considered that in general diluted honey gives a better result if compared to undiluted. This is due to dilution, where honey becomes strong in enzymes, producing hydrogen peroxide, which is otherwise crucial for successful inhibition of microbes [13, 14].

Antioxidant activity

Results of antioxidant activity are shown in **Figure 3**, in which chitosan-functionalised non-woven viscose shows very poor antioxidant action. Therefore it can be stated that there is insufficient antioxidant activity, the same as with the reference, since these substrates have no attributes to be considered as antioxidant-active. In addition, the antioxidant activity of all other samples of functionalized non-woven viscose where honey is part of the functionalisation formulation is significantly high. Such a high antioxidant activity does not arise from the inclusion of chitosan in the system with honey, because chitosan is not an antioxidant; however, this phenomenon seems to be attributed to honey in diluted form, which is the main point of maintaining antimicrobial properties [13, 14]. Much more effective diluted honey activity than undiluted is also seen in the case of the antioxidant action of non-woven viscose treated with chitosan:honey-based functionalization formulations, where honey is diluted with chitosan [15, 16]. In terms of an optimal ratio of chitosan and honey, assuming 100% antioxidant activity, it is possible to determine an optimal ratio of honey in combination with chitosan, which in this case is achieved with a ratio of chitosan:honey of 1:2.

Desorption

Polyelectrolyte titration

Figure 4.a shows an example of a typical polyelectrolyte titration curve in the case of chitosan desorbed from the surface of non-woven viscose. The red circle in diagram 4a indicates the point at which it is calculated and the actual volume of titrant PES-Na needed to establish equilibrium between the solution and the material [11]. On the basis of the actual volume of PES-Na used, the proportion of amino groups in dependence on the desorption bath was calculated afterwards (see **Figure 4.b**). As can be seen from **Figure 4.b**, the proportion

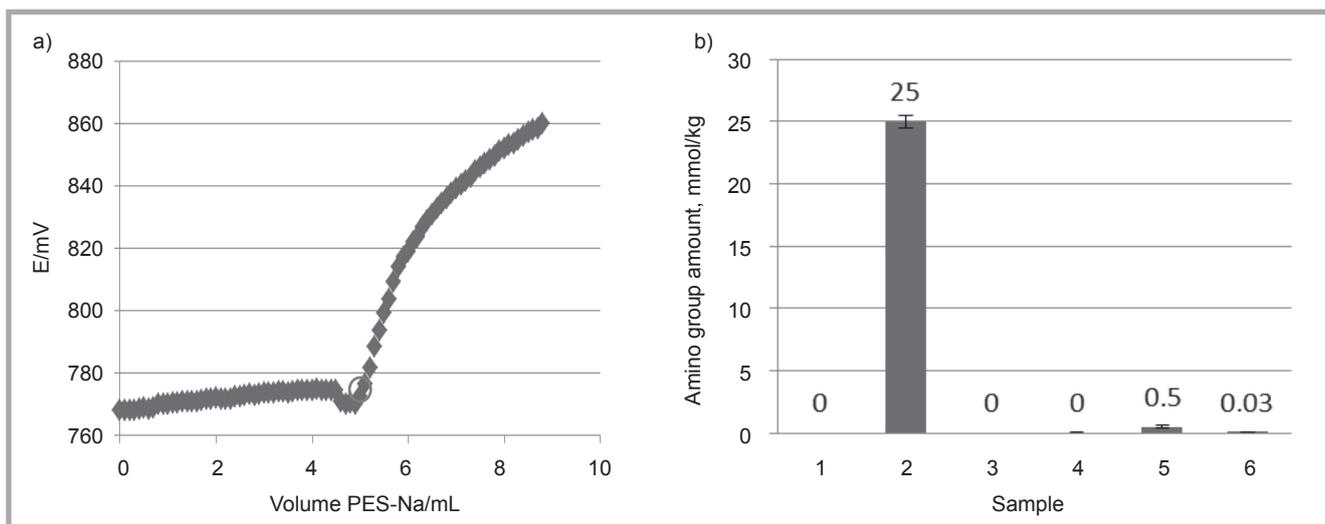


Figure 4. a) Example polyelectrolyte titration curve of chitosan desorbed from the surface of functionalized non-woven viscose, b) Proportion of amino groups depending on the functionalization formulation determined with polyelectrolyte titration.

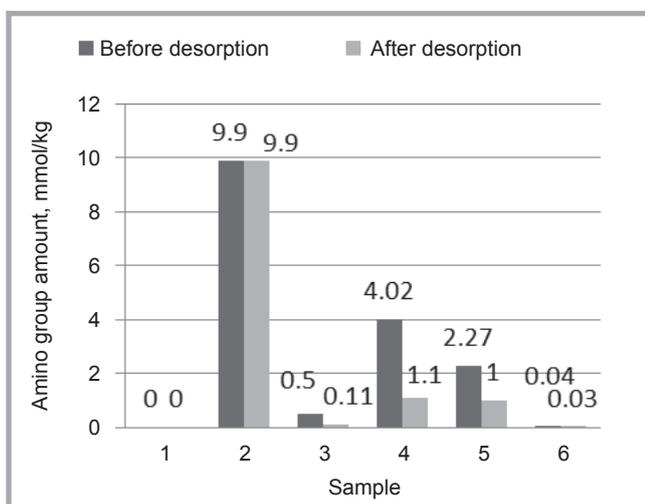


Figure 5. Proportion of amino groups of differently functionalized non-woven viscose before and after desorption.

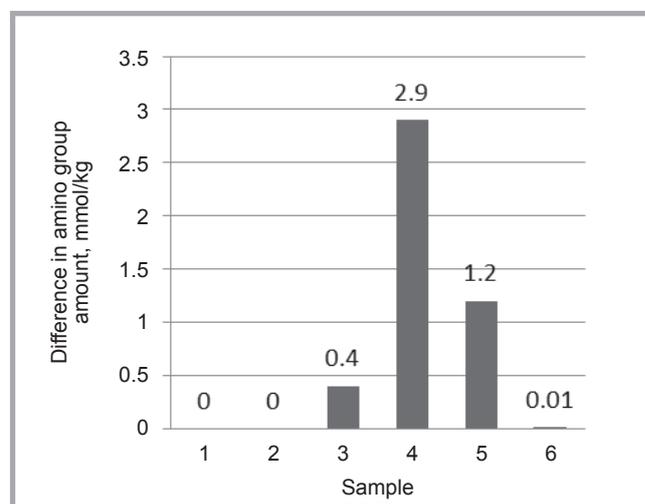


Figure 6. Proportion of amino groups in desorption bath after desorption.

of chitosan desorbed from the surface of chitosan-functionalized non-woven viscose determined with polyelectrolyte titration is 25 mmol/kg, and in the case of chitosan:honey-1:2 functionalized non-woven viscose – a modest 0.5 mmol/kg. In all other cases, by using polyelectrolyte titration, potential release of chitosan into the desorption bath was not seen.

Method Acid Orange VII

From the point of view of the stability of the functionalization formulation applied onto the substrate, there is very interesting data on the proportion of the compounds that possibly migrate from the surface into the desorption bath. In this case, the desorption bath represents de-ionized water, where pH was set to 3.7 to protonate amino groups of chitosan.

By using the Acid Orange VII method, it is possible to calculate the presence of amino groups of chitosan that are still accessible in the substrate after desorption (**Figure 5**). Thus it is necessary to calculate the difference in amino groups before and after desorption (i.e. the amount of amino groups in the desorption bath), which is represented by **Figure 6**. From the results it can be seen that chitosan-functionalized non-woven viscose shows no migration of chitosan from the surface of the functionalized non-woven viscose; the same trend is seen with sample 6 (chitosan:honey 1:3). Furthermore a relatively small proportion of desorbed chitosan (2.9 and 1.2 mmol/kg) is seen for samples 4 and 5.

Thus this example shows a situation where a relatively high proportion of honey

in the mixture with chitosan potentially covers the chitosan, thus preventing the accessibility of amino groups to be determined with the Acid Orange VII method. In the case of sample 1, there is no sign of amino groups within the desorption bath because in this case this is reference non-woven viscose without any application of functionalization formulation onto it at all. Results of Acid Orange VII (indirect method) are not exactly the same as with polyelectrolyte titration (direct method), which is, on the other hand, a very precise technique. Nevertheless in the case of both methods the same trend is seen in some cases, indicating the fact that in the case of the Acid Orange VII method it is possible to conclude that this is a good supporting and quick technique to evaluate the amino group presence of functionalized non-woven viscose.

■ Conclusion

The effectiveness of separate functionalisation formulation was evaluated systematically, which is also reflected in the scheme of selected experimental techniques. ATR FT-IR analysis confirmed the coating of chitosan and honey to the non-woven viscose substrate, but the coating of non-woven viscose with chitosan:honey formulation cannot be confirmed. The zone of inhibition shows good inhibition of *E. coli*, and *S. aureus*, while a reduction in *Pseudomonas aeruginosa* is possible to achieve only with honey-functionalized non-woven viscose. The presence of honey within the functionalization bath is reflected in very high antioxidant action, especially in the case of combining honey and chitosan. Acid Orange VII results showed no migration of chitosan from the surface of the functionalized non-woven viscose. Furthermore a smaller proportion of honey in combination with chitosan resulted in a higher proportion of chitosan desorbed from the functionalized non-woven viscose surface. The best result in terms of antioxidant and antimicrobial activity is shown by honey-functionalized non-woven viscose. However, the inclusion of chitosan in the treatment system with honey contributed to an increase in its anti-oxidant activity due to honey in a diluted form, which in terms of maintaining antimicrobial properties is much more effective undiluted. Results confirmed the situation that the properties of the functionalized viscose are dependent on the functionalization formulation used.



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