Modified with chitosan cotton fabric for control release of indomethacin

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Abstract. The study aims to obtain and characterize new composite materials with potential application as antimicrobial wound dressings releasing an anti-inflammatory biologically active substance indomethacin. The preparation of the material involves an impregnation of the cotton fabric with chitosan crosslinked with citric acid. Indomethacin interacts with the chitosan by ionic bonds at the appropriate pH. The obtained materials were characterized by infrared spectroscopy and optical microscopy. The mechanism of the indomethacin release from composite materials in phosphate buffer pH 7.4 at 37 °C was examined using different kinetics models. The concentration of citric acid affects the release of the drug, and the material with the highest concentration of citric acid has more antimicrobial activity than the other samples. The composites showed a slightly higher antimicrobial effect against Gram-negative Pseudomonas aeruginosa than against Gram-positive Bacillus cereus.

1. Introduction
Textile materials modified with high molecular weight biologically active substances find application in medical practice in the local treatment of the skin and transdermal therapy [1]. One of the advantages of these materials is their active interaction with the environment [2]. They can react to changes in external conditions with controlled biomedical action (release of biologically active substances, absorption of substances released during sweating, sensor properties, antimicrobial activity, etc.) [3].

In recent years, polysaccharides are a subject of intensive study due to their numerous advantages: biocompatibility, biodegradability, good permeability, wide distribution in nature, low cost and others [4,5]. Starch, carboxymethylcellulose, alginate, carrageenan, and chitosan are polysaccharides utilize in textile processing. They are also used for hydrogel preparation with a biomedical application [6].

Chitosan has many applications in the textile industry, such as wastewater treatment, odour removal, antistatic, and crease-resistant finishing. It also enhances textile dyeability, printability, and increase the obtained colour strength. In the medical textile, chitosan finds application as wound dressing and sutures as it accelerates wound healing, blood coagulation and has antimicrobial properties. [7,8].

Textile materials modified with chitosan hydrogel are suitable composites for the gradual release of a biologically active substance. Chitosan is a natural stimuli-responsive polymer that is capable of showing both thermo-responsive and pH-responsive properties. The presence of free amino and
hydroxyl groups in its structure allows it to be modified and interact with various inorganic and organic compounds [9].

Usually, the chitosan dissolved in a dilute solution of acetic acid has applied to the treatment of cotton fabric. However, this method does not create any strong chemical bonds between chitosan and cotton fabrics. As the chitosan is water-soluble, the obtained modification of fabric is not durable in repeatable washing. Therefore, it is necessary to use a crosslinking agent to link the reactive groups of chitosan and cellulose to improve the durability and efficiency of the resulting material [10]. Citric acid can interact with the hydroxyl functional groups of chitosan and cellulose and form covalent bonds with heat treatment and in the presence of catalyst [11]. Another possible interaction is through ionic bonds between protonated amine groups and deprotonated carboxylate ions [12].

The chitosan-treated cotton fabrics have antimicrobial properties when the amino groups of chitosan are ammonium salts in dilute acid solution. The salt then binds to the negatively charged surface of the microorganism. Protonated amino groups of chitosan can also form ionic bonds with the groups of biologically active substances. This pH-dependent interaction and subsequent separation is a way to obtain controlled drug release.

Indomethacin is a non-steroidal anti-inflammatory drug. It acts by blocking in the body an enzyme called cyclooxygenase that is involved in the production of irritating chemicals in response to surgery or illness [13]. Its controlled release has shown the potential to accelerate wound healing and the inflammatory processes [14,15]. However, its disadvantage is its low solubility in water. Therefore, it is not enough for the hydrogel alone to swell to release the drug, but the process of its release can be aid by hydrogel erosion. Indomethacin is acidic due to the ionization of the carboxylic acid group and has a pKa value of 4.5. The molecules of indomethacin and chitosan can interact with electrostatic bonds, which association and dissociation are pH-dependent.

The study aims to obtain new composite materials with potential application as antimicrobial dressings for wounds that released an anti-inflammatory biologically active substance (indomethacin). The cotton fabric was modified with chitosan, indomethacin and citric acid bonded via electrostatic bonds. The influence of citric acid on the indomethacin release and antimicrobial activity of the composite materials were investigated.

2. Materials and methods

2.1. Materials

Chitosan with molecular weight ranging from 600 000 to 800 000 was purchased from Acros organics. Citric acid, indomethacin and glacial acetic acid were used as obtained from Sigma Aldrich. A cotton fabric with a surface weight of 135±5 g/m² was used throughout the work. Sodium dihydrogen phosphate dehydrate and di-sodium hydrogen phosphate dodecahydrate were obtained from Merck. All solutions were made with distilled water.

2.2. Preparation of composite materials

Chitosan (2.7% w/v) was dissolved in water with stirring, gradually adding glacial acetic acid (1% v/v) till obtaining a clear, viscous solution. The solution was then left at room temperature for 24 hours to remove air bubbles formed during stirring. The indomethacin was dissolved in ethanol and the corresponding quantity (26 mg/g chitosan) was added to the chitosan solution. The pH of the solution was measured as pH 5.5. The cotton fabric was impregnated with the solution of the citric acid in different concentrations (1%, 5%, and 10% w/w chitosan) and dried at room temperature. The obtained samples were named m1, m2, and m3, respectively. Next, the samples were impregnated with the solution of chitosan and indomethacin. The volume of solutions was of liquor to goods ratio 2:1. The samples were dried at 50 °C for 40 minutes.
2.3. Analysis
The cotton fabric and the composite material m3 were analyzed by Optical microscope Levenhuk Rainbow D50L PLUS 2M Digital Microscope, Moonstone with 2 Mpx Digital Camera. The magnification of the pictures was x40. IR analysis was carried out on an Infrared Fourier Transform spectrometer (IRAffinity-1, Shimadzu) equipped with a diffuse-reflectance attachment (MIRacle Attenuated Total Reflectance Attachment). Measurements were done using spectral range 600–4000 cm\(^{-1}\). The pH of the prepared solution was measured with Hanna instruments, Microprocessor pH Meter. The release of the indomethacin from composite materials was followed with ONDA Spectrophotometer UV-31 SCAN, 190-1100 nm.

2.4. In Vitro Drug Release Studies
The indomethacin release has been following in pH 7.4, 0.01 M phosphate buffer at 37± 0.5 °C by measuring the absorbance at λ=320 nm. Each sample was irrigated at 20 ml buffer. 5 mL of solution was withdrawn from the vessel and replaced with 5 mL new amount of phosphate buffer at 37 °C. The concentration of withdrawn solution was determined by UV–vis spectrometry at 320 nm against a standard curve obtained by measuring indomethacin solutions of known concentrations. Each determination was carried out in triplicate.

To identify the mechanism for the release of indomethacin from crosslinked chitosan, the suitability of four kinetics models were evaluated according to the equations presented in Table 1. \(Q_t\) is the cumulative amount of drug release at time \(t\), \(Q_0\) is the initial amount of drug in the solution. \(K\) is the release constant of the relevant model. \(M_t/M_\infty\) is a fraction of drug release at time \(t\). \(n\) is an exponent. The value of \(n\) characterized the release mechanism of the drug: \(0.45 \leq n\) corresponds to a Fickian diffusion mechanism; \(0.45<n<0.89\) to non-Fickian transport, \(n=0.89\) to Case II (relaxational) transport; \(n>0.89\) to super Case II transport.

| Kinetic model            | Equation                        |
|-------------------------|--------------------------------|
| Zero order              | \(Q_t = Q_0 - kt\)              |
| First order             | \(\log Q_t = \log Q_0 - kt/2.303\) |
| Higuchi                 | \(Q = kt^{1/2}\)                |
| Korsmeyer-Peppas        | \(M_t/M_\infty = kt^n\)         |

2.5. Antimicrobial assay of the treated cotton fabrics
Agar diffusion assay was firstly used for testing the antimicrobial activity of the treated cotton fabrics against Gram-positive \(B.\ cerus\) and Gram-negative \(P.\ aeruginosa\) as model bacterial strains. Petri plates with Mueller-Hinton agar were seeded with aliquots of cell suspensions of the test cultures. Cotton specimens (10 mm x 10 mm) were placed onto the agar surface and the plates were incubated at appropriate temperature and microbial growth was monitored for 24 h. The antimicrobial activity was indicated by the presence of clear zones around the specimens.

The antimicrobial effect of the treated cotton fabrics has also been tested in meat-peptone broth (MPB) by the reduction of the growth of the model strains. Tubes containing sterile MPB and square cotton specimens (10 mm x 10 mm) were inoculated with each bacterial suspension. Tubes with untreated cotton samples and without specimens were also prepared as controls. After incubation for 24 h at appropriate temperature, microbial growth was assessed by measuring the optical density at 600 nm (\(OD_{600}\)). The antimicrobial activity of the treated cotton samples was evaluated by the reduction in cell density after incubation. All antimicrobial tests were done in triplicate.
3. Results and discussion

Chitosan is a polyelectrolyte with cationic charged amino groups in a water solution under pH 6.5. The ability of chitosan-based drug carriers to sorb and release active substances is influenced by their preparation conditions involved in the crosslinking process, such as the ratio of chitosan to crosslinker, pH, and temperature. Citric acid is a weak polycarboxylic acid. It is suitable as a crosslinking agent for polysaccharides as chitosan. The deprotonated carboxylic groups of citric acid can form ionic bonds with the ammonium groups of chitosan, as has shown in Figure 1. Indomethacin has a pKa of 4.5, and at pH above the pKa, its molecules dissociate into ionized form. In the pH range 4.5 - 6.5, the electrostatic interaction is possible between negatively charged indomethacin and positively charged chitosan [16]. The indomethacin and chitosan interaction, as well the chitosan and citric acid crosslinking, depend on pH.

![Fig. 1. Interaction of chitosan with citric acid and with indomethacin](image1)

Figure 2 presents the photographs taken with a digital camera under optical microscope observation of the cotton fabric and composite material m3, obtained with the highest quantity of citric acid. The chitosan layer covers the fibre surface and glues some of them together.

![Fig. 2. Optical microscope photographs of cotton fabric and composite materials m3 (magnification of the pictures is x 40)](image2)

FTIR experiments were performed to study the interaction between chitosan and citric acid. The FTIR spectra of the cotton fabric and samples m1, m2 and m3 are shown in Figure 3. The main component, cotton, has approximately the same functional groups as the chitosan. The quantity of the citric acid and the indomethacin is small, and this complicated the FTIR analysis. The tool for manage this problem is spectral subtraction of the main component, cotton fabric, from the others. It makes it easy to see and discuss peaks of citric acid and indomethacin.
Figure 3 shows the FTIR spectra of cotton fabric (co) and composite materials (m1, m2, and m3). The non-appearance of peaks in the region from 1680 cm\(^{-1}\) to 1750 cm\(^{-1}\), indicates the absence of free carboxyl groups in composite materials. A broad band, with increasing intensity, appears at 1570 cm\(^{-1}\) depending on the amount of citric acid. The bands in this region are characteristic of the protonated amino groups of chitosan and the carboxylate ion \(-\text{COO}^-\). The IR band at 1380 cm\(^{-1}\) corresponds to vibrations of the carboxylate ion also. This result indicates that all carboxyl groups of citric acid participate in the electrostatic interaction with chitosan [11, 17]. The other characteristic bands for polysaccharide structure appear at 1150 cm\(^{-1}\) due to the C-O-C connection of glycosidic linkage and at 1082 cm\(^{-1}\) and 1020 cm\(^{-1}\) for C-O stretching skeletal vibrations.
The concentration of citric acid as a chitosan cross-linker on the release of indomethacin in phosphate buffer pH 7.4 at 37 °C for 30 hours was compared. The cumulative indomethacin release from samples m1, m2, and m3 has shown in Figure 5. The initial burst release has been observed due to molecule desorption from the surface for the first 100 minutes. Next, the release has slowed by the more difficult detachment of the molecules inside the chitosan hydrogel. After 24 hours, the released quantity of indomethacin is approximately constant. The citric acid quantity influences the detachment of the drug molecules from composite materials. The released indomethacin increases with the increase of the quantity of the cross-linker. The reason can be the difference in the hydrogel structure and in the drug distribution in the matrix.

![Graph showing cumulative drug release](image)

**Fig. 5.** Release of indomethacin in phosphate buffer pH 7.4 at 37 °C from composite materials (m1, m2, m3) with varying citric acid concentration (at λ =320 nm)

Different kinetic models have been applied to analyze the drug release mechanism from composite materials. Table 2 summarizes the data. The results have shown that the Korsmeyer-Peppas equation describes better the indomethacin release from composite materials.

The obtained correlation coefficient is the highest of the other model equations. Release constant (n) is smaller than 0.89 for samples m1 and m2, and the release mechanism corresponds to the non-Fickian diffusion mechanism. For sample m3 its value is higher than 0.89 and corresponds to super II case transport. In this case, the process is not only diffusion-controlled. The release of the poorly water-soluble drug indomethacin also acquires by swelling and erosion control of chitosan gel.

| Sample | Zero-order r² | First-order k | Higuchi r² | k | Korsmeyer-Peppas r² | k | n |
|--------|---------------|---------------|------------|---|---------------------|---|---|
| m1     | 0.8817        | 0.3809        | 0.7572     | 0.0108 | 0.9565              | 5.975 | 0.9960 | 0.0302 | 0.8016 |
| m2     | 0.9028        | 0.6223        | 0.7572     | 0.0134 | 0.9685              | 9.7083 | 0.9958 | 0.0182 | 0.8707 |
| m3     | 0.8914        | 0.7903        | 0.7578     | 0.0120 | 0.9623              | 12.368 | 0.9962 | 0.0108 | 0.9856 |

In the agar assay, small zones (about 1.0 - 1.5 mm) were observed around the specimens against both tested strains (Figure 6), which could be explained by slow diffusion due to low hydrophilicity of the agar surface. In liquid medium, the results demonstrated reduction in the cell growth of both strains by all the tested specimens as compared to the negative control. Samples exhibited slightly higher antimicrobial effect against Gram-negative P. aeruginosa than against Gram-positive B. cereus, and m3
sample was found slightly more active than m1 and m2 samples (Figure 7). As can be seen, m3 inhibited about 41% of the growth of P. aeruginosa and about 32% of the growth of B. cereus. Growth reduction of P. aeruginosa by m1 and m2 samples was 35-38% and 27-29% towards B. cereus.

Fig. 6. Agar diffusion test of cotton samples against the model strains B. cereus and P. aeruginosa

It is hypothesized that, the direct contact of bacterial cells with cotton surface contributes to the antimicrobial effect of the treated cotton fabrics. Low water solubility of bioactive compounds does not allow their easy release from the composite materials surface into solution to contact directly with bacterial cells. That explains relatively low growth inhibition of the tested bacteria in solution. The modification of cotton fabric increases its hydrophobicity, thus preventing bacterial growth and bacterial biofilm formation onto the fabric surface.

Figure 7. Reduction of the growth of the indicated model cultures by treated cotton fabrics

4. Conclusion

New composite materials have been obtained, with potential application as antimicrobial dressings for wounds, releasing an anti-inflammatory biologically active substance (indomethacin). It has been found that as the amount of citric acid used in cotton fabric processing increases, more indomethacin is initially released. After this initial effect, the release of the biologically active substance continues for more than 30 hours in phosphate buffer pH 7.4 at 37 °C. The Korsmeyer-Peppas equation describes the process of the drug diffusion combined with the chitosan gel swelling and erosion. This effect intensifies with the citric acid concentration enhancement. The obtained materials show moderate antimicrobial activity against Gram-positive Bacillus cereus and Gram-negative Pseudomonas aeruginosa bacteria.

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