OBTAINING OF MATERIALS WITH ANTIBACTERIAL ACTIVITY BY BONDING OF CEFAZOLINE ON MODIFIED CELLULOSIC BANDAGE

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Abstract: Obtaining of biologically active cellulose bandage with antimicrobial activity is one of the most interesting trends in the development of new medical materials. This paper investigates the effect of reaction conditions on oxidation of cellulose fiber in a form of bandage with an aim to obtain a material suitable for chemical binding of antibiotics. Also, the influence of structure of cefazoline on sorption on oxidized cellulose fiber is investigated. The chemical bonding of the drug was performed on oxidized cellulose samples (OC) with different carboxylic groups content in cefazoline water solution concentration with c = 1.7 · 10^{-3} \text{mol/L}, while the desorption of bonded antibiotic was done in physiological solution. The amounts of bonded and released drug were determined spectrophotometrically in UV range. It was found that carboxylic groups content on OC and solution pH during the sorption process had a significant influence on the amount of bonded antibiotics. Antimicrobial activity of fiber samples with bonded cefazoline was tested in vitro in relation to gram positive and gram negative microorganisms (Staphylococcus aureus, Bacillus subtilis, and Escherichia coli). The paper showed that the optimal ratio of cefazoline consumption versus the amount of released antibiotic was obtained for the sample with 6.48 % of the carboxylic group.

Keywords: modified cellulose, cefazolin, biologically active materials.

1. INTRODUCTION

Producing new biologically active fibers with a therapeutic effect has been the most prominent trend in the field of biomedical materials in the past twenty years. Biologically active materials represent different types of materials compatible with human tissues and/or biological phenomena. Numerous studies [1−3] have shown that the binding of different homeotherapeutics with different structure polymer matrices resulted in biologically active fibers for local application, which are widely used in medical practice. The binding of the drug by different chemical bonds to the polymeric carriers enables continuous, targeted drug release in optimal concentration, at a controlled rate over a longer period of time, which increases the effectiveness of the therapy [4−8]. As a polymeric carrier in this study we used oxidized cellulose. It is obtained by modifying the cotton bandage through selective oxidation process which results in products (OC) with different content of carboxyl groups. For incorporation into OC we used cefazolin, a cephalosporin antibiotic of the first generation (Figure 1). It is bactericidal for a large number of Gram-positive and Gram-negative bacteria [9].

Figure 1. Chemical formula of cefazolin

The aim of this study was to get a biologically active material through binding cefazolin to oxidized cellulose fiber in form of a bandage, to determine the optimal amount of used and desorbed drug and examine the possibility of its antimicrobial activity in vitro against cultures of Staphylococcus aureus, Bacillus subtilis, and Escherichia coli.

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2. EXPERIMENTAL PART

Modified cellulose fiber was obtained by oxidation of cotton bandage with a mixture of HNO₃/H₃PO₄/NaNO₂ during 12, 24 and 48 hours by the procedure described in literature [10]. The content of carboxyl groups of the oxidized cellulose bandage was determined by using the calcium acetate method [11], and the content of carbonyl groups was determined by hydroxylamine method [12].

The material with antimicrobial activity was obtained by immersing the bandage in an aqueous solution of cefazolin in the following conditions:
- drug concentration 1.7 × 10⁻³ mol/L
- sorption duration 5, 15, 30, 60 minutes and 24 hours
- bath module 1 : 200
- sorption temperature 20 ± 2 °C

After the end of sorption the samples of cellulose material were washed out with distilled water, drained and dried at room temperature.

Desorption of the bound cefazolin from the oxidized cellulose was tested in saline solution, under the following conditions:
- desorption time 1 – 24 hours
- bath module 1 : 100
- temperature 20 ± 2 °C

Quantities of sorbed and desorbed antibiotics from the oxidized cellulose fiber were determined by using UV-VIS spectrophotometer Model 6315 Jenway, at the wavelength of λ_max 272 nm characteristic for cefazolin.

The samples of the biologically active material were subjected to the testing for the antimicrobial activity against cultures of Staphylococcus aureus, Bacillus subtilis and Escherichia coli.

We also determined the durability of antimicrobial activity by placing the samples of oxidized cellulose treated with antibiotic in a moist nutrient medium under sterile conditions, stored for 24 hours and subsequently transferred to Petri plates seeded with the test microorganism. After incubation at 37 °C for 24 hours, measuring of the inhibition zone in Petri dishes was performed [13].

3. RESULTS AND DISCUSSION

Modified cellulose fiber was obtained by selective oxidation of cellulose bandage with the mixture of HNO₃/H₃PO₄/NaNO₂ during 12-24 hours. The effects of the oxidation process on the content of carboxyl and carbonyl groups were given in the table I.

![Table 1. The effects of oxidation reaction on the content of carboxyl and carbonyl groups of the oxidized cellulose fiber in the form of bandage](image)

<table>
<thead>
<tr>
<th>Reaction system HNO₃/H₃PO₄ 2:1(vol) and 1.43 % NaNO₃(weight/vol)</th>
<th>temperature 25 ± 2 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time, h</td>
<td>Content COOH %</td>
</tr>
<tr>
<td>12</td>
<td>2,376</td>
</tr>
<tr>
<td>24</td>
<td>6,480</td>
</tr>
<tr>
<td>48</td>
<td>9,751</td>
</tr>
</tbody>
</table>

Through selective oxidation of cellulose fibers in the form of bandage we obtained carboxylic groups, which, combined with hydroxyl groups of cellulose, represent a good basis for the binding of the antibiotic cefazolin. The results of sorption of antibiotics on the OC are shown in Table 2 and Figure 2.

![Table 2. The quantity of cefazolin bound to OC (mg/g), bath module 1:200, temperature 22±3°C](image)

<table>
<thead>
<tr>
<th>% COOH in OC</th>
<th>Duration of sorption, min (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>2,376</td>
<td>3,812</td>
</tr>
<tr>
<td>6,480</td>
<td>4,290</td>
</tr>
<tr>
<td>9,751</td>
<td>10,010</td>
</tr>
</tbody>
</table>

Table 2 shows that the biggest amount of cefazolin (13.941 mg/g) was tied by the OC sample with the highest content (9.751%) of the carboxyl groups. The amount of bound cefazolin increases with the content of carboxyl groups in the sample of OC and with duration of sorption.

Cefazolin in their structure contains an amide group and a carboxyl group and two heterocyclic rings with the nitrogen, tetrazole and thiadiazole ring, so it has a possibility of forming bonds which can be coordinative, hydrogen and Van der Waals. Results of sorption of OC in antibiotic solution in the concentration of 1.7 × 10⁻³ mol/L given in the
Table 2 indicate that the drug was quite weakly bound to the fiber.

It can be assumed that such weak binding of antibiotics happened due to the structure, or the absence of basic functional groups such as amino groups, which prevents the formation of relatively strong bonds as ionic, between OC and antibiotics. Instead of ionic bonds the structure of cefazolin enables formation of a larger number of hydrogen bonds with the carboxyl and hydroxyl groups of oxidized cellulose.

In addition to the hydrogen bonds between the carboxyl groups of OC and carboxyl groups of antibiotics, cefazolin has an ability to build a large number of hydrogen bonds with the OH groups of oxidized cellulose. These hydrogen bonds may be formed via the nitrogen atom in the thiadiazole ring and a tetrazole ring. Thiadiazole ring of cefazolin is bonded to the rest of the molecule via a thioether group, while the tetrazole ring is bonded via an amide group. In both cases, the unpaired electron located on nitrogen atoms in heterocyclic rings are localized and placed in a sp^2 hybrid orbitals placed in the plane of the ring. These unpaired electrons can participate in the interaction with the H atoms of OH groups of oxidized cellulose and produce relatively strong hydrogen bonds, Figure 3.

![Figure 2. Quantity of bound cefazolin to OC (mg/g), h](image)

![Figure 3. Hydrogen bond between the modified cellulose bandage and antibiotic](image)

However, a relatively small amount of antibiotics is released, which can be explained by a large number of hydrogen bonds that the medicine builds with OC. It can also be assumed that due to a large number of nitrogen atoms in the structure of the antibiotic through interaction with hydroxyl groups of OC the networking of cellulose macromolecules occurs, which complicates and slows down the release of antibiotics.

The networking happens because of the spatial orientation of the molecule of cefazolin which is such to allow unrestricted access to the heterocyclic rings within the structure of antibiotic from different sides and the creation of hydrogen bonds with chains of oxidized cellulose.

Given that the binding of the antibiotic to the OC was performed in an aqueous solution which was not buffered, that is, at a pH which is not optimal for the ionization of the drug, the satisfactory quantity of sorbed drug was obtained, as well as a release rate corresponding to the potential use of the obtained cotton bandage.
The antimicrobial activity of the samples of oxidized cellulose fiber bonded with cefazolin on growth of gram positive and gram negative bacteria (*Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*) was determined by measuring the inhibition zone in the vicinity of the samples. Samples of OC with bounded cefazolin, which belongs to the first generation of cephalosporins, which have a more pronounced effect on gram positive bacteria, had accordingly the highest zone of inhibition with the culture of *S. aureus*. The inhibition zone with the culture of *B. subtilis* is somewhat smaller (although it does not belong to the gram-positive bacteria) which is attributed to its ability to spore formation, while, as expected, the least effect was with the culture of *E. coli* (Figure 5).

After removing the samples of the OC with the bounded cefazolin from the sterile substrate and being inoculated with the test microorganisms and after 24 hours of incubation, the media inoculated with *S. aureus* and *B. subtilis* showed no microorganisms growth, while the substrates inoculated with *E. coli* showed little growth which could be explained by cefazolin being less sensitive to Gram-negative bacteria. After transferring the samples from a sterile to a substrate inoculated with bacterial cultures, the inhibition zones were reduced in comparison to the results obtained by measuring the inhibitory activities of the bandage samples directly placed onto the inoculated medium. It is possible that the amount of residual antibiotics in samples of OC, after being held on a sterile medium for 24 hours, was not sufficient to demonstrate a stronger antibacterial activity after switching to fresh inoculated medium.

Based on the results of sorption and desorption of the drug from the OC given in percentages in Table 5, an optimum ratio between used and desorbed drug was determined for the potential use of the biologically active material with the bound antibiotic.

It can be observed from Table 5 that the largest amount of the drug was bonded to the OC with the highest content of carboxyl groups as well as that a significant amount of bound drug was released. On this basis, it can be concluded that the sample with the highest content of carboxyl groups has an optimal ratio of bound and released drug.
4. CONCLUSION

Oxidized cellulose fiber (OC) with 2.38, 6.48 and 9.75 % of carboxyl groups was obtained through the process of selective oxidation of the cellulose fiber in the form of bandage. A biologically active material with antimicrobial effects can be obtained by using sorption of cefazolin to OC. The maximum quantities of cefazolin on OC after 24 hours of sorption in aqueous solutions of antibiotics were 13,941 mg/g OC. An increase in the quantity of the bound drug to OC was noticed to occur as the content of the carboxyl groups of OC and the sorption time increased. Since the binding of the antibiotics was performed in aqueous solutions which are not buffered, satisfactory quantities of sorbed drugs were obtained, as well as the release rate corresponding to the potential use of the bandage. The optimal ratio of the bound and released drug was found in the sample with the highest content of carboxyl groups.

Antimicrobial activity of the samples of OC with the bound antibiotic was tested by the method of diffusion on agar plate against the cultures of E. coli, S. aureus and B. subtilis. The best antibacterial activity in OC was observed on the bandage with the bound cefazolin against B. subtilis. After testing by the same method the durability of the bonds between OC and the bound antibiotics it was shown that the tested samples provided a sustained antibacterial activity even after spending 48 hours on a nutrient medium under the conditions most similar to the conditions of the moist environment of the wound in human skin.

5. REFERENCES


Сажетак: Добијање завоја са биолошком активношћу представља један од најинтересантнијих тренда у развоју нових медицинских материјала. У овом раду испитује се утицај реакцијних услова на оксидовање целулозног влакна у форми завоја ради добијања материјала погодног за хемијско везивање антибиотика. Такође, испитује се утицај структуре антибиотика цефазолина на сорпцију на оксидованом целулозном влакну. Хемијско везивање лијека вршено је на узорцима оксидоване целулозе (OC) са различитим садржајем карбоксилних група из воденог раствора цефазолина концентрације с = 1,7·10⁻³ mol/L, док је десорпција вршена у физиолошком раствору. Количине везаног и отпушеног лијека одређиване су спектрофотометријски у UV подручју. Утврђено је да садржај карбоксилних група на OC и рН вриједност раствора приликом сорпције имају битан утицај на количину везаног лијека. Антимикробно дјеловање узорака завоја са везаним цефазолином тестирано је in vitro на грам-позитивне и грам-негативне микроорганизме (Staphylococcus aureus, Bacillus subtilis и Escherichia coli). У раду је показано да се оптимални однос утрошка цефазолина у односу на количине отпушеног антибиотика добија за узорак OC са 6,48% карбоксилних група.

Кључне ријечи: модификована целулоза, цефазолин, биолошки активни материјал.