The Release of Tacrolimus from a Cotton Biomaterial to Dermis
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Abstract
Objective: The paper describes the creation of a delivery system for a drug with high dimensions, as tacrolimus.
Methods: The achievement of a biomaterial manufactured of alternative layers of chitosan (CS) and sodium alginate (Alg) with the inclusion of tacrolimus (Ta) between layers. By the gradual dissolution of layers, Ta is released.
Results: On a cotton fabric, it has been performed a biomaterial consists of 10 layers of CS and Alg. The biomaterial can sustain and then release Ta according with a specific release kinetics and by avoiding “burst effect”. The following have been evaluated: coated fabric loading degree, the number of charges on the specific surface, the dyeing tests, the Ta kinetic release and the elemental analysis (EDAX). The method proposed describes the advantages of releasing the drug as well as the limits imposed by using other systems for controlled drug release, such as cyclodextrins or hydrogels.
Conclusion: In paper, the proposed biomaterial is a viable alternative for regular treatments of cutaneous diseases with a Ta formulation supported from a textile fabric for psoriasis.
Keywords
Multilayer; Tacrolimus; Chitosan; Sodium alginate; Release kinetics

Introduction
Drug-loaded textiles have certain advantages compared with the conventional routes of administration of various pharmaceutical formulations [1-3]. Although oral administration is characterized by a high degree of therapeutic compliance, it cannot be used in patients who have problems swallowing, who are noncooperative, or are diagnosed with serious medical disorders. In this paper, compliance refers to the patients’ adhesion to the therapeutic means necessary to improve their medical condition. The topical administration of the drug, while possible, would cause some discomfort for patients due to the repeated procedures entailing interrupted work, dirty hands, time spent dressing and undressing. By using a piece of medical fabric as a spent dressing and undressing. By using a piece of medical fabric as a

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thermal properties, under strictly controlled conditions [14]. Most studies on multilayer constructions do not have medical purposes, despite the fact that their characteristics could also be useful in evaluating biomaterials for drug delivery [15-17]. The novelty of this paper lies in the release of Ta through the dissolution of the soluble CS and Alg layers applied over the cotton material under the action of an alcoholic solution. The layers acted as coatings, fixing the drug onto the fabric. The aim of this paper is to evaluate the performance and limits of the new Ta release system. The procedure proposes the application of a medical textile in the treatment for psoriasis.

Materials and Methods

The textile substrate used in this study was a 100% cotton woven fabric supplied by SC Iasitex SA company, with yarn fineness Nm = 34/1, in warp and weft, density 225 yarns/10 cm in warp and 185 yarns/10 cm in weft and specific weight 130 g/square meter. The fabric was desized, alkaline boiled and bleached with hydrogen peroxide, washed and dried in industrial conditions. The triplicate samples with dimensions of 10 cm × 50 cm were then subjected to additional preparatory operations in the laboratory, according to protocol 1 presented in the page 3. The CS with a de-acetylation degree of 75-85% and molecular weight ∼ 600 kDa was delivered by Fluka, as a white powder forming low viscosity solutions. The reagents NaOH, Na2CO3, H2O2, KCl, HCl and CH3COOH, purchased as a white powder forming low viscosity solutions, were used without any alterations. Figure 1 illustrates the chemical structure of Ta.

Figure 1: Chemical structure of Ta.

Obtaining the multilayer biomaterial

The cotton samples obtained by courtesy SC Iasitex SA were subjected to additional preparatory operations in the laboratory, according to protocol 1.

Protocol 1

1. Cleaning in distilled water for 8 hours using a Soxhlet;
2. Activating the surface fabric by: a) treatment with a solution containing 12 m/L H2O2; 2 g/L NaOH; 2 g/L Na2SiO3 at boiling point 1 hour, at liquor ratio 1:10; b) ionization of the carboxylic acid groups according to protocol 2 were subsequently subjected to coating with alternative layers of CS and Alg in compliance with protocol 2.

Protocol 2

1. Padding on a Benz machine with a 5% solution of CS (reported on fabric weight) dissolved in solution of 2 g/L CH3COOH;
2. Drying 10 min at 80°C; 3. Curing 30 seconds at 130°C; 4. Padding with a 5% solution of Alg (reported on fabric weight);
5. Drying 10 min at 80°C; 6. Curing for 30 seconds at 130°C. In creating the biomaterial according to protocol 2, ten alternating layers of CS and Alg were applied, as shown in Figure 3. Ta layers were inserted between the layers, as illustrated in Figure 3.

Figure 1: Chemical structure of Ta.

AMETEC EDAX equipment, coupled with SEM Quanta 302D and Genesis Software, were used in elemental analysis. The size of drug molecules was measured using ChemAxon software Marvin Space 5.4.0.0.
Loading the drug onto the multilayered biomaterial

In order to incorporate the drug between the CS and Alg layers, a solution containing Ta 0.010 g / 20 ml ethanol was used. The alcoholic solution based on Ta was sprayed onto the surface of the biomaterial. The material was dried for 4 hours at 20° C, after which the following layer of CS or Alg was deposited according to the procedure described above. Two Ta inclusion procedures were used, as presented in recipe 1 and 2 (from left to right), shown schematically in Figure 3 above. According to recipe 1, the drug was introduced in four batches containing 10 mg Ta, with a total amount of 40 mg. According to formulation 2, a first portion of the product (10 mg) was inserted between the last and the last but one layer of polymer and a second portion (10 mg) was laid onto the outer surface. A total amount of Ta 20 mg was inserted. There are various ways in which the drug can be loaded into the biomaterial. This study used 10 layers in order to be able to observe the multilayer behavior based on a considerable number of layers. On the other hand, the drug was inserted on the surface, and between the 6th and 10th layers, with the purpose of determination of the release profile. As no specific therapy was targeted, we did not attempt to establish a therapeutic dose. The number of biomaterial layers depends on the required therapeutic dose to be released to the dermis.

Tests of dimensional size of Ta

The measurements used the Marvin Space software 5.4.0.0., courtesy of Prof. E. Dumitriu. The data show that the Ta molecule presents maximum dimensional values (length=13.99-14.65 Å and width=10.17-12.29 Å) compared to hydrocortisone acetate and Advantan, medicines used in allergic dermatitis. For β-CD, the size of 6.2 Å is the value of the internal diameter of the molecule, and 7.8 Å is cavity height. By comparing Ta sizes with β-CD sizes, it becomes clear that the drug molecule size significantly exceeds the dimensions of the CD inner cavity. This is the reason why Ta cannot be complexed in β-CD. This detail has been verified experimentally. Furthermore, under the experimental conditions tested by the authors, Ta cannot be adsorbed in the interstices of the CS-based hydrogel deposited on a cotton fabric and ionically crosslinked with Na2SO4. Under the experimental conditions of our research, as Ta has a large molecule, it probably forms associates with dimensions that do not allow inclusion and drug release from the hydrogel mass, respectively.

Drug release from the multilayered material

In order to release the drug, the textile samples (each weighting approximately 9 g) containing the drug (20 or 40 mg) at 20° C were immersed in 200 ml of 50% ethanol solution (Ta is insoluble in water but soluble in ethyl alcohol) at 400 rpm on magnetic stirring. Samples were collected from this solution after 24 hours, and the multilayer samples containing the drug were later introduced in a fresh aqueous solution based on ethyl alcohol (50%). The procedure was repeated every 24 hours. The Ta concentration was determined through photocolorimetation at a wavelength of 194 nm. The calibration curve and the straight line equation $y = 0.0606x + 1.4307$, where $y$ is absorbance and $x$ the Ta concentration (mg/mL) was calculated by introducing the experimentally determined absorbance values, obtaining the value of the Ta concentration.

Results

This study presents the results obtained in developing a multilayer system consisting of 10 alternating layers of biocompatible CS and Alg and water-soluble polymers. In the first stage of research, we studied the formation and features of the layers deposited onto a 100% cotton fabric. In the second we tested the characteristics of the Ta release.

Coated fabric loading degree

After padding with CS or Alg, drying and curing, the samples were conditioned and weighted in order to determine the degree of loading. The results are shown in Table 1.

Table 1 shows the specific mass values (mg/cm²) based on the number of layers. Prior to the application of the CS and Alg layers, the fabric had a specific weight of 12.9 mg/cm². According to Table 1, the correspondence between the number of layers provided (2, 4, 6, 8 and 10) and the layers is as follows: in the case of 2 layers, the 1st layer - CS and the 2nd layer - Alg; in case of 4 layers, the 1st layer - CS, the 2nd layer - Alg, the 3rd layer - CS and the 4th layer – Alg and so on for other values. The values of loading degree illustrated show only the contribution of the CS and Alg layers without Ta. The loading degree depends on the number of layers adding a new amount of polymer to the next layer. The specific pressure on the contact line, as squeezing parameter, was maintained constant at 1.1 N/cm.

Surface charges

In order to acquire additional information on surface behavior, the number of charges on the surface of the samples was determined with the addition of each layer. The variation in the number of charges on the surface of the biomaterial samples depended on the number of layers applied to the cotton surface. The values obtained show a general variation from $10^{2}$ positive charges/25 square cm up to $85.10^{2}$ negative charges/25 square cm of the biomaterial as was
The colour differences determined for the same samples dyed with Acid Red 26 had the following values: $\Delta E_{C2S1,1}=21.991$ AN; $\Delta E_{C2S1,2}=19.191$ AN; $\Delta E_{C2S1,3}=17.828$ AN; $\Delta E_{C2S1,4}=17.488$ AN; $\Delta E_{C2S1,5}=16.247$ AN. The rating values obtained ($\Delta E_{C2S1,1} > \Delta E_{C2S1,2} > \Delta E_{C2S1,3} > \Delta E_{C2S1,4} > \Delta E_{C2S1,5} > \text{Witness}$) are overlapped with the K/S rating. In cases in which other methods are unavailable (Zeta potential, Number of surface charges, etc.) dyeing tests are a qualitative method that can be used to evaluate the behavior of ionic interactions between a dyestuff and a polymer layer having opposite charges. Even in this case, the interactions between layers form a system that is too complex for an accurate characterization.

### Biomaterial Ta release

As Ta is insoluble in water but soluble in ethyl alcohol, it was dissolved in ethyl alcohol for spray application between CS and Alg layers. In order to determine the kinetics of Ta release, the drug was deposited in two variants by inserting it between the layers without monitoring the therapeutic dose. Water soluble polymers were chosen in order to activate the drug substance through solubilization (one has in view to mimic the perspiration action). The results of this study reveal the load capacity of Ta on CS and Alg layered textile materials. Based on the results obtained, the drug load of a biocompatible system with topical application and transcutaneous release of Ta can be modulated, depending on the required therapeutic dose. Furthermore, the choice of the two polysaccharides (CS and Alg) was justified by their polyelectrolyte character with opposite electric charges, as well as by their biocompatible, biodegradable and nontoxic nature, given that the final material is meant to act by direct skin contact. Another argument was the hydrogel nature of the multilayer deposited onto the fabric allowing the gradual release of the included Ta by diffusion, triggered by the swelling of the material in contact with the perspiration drawn from the skin. The data on Ta release kinetics, for recipe 1 and 2, are illustrated in Figure 5. The drug release was determined for ten-day observation. The efficacy of drug release in case of formulation 1, calculated as the amount of drug released relative to the total amount of Ta introduced in the biomaterial, is $\left(27.95/40 \right) \times 100 = 69.8\%$, while in the case of formulation 2, it is $\left(18.43/20 \right) \times 100 = 92.15\%$. It follows that the more dispersed the drug is among multiple layers, the lower its release. In recipe 1, four doses were introduced between the last 5 layers, while in recipe 2, two doses were released, the first on the outer layer and the second between the last and the last but one layers. The kinetics revealed a similar general profile, except for the fact that the release in case of the first formulation started after the first day of

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**Table 1: Values of loading degree with CS and Alg.**

<table>
<thead>
<tr>
<th>Number of layers</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton fabric</td>
<td>12.9</td>
<td>13.1</td>
<td>13.4</td>
<td>13.5</td>
<td>13.7</td>
<td>14.0</td>
</tr>
</tbody>
</table>

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**Figure 4: Values of the number of surface electric charges obtained on each layer of biomaterial.**

- Layer 1 = CS 1.1; Layer 2 = Alg 1.2; Layer 3 = CS 2.1; Layer 4 = Alg 2.2; Layer 5 = CS 3.1; Layer 6 = Alg 3.2; Layer 7 = CS 4.1; Layer 8 = Alg 4.2; Layer 9 = CS 5.1; Layer 10 = Alg 5.2.

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**Figure 5: Ta release kinetics.**

- Layer 1 = CS 1.1; Layer 2 = Alg 1.2; Layer 3 = CS 2.1; Layer 4 = Alg 2.2; Layer 5 = CS 3.1; Layer 6 = Alg 3.2; Layer 7 = CS 4.1; Layer 8 = Alg 4.2; Layer 9 = CS 5.1; Layer 10 = Alg 5.2.

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**Figure 6: The character of Ta release.**

- Layer 1 = CS 1.1; Layer 2 = Alg 1.2; Layer 3 = CS 2.1; Layer 4 = Alg 2.2; Layer 5 = CS 3.1; Layer 6 = Alg 3.2; Layer 7 = CS 4.1; Layer 8 = Alg 4.2; Layer 9 = CS 5.1; Layer 10 = Alg 5.2.
The nitrogen content (4.97%) in the inner layer of the biomaterial is higher than that in the outer layer (3.61%). As illustrated in Table 2, Ta contains a certain amount of nitrogen in its structure, while in CS, an amino group is present. It follows that there is a higher nitrogen level in the inner surface, where both Ta and CS are present. In the outer layer of the biomaterial, however, Ta is the only source of nitrogen, determining a lower nitrogen content. The nitrogen content, therefore, confirms the presence of Ta on the structure of the biomaterial. On the other hand, concerning textile support, either a piece of 100% cotton knitted or woven fabric can be used as substrate. In our case, the latter was preferred in order to fulfill the need to maintain a flat structure, which would have been difficult to obtain had we used knitted fabric due to its tendency to bend or roll. When designing a textile fabric, the number of layers deposited onto the fabric surface can be adjusted depending on the amount of drug used for therapeutic purposes, an issue that was not taken into consideration in this study. The ten layers used in this paper allowed us to obtain a general characterization of a multilayer biomaterial. As noted in Figure 4, the multilayer system forms a complex system of electrostatic interactions. CS and Alg were chosen due to their good solubility properties as well as to their biocompatibility with the skin, being produced from natural materials. While, after depositing the first 4 layers of CS and Alg, the biomaterial obtained still retained the flexibility specific to textiles, after depositing the additional layers, the biomaterial became rigid.

**Conclusion**

The alternative deposition of polyelectrolyte layers of opposite electric charges can be a viable and attractive option in creating a drug reservoir on a textile substrate. Ta, a water-insoluble drug, can be included in the multilayered material obtained to be gradually released by diffusion under the action of the perspiration. The system proposed in this paper can be an alternative to existing treatments of cutaneous diseases, especially of psoriasis.

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