Specific Characterization of a Multilayer Biomaterial Controlled Release of Tacrolimus

CEZAR-DORU RADU[1], OANA PARTENI[1*], IOAN GABRIEL SANDU[2], GABRIELA LISA[3], CORNELIU MUNTEANU[4], VASILE VALERIU LUPU[5]

[1] Gheorghe Asachi Technical University Iasi, Department of Textile, Leather and Industrial Management, 29 Mangeron Blvd., 700050, Iasi, Romania

[2] Gheorghe Asachi Technical University Iasi, Faculty of Chemical Engineering and Environmental Protection, 73 Mangeron Blvd., 700050, Iasi, Romania

[3]Gheorghe Asachi Technical University Iasi, Faculty of Chemical Engineering and Environmental Protection, 73 Mangeron Blvd., 700050, Iasi, Romania

[4] Gheorghe Asachi Technical University Iasi, Faculty of Mechanical Engineering, 61-63 Mangeron Blvd., 700050, Iasi, Romania

[5] Grigore T. Popa University of Medicine and Pharmacy Iasi, Mother and Child Medicine Department, 16 Universitatii Str., 700115, Iasi, Romania

The article presents thermogravimetric (DTG and DSC) and dyeing data (color strength index, K/S and color difference, ΔE) as well as SEM micropictures of the layers of a biomaterial composed of chitosan (CS) and sodium alginate (Alg) films, alternatively deposited onto a cotton woven fabric. The biomaterial having CS as the last applied layer was dyed with an acid dyestuff, and the one having Alg as the last applied layer was dyed with a basic dyestuff, respectively. Between the last layers of CS and Alg, an amount of tacrolimus (Ta), a drug used for the treatment of psoriasis, was also applied.

Keywords: multilayer, chitosan, sodium alginate, tacrolimus, thermogravimetric behavior, dyeing tests

The multilayer technique is used for several purposes, either for the achievement of a polyelectrolyte system used for the treatment of the wastewater from different industries [1], either for the increase of the storage capacity of a drug on a polymer surface [2, 3] or for the procurement of a biomaterial with properties specific to a certain therapy [4, 5].

The research focused on the formation of a biomaterial from two biocompatible polymers soluble in water, such as CS and Al. The alternative deposition of CS and Alg on a 100% cotton woven fabric determines a packaging carried out through ionic interactions; subsequently, the drug is released through the layers dissolution under transpiration action.

This paper aims at assessing the features induced by layer application through gravimetric measurements (DTG and DSC), morphological observations (SEM micro pictures) and tinctorial tests (K/S and ΔE).

Experimental part

Materials and methods

In the paper, 5 layers of CS and 5 layers of Alg were alternatively deposited through the pad-dry-cure technique on 100% cotton woven fabric. The figure 1 shows layers arrangement in the biomaterial.

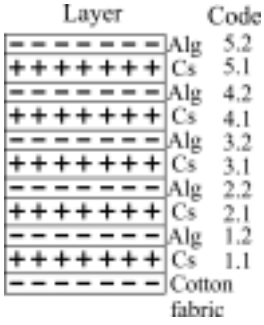


Fig. 1. The scheme of CS and Alg layer disposal within the biomaterial

Upon the drug (Ta) inclusion in the biomaterial, the following recipes were used: between the layers from Alg 3.2 up to Alg 5.2 - a batch of 10 mg Ta was applied; a batch of 10 mg Ta between layers CS 5.1 and Alg 5.2 and a second batch of Ta on the external surface of the biomaterial. The batch of 10 mg Ta is dissolved in 20 mL ethylic alcohol, the solution being pulverized on both sides of the biomaterial, having the dimensions of 10 x 50 cm. We worked in triplicates.

The textile underlayer used in this study was a 100% cotton woven fabric, with a fineness of yarn Nm = 34/1, in warp and weft, with density 225 yarns/10 cm in warp and 185 yarns/10 cm in weft, and a specific weight 130 g/square meter.

The CS with a degree of deacetylation of 75-85% and molecular weight 600 kDa was delivered by Fluka, as a highly viscous form of CS. The Alg was provided by Sigma Aldrich (CAS number: 9005-38-3) as a white powder forming low viscosity solutions. The reagents: NaOH, Na_2CO_3, H_2O_2, KCl, HCl and CH_3COOH, purchased from Sigma Aldrich, were used without any alterations. The fabric was desized, alkaline boiled and bleached with hydrogen peroxide, washed and dried in industrial conditions.

The triplicate samples with dimensions of 10x50 cm were then subjected to additional preparatory operations in the laboratory additional bleaching with hydrogen peroxide and treatment with Na_2CO_3.

The textile material was covered with alternating films of CS and Alg using a pad-dry-cure technique. The conditions for depositing the layers were: padding on a Benz machine with a 5% solution of CS (reported on fabric weight) dissolved in a solution with 2 g/L CH_3COOH; drying 10 min. at 80°C; curing 30 s at 130°C; padding with a 5% solution of Alg (reported on fabric weight); drying 10 min at 80°C; curing for 30 s at 130°C.

* email: oanaparteni@yahoo.com; Tel.: +40755866925

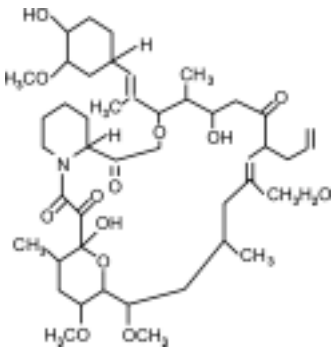


Fig. 2. Chemical structure of Ta

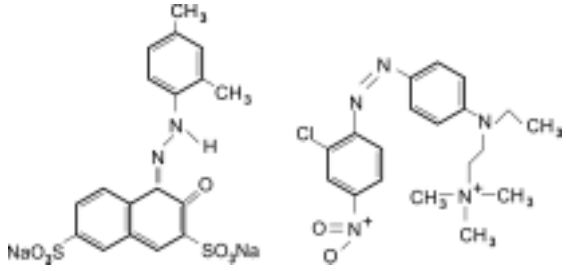


Fig. 3. Structure of dyestuffs
a. Acid Red 26 for dyeing the biomaterial with CS as the final layer;
b. Basic Red 18 for dyeing the biomaterial with Alg as the final layer

Sample	Stage I				Stage II				Residue
	T_{onset} (°C)	T_{peak} (°C)	T_{endset} (°C)	W (%)	T_{onset} (°C)	T_{peak} (°C)	T_{endset} (°C)	W (%)	(%)
Fabric	45.1	76.9	86.7	3.21	318.2	364.3	382.1	9.62	17.17
CS 1.1	47.5	59.6	95.9	3.78	333.8	361.4	382.6	86.00	10.22
Alg 1.2	47.9	59.4	89.1	2.99	332.9	361.9	382.2	87.13	9.88
CS 5.1	48.1	62.3	96.6	3.81	322.4	368.2	387.4	79.31	16.88
Alg 5.2	48.3	62.6	94.1	3.57	315.1	362.1	380.6	81.16	15.27

Table 1
THERMOGRAVIMETRIC DATA OF SOME LAYERS FROM BIOMATERIAL

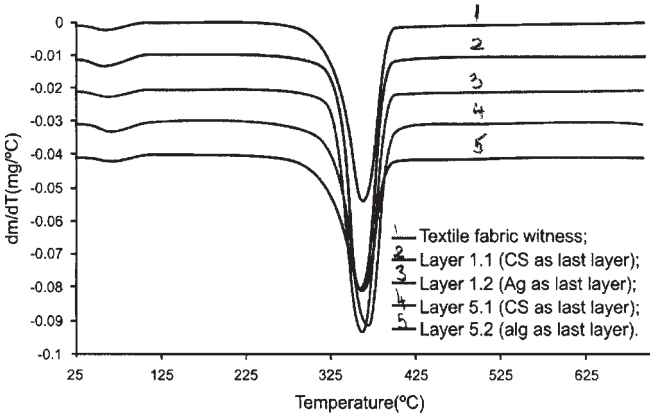


Fig. 4. DTG curves of biomaterial samples

10 layers were intercalated, comprising 5 layers of CS and 5 layers of Alg, squeezing constantly at a specific pressure on the contact line of padder of 1.1 N/cm.

The thermogravimetric study was performed using a Mettler Toledo TGA-SDTA851^e derivatograph. The recordings were made at a heating rate of 10°C/min, under constant nitrogen flow (20 mL/min), within the temperature range 25-700°C. The mass of the samples ranged between 2.9 and 3.8 µg. ME-24123 aluminum oxide crucibles 70mL was used. Curve processing, in order to obtain thermal characteristics, was carried out using STAR software. The DSC curves were recorded by means of a Mettler Toledo DSC1 device, in an inert atmosphere, at the heating speed of 10°C/min. Scans in the temperature interval 25 – 300°C, two heating and one cooling processes, were performed. The analyzed sample quantity varied between 2.9 and 6.5 mg.

The biomaterial samples with CS and Alg films of the order of mg were subjected to SEM tests. SEM Quanta 302D and Genesis Software, was used for this purpose, at a magnitude of 5,000.

Dyeing tests were performed on biomaterial samples of 5 x 5 cm with a different number of layers of CS and Alg in order to differentiate them from a colour perspective. Dyeing tests were performed on a Mesdan Lab dyeing machine (Italy), 2008. If the last layer deposited was CS, the multilayer material was dyed using acid dyestuff (CI Acid Red 26) in the following formulation: (2%) Acid Red 26, liquor ratio = 1:200, 5 min. at the boiling point of the solution; cold wash for 10 min. In cases in which the last layer was Alg, the material was dyed using basic dyestuff (C.I. Basic Red 18) in the following formulation: (1.2%) Basic Red 18, liquor ratio = 1:150, 5 min. at the boiling point of the solution, cold wash 10 min. After washing and drying, the samples were photocolorimetered using Data Color equipment, with software included, determining and calculating K/S and ΔE values and their corresponding items, both in graphical and tabular form. Figure 2 illustrates the chemical structure of Ta.

The dyestuffs used (Acid Red 26 and Basic Red 18) were supplied by Bezema. The structures of the dyestuffs are shown in figure 3.

Results and discussions

Thermogravimetric measurements

The derivative thermogravimetric (DTG) curves recorded are presented comparatively in figure 4. The thermal degradation of the samples analyzed was performed following a two-stage process [6, 7]. The main thermogravimetric characteristics are: T_{onset} - onset temperature at which thermal degradation starts, T_{peak} – temperature at which the degradation rate reaches its maximum, T_{endset} – temperature at which the degradation process ends, in either of the two stages, W% - mass percentage loss per stage and the amount of remaining residue at a temperature of 700°C, as shown in table 1.

Figure 4 and table 1 show differences with regard to the gravimetric behavior between the textile fabric used as support and the biomaterial formed of the fabric and the deposited layers. Therefore, there are differentiations both for stage I and for stage II of heating. The differences manifest for all the thermogravimetric assessment criteria presented in table 1. They are caused by the increase of biomaterial thickness, caused by the addition of new

Sample	T_{peak} (°C)	ΔH (J/g)
Textile	0.93	-99.39
1.1	4.25	-132.54
1.2	6.91	-121.30
5.1	2.23	-129.41
5.2	1.20	-119.44

Table 2
THE MAXIMUM TEMPERATURE OF ENDOTHERMIC PEAK AND HEAT OF DEHYDRATION

layers, the different structure of the CS and Alg layers and the packaging manner of the polymer material.

The DSC curves obtained from the first heating cycle led to the establishment of the maximum temperature values of the endothermic peak and heat of dehydration, [7] presented in table 2.

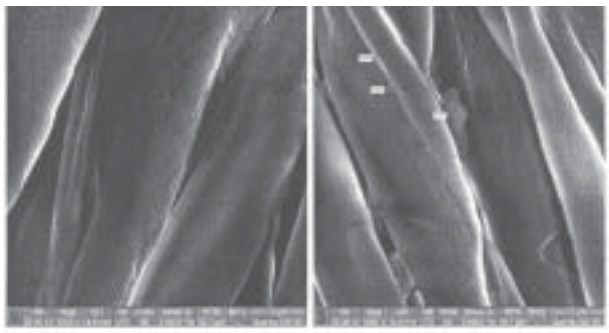
The absorption capacity of the environmental humidity influences the thermogravimetric behavior of the biomaterial. Therefore, water binding capacity on biomaterial surface is the result of two tendencies. CS and Alg are hydrophilic polymers, by the increase of the number of layers, the absorbed water modifies the thermogravimetric behavior as a result of the increase of the number of humidity absorption active centers (oxidrilic groups), but also of the layers coverage effect, which, on the other hand, limits the accessibility of the humidity absorption hydroxyl groups.

Implicitly, the thermal insulation capacity of the biomaterial increases once with the increase of the added material quantity. Even though this feature was not the object of this research, we can obtain useful data regarding the comfort of a biomaterial which can partially intervene in the construction of a clothing assembly for the patients with psoriasis.

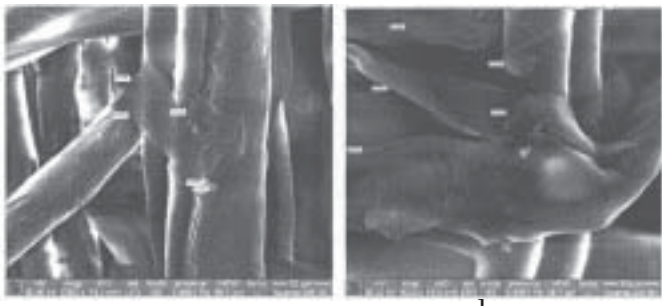
Micropictures of the multilayer biomaterial

The micropictures illustrated in figures 5a up to figure 5g show the morphological appearance of fibers, CS and Alg layers and Ta. Thus, the image of the individual cotton fibers (fig. 5a) shows the organization of the fibers from the basic fabric, over which the layers of CS and Alg were deposited, as illustrated in the following figures. Figure 5b shows the structural appearance of the CS film and figure 5c illustrates the Alg film deposited onto the CS film. Figures 5d and 5e, represent layers 5.1 and 5.2 respectively, the CS film on the outside, and figure 5f showing the Alg film. Figure 5g illustrate the location of Ta (indicated by arrows) between the layers of CS and Alg (fig. 5f) and respectively, located on the exterior surface of the biomaterial (fig. 5g).

The presented micropictures show a higher weight of CS and Alg in the image surface with the increase of the number of deposited layers. The layers realize at the end (fig. 5f and fig. 5g) a matrix encompassing the fibers and the yarns. The presented images do not show remarkable morphological differentiations between the CS layers and the Alg ones. The polymer depositions form connection points between the fabric threads which determine a better



a b



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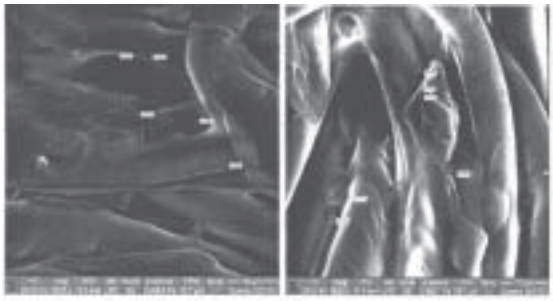

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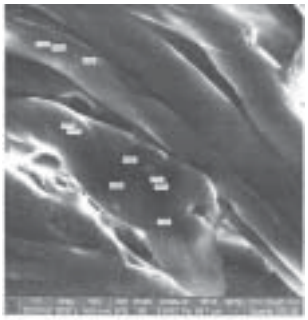

g

Fig. 5. Micropicture of the witness sample:
a - Cotton fibers of the woven fabric; b - Layer 1.1 - the arrows indicate the deposition of the CS; c - Layer 1.2 - the arrows indicate the CS and Alg layers; d - Layer 5.1 - the arrows indicate the multilayer material having CS as the last layer; e - Layer 5.2 - the arrows indicate the multilayer material having Alg as the last layer; f - Layer 5.1 where Ta located between last layers of CS and Alg, recipe 1; g - Layer 5.2, Ta located on the outside surface of the last layer of Alg, recipe 2

continuity of the surface of the obtained biomaterial than in case of the cotton woven fabric. Moreover, the images show the drug inclusion between the Alg and CS layers (fig. 5f, recipe 1) or on the external surface of the biomaterial (fig. 5g, recipe 2).

Dyeing tests

Figure 6 illustrates the variation of the K/S index for the layers 1.1, 2.1, 3.1, 4.1 and 5.1, with an outer film of CS. The samples were dyed with an acid dyestuff (Acid Red 26) of a negative charge, which have an ionic interaction with the positive charge of the amine group of CS.

Table 3 presents the color difference data, ΔE, of the samples with an outer film of CS and dyed with Acid Red 26.

The relation between color items are the following [8-16]:

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2} \quad (1)$$

$$C = (a^2 + b^2)^{1/2} \quad (2)$$

$$H = \text{arctg}(b/a) \quad (3)$$

$$\Delta = \text{batch-standard} \quad (4)$$

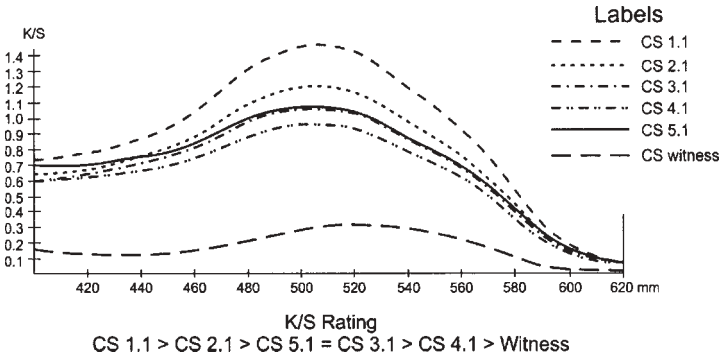

Fig. 6. Color strength, (K/S), for the biomaterial with the outer film of CS dyed with Acid Red 26, where: K = Kubelka Munck index; S = scattering light

Table 3
COLOR DIFFERENCE VALUES OF THE BIOMATERIAL SAMPLES WITH AN OUTER FILM OF CS

Illuminant	Batch CS 11			Standard CS Witness			
	ΔE	ΔL	Δa	Δb	ΔC	ΔH	Batch is
D65	21.991	-5.636	11.7	10.0	13.1	8.14	Darker, redder, less blue
	Batch CS 21			Standard CS Witness			
D65	19.191	-13.48	9.442	9.866	10.88	8.245	Darker, redder, less blue
	Batch CS 31			Standard CS Witness			
D65	17.488	-12.39	7.41	9.85	8.95	8.48	Darker, redder, less blue
	Batch CS 41			Standard CS Witness			
D65	16.247	-11.36	5.99	9.94	7.64	8.74	Darker, redder, less blue
	Batch CS 51			Standard CS Witness			
D65	17.828	-12.66	6.56	10.6	8.43	9.28	Darker, redder, less blue

Table 4
COLOR DIFFERENCES OF THE BIOMATERIAL SAMPLES WITH AN OUTER LAYER OF ALG

Illuminant	Batch Alg 12			Standard Witness			
	ΔE	ΔL	Δa	Δb	ΔC	ΔH	Batch is
D65	1.678	1.163	- 0.434	-1.129	-0.86	-0.81	Lighter, less red, less yellow
	Batch Alg 22			Standard Witness			
D65	5.881	- 2.863	2.384	4.550	4.236	2.905	Darker, redder, yellow
	Batch Alg 32			Standard Witness			
D65	5.093	- 3.043	1.330	3.861	2.979	2.793	Darker, redder, yellow
	Batch Alg 42			Standard Witness			
D65	8.089	- 4.579	2.212	6.291	4.992	4.422	Darker, redder, yellow
	Batch Alg 52			Standard Witness			
D65	5.334	0.753	1.184	5.146	3.513	3.942	Darker, redder, yellow

Table 4 illustrated color differences data from samples with an outer film of Alg, dyed with Basic Red 18.

The tinctorial tests aim at identifying certain color differentiation criteria obtained through the color strength and color difference indicators, in order to advance biomaterial construction. The procedure is based on the ionic attraction interactions between the negative sulphonate groups of the Acid dyestuff and the positive amino group of the CS and respectively in case of the cationic groups of the basic dyestuff with the negative groups of the Alg. Figure 6 illustrated the values of color strength (K/S) for the biomaterial with the outer film of CS and dyed with Acid Red 26. Based on the maximum K/S values one established the following ranking (5):

CS 1.1 > CS 2.1 > CS 3.1 = CS 5.1 > CS 4.1 > witness (sample without CS layer) (5)

Based on ΔE values presented in table 3, the ranking (6) below was determined:

CS 1.1 > CS 2.1 > CS 5.1 > CS 3.1 > CS 4.1 > witness (sample without CS layer) (6)

Based on ΔE values presented in table 4 obtained for the biomaterial with Alg outer layer and dyed with Basic Red 18, the following ranking (7) has been established:

Alg 4.2 > Alg 2.2 > Alg 5.2 > Alg 3.2 > Alg 1.2 > Witness (without Alg layer) (7)

Conclusions

The level of ionic interactions between positive and negative charges is frequently affected by conformation restrictions of dyestuff structure, reason for which the established order must be qualitatively considered. Despite of the mentioned deficiencies, the tinctorial method is an indicator of the interactions with the biomaterial layers.

The presented system is a complex one, due to the electrical charges between the layers and the method of packaging of the layers;

The thermogravimetric items reveal a specific behavior of the biomaterial layers;

The SEM images show the way of packaging CS and Alg layers between fibers, as well as the method of packaging Ta between layers or on the exterior surface of the biomaterial;

The dyeing data signal the possibility of using K/S and ΔE as a qualitative criteria, accessible as a means of differentiating layers on the dyeing principle with a dyestuff having a charge opposite to that of the last layer.

Acknowledgements: The work was supported by the strategic grant POSDRU/159/1.5/S/133652, co-financed by the European Social Fund within the Sectorial Operational Program Human Resources Development 2007–2013.

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Manuscript received: 26.10.2015