Improving the Obtaining Factors of a Chitosan Hydrogel Based Biomaterial

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The paper aims to optimize the experimental conditions of a hydrogel preparation applied on a textile knitting fabric made of 100 % cotton yarns; one used as input variables: i) the volume of chitosan (CS) solution, ii) lasting time of CS sorption on cellulose and the amount of CS, which correlates with the amount of drug that is included and by default the amount released to the skin. In this study hydrocortisone acetate (HCr) was chosen as drug for coetaneous pathologies. The procedure was applied in the following steps: 1) the application of CS-based hydrogel on knitted fabric; 2) the inclusion of HCr and cross-linker (Na_2SO_4) in the hydrogel mass deposited on knitted fabric. To release the drug, the assembly of textile support + drug + hydrogel was immersed in a perspiration kit at pH = 5.5 and T = 37 °C, which mimics the physiological conditions on the skin. The degree of fabric loading with hydrogel was determined; inclusion ability at 20°C and 50°C and then the kinetics of HCr release at 37°C under the action of a perspiration kit, visual SEM micro photograph of morphological components of fiber with hydrogel and elemental analysis (EDAX).

Keywords: cotton knitting fabric, hydrogel, hydrocortisone acetate, inclusion, drug release

The aim of the work is to determine the correlation between the preparation factors of the hydrogel deposited on interlock knitting and the structural response of the biomaterial consisting of knitting fabric + hydrogel + Na_2SO_4 concerning the sorption and release of HCr while depositing hydrogel on the knitting fabric.

Chitosan (CS) is a biopolymer of natural genesis with significant properties concerning biocompatibility, antifungal and antibacterial action, anti-cancer effects and capacity to heal the wounds [1-5]. Based on its good compatibility and degradability in biological medium, CS was frequently proposed for pharmaceutical applications [6]. In medical emergencies, it is inserted in bandages to reduce bleeding and as anti-bacterial agent, being used more and more as support for drugs transport from a polymeric surface to the skin [7]. For instance, CS was used as dispersion medium of drug at its release and to improve the bioavailability of hydrophobic drugs [8]. The poly-cationic character, as well as the presence of functional reactive groups, offers CS the possibility to be used in application with controlled release [7-9].

CS application on textile support was paid much attention recently [10]. The cotton fabrics are a medium favorable for microorganism development, such that the application of a CS treatment improves the behavior of fibrous polymers [11, 12] from this standpoint. Given its good biocompatibility, CS is used as hydrogels, films, fibrous and absorbent structures with applications in various pathologies [13-17].

The hydrogels consist of hydrophilic polymer chains linked with hydrogen bonds, which determines a certain packing degree and places the behavior of these entities somewhere between solids and liquids. Through the action of physiological liquids, the water molecules, having a much smaller volume than the size of macromolecular chains, are diffusing among polymer chains, breaking the polymer-polymer hydrogen bonds, which they replace with hydrogen bonds of polymer-water type. Its specific swelling characteristic is an advantage in medical applications. A significant drug sorption and release potential is thus created [18, 19]. Physical cross-linking has an applicative potential, as it avoids the utilization of chemically-grafted cross-linking agents, which are toxic and imply subsequent complicated purification operations.

HCr is a drug administrated in the treatment of skin diseases, rectal affections and immunity system. It is traded in various forms or formulas, being also topically administrated at patients with allergic eruptions, eczemas, psoriasis and dermatitis [20].

Experimental part

Materials and methods

The textile support is an interlock knitting fabric of 100% cotton yarns with yarn count of Nm = 60/1. CS with deacetylation degree of 75–85% was supplied by Fluka under the name highly viscous CS. HCr is purchased from pharmaceutical market, and ferric chloride, potassium hexacyanoferrate, sulphuric acid and the other chemical

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reagents were used without modifications.L-histidine, and dehydrated natrium orthophosphate were supplied by Merk KGaA. Humidity was removed from cotton knitting using the Vacuum Dryer, Control AG-System Fratelli Galli, 1995.

CS–based hydrogel of the coated knitting fabric was tested by the SEM and EDAX using AMETEK EDAX equipment, coupled with SEM Quanta 203D and Genesis Software and by weighting the knitting fabric with analytical balance with an accuracy of $\pm 1.1 \cdot 10^{-4}$g.

HCr calibration curve, the inclusion and release tests were performed using S-22 UV/VIS Boeco Spectrophotometer, Germany. *In vitro* release at 37°C was performed using the equipment Veticell 55, 2009.

Determination of loading degree

The textile samples with hydrogel are conditioned and weighted with analytical balance. The degree of textile material loading with hydrogel is determined from the formula:

$$\text{Loading degree (\%)} = \frac{(Mf - Mi)}{Mi} \times 100$$

where: M_f is the mass of textile material with hydrogel;
M_i is the mass of textile material without hydrogel.

The work is focused on the aspects of hydrogel preparation, which implies getting an optimum flux for the inclusion and release of a maximum drug quantity.

Preparation of textile support

The knitting fabric was at first subjected to alkaline boiling (solution of NaOH, Na_2CO_3, surfactant and reducing agent) for 2 h to remove the natural cotton impurities, and to bleaching with hydrogen peroxide (0.1 M H_2O_2, 1g/L, *p*H= 11.2 at 95°C for 30 min. The knitting fabric samples are dried in vacuum dryer at 50°C, 3h, and then conditioned at 20°C and 65% relative humidity.

Knitting samples (5x5 cm) are then prepared, marked as **M1- M10** for the inclusion of HCr at 20°C, and samples marked as **M1'- M10'** for the inclusion of HCr at 50°C; each variant is a triplicate.

Hydrogel preparation

The working protocol includes: CS dissolution through magnetic stirring (100 rpm) and heating (70°C) in acid medium realized with 2 mL CH_3COOH (99.6%)/100 mL. The following working parameters were used : CS sorption duration, volume of CS solution ; CS quantity. For the tests concerning sorption duration, CS mass (0.5g) and volume (50 mL) are maintained constant, the durations changing in four steps: 3; 6; 12 and 24 h. The experimental conditions are illustrated in table 1.

Table 1
THE INFLUENCE OF IMMERSION TIME OF KNITTING FABRIC

Sample code	Textile mass (g)	CS mass (g)	Solution volume (mL)	Time (h)
M1	0.5732	0.5	50	3
M1'	0.5434	0.5	50	3
M11	0.4434	0.5	50	3
M2	0.5230	0.5	50	6
M2'	0.5293	0.5	50	6
M12	0.4575	0.5	50	6
M3	0.5270	0.5	50	12
M3'	0.5174	0.5	50	12
M13	0.4079	0.5	50	12
M4	0.5006	0.5	50	24
M4'	0.5122	0.5	50	24
M14	0.3753	0.5	50	24

Table 2
THE INFLUENCE OF THE VOLUME OF CS SOLUTION

Sample code	Textile mass (g)	CS amount (g)	Time (h)	Solution volume (mL)
M5	0.5000	0.5	12	60
M5'	0.4995	0.5	12	60
M15	0.3880	0.5	12	60
M6	0.5950	0.5	12	80
M6'	0.5434	0.5	12	80
M16	0.3618	0.5	12	80
M7	0.5072	0.5	12	100
M7'	0.5194	0.5	12	100
M17	0.3832	0.5	12	100

For the tests on the influence of the volume of CS solution, the CS quantity (0.5g) and CS sorption duration (12 h) are maintained constant, while the solution volumes of 60, 80 and 100 mL are applied, as illustrated in table 2.

For the influence of CS quantity solution volume (50 mL) and maintaining duration (12 h) are kept constant, while CS quantities of 0.3, 0.75 and 1.0 g are tested, as illustrated in table 3.

After performing the experiments illustrated in tables 1-3, the textile samples are introduced in hydrogel, then taken off and let them drip out for 3 h at room temperature. Only the samples M11- M20 are dried, conditioned and weighted to determine the loading degree.

Table 3
THE INFLUENCE OF CS AMOUNT

Sample code	Textile mass (g)	Solution volume (mL)	Time (h)	CS amount (g)
M8	0.5442	50	12	0.3
M8'	0.5320	50	12	0.3
M18	0.4138	50	12	0.3
M9	0.5091	50	12	0.75
M9'	0.5132	50	12	0.75
M19	0.4868	50	12	0.75
M10	0.4928	50	12	1.0
M10'	0.5004	50	12	1.0
M20	0.4067	50	12	1.0

Calibration curve

A solution of 20 mgHCr/100 mL ethyl alcohol 25% (v/v) was prepared. The solution for the calibration curve for HCr release contains 2mL sulphuric acid (4N), 2mL ferric chloride solution (0.5% v/v), 0.5 mL potassium hexacyanoferrate (0.5% v/v) and increasing concentrations of HCr from the basic solution (0.5, 1.0, 1.5 and 2.0 mL), and a reference solution made of 2 mL H_2SO_4 (4N), 0.5 mL potassium hexacyanoferrate (0.5% v/v), 3 mL perspiration kit and 2 mL ferric chloride solution (0.5% v/v). The absorbance value was determined in comparison with the reference solution, at 780 nm. The prepared solutions are thermostated at 70°C, 15 minutes, after which they are cooled and brought to 25 mL constant volume with distilled water.

HCr inclusion

The basic solution contains 0.02% (w/v) HCr (in 100 mL water/ethanol solution (75/25%). Each knitting sample sized 5 x 5 cm weights 0.5 g and is immersed in 20 mL drug solution (0.02% HCr), for 24h, under magnetic stirring (100 r.p.m.). The solution also contains the crosslinking agent, Na_2SO_4, in amount computed in terms of CS quantity from the network. The samples M1- M10 were introduced in the drug solution at 20°C, while the samples M1'- M10' were introduced at 50°C. After 24 h the fabric samples are dried, and the residual solutions are filtered and the absorbance of each solution is determined at 780 nm. In order to determine HCr in solution more solutions were

prepared, containing 2 mL sulphuric acid 4N, 2 mL ferric chloride solution, 2 mL potassium hexacyanoferrate, 3 mL perspiration kit and 2 mL from the filtered solution remained after the inclusion of textile material. The solution was heated at 70°C for 15 min, then cooled and brought to 25 mL constant volume with distilled water. The solution absorbance was measured by spectrophotometry, at 780 nm, in comparison with the reference solution (without HCr). The quantity of HCr included on the textile samples is computed in terms of measured absorbancies.

In vitro release

After including the drug, the samples are dried at 20°C. A perspiration kit was prepared at *p*H = 5.5, containing: sodium chloride, monochlor-L- histidine and dehydrated natrium diacid orthophosphate. Each material sample was included in 20 mL perspiration kit at t = 37°C for 1; 3; 6; 12 and 24h. The absorbance of each solution was determined for each time interval.

Results and discussions

Determination of loading degree

After being immersed in chitosan solution and then dried, the samples M11- M20 are conditioned and weighted, the loading degree being then determined. The data are presented in table 4.

Sample	Final mass (g)	Loading degree (%)
M11	0.5086	14.7
M12	0.5243	14.6
M13	0.4589	12.5
M14	0.4258	13.4
M15	0.4376	12.7
M16	0.4006	10.7
M17	0.4190	9.4
M18	0.4498	8.69
M19	0.5898	21.1
M20	0.5417	33.1

Table 4
VALUES OF LOADING DEGREE OF THE SAMPLES WITH HYDROGEL

From the values presented in table 4, one can notice that for the samples M11-M18 the hydrogel loading degree ranges between 8.69 and 14.7%, while for the samples M19 and M20 the loading degree exceeds 20%; this happens because CS weight at least 25% more than at the other experimental variants.

Morphological elements of textile material surface

Figure 1 illustrates the morphological elements of the surfaces of the fibres from the knitting samples.

From the images illustrated in Figure 1 one can notice that for the treated sample adherent hydrogel formations appear, surrounding the fibres like a sheath. In this way the CS treated cellulose samples are no longer individualized as fibres from reference sample.

EDAX elemental analysis

Figure 2 illustrates the elemental analysis of the sample M10.

The EDAX tests from figure 2 reveal the presence of the elements: C, O, N and S. The first elements, C and O are the result of the hydrocarbon structure of CS, while the nytrogen reveals the amine group specific for CS. Sulphur indicates the presence of natrium sulphate used as crosslinking agent between the CS amine groups and sulphate anions.

Calibration curve of HCr

The calibration curve was determined in coordinates of absorbance coefficients - mg HCr /mL using an ethyl alcohol solution (25%). A straight line intersecting the origin was obtained, with the equation: y = 0.0275x + 0.0132 and the regression factor R^2 = 0.9942.

The performed calculations were reported to a mass of 0.5g knitting fabric. The initial drug mass was of 8g HCr/g material ; the inclusion results are presented in table 5.

As the result of the determinations presented in table 5, a maximum of the amount of absorbed drug was obtained

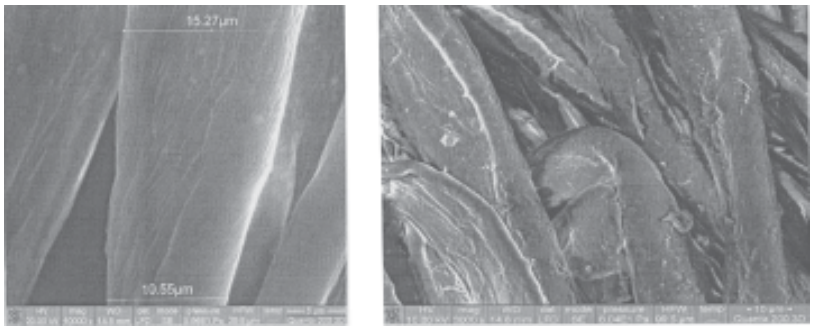

Fig. 1. SEM microphotograph;
a) reference cotton knitting fabric
b) cotton knitting fabric grafted with CS-hydrogel

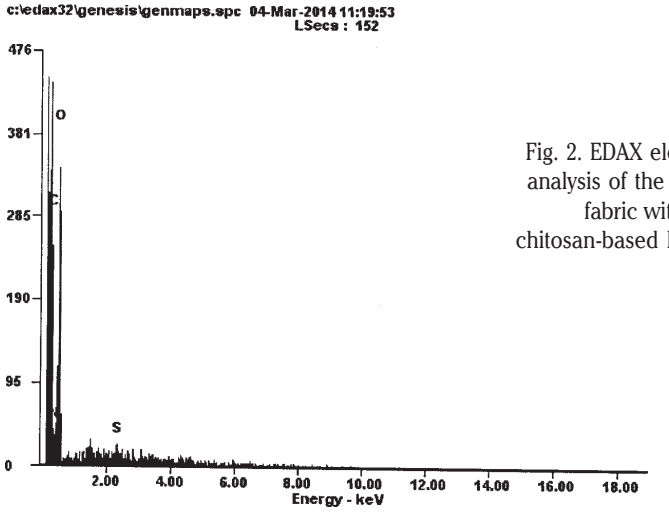

Fig. 2. EDAX elemental analysis of the knitting fabric with chitosan-based hydrogel.

Table 5
THE MASS VALUES OF THE INCLUDED DRUG

Sample	Amount included (HCr mg/g knit)	
	20^0C (M1-M10)	50^0C(M1'-M10')
M1/M1'	**1.74**	**1.05**
M2/M2'	2.05	1.14
M3/M3'	3.46	2.41
M4/M4'	3.42	2.14
M5/M5'	2.36	1.69
M6/M6'	**4.20**	**3.23**
M7/M7'	3.23	1.96
M8/M8'	3.94	3.07
M9/M9'	2.74	2.10
M10/M10'	1.96	**0.87**

Sample	Included drug (mg HCr/g knit.)	Amount released, mg HCr/g mat 1h	3h	6h	12h	24h	Total amount (mg HCr/g knit.)	Release (%)
M1	1.74	1.17	0.26	0.08	-	-	1.51	86.7
M1'	1.05	0.74	0.10	-	-	-	0.84	80.0
M2	2.05	1.24	0.40	0.10	-	-	1.74	84.8
M2'	1.14	0.79	0.17	-	-	-	0.96	84.2
M3	3.47	2.10	0.97	0.10	-	-	3.17	91.3
M3'	2.41	1.42	0.76	0.04	-	-	2.22	92.1
M4	3.42	1.97	0.90	0.10	-	-	2.97	86.8
M4'	2.14	1.26	0.42	0.04	-	-	1.72	80.3
M5	2.36	1.47	0.58	0.15	-	-	2.2	93.2
M5'	1.69	1.04	0.35	-	-	-	1.39	82.2
M6	4.20	2.31	1.17	0.42	-	-	3.9	92.8
M6'	3.23	1.83	0.92	0.15	-	-	2.9	89.7
M7	3.24	1.85	0.97	0.15	-	-	2.97	91.6
M7'	2.78	1.65	0.67	0.15	-	-	2.47	88.8
M8	4.11	2.22	1.17	0.4	-	-	3.79	92.2
M8'	3.12	1.85	0.81	0.15	-	-	2.81	90.0
M9	2.74	1.58	0.72	0.10	-	-	2.4	87.5
M9'	2.15	1.22	0.47	0.10	-	-	1.79	83.2
M10	1.96	1.24	0.4	-	-	-	1.64	83.6

Table 6
THE VALUES OF HCr INCLUDED AND RESPECTIVELY RELEASED FROM THE HYDROGEL AND THE TEXTILE MATERIAL

at the formula M6/M6', meaning 80 mL distilled water and 0.5 g chitosan, t = 12h, T = 20°C, and a minimum at the recipe M1, with 0.5 g CS, 50 mL solution and t = 3h. At the same time, there is a relation according to which the experiments carried out at 50°C imply, with no exception, a smaller quantity of absorbed drug than at 20°C under the same experimental conditions. A high value of the amount of absorbed HCr close to maximum was also obtained for M8, namely 0.3 g chitosan in 50 mL distilled water and 12 h absorption time at 20°C. The inclusion mechanism is an absorption mechanism which, being exothermal, is favored by low temperatures and longer immersion interval. A higher temperature intensifies the thermal agitation and missfavors the drug sorption.

HCr release

After inclusion, the knitted samples were immersed in perspiration kit solution for 1; 3; 6; 12 and 24 h. The solution absorbance is determined instrumentally, while by means of the calibration curve one can determine the amount of released HCr for each recipe. The obtained values are presented in table 6.

The values illustrated in table 6 show that drug release extends on a duration of up to 6 h, even if the experimental period included tests up to 24 h.

In all the experiments of HCr release a « burst effect » is manifested. In most of the experimental variants which implies drug sorption at 50°C the « burst effect » is raised as compared to the variant at 20°C.

The last column, i.e. Release (%) shows the percentage of released drug as compared to the included drug ; i.e. this parameter is a measure of the efficiency of releasing process. From this point of view, the M3, M3' and M5-M8 experiments show a releasing efficiency higher than 90%, determining an experimental area of HCr release efficiency.

The optimal range of HCr absorption is the working protocol which implies the maximum drug absorption. The experimental parameters are those of the variant M6 carried out at a volume of CS solution of 80 mL, which contains 0.5 g CS, with the generation of a hydrogel deposited on the textile material for a duration of 12 h, and absorption at 20°C for which the release efficiency is 92.8%.

Figure 3 illustrates the HCr release curve for the experimental parameters of the sample M6, which represents the maximum amount of HCr absorbed at 20°C from the drug existing in hydrogel.

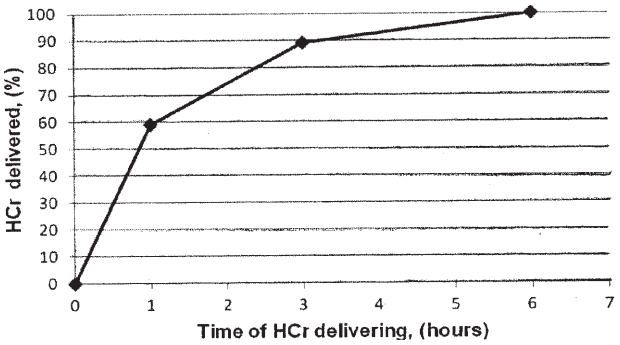

Fig. 3. Curve of HCr release for variant M6

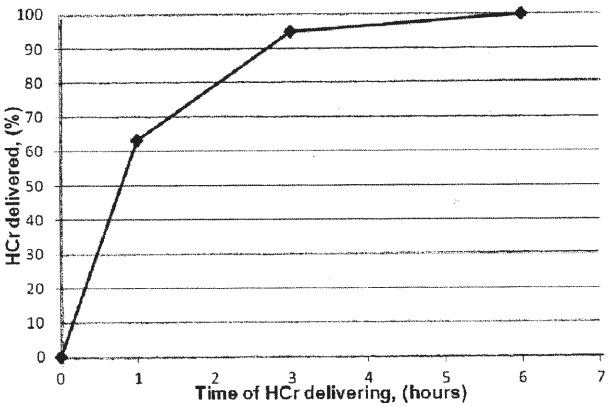

Fig. 4. Curve of HCr release for variant M6'

The presented experimental variants and the optimum drug absorption/release domain are useful to the extent to which they create punctual conditions to realize the therapeutic doses for a specified coetaneous pathology for which the drug release from a textile support reduces to minimum the patient effort for drug administration.

Conclusions

Using as material support a 100% cotton knitted fabric, one can achieve a biomaterial consisting of CS gel and HCr.

The formed biomaterial can provide a flux of HCr released to the dermis for a duration of 6 h, with a marked burst effect during the first hour of release, with a releasing efficiency of about 90%.

The presented working protocol has a potential for coetaneous application due to the utilization of a biocompatible system of drug inclusion and release.

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