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Cotton fabric: A natural matrix suitable for controlled release systems

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Abstract

The possibility to use cotton as a matrix for controlled release systems was studied by covalently attaching a model compound, specifically the reactive dye Remazol Brilliant Blue R to its surface. Afterwards the fabric was coated with a commercial cellulase. The release of the dye, obtained by the hydrolysis of cotton fibres in sweat buffer, was monitored. The reducing sugars concentration increased for both fabrics (with and without the dye covalently fixed) while the increase in the absorbance was only attained for the dyed cotton, as expected. These results confirm the viability of using cotton as a natural matrix for controlled released systems while presenting a promising approach to immobilize covalently other substances in cotton garments, like fragrances, which could be released by the action of human sweat.

Keywords: Cellulases; Controlled release systems; Cotton fabric; Glutaraldehyde

1. Introduction

Cellulases are a group of hydrolytic enzymes able to hydrolyse cellulose that play an important role in maintaining the carbon balance in nature. There are three types of enzymes which have been traditionally assigned to the cellulase system: endoglucanases (EG) which cleave randomly cellulose chains (EC 3.2.1.4), cellobiohydrolases (CBH) which cleave cellobiose from the chain ends (EC 3.2.1.91), and β-glucosidases which hydrolyses cellobiose into glucose (EC 3.2.1.21). Of the three classes of enzymes, EG are the less specific ones, since they can also hydrolyse cellulose derivatives like carboxymethyl cellulose and reactive dyed cotton. This fact is mainly due to the geometry of the active site of those enzymes which is of a tunnel shape for CBHs and of a cleft shape for EGs [1].

Considering those fundamental facts, our research group conjectured the hypothesis that cellulases could be used as an agent to release drugs linked to cotton fabrics, where the addition of the enzyme could be the agent to "switch on" the release of the drug in an aqueous medium. The release of the enzyme is not however desirable in the environment where the drug should be released to. Therefore, another possibility to allow for the drug delivery is to coat the fibres with the enzyme, where the drug had been previously covalently bound. The coating process could be performed using glutaraldehyde at low temperatures, inhibiting in this way the catalytic action of the enzyme. It has been reported that cellulases are stable when immobilized closely by their substrates, especially in dry conditions [2–4]. Consequently, if the enzyme is crosslinked around the fibre containing the drug, the water environment could be the "switch on" factor for the release of that specific drug.

In this study a reactive dye was used as a drug model and cotton fabric was used as the support for a potential application into a drug release system, as shown in Fig. 1.

To provide evidence of the concept, the dye was covalently attached to the fibre and subsequently a cellulase was cross linked to the fibre surface using glutaraldehyde. The water environment was used as the "switch on" agent to release the dye. The mechanism for the release of the drug proceeds via the hydrolysis of the substrate (cellulose) with the concomitant release of the covalently attached dye and of soluble reducing sugars.

2. Material and methods

2.1. Enzyme, chemicals and fabric

The enzyme Cellusoft L, a total cellulase from *Trichoderma* origin, was a gift from Aquitex (Porto, Portugal). All other chemicals used were of

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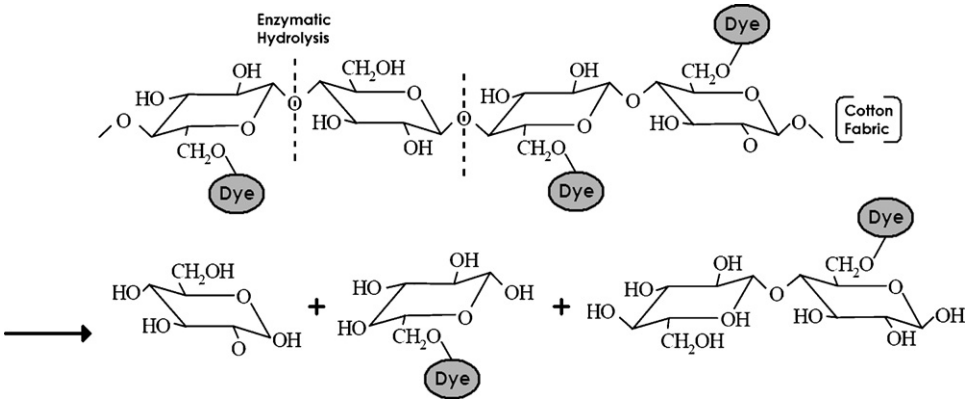

Fig. 1. Schematic representation of the release of the reducing sugars covalently linked with dye molecules from the enzymatic hydrolysis of cotton matrix (adapted from [5,6]).

analytical grade. The fabric used was alkaline scoured and bleached 100% cotton.

2.2. Covalent fixation of a reactive dye onto cotton

A standard dyeing protocol was used. The cotton fabric was dyed with the reactive dye Remazol Brilliant Blue R (C.I. no. 61200). In the first step, cotton was treated with 50 g NaCl/l and 3% (w/v) of the dye solution for 15 min. The dyeing proceeded with the addition of 5 g Na_2CO_3/l solution and 1 ml NaOH/l for 30 min at 60°C. The dyeing finished after 70 min of treatment and then the fabric was washed three times with 20 g Lutensol AT25/l for 30 min in boiling water. The maximum wavelength of Remazol Brilliant Blue R (1 g/l) was determined spectrophotometrically and it was found to be 570 nm.

2.3. Cotton fibres coating with the enzyme

The dry cotton samples (dyed and undyed) were weighted and placed to boil in distilled water for 15 min. The boiled fabric was left overnight at 4 °C in acetate buffer pH 5. The fabrics were then squeezed and treated with a cold cellulase solution (pure Cellusoft L) on a Foulard machine (Roaches England, Padder BHP). The fabric was impregnated three times with the enzyme on the Foulard. The enzyme solution was removed from the Foulard, a 2% glutaraldehyde solution (v/v) was introduced and the fabric was again impregnated three times on the Foulard machine with this solution. Subsequently, the fabric was squeezed and left overnight at 4 °C. The last step of the process, before the controlled release tests, was to rinse the fabric well with cold water.

2.4. Controlled release of dye in sweat buffer

Cotton fabrics (1 g) were treated in sweat buffer using a bath ratio of 1:100, in a shaker bath at 37 °C and 70 rpm. Aliquots were taken and several parameters were measured (reducing sugars concentration, absorbance at 570 nm and protein concentration, by the Lowry method [7]). The sweat buffer (pH 5.5) was prepared with 2 M L-histidine monohydrochloride monohydrate, 1 M sodium phosphate and 8 M sodium chloride. The control sample was dyed cotton fabric without enzymatic and glutaraldehyde coating.

2.5. Fluorescence microscopy

The cotton fibres were embedded in an epoxy resin and transversal cuts of the fibres with 10 μm were prepared using a microtome. Fibres cross-sections were analyzed by a transmission optic microscope (Olympus BH2) with a magnification of 40×.

2.6. Reducing sugars concentration

The reducing sugars concentration was determined spectrophotometrically at 475 nm using the neocuproine-Cu method as previously referred [8].

3. Results

To investigate the possibility for the use of cotton fabric as a matrix for controlled release systems, a reactive dye was covalently attached to its surface, as previously stated. The release of the model drug was induced by the hydrolysis of cotton in a simulated sweat buffer by the previously immobilized cellulases. This release was monitored by the increase of reducing sugars concentration and also by the increase of absorbance, as described below.

3.1. Reducing sugars concentration

The results for the reducing sugars concentration are shown in Fig. 2. The concentration of reducing sugars increased with time for all samples, except for the control sample, which is dyed fabric without enzymatic coating. The behaviour of the undyed cotton (representing the fabric without drug) and of the dyed cotton (representing the fabric with drug) are quite similar. Nevertheless, it seems that the hydrolytic attack to cellulose started later in the dyed fabric, which can be explained by the lower accessibility of the enzyme to the fabric having the dye covalently fixed.

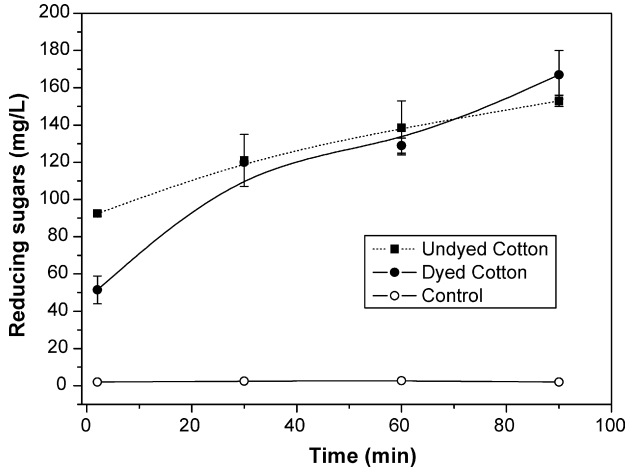

Fig. 2. Reducing sugars concentration attained for the treatment of cotton fabrics in sweat buffer, at 37 °C and 70 rpm. Control sample is dyed fabric without enzymatic coating.

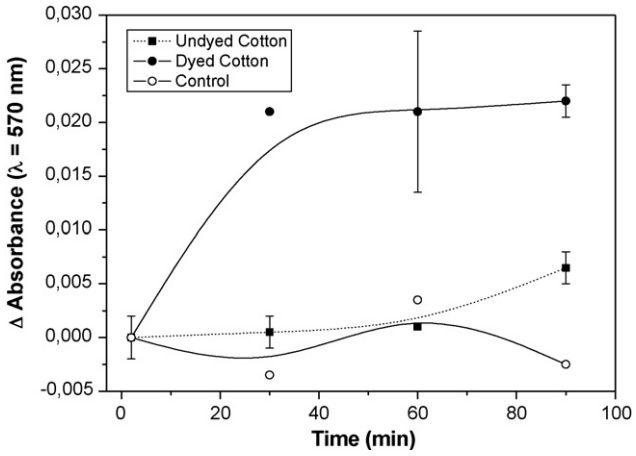

Fig. 3. Release of Remazol Brilliant Blue R, measured by the absorbance at 570 nm, for the cotton fabrics in sweat buffer, at 37 °C and 70 rpm. Control sample is dyed fabric without enzymatic coating.

3.2. Absorbance on solution

After the covalent fixation of the dye and the enzymatic coating of the cotton fabric, the absorbance at 570 nm was measured in the remaining solution after the sweat buffer treatment. The results (Fig. 3) show that the absorbance increased only for dyed cotton, as expected. This fact indicates that the drug (reactive dye) was released by the action of the enzyme in the sweat buffer. This buffer simulates normal human sweat, having a pH of 5.5, which stands in the range for the optimum pH of cellusoft L. It seems that cellulases were not affected by this buffer system, although it is very rich in salts. Continuing with this study, the stability of cellusoft L in the sweat buffer will be determined.

3.3. Fluorescence microscopy

To visualize the dye penetration into the cotton fibres, transversal cuts were performed and analysed by fluorescence microscopy. The photographs (Fig. 4) show that for dyed and enzymatically coated cotton (B) some of the interfibrillar space is filled, probably with enzyme and glutaraldehyde, confirming the covalent attachment of the enzyme to the fabric. Also, an increase in fibres thickness seems to occur when compared with the control sample (A).

4. Discussion

Controlled release technology is recent and it has considerable potential in the fields of medicine, pharmacy and agriculture [9]. The parameters that affect the properties of controlled release formulations depend upon the nature and type of the polymer used. Natural polymers are often preferred to synthetic polymers because of their non-toxic, low cost, free availability, and biodegradability characteristics [10]. Amongst the natural polymers, cellulose, the ubiquitous homopolysaccharide made up of β-D-glucose, is particularly abundant, inexpensive and available [11]. Several authors investigated the crosslinking potential of cellulose or cellulose derivatives with glutaraldehyde. Xu et al. [12,13] reported the use of glutaraldehyde and glyoxal to impart higher levels of wet strength to paper, comparing their different reactivity with cellulose. Choi et al. [14] tested the use of these two aldehydes for replacement of formaldehyde in the durable press finishing of cotton, reporting the higher efficiency of glutaraldehyde in the crosslinking with cellulose. In another study, Işiklan [9] tested the potential of blends of carboxymethyl cellulose and gelatin for the controlled release of an insecticide.

In this study, a cotton fabric was tested as a matrix for the controlled release of a model compound, by crosslinking to cellulases with glutaraldehyde. Glutaraldehyde is one of the best known crosslinking agents, being extremely efficient for crosslinking proteins. Glutaraldehyde can react with several functional groups of proteins and generally the crosslinking of proteins, either to a carrier (solid support) or between protein molecules (carrier-free), implies the ε-amino group of lysyl residues [15,16]. Most proteins, including cellulases, contain many lysine residues that are usually located on the protein surface (i.e., exposed to the aqueous medium) because of the polarity of the amine group. Furthermore, lysine residues are generally not involved in the catalytic site, which allows moderate crosslinking to preserve protein conformation and thus biological activity [16–18].

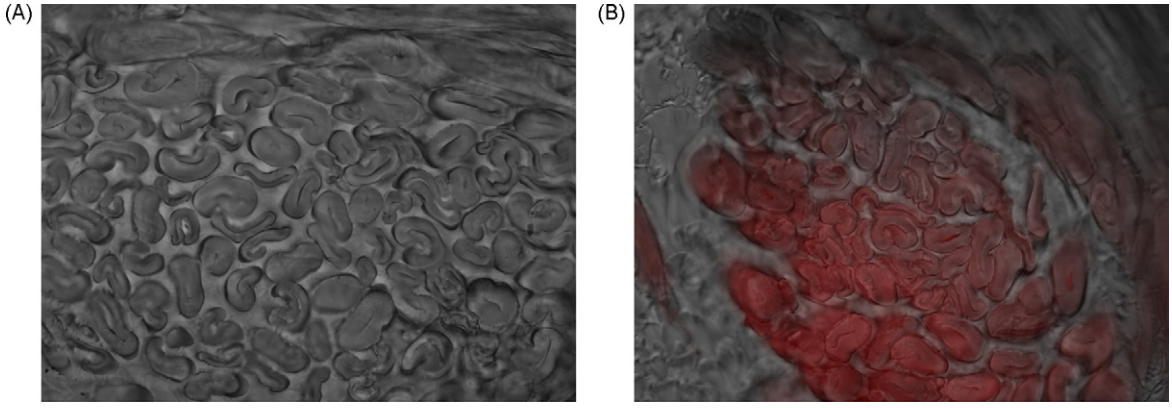

Fig. 4. Fluorescence microphotographs of fibre cross-sections of untreated cotton (A) and after the treatment of dyed cotton fabric (B).

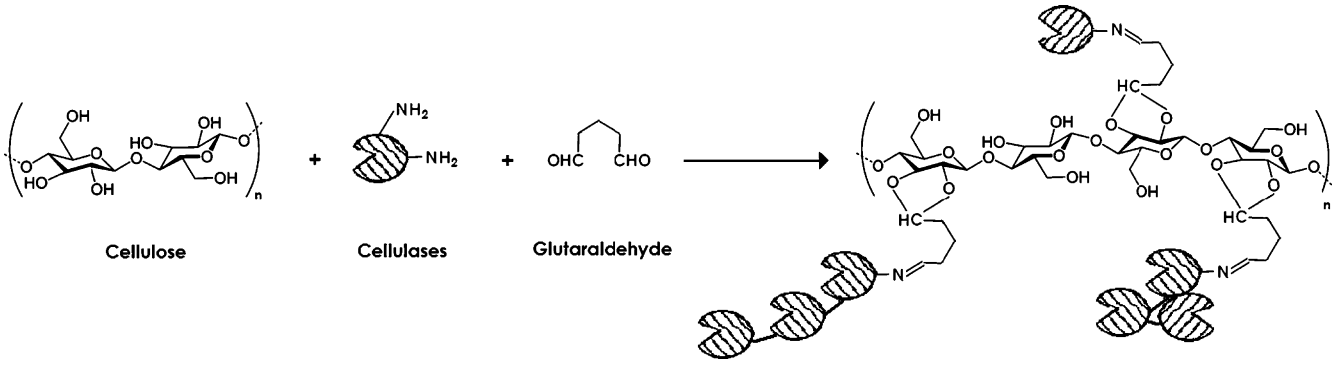

Fig. 5. Possible crosslinking reaction scheme between cellulose, enzyme and glutaraldehyde (adapted from [12–16]).

Walt and Agayn [19] proposed multiple reaction products for the different glutaraldehyde structures in solution, depending on the pH conditions because each form of glutaraldehyde might participate differently in crosslinking reactions with proteins. Thus, under acidic or neutral conditions, glutaraldehyde exists as a mixture of monomers. Each of these structures would be expected to form Schiff bases upon nucleophilic attack by lysine residues in a protein. Under basic conditions, the reaction of α,β-unsaturated oligomeric aldehydes with amine can give two robust products to acid hydrolysis: a Schiff base, which is more stable because of the conjugation of the internal aldehyde group with the C–C double bonds and a Michael addition product [16].

Accordingly, it was our assumption that glutaraldehyde could link the enzyme to the cotton surface via an acetal mechanism, i.e., the formation of a Schiff base (based on references [12–16]). The reaction was performed at low temperature, to minimize activity loss [16–18]. Fig. 5 shows the schematic representation of the possible reaction mechanism between glutaraldehyde, cotton and cellulases.

The enzymes represented in the above scheme might react further with other enzymes, creating multi-enzymatic aggregates of cellulases. Therefore, the cotton fabric might be potentially coated with these multi-enzymatic aggregates of cellulases generated during the crosslinking procedure.

It is our conviction that the crosslinked enzyme has enough mobility to interact with cotton surface, therefore releasing the dye molecules previously covalently attached to it. This assumption was confirmed by the two methodologies used to monitor the controlled release of the dye. First, the measure of the absorbance at 570 nm, which represents the release of the dye into the solution, showed that for dyed samples in a sweat buffer environment, the dye was released in a controlled mode, proving the concept. The other method used, the amount of reducing sugars released to the media, indicates the degradation of the cotton fabric. Confirming the other results, the degradation of the cotton fabric was increasing with time, indicating that the enzyme was covalently attached to the fibre surface and at least part of it was in an active form.

The values for reducing sugars concentration were higher for the undyed samples, indicating as previously stated a higher accessibility of the enzyme to the substrate in the undyed samples. In a previous study, the use of water to replace the sweat buffer as the aqueous environment for the release of substances was also attempted and the results were quite similar (data not shown). Since distilled water pH is close to that of the buffer (approximately 5.5) it is our assumption that the enzyme is also quite stable in water. In a future work, the stability of cellusoft L will be measured in both environments as to better understand the enzyme mechanism as a "switch on" agent on controlled release systems.

5. Conclusions

The attained results show that the release of several compounds can be accomplished when using cotton fabrics as a matrix. In this particular study, the exploitable substance was a reactive dye, Remazol Brilliant Blue R, which was covalently fixed to the cotton surface. After the dyeing (covalent fixation of the substance to the matrix), the fabric was subsequently coated with the enzyme using a common crosslinking agent, glutaraldehyde, which allowed for the enzyme cellusoft L to be linked covalently to the fabric surface. The crosslinked enzyme had enough mobility to interact with cotton surface, therefore releasing the dye molecules previously covalently attached to it.

This work will be continued by studying other compounds that could be effectively linked to cotton fabric or clothing. It seems a promising approach to immobilize covalently fragrances, in cotton garments, for instance, which could be released by the action of human sweat. Also, it is our intent to use modified cellulosic fibres, such as carboxymethyl-cellulose (CMC) fibres, for the enhanced entrapping and release of chemicals [20]. Another approach will be the study of blends with other polymers for an effective release of drugs [9,21].

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