Membranes of Cellulose Triacetate Produced from Sugarcane Bagasse Cellulose as Alternative Matrices for Doxycycline Incorporation

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ABSTRACT: Cellulose triacetate (CTA) membranes were prepared using polyethylene glycol, 600 g mol^{-1}, (PEG) as additive and were utilized in essays of doxycycline (DOX) incorporation using two different procedures: (i) incorporation of the drug during the membrane preparation and (ii) incorporation of the drug to a previously prepared membrane. In the first, the produced membrane presented high compatibility between DOX and CTA, what was evidenced by analyzing the DSC curve for a CTA/PEG 50%/DOX system. Results showed that the drug is homogeneously distributed throughout the matrix, molecularly. In the second method, the drug was molecularly and superficially adsorbed, as seen through the DSC curve for the system CTA/PEG 10%/DOX, which nearly does not present alterations in relation to the original material, and through the isotherm of drug adsorption that follows the Langmuir model. Results showed that the membranes produced from sugarcane bagasse are adequate to produce matrices for drug-controlled release, both for enteric use (Method (i)) and topic use (Method (ii)). © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 113: 3544–3549, 2009

Key words: cellulose acetate; sugarcane bagasse; controlled drug release; doxycycline

INTRODUCTION

Brazil is the world's major sugarcane producer, followed by India and Australia. In average, 55% of Brazilian sugarcane is used to produce alcohol and 45% for producing sugar. Brazilian sugarcane production in 2007 was 515.8 million metric tons and a 589.2 million metric ton production is expected for 2008, 14.2% higher than in 2007.[1] For each sugarcane metric ton used to produce sugar and alcohol, 280 kg bagasse is produced as residue. Although a great amount of this residue is used for producing energy, some of it is still discarded and, thus, could be used as an alternative source of raw materials. In the last decades, studies on the use of residues of several agroindustrial activities have increased.[2] Considering that sugarcane bagasse is composed by about 30–50% cellulose and 20–24% lignin, alternatives to use these components in the production of lignin[3–5] and cellulose[6–12] derivatives have been investigated.

Cellulose esters are cellulose derivatives of great commercial importance, particularly cellulose acetates. These derivatives are usually produced through acetylation reactions using acetic acid as solvent, sulfuric acid as catalyst and nitric acid as acetylating agent. The process may be carried out through two routes: homogeneous and heterogeneous.[12–14] In the homogeneous route, cellulose fibers are dissolved in the reactional medium, destroying the crystalline structure, while in the heterogeneous route, toluene is used during the synthesis as a non-swelling agent.[13] The choice of one of these processes is related to the properties of the intended final material, as the heterogeneous route leads to a more fibrous and crystalline material, what may lead to the production of a less biodegradable matrix.[15]

The wide range of application of cellulose esters has been constantly renewing the interest in these systems. Particularly, in the production of matrices for the controlled release of drugs, where cellulose esters have been assuming a vital role owing to some of their properties such as low toxicity, high permeation to water, high glass transition temperature, production of resistant films, compatibility with several active agents and ability to form micro- and nano-particles.[16] Physical phenomena such as dissolution and diffusion, as well as the chemical degradation of these matrices are very important for

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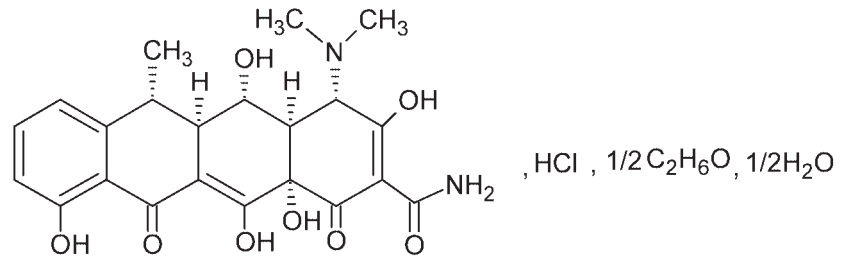

Figure 1 Chemical structure of hydrochloric doxycycline.

the release of bioactive agents. Besides, the route through which the drug is incorporated is very important, since it may be either molecularly disperse in the polymer,[16–18] or superficially adsorbed.[19] In this case, the kind of observed interaction is fundamental on predicting the profile of the drug release, besides being a parameter for choosing the kind of developed therapeutic system (topic administration, enteral and parenteral).[18]

In a previous work,[8] cellulose triacetate (CTA) membranes produced from sugarcane bagasse cellulose were prepared using polyethylene glycol (PEG), 600 g mol^{-1}, PEG as admixture. DSC results showed that the matrices presented morphological modifications, which were intensified for high PEG content (CTA/PEG 50% w/w). Furthermore, the produced membranes did not present toxicity according to the cellular viability tests. These morphological changes on the matrix, as well as the non-toxicity are important on the development of systems for drug release. The matrix modification because of the presence of plasticizing admixtures tends to increase the rate of drug release, especially if the plasticizing is hydrophilic such as PEG.[16] Considering these aspects, CTA produced from an alternative cellulosic source (sugarcane bagasse) was used in the production of matrices for incorporating an antibiotic, doxycycline (DOX).

DOX, shown in Figure 1, is a synthetic bacteriostatic derivative from oxytetracycline, which inhibits the synthesis of the bacterial protein owing to the perturbation of the transfer RNA and messenger RNA in the ribosomic sites.[20] This antibiotic is often used in the treatment of several infections such as periodontal diseases.[21,22] Periodontal diseases are a group of inflammatory conditions that affect the secondary teeth and are started by microorganisms that colonize the teeth surface and infect the surroundings. The inflammation extension of the marginal gum on the secondary periodontal tissues delimit the transition from gingivites to periodontitis.[23,24]

In vitro tests have showed that *Porphyromas gingivalis, Prevotella intermedia, Campylobacter rectus* and *Fusobacterium nucleatum*, bacteria that are associated with periodontal diseases, are susceptible to 6.0 μg mL^{-1} DOX.[25]

In this work, CTA produced by homogeneous acetylation of cellulose extracted from sugarcane bagasse, was used in the production of membranes using PEG as plasticizing additive. DOX was incorporated to the membranes in two different ways: *Method (i)* by dissolution of the drug in the polymeric solution and consequent dispersion of the drug in the matrix during the membrane formation; and *Method (ii)* by adsorption of the drug acid solution to a previously prepared membrane. The incorporation test was performed using spectrophotometric analysis on the ultraviolet-visible range (UV–vis). The investigation of the compatibility between the drug and cellulose acetate was performed by differential scanning calorimetry (DSC).

EXPERIMENTAL

Materials

Sugarcane bagasse was provided by usina Delta, from Caeté- MG, Brazil. Sodium hydroxide, acetic anhydride, dichloromethane were purchased from VETEC, ethanol and PEG 600 g mol^{-1} from SYNTH, nitric acid, acetic acid and methanol from ISOFAR, sulfuric acid from QUIMEX and doxycycline from GENIX.

Extraction of cellulose from sugarcane bagasse

The extraction of cellulose from sugarcane bagasse was carried out as described by Filho et al.[12] Sugarcane bagasse was washed with water and then immersed in sodium hydroxide 0.25*M* for 18 h at room temperature. Next, the material was washed, filtered and put into reflux with nitric acid/ethanol solution, 20% (v/v), for 3 h, changing the solution at each 1 h. Finally, the material was washed to remove the acid and dried at 105°C for 3 h.

Synthesis of cellulose triacetate

Cellulose triacetate was produced according to the methodology described elsewhere.[12] The chosen route was the homogeneous,[8,13] in which cellulose was

acetylated using a mixture of acetic anhydride in acetic acid, and sulfuric acid was used as catalyst. The produced material presents degree of substitution (DS) of 2.80, which was determined by titration, using an acid–base reaction, according to Myamoto et al.[26]

Membrane preparation and study of doxycycline incorporation

Doxycycline was incorporated to the membranes in two ways: Method (i), incorporation of DOX during membrane preparation, in which the drug was dissolved in the polymeric solution (0.05 g DOX, 1 g CTA, and 1 g PEG (600 g mol^{-1}) were dissolved in the solvent mixture dichloromethane/methanol (9/1 v/v)). The mixture was deposited in a Petri dish, the solvent evaporated for 2 min and the process was finished with the immersion of the system into a distilled water bath at 5°C. The bath solution was analyzed by electronic spectroscopy in the UV–vis region, at 275 nm, using a UV-2501PC spectrophotometer (Shimadzu), for measuring the amount of drug that remains incorporated to the membrane, in relation to a calibration curve built using DOX solutions (1.0×10^{-5} to 5.5×10^{-5} mol L^{-1}).

In the second methodology, Method (ii), CTA membranes were previously prepared by casting,[8] using dichloromethane as solvent (10% w/w) and PEG in the proportion from 0 to 50%. These membranes were used in the assays for DOX incorporation, which were carried out by dipping the membrane in a drug aqueous solution (2 g/L) for 12 h at 10°C. After removing the membrane, the solution concentration was determined by electronic spectroscopy in the UV–vis region, as in Method (i).

In both methodologies, membranes without DOX were produced as standard.

Differential scanning calorimetry

DSC analyses were carried out in a Rheometric Scientific DSC-SP equipment. About 10 mg of membranes were sealed in an aluminum pan with lid, and purged with ultra-pure dry nitrogen at a flow rate of 20 mL/min. The temperature ramp was set at 20°C/min and the heat flow was recorded from 25 to 350°C. Indium and zinc were used as standards to calibrate the temperature and the energy scales of the DSC instrument. The DOX melting point is about 210°C as observed in DSC curve shown by Mundargi et al.[17]

Production of the adsorption CURVES of DOX onto cellulose triacetate membranes

Membrane samples containing 10% PEG, weighting $\sim$ 0.025 g and measuring 2.0 cm^2, were immersed in solutions with different DOX concentrations, which were prepared using a dilution series of the standard acid solution (pH = 2.0) with original concentration of 2.0 g/L. The solutions were stirred for 12 h in a thermostated environment at $\sim$ 4°C, and the concentrations were determined by UV–vis spectrometry, using a previously prepared calibration curve, which was built using standard DOX solutions with known concentrations. The values for the initial (C_o) and final (C_e) concentrations were used to calculate the incorporation capacity (q_e), which is the amount of adsorbed DOX in equilibrium to the membrane weight (mg/g). The analyses were performed in triplicate. The experimental data were tested for the Langmuir isotherm. In this model, the drug adsorption must follow eq. (1), where the surface saturation (Q_{max} [mg/g]) implies on the saturation of the monolayer sites, and the constant b (L/mg) is related with the free energy of adsorption. In this case, the adsorption must follow the profile of chemical adsorption, with the absence of lateral interactions, and existence of surface homogeneity, i.e. all the adsorption sites have the same free energy of adsorption (ΔG°).

$$q_e = \frac{bQ_{max}C_e}{1 + bC_e} \quad (1)$$

To obtain the parameters for the Langmuir isotherms (1), the data were adjusted in a nonlinear regression using the software STATISTIC.

RESULTS AND DISCUSSION

The drug incorporation into the cellulose acetate membrane, Method (i), was determined considering the total weight of the added DOX to the solution for the membrane preparation. After the production of the membranes, the spectroscopic determination of the drug showed that 90% of the added DOX was incorporated to the polymeric matrix (36.04 mg DOX/g membrane).

The matrix properties are important when assembling systems for controlling drug release the rate of drug dissolution in the medium. However, this process depends on the interaction between the drug, the polymer and the other blend compounds such as the additives, i.e. the compatibility between the drug and the polymeric matrix may favor the drug bioavailability.[19] In this sense, the compatibility of the system CTA/PEG 50% /DOX was studied by DSC.

Figure 2 shows the DSC scans of the CTA membranes prepared using the system dichloromethane/methanol as solvent and PEG as additive. The first membrane was prepared without the drug (CTA/PEG 50%) and the second with its incorporation (CTA/PEG 50%/DOX).

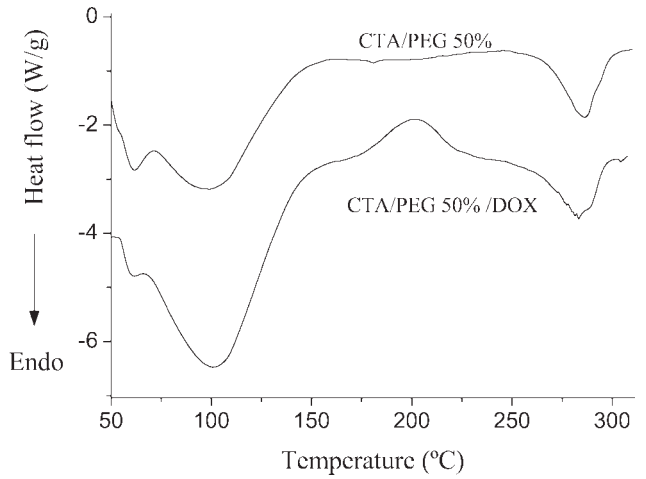

Figure 2 DSC first scans of CTA/PEG and CTA/PEG 50%/DOX membranes.

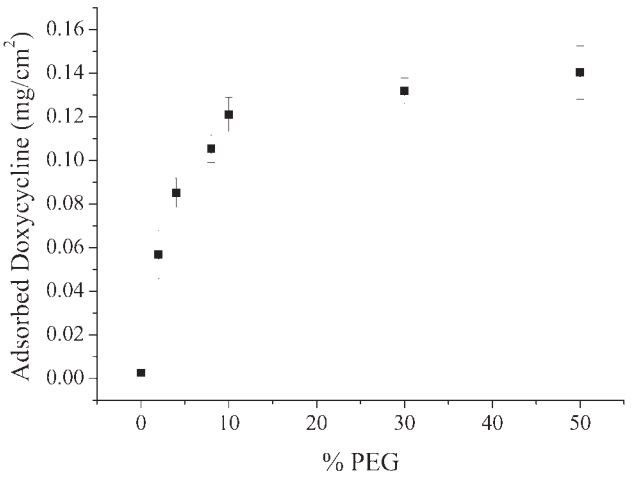

Figure 3 DOX adsorption in function of PEG content used for preparing the membranes.

Some aspects should be emphasized in relation to the DSC curves: (i) CTA/PEG 50% presents an endotherm of fusion at 286.2°C and enthalpy of fusion of 16.37 J g^{-1}. Because the crystallization process is not observed during the DSC run, the presence of melting endotherm indicates that CTA/PEG membrane is a semicrystalline material. It is possible that the high PEG content leads to a decrease in the polymer T_g and favors the crystallization during the processing. This has already been observed in a previous work, in which the interaction between the additive and the matrix was investigated.[8] (ii) the DSC scan of CTA/PEG 50% /DOX presents a crystallization exotherm at 202.30°C and the endotherm of fusion in 283.27°C. Moreover, the endotherm of fusion and the exotherm of crystallization present very similar enthalpy values. The presence of an exotherm of crystallization indicates a crystallization process during the scanning, which melts as the temperature increases, giving rise to the endotherm of fusion. The similar values of these two thermal events indicate that the crystallinity of this new CTA/PEG 50%/DOX material is very small, and it can be considered as being fundamentally amorphous.

The modification of DSC patterns of the membrane with DOX (Fig. 2) compared with the original membrane with PEG, as well as the absence of a melting endotherm for DOX indicate that the drug was molecularly dispersed in the polymer matrix. According to Edgar, the proper polymer/drug ratio in membrane preparation can result in no drug crystallinity observable by X-ray or DSC in formulations.[16] Therefore, these results show good compatibility between the polymer and DOX at the evaluated concentration. This kind of association can enhance the solubility and bioavailability of drug.

With the other methodology, i.e. the incorporation of the drug in the previously prepared membrane, Method (ii), the incorporation was evaluated in function of the PEG content used for preparing the membranes, according to what is presented in Figure 3. The curve may be divided in two regions: in the first, there is an increase in the concentration of adsorbed DOX as PEG content increases up to 10%; in the second part, as PEG content increases up to 50% the adsorption capacity of the polymer practically does not change.

According to Figure 3, a 10% PEG content is enough for reaching the maximum drug adsorption through this methodology (0.12 mg/cm^2 of membrane). Considering this result, the interaction between polymer and drug was evaluated through the DSC scans, presented in Figure 4.

The DSC scans show that the drug adsorption practically does not modify the morphology of the polymeric matrix. This indicates that the adsorption process occurs predominantly on the membrane surface, differently of what was observed in Method (i),

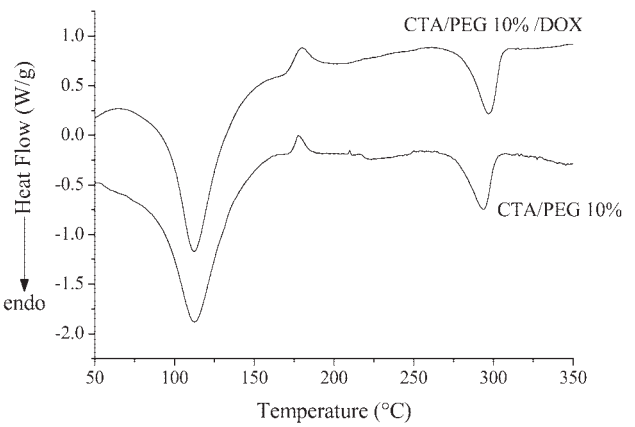

Figure 4 DSC first scans of CTA/PEG 10% and CTA/PEG 10%/DOX membranes.

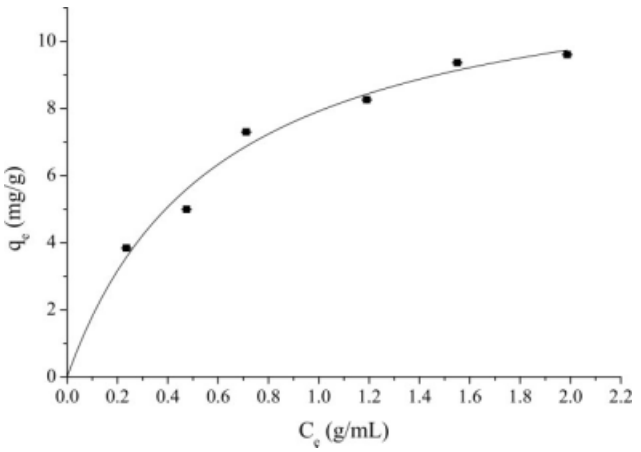

Figure 5 Isotherm of DOX adsorption on CTA membranes prepared with 10% PEG.

in which the incorporation is performed by the addition of the drug in the polymeric solution. In Method (i), DOX is homogeneously dispersed throughout the matrix and acts as if it were a plasticizer.

DOX incorporation in membranes produced with 10% PEG was also evaluated through the isotherm of adsorption. In this study, the adsorption capacity (q_e) of the membranes was analyzed in function of DOX concentration (C_e). Figure 5 presents the obtained isotherm.

The experimental results, shown in Figure 5, showed to be well adjusted to the model of the Langmuir isotherm, what could be confirmed through the value of the correlation coefficient (R^2), presented in eq. (2).

$$q_e = 21.03C_e/(1 + 1.66C_e) \quad R^2 = 0.974 \qquad (2)$$

As the CTA/10% PEG/DOX system fits well to the Langmuir isotherm, it corresponds to a homogeneous monolayer covering of the adsorbent surface, without lateral interaction, i.e. the drug is dispersed only on the membrane surface. This dispersion is molecular and homogeneous, what hampers DOX crystallization.[27] This is confirmed by DSC scan of CTA/10% PEG/DOX, shown in Figure 4, which does not present an endotherm of fusion around 210°C, DOX melting temperature.

Using the Langmuir Isotherm model, the monolayer covering (Q_{max}) for CTA/10% PEG DOX membrane is estimated to be 12.2 mg of DOX adsorbed per gram of CTA/10% PEG.

In membranes prepared by either processing methods; Method (i) incorporation during the membrane preparation, and Method (ii), adsorption on the previously prepared membrane, DOX was homogeneously dispersed in the polymeric matrix. The differences in processing lead to the differences in the incorporation efficiency and drug distribution throughout the matrix. In Method (i), DOX incorporation occurs throughout the matrix with an incorporation efficiency of 90%, while in Method (ii), the incorporation is superficial and the incorporation efficiency is small, around 1%. Regarding the application of these systems, it is important to emphasize that because of the incorporation characteristics and to the high drug content in the matrix, prepared by Method (i), the administration route for the system CTA/DOX can be enteric, where the action effect is systemic. Cellulose esters have good resistance to low pH (stomach condition) and are sensitive to high pHs. High pHs increase the solubility and degradability of the membrane in aqueous medium, which may cause an increase on the drug release in the intestine.[16] The CTA/DOX system with superficially incorporated drug, as produced by Method (ii) may have topic (local) use, which demands a lower amount of drug and make superficially adsorbed drug available in the place where it is requested.

CONCLUSION

CTA produced from sugarcane bagasse is an adequate matrix for DOX incorporation. The process of incorporation was carried out using two different procedures: (i) incorporation of the drug during the membrane preparation process and (ii) incorporation of the drug to a previously prepared membrane. In the first, the produced membrane presented high compatibility between DOX and CTA as confirmed by DSC because drastic modifications were observed in the scan patterns of the CTA/PEG 50%/DOX in relation to CTA/PEG 50% curve, indicating plasticizing phenomena with the drug homogeneously distributed throughout the matrix. In the second method, the drug was molecularly and superficially adsorbed, as seen through DSC for the system CTA/PEG 10%/DOX, which nearly does not present alterations in relation to the original material, and through the isotherm of drug adsorption that follows the Langmuir model.

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