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In vitro release and characterization of chitosan films as dexamethasone carrier

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ABSTRACT

Chitosan, a biodegradable and biocompatible polysaccharide, is a potentially useful material in various fields. We produced mono and bilayer chitosan films containing dexamethasone as a drug carrier for controlled release. The chitosan drug-loaded films were produced by a casting/solvent evaporation technique using 2 wt% acetic acid solution and distilled water and they were dried at room temperature. These films were characterized by release and swelling studies, DSC and ATR-FTIR. The total profile for water absorption was similar for the types of films developed. ATR-FTIR analysis showed little change in the band position of the O—H and N—H stretching from dexamethasone and chitosan, respectively. DSC analysis from bilayer film indicates that the dexamethasone peak was shifted from 256 to 240 °C. These results suggested an interaction between hydroxyl and amino groups of chitosan and hydroxyl groups of dexamethasone. In the drug release studies it was observed 89.6% release from the monolayer film in 8 h and 84% from the bilayer film in 4 weeks. These results suggested that the chitosan sheet prepared in this study is a promising delivery carrier for dexamethasone.

1. Introduction

Biodegradable polymers have been explored as biomaterial in the field of drug delivery system. They can be used to form solid or injectable implants or they can be used to encapsulate particular system as nano and microparticles (Borges et al., 2006). The search for ideal biomaterials is still on-going for ocular drug delivery where the properties of the device are dictated, for example, by biocompatibility and biodegradability, physicochemical properties, non-toxicity and others. Chitosan, a biodegradable polysaccharide, comprising glucosamine and *N*-acetylglucosamine residues, is an alkaline-deacetylated chitin derived from the exoskeletons of insects and shells of crustaceans (Felt et al., 1999; Ludwig, 2005). Chitosan has been used in a wide variety of biomedical applications like sustained-release of drugs (Feng, 2004; Ugwoke et al., 2005; Yu et al., 2008) and ocular disorders (Felt et al., 1999; Yuan et al., 2008). On the other side, films, erodible and non-erodible inserts, rods and shields are the most logical delivery systems aimed at remaining for a long period of time in contact with eye (Ludwig, 2005). These delivery systems sustain and control drug release and thus avoid pulsed entry characterized by a transient overdose, followed by a relative short period of acceptable dosing, which is in turn followed by a prolonged period of under dosing (Ludwig, 2005). Therefore, if a chitosan carrier containing drugs can be applied to the focuses of a disease, drugs can be released gradually and improve therapeutic efficiency (Felt et al., 1999; Lang, 1995; Sarasam and Madihally, 2005). In order to exert notable drug activity in ocular topical diseases without any side effects induced by biomedical materials, the quality and form of the material is very important (Calvo et al., 1997). Dexamethasone is a corticosteroid and its most common use in eye drops is for inflammation following eye surgery, such as after cataract surgery and corneal operations (Wilson, 2004) and others applications.

In the present study, we have used a simple technique to make chitosan films with a flexible flat shape, with one and two layers and furthermore, we have investigated the release of dexamethasone from the film in order to explore the feasibility of chitosan as a drug carrier.

2. Materials and methods

Chitosan with medium molecular weight was supplied by Polymar (Brazil) with a deacetylation degree of 85% according to the supplier. Dexamethasone (Dx) was purchased from Henrifarma and

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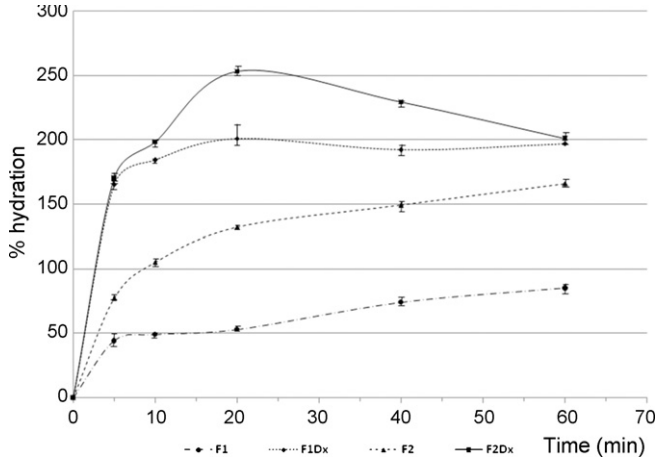

Fig. 1. Hydration of films in medium buffered solutions (PBS; pH 7.5): (F1) blank matrix monolayer chitosan film, (F1Dx) drug-loaded monolayer film, (F2) blank matrix bilayer chitosan film, (F2Dx) drug-loaded bilayer film.

was used as model drug. Others reagents were all analytical grade and were used without further purification.

2.1. Chitosan films preparation

Initially, films were prepared using only chitosan (blank chitosan films). For their preparation the casting/solvent evaporation technique was employed. Solution of chitosan, 2 wt%, was prepared with 2 wt% acetic acid solution and distilled water. This solution was prepared with a magnetic stirrer and was dried in glass Petri dish at room temperature for 1–3 days until monolayer film formation (F1). Then a new solution of chitosan, as previously described, was added on the preformed film and dried, as described, until bilayer film formation (F2). The chitosan films were accurately observed and checked for possible imperfections by visual monitoring.

Later, chitosan-dexamethasone loaded films were produced using steps as described previously. The solution of chitosan was mixed with dexamethasone (1.5 wt%) using a syringe as homogenizer and was dried in glass Petri dish at room temperature for

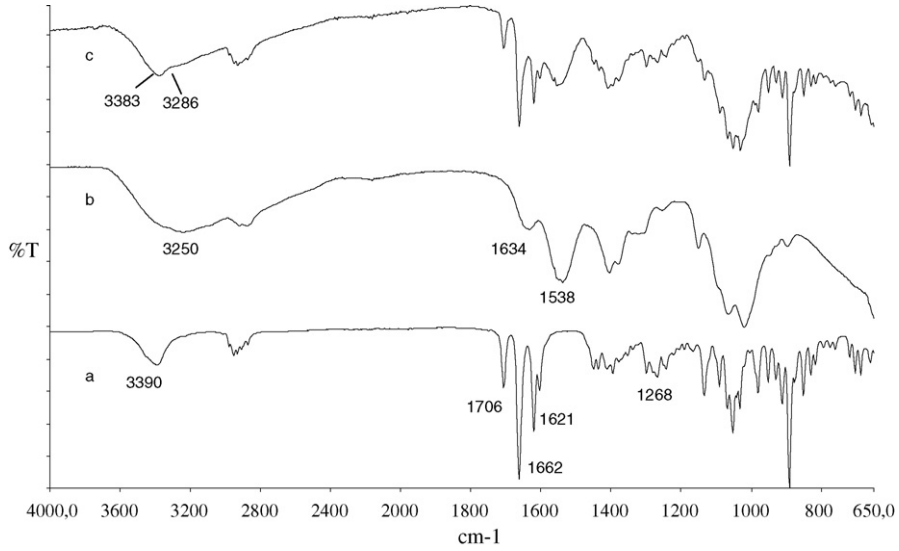

Fig. 2. ATR-FTIR spectra of dexamethasone - Dx (a); blank matrix monolayer chitosan film, F1 (b) and drug-loaded monolayer film, F1Dx (c).

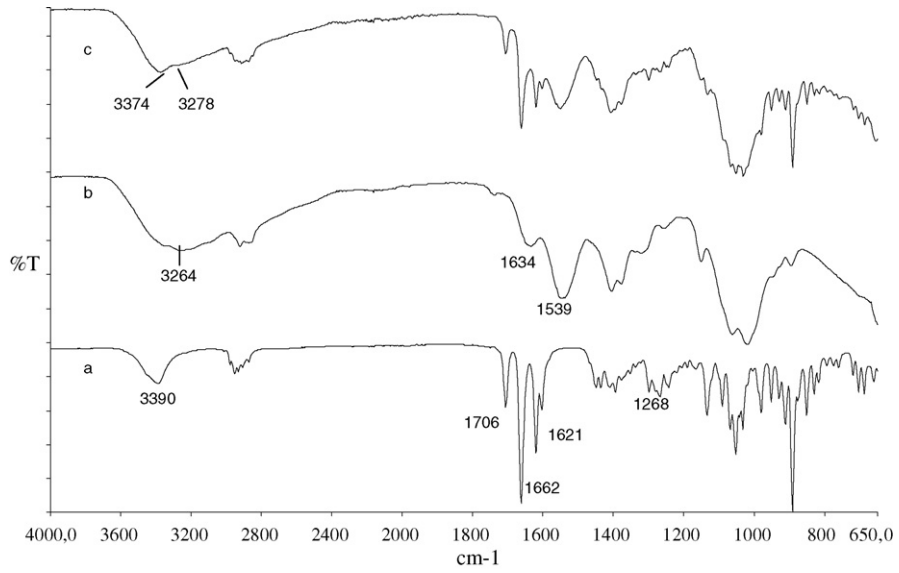

Fig. 3. ATR-FTIR spectra of dexamethasone - Dx (a); blank matrix bilayer chitosan film, F2 (b) and drug-loaded bilayer film, F2Dx (c).

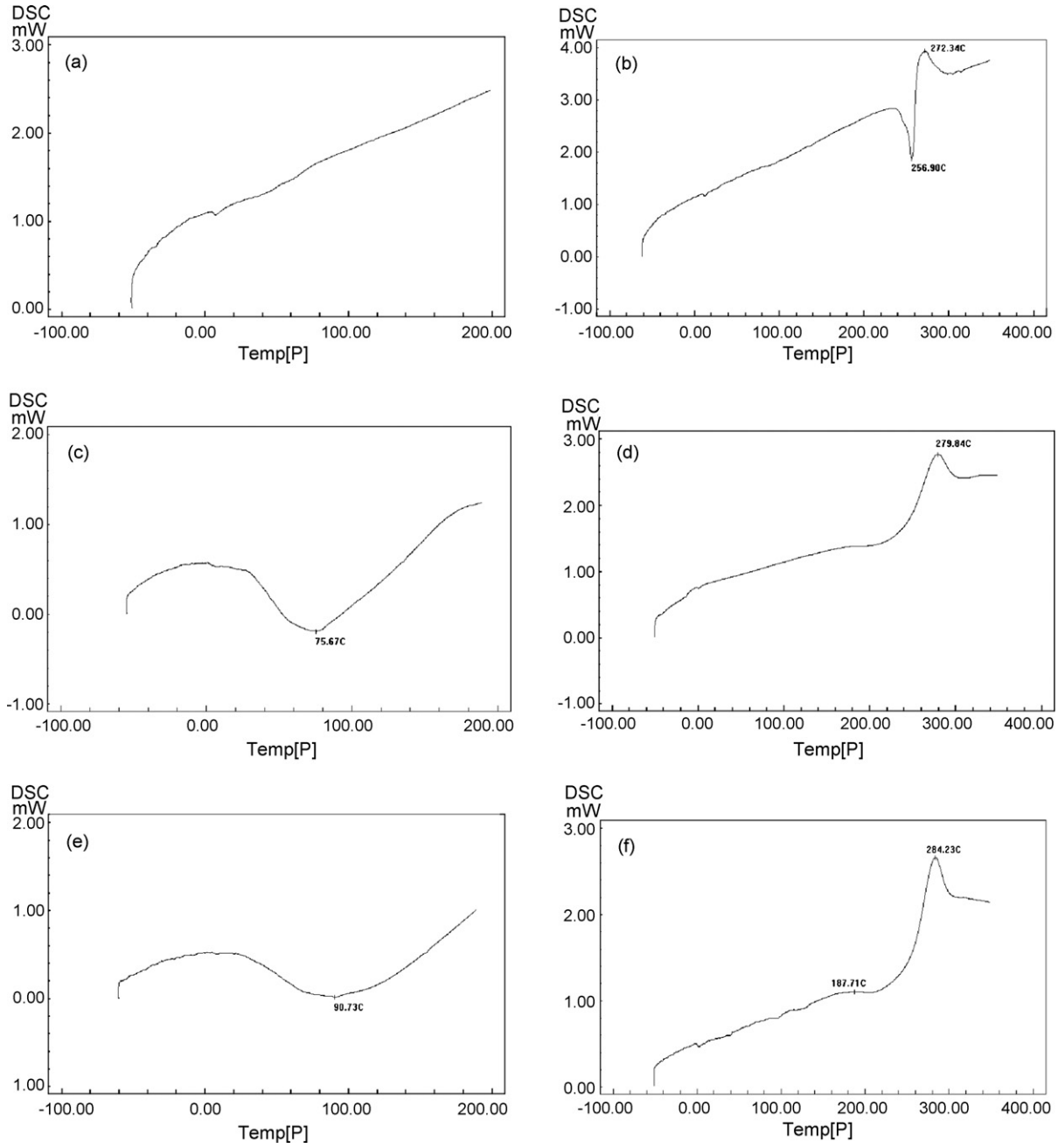

Fig. 4. DSC thermoanalysis of pure dexamethasone, Dx (a and b); blank matrix monolayer chitosan film, F1 (c and d) and drug-loaded monolayer film, F1Dx (e and f).

1–3 days until monolayer film formation (F1Dx). Analogue procedure was made to get bilayer film formation with dexamethasone (F2Dx).

2.2. Swelling studies

Films swelling properties were evaluated by determining the percentage of hydration. The hydration capacities of the chitosan film formulations were determined by weighing the film pieces before and after placing in pH 7.4 phosphate buffer solution. Each film was divided in portions of 1 cm^2 (1 cm × 1 cm) and cut, weighed and placed in buffer solution for predetermined periods of time (5, 10, 20, 40, 60 and 90 min) as described by Öner et al. (2007). After immersion, the films were removed from the medium and weighed after removal of the excess surface water using filter paper. The percentages of hydration were calculated by using Eq. (1).

$$\%\,\text{hydration} = \left[\frac{X_t - X_0}{X_0}\right] \times 100 \quad (1)$$

where X_t is the weight of the swollen film after time t and X_0 is the original film weight at zero time.

This experiment was performed in triplicate.

2.3. Determination of dexamethasone

UV spectroscopy was the methodology chosen to quantify the drug contained in the polymeric system. A Shimadzu Ultraviolet spectrometer was used at a wavelength of 254 nm. The method was validated with a known amount of Dx diluted in PBS (phosphate

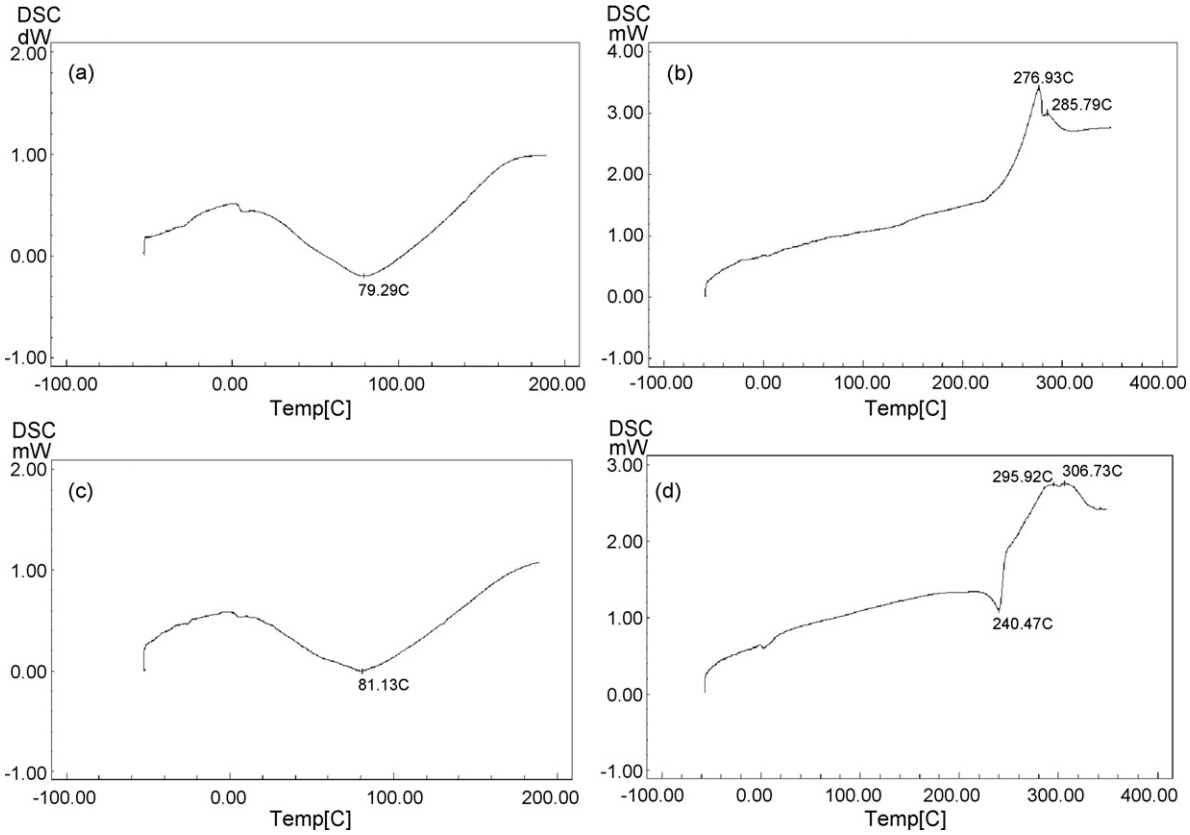

Fig. 5. DSC thermoanalysis of blank matrix bilayer chitosan film, F2 (a and b) and drug-loaded bilayer film, F2Dx (c and d).

buffer solution, pH 7.5) in the concentration range of 2.5–50 μg/mL ($y = 0.028x + 0.004$, $R^2 = 0.997$; $n = 5$).

2.4. In vitro drug release

The drug-loaded films with constant exposed area were soaked in a suitable volume of dissolution medium to maintain "sink conditions" in glass vessels, as specified below, in a horizontal laboratory shaker with five replicates. These glass vessels were incubated at 37 ± 0.5 °C shaking the glass at 50 rpm. At appropriate time intervals all the solution was withdrawn from the glass vessels and the amount of dexamethasone released from the drug-loaded films were evaluated by UV spectrophotometry at 254 nm. Then an equal volume of the same dissolution medium was added back to maintain a constant volume. The medium for the controlled release studies was typical pH 7.5 solutions (10 mM NaH_2PO_4–Na_2HPO_4-buffered solution).

2.5. ATR-FTIR analysis

Attenuated total reflectance Fourier transformed infrared spectroscopy (ATR-FTIR) spectra of blanks matrix chitosan films (F1 and F2), dexamethasone (Dx), monolayer drug-loaded film (F1Dx) and bilayer (F2Dx) drug-loaded film were recorded within films on a PerkinElmer FTIR spectrometer, Model Spectrum One (USA).

2.6. Differential scanning calorimetry (DSC) analysis

Thermal properties of blanks matrix chitosan films (F1 and F2), dexamethasone (Dx), monolayer drug-loaded film (F1Dx) and bilayer (F2Dx) drug-loaded film were characterized using a DSC50 from Shimadzu. Nitrogen at the rate of 20 mL/min was used as purge gas. The specimens were packed in aluminum pans, and heated from −50 to 200 °C (RUN 1) at heating rate of 10 °C/min under a nitrogen atmosphere. The specimens were cooled to −50 °C, under nitrogen atmosphere at the same rate of 10 °C/min, from where they were reheated to 350 °C at a heating rate of 10 °C/min (RUN 2).

3. Results and discussion

3.1. Swelling studies

All the films have hydrated very quickly, reaching 70% hydration after a few minutes. Proportionally maximum hydration was obtained with formulations containing two chitosan layers. The effects of dexamethasone on the swelling behavior of chitosan films are presented in Fig. 1. The water-insoluble drug addition increased the water uptake. This fact is due to possible existence of micronized particles between the polymer chains allowing each chain to hydrate freely. These areas may increase the strength of the swollen layer followed by an obvious increase in the amount of penetrated water (Panamsuk et al., 1996). Miconazole, a water-insoluble drug, was found to increase the swelling behavior of chitosan matrices (Nafee et al., 2003). The addition of dexamethasone increased surface wettability and, consequently, water penetration within the matrix. The monolayer chitosan film formulation with dexamethasone absorbed water up to 200% of its initial weight. This is approximately two times higher than monolayer blank. On the other hand, formulations with chitosan bilayer films absorbed water up 253% of its initial weight. The total profile for water absorption was similar for the types of film obtained in this study. During the tests the integrity of the films was not lost. Therefore the separation of the layers was not evidenced.

3.2. ATR-FTIR analysis

Fig. 2 shows the ATR-FTIR spectra of dexamethasone (Dx), monolayer chitosan and drug-loaded films (F1 and F1Dx, respectively). In pure dexamethasone spectra, the characteristic absorption bands at 3390 and 1268 cm^{-1} were due to the stretching vibration of O—H and C—F bonds, respectively; the stretching vibration at 1706, 1662 and 1621 cm^{-1} were due to —C=O and double bond framework conjugated to —C=O bonds. In pure chitosan, two characteristic absorption bands at 1634 and 1538 cm^{-1} were detected and attributed to amide I (C=O) and N—H (amine) vibration overlapping the amide II vibration, respectively. Finally, the overlapped wide absorption band around 3250 cm^{-1} was due to the stretching vibration of O—H bonded to N—H in chitosan film (Lawrie et al., 2007).

From the FT-IR spectra of F1Dx film, it can be seen that the characteristics absorption bands at 3250 cm^{-1} of chitosan and 3390 cm^{-1} of dexamethasone shifted to wave number at 3286 and 3383 cm^{-1}, respectively, suggesting an increase in the hydrogen bonding. In this case, those changes give an evidence for the intermolecular interaction between the lipophylic drug and the polymeric matrix. No significant alteration was observed on the other regions the F1Dx film spectra.

Fig. 3 shows the ATR-FTIR spectra of dexamethasone (Dx), bilayer chitosan and drug-loaded film (F2 and F2Dx, respectively). In pure dexamethasone spectra the characteristic absorption bands can be observed like previously described. In bilayer chitosan film (F2) spectra can be observed that the characteristics absorption bands at 3250 cm^{-1} of blank monolayer chitosan film were shifted to wave number at 3264 cm^{-1} in the bilayer blank. These results suggest that larger water absorption leads the chains of polymer to interact more with the water molecule than with the intramolecular groups in the polymeric matrix. No significant alteration was observed on the other regions the F2 film spectra.

F2Dx spectra showed that characteristic peaks at 3264 and 3390 cm^{-1} assigned to O—H and N—H stretching from chitosan and OH stretching of dexamethasone, respectively, were broadened and shifted to 3278 and 3374 cm^{-1}, respectively. This implies, similarly to F1Dx sample, an increase in the hydrogen bonds between drug-polymeric chain. These results can be explained by the increase in the number of tight water-binding sites with the incorporation of drug (Dx) in the film because it could inhibit the formation of intermolecular hydrogen bonding in chitosan film. No significant alteration was observed on the other regions the F2Dx film spectra.

These observations in the ATR-FTIR spectra suggest that the intermolecular hydrogen bonding in chitosan chains was inhibited to a certain extent, since the dexamethasone, an apolar drug, was introduced, resulting in extensive water-polymer interactions and higher water-absorption ability. Carboxymethyl hexanoyl chitosan, a hexanoyl derivate from chitosan, was found to increase the swelling when the hexanoyl group (apolar) was introduced in chitosan polymer (Liu et al., 2006).

At the same time, the analysis in ATR-FTIR showed that there were no new characteristics absorption bands on either drug-loaded films leading to the conclusion that there was no obvious chemical reaction between the drug and the matrix (Figs. 2 and 3). As an important result, dexamethasone probably did not loose its activity in the drug-loaded films.

3.3. Differential scanning calorimeter (DSC) analysis

DSC is a possible method to determine the modification of a drug. Fig. 4 shows DSC thermograms of pure dexamethasone, mono and bilayer chitosan films and mono and bilayer drug-chitosan-loaded films. DSC second scan curve of Dx crystals (Fig. 4b) exhibits an endothermic peak at 256.9 °C due to crystallization peak (Gómez-Gaete et al., 2007). Blank chitosan monolayer films showed a broad endothermic peak at about 75.67 °C (Fig. 4c) and broad exothermic peak in 279.84 °C (Fig. 4d) on the first and second scan curves, respectively. In the case of chitosan film the endothermic peak is extremely irregular. These results could be attributed to evaporation of residual water and degradation of the main chain (Mucha and Pawlak, 2005; Neto et al., 2005), respectively.

No significant difference could be observed in first scan to the DSC curve of a dexamethasone-chitosan film (Fig. 4e). One chitosan peak was shifted from 279.84 to 284.23 °C in the second scan curve (Fig. 4f). This observation indicates a weak interaction between the amino group of chitosan and the hydroxyl group of the drug. In this case, micronized drug particles may exist between the polymer chains. These results suggest that the film swelling is larger due to surface wettability and, consequently, there is higher water penetration within the matrix. The water molecules in such a system are an active compatibilizer which works as a "glue" acting by formation of additional hydrogen bond (Mucha and Pawlak, 2005). Therefore, the chitosan, in the drug-loaded film, degrades in higher temperature than in the blank chitosan film. This observation is in accordance with ATR-FTIR analysis and swelling studies.

Blank chitosan bilayer films showed a broad endothermic peak at about 79.29 °C (Fig. 5a) and two broad exothermic peaks in 276.93 and 285.79 °C (Fig. 5b) on the first and second scan curves, respec-

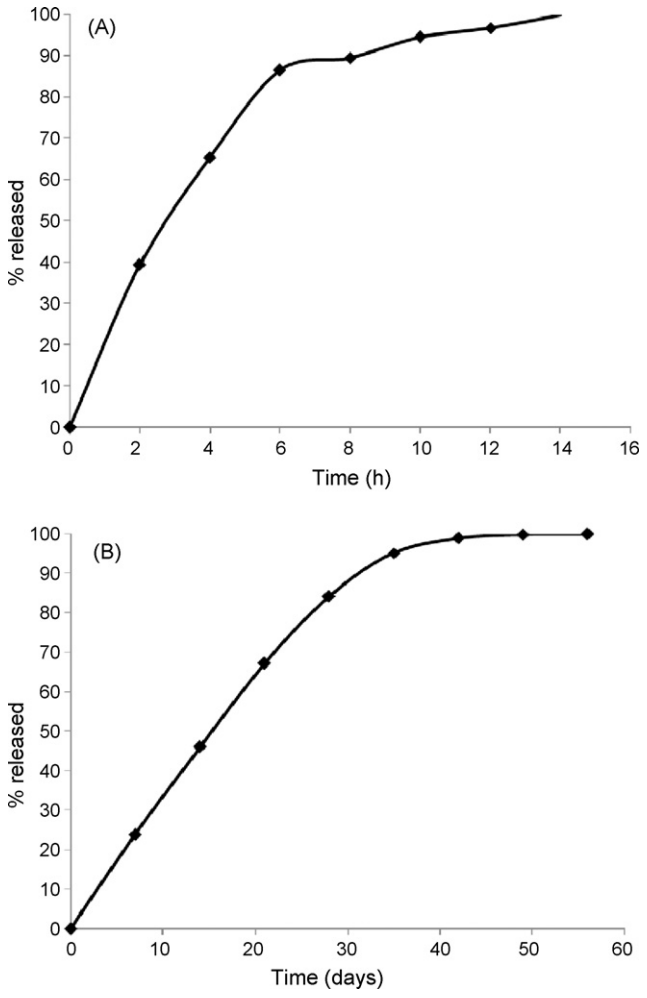

Fig. 6. *In vitro* release profiles of Dx from film formulations: (A) drug-loaded monolayer film, F1Dx; (B) drug-loaded bilayer film, F2Dx.

tively. These results could be attributed to evaporation of residual water and degradation of the main chain in bilayer film, respectively.

No significant difference could be observed in first scan of the DSC curve of a dexamethasone-chitosan film (Fig. 5c). In contrast to the DSC curves for the monolayer, the DSC curves of the bilayer films showed that the two exothermic peaks of chitosan were shifted from 276.93 and 285.79 °C to 295.42 and 306.73 °C (Fig. 5d). Furthermore, is possible to observe that one endothermic peak of dexamethasone was shifted from 256.90 to 240.47 °C. The observations in DSC analysis are similar to ATR-FTIR and swelling studies. These analyses suggest that there is more interaction in bilayer film between the drug and the polymer than in monolayer film. Micronized drug particles, in both cases (mono and bilayer drug-loaded films), may exist between the polymer chains and increase the water penetration within the matrix. Consequently, the chitosan, in bilayer film, degrades in higher temperature than monolayer drug-loaded films.

3.4. In vitro release studies

Chitosan films have produced sustained-release in all formulations. The maximum release rate was observed in the system containing one layer of chitosan film where 39.3% Dx was released in the first 2 h and progressively to 89.6% after 8 h (Fig. 6). The bilayer chitosan film release was much more slower than monolayer film release (23% Dx was released in the first week and slowly progressed to 84% after 4 weeks).

4. Conclusions

Mono and bilayer dexamethasone-chitosan films were successfully obtained and release tests suggest that these films are potential sustained-release carrier for dexamethasone. Their release time is longer than conventional ocular topical delivery dosage forms. Incorporation of a second layer of chitosan film modifies significantly the drug release profile. Therefore, the monolayer Dx-chitosan film might be a promising ocular delivery carrier for dexamethasone in few hours and bilayer Dx-chitosan film in weeks.

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