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Research paper

Characterization of a polyurethane-based controlled release system for local delivery of chlorhexidine diacetate

Truc Thanh Ngoc Huynh [a,b], Karine Padois [a,*], Fabio Sonvico [b], Alessandra Rossi [b], Franca Zani [b], Fabrice Pirot [a], Jacques Doury [a], Françoise Falson [a]

[a] Laboratoire de Recherche et Développement de Pharmacie Galénique Industrielle, Université de Lyon, Lyon 08, France
[b] Dipartimento Farmaceutico, Università degli Studi di Parma, Parma, Italy

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ABSTRACT

Conventional formulations of chlorhexidine usually provide short-term efficiency, requiring repeated applications to maintain antibacterial activity. Therefore, appropriate release system of chlorhexidine controlling local drug delivery would reduce the number of applications and enhance patient compliance.

The aim of this study was to develop a controlled release system based on medical polyurethane for the local delivery of chlorhexidine diacetate (CDA). CDA-loaded polyurethane films (CDA-Films) and CDA-loaded polyurethane sandwiches (CDA-Sandwiches) were obtained by casting and solvent evaporation.

The physico-chemical aspects of CDA-loaded polyurethane systems were investigated, and the crystalline state of CDA in the polymeric system was highlighted. CDA-Films exhibited appropriate mechanical properties for further applications. Drug release was measured in two different media: (i) distilled water and (ii) physiological saline solution to mimic in vivo conditions. Drug release studies were performed up to 11 days on CDA-Films and 29 days for CDA-Sandwiches. Release of CDA depended on drug loading and the structure of the system. In particular, release of CDA from the sandwich system followed zero-order kinetic. The release rate was significantly lower in physiological solution. Antibacterial studies were carried out on CDA-Films against *Staphylococcus aureus* and *Staphylococcus epidermidis* showing 35 days persisting antibacterial activity.

In conclusion, the polyurethane-based system developed in this study is potentially useful as a local delivery system for CDA and could be used not only in surgery but also in dental and clinical applications.

1. Introduction

Chlorhexidine, a bis-biguanide antiseptic, is widely used to treat and prevent skin and mucosal infections with a low topical toxicity. In addition, it has been demonstrated that chlorhexidine has a strong substantivity within the oral cavity, making it efficient for antiplaque activity [1,2]. Since last two decades, chlorhexidine is recognized in dentistry as gold standard against antiplaque and gingivitis [2]. In this field, chlorhexidine is used either as rinse solution between 0.2% and 0.5% (w/v). Furthermore, several formulations containing chlorhexidine are available for topical delivery (e.g. scrub and rubs) and urinary or central venous catheter impregnation [1].

However, due to uncontrolled release, chlorhexidine activity might not be sustained in conventional formulations. Consequently, repeated applications needed to maintain its antibacterial efficacy might result in patient discomfort due to bitter taste, teeth discoloration and occasional mucosal irritations [3]. A controlled release system for antiseptic will prolong antimicrobial activity locally in order to reduce the number of applications or lower dosage form and enhance patient compliance.

Taking into account these potential adverse effects, sustained released delivery system based on ethylcellulose [4,5], cross-linked protein matrix [6], acrylic strip [7] and tooth-bonded chlorhexidine delivery based on poly (ε-caprolactone) [8] were proposed. More recently, innovative systems based on chlorhexidine-loaded chitosan microspheres [9], inclusion of chlorhexidine in cyclodextrin [10,11], poly (ε-caprolactone) nanocapsules (Nanochlorex®) [12,13], ethylene vinyl acetate copolymer containing chlorhexidine [14–16], methacrylate systems [17–19], and thermosensitive vinyl ether-based hydrogel were reported [20].

Polyurethanes are thermosetting polymer products of a step-reaction polymerization process. These synthetic polymers have been found in many applications as biomaterials due to their excellent physical properties and good biocompatibility. It is clinically used in central venous catheters, vascular grafts, mammary

* Corresponding author. Laboratoire de Recherche et Développement de Pharmacie Galénique Industrielle, Université de Lyon, "Fonctions normales et pathologiques de la barrière cutanée", EA 4169, ISPB – 8, Avenue Rockefeller, 69373 Lyon 08, France. Tel.: +33 04 78 77 71 13; fax: +33 04 78 77 72 47.
E-mail address: karine.poret-padois@univ-lyon1.fr (K. Padois).

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prostheses, implants and drug delivery systems [21]. Over the past few years, the development of antimicrobial-loaded polyurethane catheters has been reported as an approach for the prevention of nosocomial infections [22,23].

In the present study, polyurethane-based controlled release systems were developed in order to provide prolonged and controlled release of chlorhexidine diacetate for antiseptic activity. Medical polyurethane was evaluated as a drug carrier material to modulate the release profile. The systems prepared by solvent evaporation were characterized for (i) solid state of chlorhexidine and (ii) drug–polymer interaction by Differential Scanning Calorimetry, Hot-stage Microscopy and Attenuated Total Reflection Fourier Transform Infrared Spectroscopy. Complementary analysis of morphology and mechanical properties was conducted by Scanning Electron Microscopy and tensile test. Finally, antibacterial efficiency of chlorhexidine-loaded polyurethane system was studied against two Staphylococci strains and discussed from drug release profile.

2. Materials and methods

2.1. Materials

Chlorhexidine diacetate dihydrate (CDA), sodium chloride and sodium acetate were purchased from Sigma–Aldrich (Lyon, France). Tetrahydrofuran, glacial acetic acid and acetonitrile were supplied from Carlo Erba (Val de Reuil, France) at HPLC grade. Medical grade polyurethane (PU) (3M Unitek, Cergy Pontoise, France) was used as a polymer to incorporate CDA. Polycarbonate filters (0.45 μm) were purchased from Fisher Bioblock (Illkirch, France).

2.2. Preparation of chlorhexidine diacetate-loaded polyurethane systems

Samples were prepared by casting and solvent evaporation. Medical grade PU dissolved in tetrahydrofuran [7.5% (w/v)] was added to CDA powder under vortex agitation for one minute. Two types of PU systems were prepared. Firstly, unloaded PU-Film and 5%, 10%, 20%, 40% CDA-Films (w/w) were obtained by casting 2 mL of CDA–PU mixture in tetrahydrofuran into a flat bottom crystallizing dish (∅: 60 mm; 10-mm height). The solvent was allowed to evaporate at about 25 °C for 30 min. Secondly, 20% CDA-Film sandwiched between two unloaded PU layers (named 20% CDA-Sandwiches) was obtained by successively pouring and drying the 3 layers. The first unloaded PU layer was obtained by casting 2 mL of pure PU solution in a flat bottom crystallizing dish (∅: 60 mm; 10-mm height). The solvent was allowed to evaporate at about 25 °C for 30 min. The second layer of CDA-loaded PU was cast onto the PU layer as prepared for 20% CDA-Films described earlier. The third layer was obtained by repeating the casting as for the first PU unloaded layer.

For both systems, after solvent evaporation, crystallizing dishes were left in a hood overnight to complete drying. CDA-Films and sandwiches were checked until the weight remained constant.

2.3. Physico–chemical characterization of chlorhexidine diacetate-loaded polyurethane systems

2.3.1. Macroscopic observation

CDA-Films were thin, flexible and easy to handle. CDA-Sandwiches were thicker but still flexible. The thickness of each sample was measured in five positions by a digital thickness gauge (Mitutoyo, Tokyo, Japan).

2.3.2. Physico-chemical characterization

CDA, PU-Film, 5%, 10%, 20% and 40% CDA-Films were characterized.

2.3.2.1. Differential Scanning Calorimetry. Thermal properties of CDA-Films were characterized by Differential Scanning Calorimetry (DSC). Measurements were carried out on a Mettler Toledo DSC 812e module controlled by STARe software (Mettler-Toledo, Zürich, Switzerland). An aluminum pan was filled with 7.5 ± 0.5 mg of CDA-Films accurately weighed by a microbalance MT 5 (Mettler-Toledo, Zürich, Switzerland) and subsequently hermetically sealed. DSC analysis was performed from 25 °C to 180 °C with a heating rate of 5 °C/min, in inert atmosphere (N_2, flow 100 mL/min). The integral enthalpy of fusion (ΔH) and the normalized enthalpy of fusion (ΔH_f normalized, J/g) were calculated from peak area by STARe software. The normalized enthalpy of fusion was calculated in function of CDA amount in the sample.

2.3.2.2. Hot-Stage Microscopy. Hot-Stage Microscopy (HSM) experiments were carried out using a polarized light microscope OPTIPHOT 2-POL Nikon (Nikon, Tokyo, Japan) equipped with a Linkam HSF 91 hot-stage and a Linkam TP 93 heating system (Linkam Scientific Instruments Ltd, Tadworth, UK). Samples were subjected to a heating program from 25 °C to 160 °C at a rate of 10 °C/min in air flow. Photographs were taken at different temperatures by using a color camera Nikon Coolpix 4500 (magnification 200×).

2.3.2.3. Attenuated Total Reflection Fourier Transformed Infrared Spectroscopy. Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR) spectra of CDA, PU-Film and CDA-Films were recorded on a Thermo Scientific Nicolet iS10™ FT-IR spectrometer equipped with a Smart iTR™ ATR sampling accessory (Thermo Fisher Scientific, Illkirch, France). Samples were scanned from 4000 to 600 cm^{-1} at a resolution of 4 cm^{-1}.

2.3.2.4. Scanning Electron Microscopy. Scanning Electron Microscopy (SEM) was used to examine the surface morphology of PU and CDA-Films at 15 kV (Jeol 6400 apparatus, Jeol, Tokyo, Japan). Samples were fixed on a holder and coated with gold (10-nm thick) by metallization under vacuum.

2.3.3. Mechanical properties

The mechanical properties of films were determined from tensile test measurement, using a universal one-column electronic dynamometer (Acquati, Milan, Italy) at 25 ± 1 °C and relative humidity of 55%. Rectangular samples (45-mm length; 10-mm width) of 5%, 10%, 20% and 40% CDA-Films were tested. Samples were clamped between grips set at initial distance of 30 mm. Stress and strain were recorded during vertical extension at 50 mm/min. Tensile strength (MPa), elasticity modulus (MPa) and elongation at break (%) were determined from three replicates for each film.

2.3.4. Drug release study

Drug release was measured in two different media: (i) distilled water and (ii) 0.9% (w/v) NaCl aqueous solution adjusted to pH 7. All experiments were performed in a shaking bath thermostated at 37 °C by immersing disks (∅: 10 mm) of 5%, 10%, 20%, 40% CDA-Films and of 20% CDA-Sandwich in sealed glass vial containing 10 mL of the media. The experiments were done in triplicate. At fixed intervals, 1 mL of sample was withdrawn and replaced by pre-warmed medium. Samples were filtered through a polycarbonate membrane (0.45 μm) and analyzed by previously developed High Performance Liquid Chromatography (HPLC) [12]. Briefly, analysis was performed on Agilent 1200 series using the

following conditions: column LiChrospher 100 RP-18, 4 × 125 mm, 5 μm (Agilent, Massy, France); mobile phase: acetonitrile and 30 mM sodium acetate aqueous solution, 50:50 (v/v), adjusted to pH 3.3 by glacial acetic acid; flux 1.5 mL/min; injection volume 40 μL; UV detector at λ = 260 nm.

2.3.5. In vitro antibacterial activity

Antibacterial activity was evaluated *in vitro* by a serial plate transfer test against *Staphylococcus aureus* ATCC 6538 and *Staphylococcus epidermidis* ATCC 12228.

Mueller–Hinton milieu was used throughout the test. Molten Mueller–Hinton agar with a volume of 15 mL was poured into sterile disposable Petri dishes (∅: 100 mm; 15-mm height). Cultures were prepared in 10 mL of sterile Mueller–Hinton broth and were incubated for 12 h. Cultures were vortexed and a 1/10 dilution in 0.9% (w/v) NaCl aqueous solution was prepared. 0.5 mL of this 1/10 dilution was spread on the surface of Petri plates. The colony-forming unit (CFU/mL) of each dilution was determined after a serial dilution and count for colonies on Mueller–Hinton agar. The disks (∅: 8 mm) of PU-Film (control) and of 5%, 10%, 20% and 40% CDA-Films were placed in these Petri plates. The experiments were performed in triplicate. After incubation at 37 °C for 24 h, the inhibition zone was measured by a digital Digimatic caliper (Mitutoyo, Tokyo, Japan) from one edge of the zone of inhibition to the opposite edge, including the diameter of the disk. After that measure, all disks were transferred to new seeded Petri plates. These manipulations were repeated until 35 days.

In parallel, the minimum concentrations of inhibition (MIC) of CDA against these bacteria were determined by broth macrodilution method [24]. Briefly, tubes containing either *S. aureus* or *S. epidermidis* in Mueller–Hinton broth at approximately 10^7 CFU/mL were prepared. The bacterial concentration (CFU/mL) was determined after a serial dilution and count for colonies on Mueller–Hinton agar. CDA at 0.125–40 μg/mL in distilled water was added into these tubes. The positive control (Mueller–Hinton broth containing *S. aureus* or *S. epidermidis* without CDA) and negative control (sterile Mueller–Hinton broth) were prepared. After incubation at 37 °C for 24 h, the lowest concentration of CDA inhibiting the growth of bacteria (MIC) was determined by the lack of turbidity, matching with the negative control.

2.4. Statistics

Results are expressed as the mean ± standard deviation of three experiments. Normalized enthalpy of fusion, film thickness, tensile strength, elongation at break and elasticity modulus were evaluated by one-way ANOVA test. Kinetic parameters and diameter of bacterial inhibition were analyzed by Student's *t*-test. Significance was tested at the 0.05 and 0.001 levels of probability.

3. Results

3.1. Macroscopic observations

The thicknesses of PU-Film and of 5%, 10%, 20%, 40% CDA-Films and 20% CDA-Sandwich are reported in Table 1. Films thickness increased from 0.050 ± 0.001 mm to 0.133 ± 0.006 mm and in function of CDA concentration. 20% CDA-Sandwich was thicker than CDA-Film but still flexible.

3.2. Physico-chemical characterization

3.2.1. Differential Scanning Calorimetry

DSC analysis was performed on CDA, PU-Film, 5%, 10%, 20% and 40% CDA-Films (Fig. 1). In DSC trace of CDA, two thermal phenomena can be noted. Firstly, a shallow broad endothermic effect in the 50 °C to 100 °C range corresponded to water. Secondly, a sharp melting endothermic peak at 154.57 ± 0.15 °C corresponded to chlorhexidine diacetate, indicating its crystalline structure. The same thermal phenomena were observed in DSC traces of 5%, 10%, 20% and 40% CDA-Films.

Fusion temperature (T_f, °C) and normalized enthalpy of fusion of CDA (ΔH_f normalized, J/g) are regrouped in Table 2. There was a reduction in fusion temperature of pure CDA and CDA in 5%, 10%, 20% and 40% CDA-Films (one-way ANOVA test, $p < 0.001$), but no significant difference was observed between normalized enthalpy of fusion of pure CDA and CDA in 5%, 10%, 20% and 40% CDA-Films, respectively (one-way ANOVA test, $p > 0.05$).

3.2.2. Hot-Stage Microscopy

HSM photographs of CDA in the temperature range of 25–160 °C (Figs. 2A–C) collected at magnification 200×, showed polyhedral CDA sparkling under polarized microscopy at 25 °C (Fig. 2A). Pure CDA fusion was observed between 154 °C and 160 °C as previously demonstrated by DSC. All the investigated CDA-Films showed the same phenomena. The photographs of 10% CDA-Films are presented as an example. Fig. 2D at 25 °C showed the crystalline state of CDA in 10% CDA-Films similarly to CDA powder. The melting range of CDA in CDA-Films was observed between 151 °C and 160 °C (Figs. 2E–F). HSM photographs confirmed DSC analysis and, thus the crystalline state of CDA in CDA-Films in physiological conditions.

3.2.3. Attenuated Total Reflectance Fourier Transformed Infrared Spectroscopy

Interactions between the CDA and the PU were investigated with ATR-FTIR, and all spectra viewed from 3950 to 700 cm^{-1} were regrouped in Fig. 3.

In pure CDA spectrum, the characteristic absorption bands at about 3323 cm^{-1} and 3119 cm^{-1} were due to stretching vibration

Table 1
Mechanical properties of PU-Film, 5%, 10%, 20% and 40% CDA-Films. Each of data is the means ± standard deviation of 3 or 15 experimental determinations.

Samples	Thickness (mm) (n = 15)	Tensile strength (MPa) (n = 3)	Elongation at break (%) (n = 3)	Elasticity modulus (MPa) (n = 3)
PU-Film	0.050 ± 0.001	19.32 ± 1.53	422.48 ± 36.93	0.21 ± 0.01
5% CDA-Film	0.077 ± 0.006*	12.03 ± 0.16*	388.72 ± 31.60ns	0.16 ± 0.01*
10% CDA-Film	0.097 ± 0.006*	11.25 ± 1.52*	412.58 ± 33.59ns	0.14 ± 0.01*
20% CDA-Film	0.110 ± 0.006*	9.77 ± 0.48*	440.49 ± 23.72ns	0.13 ± 0.01*
40% CDA-Film	0.133 ± 0.006*	7.53 ± 0.71*	409.60 ± 33.50ns	0.11 ± 0.01*
20% CDA-Sandwich	0.210 ± 0.010*	–	–	–

* Significatively different compared to PU-Film group (one-way ANOVA test, $p < 0.001$).
ns: Nonsignificatively different compared to PU-Film group (one-way ANOVA test, $p > 0.05$).
(–): Not determined.

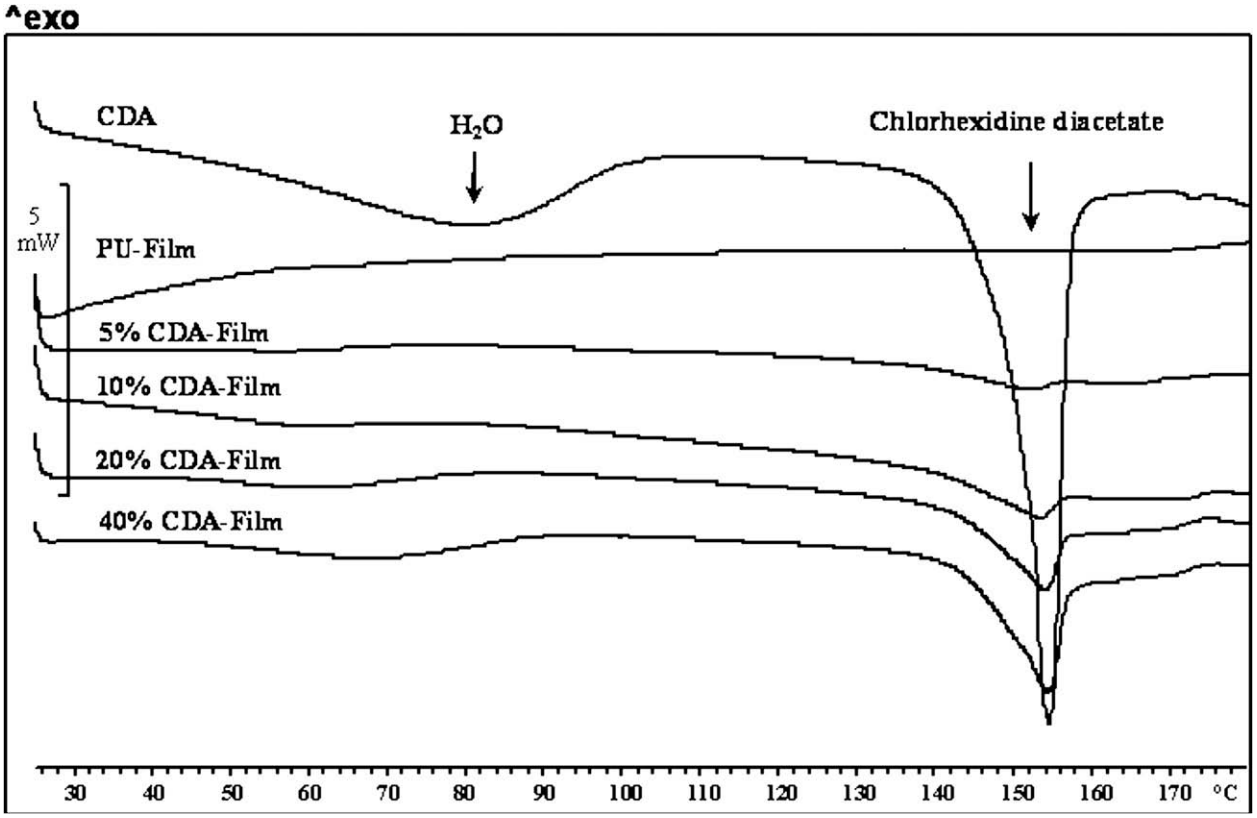

Fig. 1. Differential Scanning Calorimetry traces of CDA powder, PU and 5%, 10%, 20% and 40% CDA-Films.

Table 2
Differential Scanning Calorimetry results of CDA as pure powder and of 5%, 10%, 20%, 40% CDA-Films. Each of data is the means ± standard deviation of three experimental determinations.

Samples	T_f (°C)	ΔH_f normalized (J/g)
Pure CDA powder	154.57 ± 0.15	−95.90 ± 0.26
5% CDA-Film	151.78 ± 0.33*	−95.18 ± 0.60ns
10% CDA-Film	152.89 ± 0.16*	−95.71 ± 0.62ns
20% CDA-Film	153.57 ± 0.44*	−95.67 ± 0.15ns
40% CDA-Film	154.20 ± 0.22*	−95.39 ± 0.44ns

* significatively different compared to that of pure CDA powder (one-way ANOVA test, $p < 0.001$).
ns: Nonsignificatively different compared to that of pure CDA powder (one-way ANOVA test, $p > 0.05$).

N–H; 1610 cm^{-1} and 1489 cm^{-1} were due to C=C aromatic bending. In PU-Film spectrum, band at 3328 cm^{-1} assigned to N–H stretching, 1727 cm^{-1} peak of C=O group stretching, 1597 cm^{-1} peak of aromatic N–H bending, 1530 cm^{-1} peak N–H bending plus C–N stretching and 1221 cm^{-1} of C–O stretch were observed. All characteristic features of pure CDA and PU-Film were observed in CDA-Film spectrum.

3.2.4. Scanning Electron Microscopy

The morphologies of the PU-Film (Fig. 4A) and CDA-Films (Fig. 4B) were characterized by SEM in order to elucidate the controlled release patterns of CDA. PU-Film surface was smooth and uniform. 20% CDA-Film's surface showed the presence of poly hedral CDA with varying size.

3.3. Mechanical properties

Stress–elongation curves of PU-Films and of 5%, 10%, 20% and 40% CDA-Films are presented in Fig. 5a. Elasticity modulus (MPa) is the slope of fitting the elastic zone of stress–elongation curve to linear equation as shown in Fig. 5b. Tensile strength (MPa) and elongation at break (%) are maximum values before rupture (Table 1).

Incorporation of CDA into PU-Films (from 5 to 40% w/w) caused the decrease in elasticity modulus from 0.21 ± 0.01 MPa to 0.11 ± 0.01 MPa and in tensile strength from 19.32 ± 1.53 MPa to 7.53 ± 0.71 MPa. However, there was no change in elongation at break (one-way ANOVA test, $p > 0.05$).

3.4. Drug release study

Cumulative released amount of CDA (mg) from CDA-Films in distilled water and in 0.9% (w/v) NaCl aqueous solution as a function of time are shown in Figs. 6 and 7, respectively. Kinetic parameters of CDA from different CDA-Films and CDA-Sandwiches are reported in Table 3. Kinetic parameters were obtained by fitting the curve of cumulative released amount in function of time to linear equation (KaleidaGraph 3.6, Synergy Software). For 5%, 10% and 20% CDA-Films, the fitting was applied to the experimental curve from 0 to 1 h and 1 to 120 h (in distilled water); from 0 to 1 h and 1 to 216 h (in 0.9% (w/v) NaCl aqueous solution). For 40% CDA-Film, the fitting was applied to the experimental curve from 0 to 1 h and 2 to 120 h (in distilled water); from 0 to 1 h and 1 to 216 h (in 0.9% (w/v) NaCl aqueous solution). For 20% CDA-Sandwich, the fitting was applied to the experimental curve from 0 to 696 h (in distilled water and 0.9% (w/v) NaCl aqueous solution).

3.4.1. Release study of CDA from CDA-Films in distilled water

Release of CDA from CDA-Films in distilled water followed a bimodal release pattern (Fig. 6): during the first hour, a large amount of CDA was released corresponding to well-known burst effect and

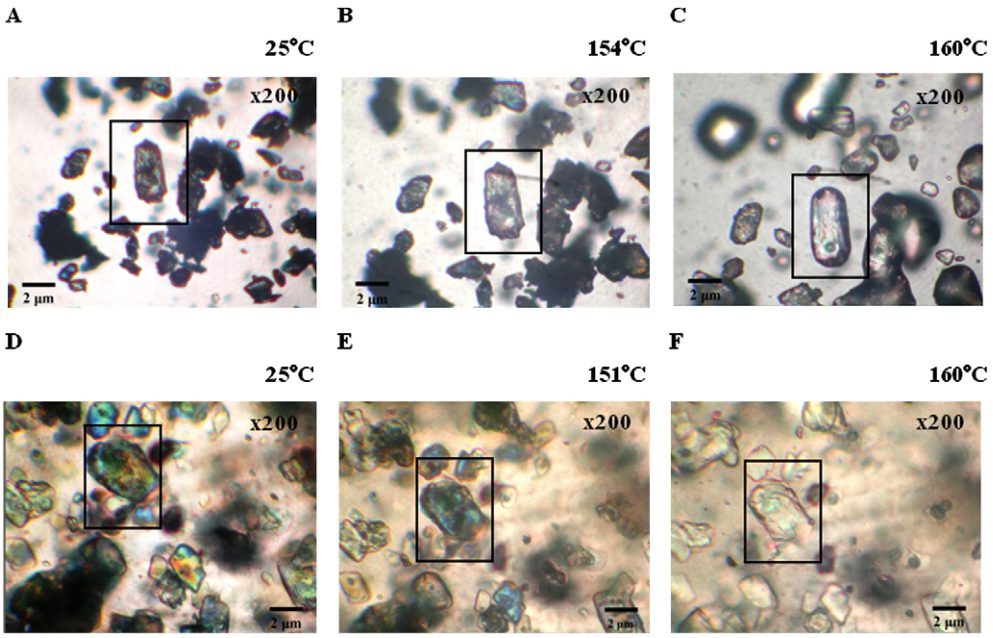

Fig. 2. Photographs of pure CDA powder at 25 °C (A), 154 °C (B), 160 °C (C) and of 10% CDA-Film at 25 °C (D), 151 °C (E), 160 °C (F) at magnification 200× obtained during HSM analysis. (For interpretation of the references in colour in this figure legend, the reader is referred to the web version of this article.)

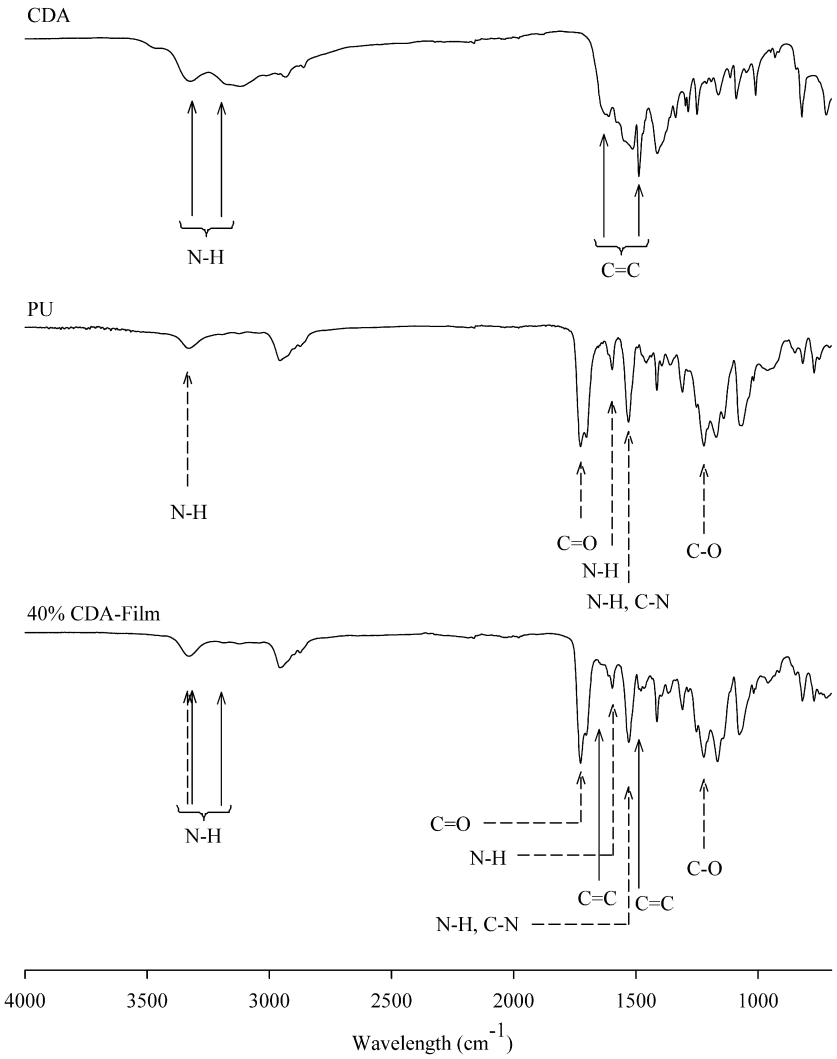

Fig. 3. ATR-FTIR spectra viewed from 3950 to 700 cm^{-1} of CDA, PU-Film and 40% CDA-Film.

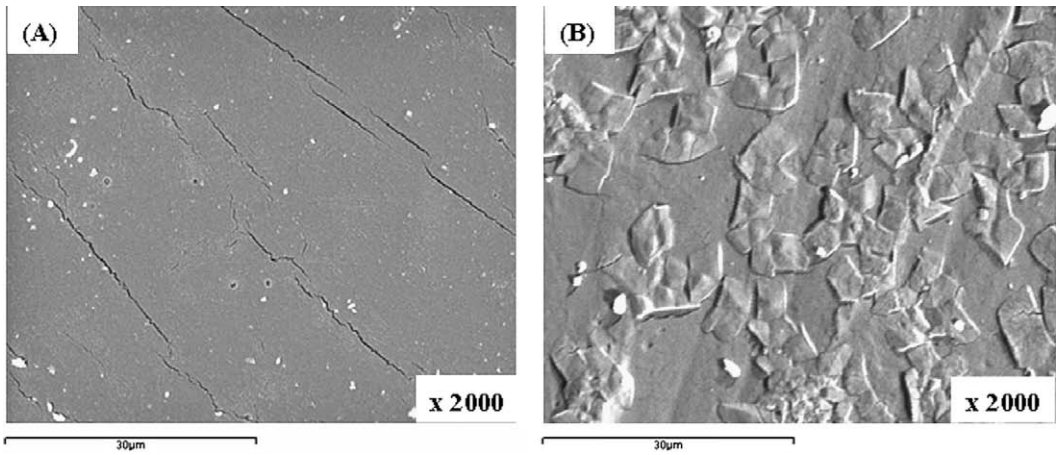

Fig. 4. Scanning Electronic Microscopy photos of (A) PU-Film and (B) 20% CDA-Film showing surface-located CDA.

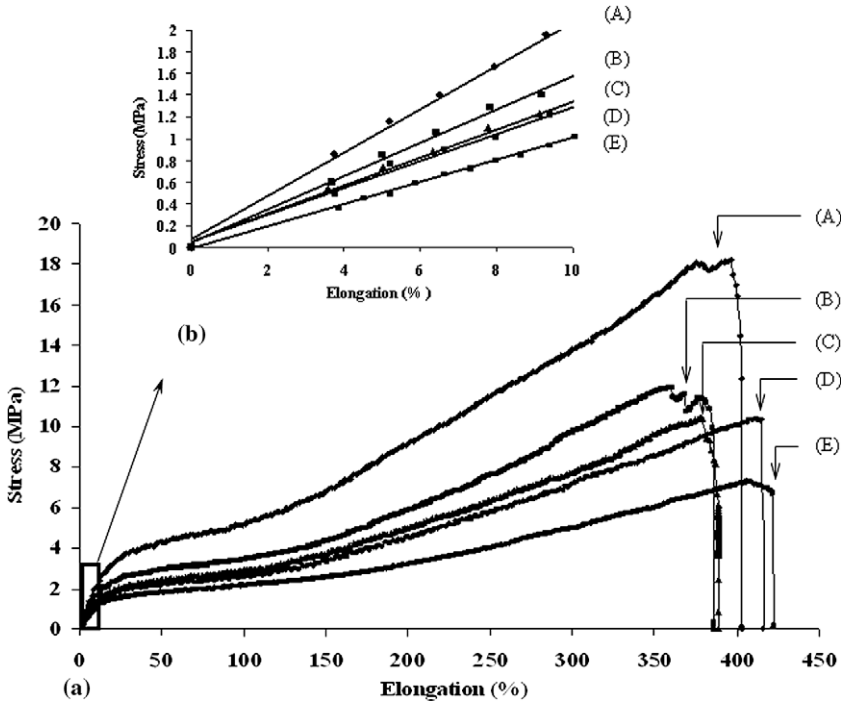

Fig. 5. Stress–elongation curves (Fig. 5a) of PU-Film (A), 5% (B), 10% (C), 20% (D) and 40% (E) CDA-Films showing their elastoplastic behavior and elastic zone zoomed from stress–elongation curves for elasticity modulus calculation (Fig. 5b).

a lower release thereafter. CDA released amounts increased with initial CDA loading in CDA-Films.

Drug study release in distilled water was carried out in sink conditions: maximum concentration achieved in distilled water at 120 h (0.18 mg/mL) was below 10% of solubility of CDA (2.3 mg/mL). Burst release in the first hour was found proportional to initial quantity of CDA. The release rates of CDA diffused after the first hour (Table 3) decreased at least 245-fold.

3.4.2. Release study of CDA from CDA-Films in 0.9% (w/v) NaCl aqueous solution

Release of CDA in 0.9% (w/v) NaCl aqueous solution was carried out for 11 days (Fig. 7). Burst release was also observed in the first hour of immerging CDA-Films in saline solution but from 2.7 to 5.3-fold lower than burst release observed in distilled water (Student's *t*-test, $p < 0.05$). After burst release, CDA was released from CDA-Films continuously, with a release rate significantly different to those obtained in distilled water (Student's *t*-test, $p < 0.05$), except for 5% CDA-Films where there was no significant difference (Student's *t*-test, $p > 0.05$) (Table 3). There was no significant difference between release rate of CDA from 20% and 40% CDA-Films (Student's *t*-test, $p > 0.05$).

3.4.3. Release study of CDA from CDA-Sandwich

The release profiles of CDA from 20% CDA-Sandwich in distilled water and in 0.9% (w/v) NaCl aqueous solution as a function of time are shown in Fig. 8. CDA releasing from 20% CDA-Sandwich was linear ($R^2 = 0.99$), indicated a zero-order kinetic, with a release rate constant of 0.67 ± 0.10 µg/h (in distilled water) and 0.68 ± 0.05 µg/h (in 0.9% (w/v) NaCl aqueous solution) (Table 3). There is no difference of release rate and cumulative released amount of CDA obtained at the end of experiment in the two studied media

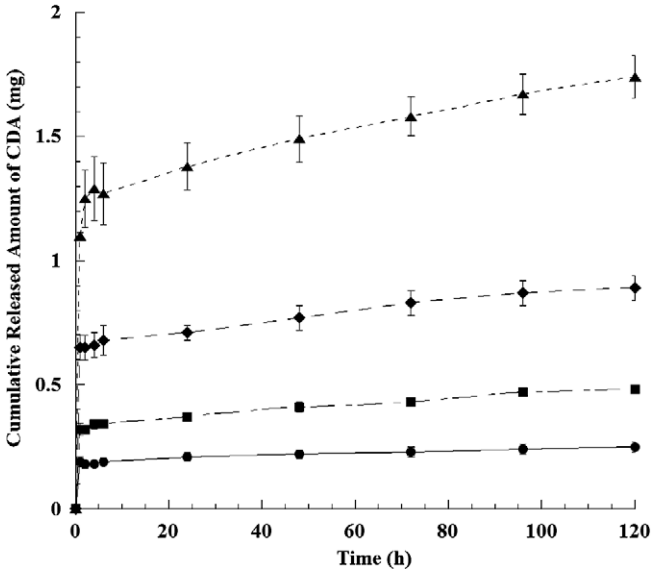

Fig. 6. Release of CDA from 5% (●), 10% (■), 20% (♦) and 40% (▲) CDA-Films in distilled water ($n = 3$).

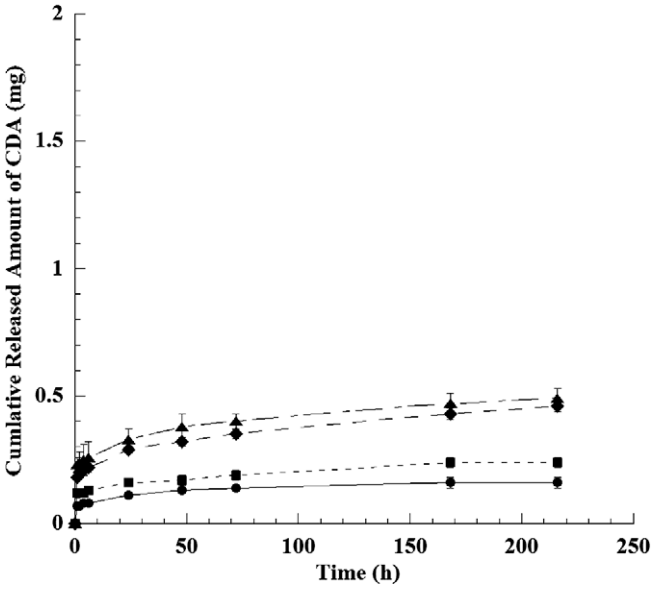

Fig. 7. Release of CDA from 5% (●), 10% (■), 20% (♦) and 40% (▲) CDA-Films in 0.9% (w/v) NaCl aqueous solution ($n = 3$).

(Student's t-test, $p > 0.05$). CDA was still released at 29th day of experiment.

3.5. Antibacterial activity

The MIC values experimentally obtained by broth macrodilution method for *S. aureus* ATCC 6538 (2.3×10^7 CFU/mL) and *S. epidermidis* ATCC 12228 (2.8×10^7 CFU/mL) were 4 μg/mL and 1 μg/mL, respectively.

Diameters of inhibition of CDA-Films were evaluated by serial plate transfer test. Inoculum of *S. aureus* ATCC 6538 was ranged from 4×10^6 to 3×10^7 CFU/mL and of *S. epidermidis* ATCC 12228 from 1×10^8 to 3×10^8 CFU/mL.

CDA-Films were active against *S. aureus* ATCC 6538 (Fig. 9A) and *S. epidermidis* ATCC 6538 (Fig. 9B) at 35th day of experiment, whereas PU-Films showed no inhibitory activity. The diameter of inhibition at the 1st day was the largest, regardless of CDA concentration and of bacteria tested. From the 2nd day to the 35th day, the diameters of inhibition were significantly shorter (Student's t-test, $p < 0.05$), but the inhibitory activity of CDA-Films was maintained.

4. Discussion

The objectives of the present study were to investigate the physico-chemical, mechanical properties and CDA release from different types of polymeric systems for a controlled antibacterial efficacy. CDA was incorporated into PU to form CDA-Films and CDA-Sandwich by casting technique and solvent evaporation.

4.1. Solid state of CDA, compatibility of CDA and PU and mechanical properties of CDA-Films

During the preparation, CDA is practically insoluble in tetra hydrofuran and therefore was dispersed in polyurethane solution. It was therefore interesting to investigate the physico–chemical properties of CDA-Films.

In this study, Differential Scanning Calorimetry analysis was used to evaluate the solid state of CDA in CDA-Films [25] and the compatibility of PU and CDA [26]. DSC results showed that CDA was in crystalline structure in CDA-Films. Interactions could be deduced from DSC by changes in thermal events such as disappearance or appearance of a new thermal event, changes in peak shape, peak onset, peak temperature and enthalpy of fusion.

Table 3
Kinetic parameters of CDA released from 5%, 10%, 20%, 40% CDA-Films and from 20% CDA-Sandwiches in two release media. Each of data is the mean ± standard deviation of 3 experimental determinations.

Sample	Initial CDA loading (μg)	In distilled water				In 0.9% (w/v) NaCl aqueous solution			
		Release rate (μg/h)		Cumulative released amount (μg)		Release rate (μg/h)		Cumulative released amount (μg)	
		0–1 h 0–696 h for CDA-Sandwich	1–120 h for CDA-Films	0–1 h	0–120 h for CDA-Films 0–696 h for CDA-Sandwich	0–1 h 0–696 h for CDA-Sandwich	1–216 h for CDA-Films	0–1 h	0–216 h for CDA-Films 0–696 h for CDA-Sandwich
5% CDA-Film	271.17 ± 22.81	188.04 ± 14.36	0.56 ± 0.08	188.04 ± 14.36	245.81 ± 20.74	69.82 ± 1.84*	0.45 ± 0.07ns	69.82 ± 1.84*	164.89 ± 15.60*
10% CDA-Film	533.57 ± 53.17	330.82 ± 5.60	1.35 ± 0.13	330.82 ± 5.60	476.29 ± 14.04	115.20 ± 2.03*	0.59 ± 0.04*	115.20 ± 2.03*	241.89 ± 17.34*
20% CDA-Film	1186.67 ± 94.15	655.25 ± 42.92	2.11 ± 0.27	655.25 ± 42.92	890.00 ± 52.95	188.94 ± 3.54*	1.23 ± 0.18*	188.94 ± 23.54*	458.27 ± 74.51*
40% CDA-Film	2650.67 ± 151.85	1228.50 ± 91.03	4.21 ± 0.37[a]	1228.50 ± 91.03	1744.07 ± 84.73	231.54 ± 9.65*	1.18 ± 0.17*	231.54 ± 29.65*	488.12 ± 38.95*
20% CDA-Sandwich	1190.00 ± 108.63	0.67 ± 0.10	–	–	458.97 ± 44.28	0.68 ± 0.05ns	–	–	470.53 ± 34.90ns

[a] Release rate calculated for 40% CDA-Film from 2nd to 120 h.
* Significatively different compared to that obtained in distilled water (Student's t-test, $p < 0.05$).
ns: Nonsignificatively different compared to that obtained in distilled water (Student's t-test, $p > 0.05$).

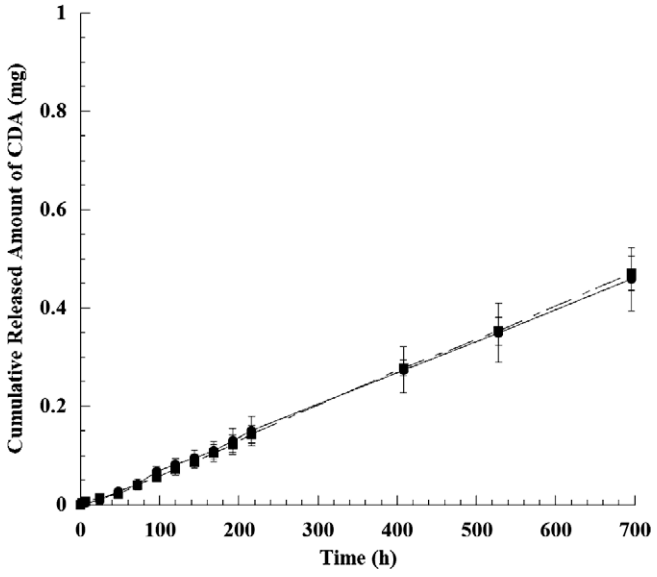

Fig. 8. Release of CDA from 20% CDA-Sandwich in distilled water (●) and in 0.9% (w/v) NaCl aqueous solution (■) ($n = 3$).

However, there should be caution that some broadening of peaks that leads to changes in area, onset or peak temperature was simply due to the mixing of the components without indicating an interaction [26]. The variation of fusion temperature of CDA in CDA-Films could be ascribed to the presence of PU, which acted as an impurity. Furthermore, similar normalized enthalpies of fusion were determined for CDA in PU-Films and that of CDA. It suggested that there was no drug amorphization during the preparation and that no interaction between CDA and PU occurred.

Hot-stage Microscopy studies were performed as a complementary technique in order to complete the DSC results [27]. Observations from HSM of CDA-Film confirmed the results obtained from DSC analysis: CDA remains in the crystalline state in CDA-Films, and the phenomenon leads to endothermic peak observed in DSC traces is the melting of CDA. In addition, Scanning Electron Microscopy observations showed polyhedral CDA crystals of varied sizes at the film surface.

In ATR-FITR, CDA features were in agreement with results previously reported [28]. PU features at 3328 cm^{-1}, 1727 cm^{-1}, 1597 cm^{-1} and 1221 cm^{-1} were in accordance to those already demonstrated in previous study [29] while the feature at 1530 cm^{-1} was ascribed to N–H bending [29] or N–H bending plus C–N stretching [30]. The superposition of pure CDA and PU-Film spectra observed in CDA-Film spectrum indicated that there was no interaction between CDA and PU.

PU-Film exhibited an elastoplastic behavior [21]. CDA-Films also showed an elastoplastic behavior in which decreases in elasticity modulus and tensile strength were noted. A previous work has demonstrated the same behavior for CDA-loaded acrylic resin tested by a bending test. The presence of CDA in the acrylic matrix could behave like a load and increase the plasticity of the system [31]. In the present study, polyhedral CDA crystals were located inside CDA-Films (observed in HSM and SEM). The sharp angles of CDA crystals could produce tips inside the polymeric film. When the tensile stress was applied, tips were propagated and cracks appeared. The higher the CDA concentration, the higher the number of cracks and the lower the tensile strength. The influence of crystals in polymeric film and consequently decrease in film's tensile strength were in accordance with a previous work reported by Crowley et al. [32]. However, tensile test was carried out until the fracture of CDA-Films, which in fact was not the real condition use *in vivo*. The observed decreases in tensile strength and elasticity modulus of CDA-Films, therefore, would not restrict the use of designed system for clinical applications.

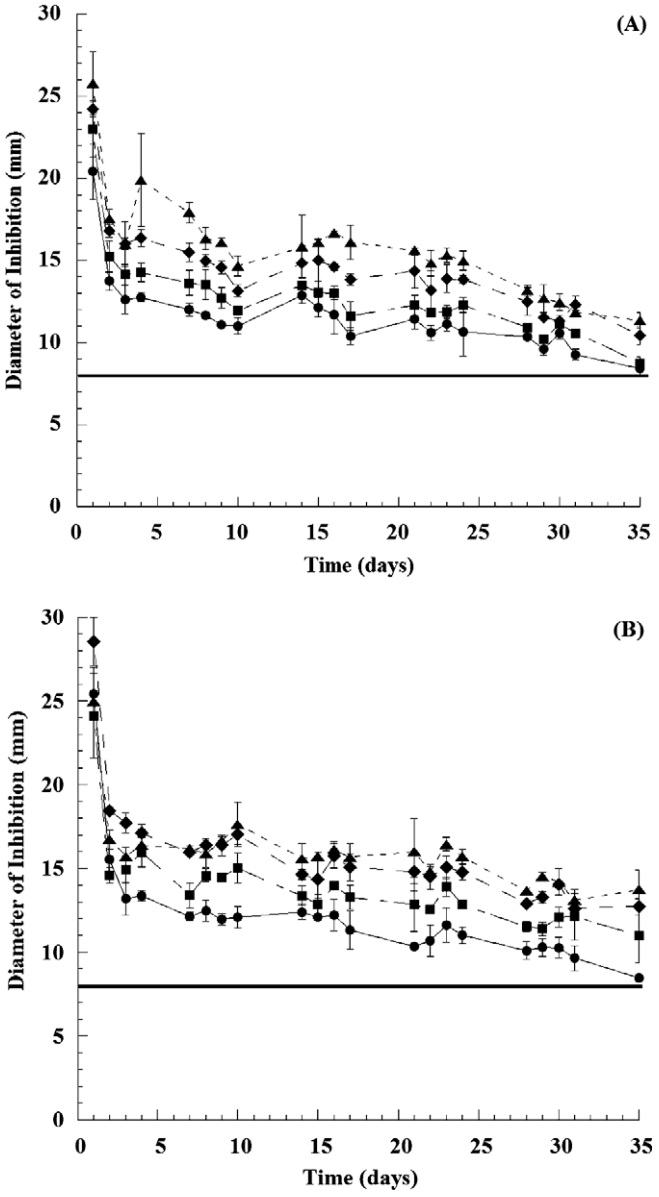

Fig. 9. Diameters of the inhibition of 5% (●), 10% (■), 20% (♦) and 40% (▲) CDA-Films against (A) *Staphylococcus aureus* ATCC 6538, (B) *Staphylococcus epidermidis* ATCC 12228 (serial plate transfer test) as a function of time. The horizontal line represents the diameter of CDA-Film disks.

4.2. Controlled release of CDA from CDA-Films and sandwiches

Earlier results showed a reduction in solubility of chlorhexidine salt in physiological saline solution [33], which should be carefully considered in the development of sustained release formulation for long-term disinfection [34,35]. The second set of drug release study in 0.9% (w/v) NaCl aqueous solution in the present work was performed in order to evaluate the effectiveness of designed system in a simple physiological solution. CDA released from designed system showed different profiles, depending on the initial drug loading and on the structure of system either film or sandwich.

More than 60% of initial quantity of CDA was released at the 5th day of experiment for 10%, 20% and 40% CDA-Films. Almost CDA loading was released within 24 h for 5% CDA-Films, which limits the use of this system for prolonged period. However, for 20% CDA-Sandwich, only around 40% of drug loading was released at the 29th day of experiment.

Under sink conditions, initial drug loadings played major roles in controlling the release of CDA from polymeric systems. In the case of CDA-Films, the higher the initial drug loading, the higher the release rate and higher the amount of CDA released. Significant lower total CDA release from sandwich compared to film containing the same drug loading can be explained by the difference of structure. Two outer polyurethane layers acted as rate-controlling barriers, which were responsible for the zero-order kinetic. CDA was supposed to diffuse through these polyurethane layers. Zero-order kinetic was described for multi-layer matrix devices, where the drug-loaded matrix core was covered by one or more modulating layers that act as rate-controlling elements [36,37].

The release profile of CDA from film in 0.9% (w/v) NaCl aqueous solution was more sustained and significantly different from that in distilled water. Lower burst release, lower total release amount and lower release rate of CDA were observed. This diminution was due to the modification of solubility. In fact, in saline solution, CDA was dissociated and transferred to chlorhexidine dihydrochloride, whose solubility is 1 mg/mL at 37 °C compared to 23 mg/mL of CDA [33]. In our cases, when the released amount of CDA was small (cases of 5% CDA-Films and of 20% CDA-Sandwich), the concentration of chlorhexidine dihydrochloride remained lower than 10% of its solubility and did not affect the CDA release. On the other hand, when the released amount of CDA was higher (cases of 10%, 20%, 40% CDA-Films), transformed chlorhexidine dihydrochloride concentration exceeded 10% of its solubility and restrained CDA release because sink conditions were not maintained.

4.3. Correlation between release profile of CDA from CDA-Films and antibacterial activity

In distilled water, burst release was correlated to initial CDA loading and could be predicted. This burst release allowed CDA to reach an effective concentration in a short period of time. Concerning CDA-Films for example, CDA concentrations in the receptor within one hour were ranged from 0.03 mg/mL to 0.11 mg/mL, respectively, which in fact cover the MICs against Gram-positive bacteria (lower than 10 μg/mL for *S. aureus*, *S. epidermidis*, *Streptococcus mutans*), some Gram-negative bacteria (5 and 12.5 μg/mL *Escherichia coli*, *Salmonella*) and against fungi such as *Candida albicans* (25 μg/mL) [1]. Antiseptic effect could be maintained during 5 days by a low CDA amount released but steady and sufficiently active. This behavior was described for numerous anti-infective substances and local controlled release system [38].

In 0.9% (w/v) NaCl aqueous solution, CDA concentrations obtained within one hour were from 6.98 μg/mL to 23.15 μg/mL, values in the same range of reported MICs against Gram-positive and Gram-negative bacteria. However, it has to be pointed out that the release profile has to be tailored and adapted to the clinical requirements. In dental use for example, the bacteriostatic concentration of chlorhexidine in the saliva has to be between 4 and 32 μg/mL [39]. In other clinical applications, a polyurethane central venous catheter impregnated extraluminally with 0.75 mg of chlorhexidine and 0.70 mg of silver sulfadiazine (CSS catheter) has been used [40,22]. This catheter proved to reduce the incidence of catheter-related bloodstream infections when was left in place less than 11 days [22]. Schierholz et al. studied the release rate of chlorhexidine from CSS catheter in same conditions as the present study [41]. The reported release rate for the first 24 h was 15 ± 3.2 μg/cm^2 which is much lower than the calculated release rate values of the present study (from 67.2 ± 3 μg/cm^2 to 210.10 ± 24.3 μg/cm^2). Sherertz et al. have reported that *in vivo* chlorhexidine release rate from catheter-bound chlorhexidine exceeded 0.36 μg/cm^2/h during the first 5 h and was lower than 0.10 μg/cm^2/h by 10 h [42]. These results are correlated to a lower risk of *S. aureus* catheter infection compared to unloaded control catheter.

4.4. Long-term antibacterial activity of CDA-Films

S. aureus ATCC 6538 and *S. epidermidis* ATCC 12228 were selected because *S. aureus* was the predominant organism cultured from infected sites, and *S. epidermidis* remained the most common opportunistic microorganism involved in catheter/implant infections [22]. MIC values determined for these strains were within the values reported in literature [1]. *S. epidermidis* ATCC 12228 is more sensitive to CDA than *S. aureus* ATCC 6538.

Serial plate transfer test was used by many authors to determine both the presence of antibacterial activity and its duration [5,6,43]. In this present work, serial plate transfer test was used to evaluate the antibacterial activity of CDA-Films against *S. aureus* ATCC 6538 and *S. epidermidis* ATCC 12228.

Under sink conditions, CDA was released continuously during 5 days. In static conditions in serial plate transfer test, against a high bacterial inoculum, all concentrations showed a remarkable antibacterial efficacy for 5 weeks of experiment.

5. Conclusions

This present work aimed at the formulation and the characterization of a controlled release system for an antiseptic, chlorhexidine diacetate. There was no interaction between the chlorhexidine diacetate and the polyurethane (proved by DSC and ATR-FTIR). Results showed that it was possible to modulate the chlorhexidine diacetate release using a polyurethane system. The chlorhexidine diacetate release depended on (i) the initial drug loading, (ii) the system structure (film or sandwich with two outer rate-controlling layers) and (iii) the nature of release medium (distilled water or 0.9% (w/v) NaCl aqueous solution). The proposed chlorhexidine diacetate-loaded polyurethane systems exhibited an antibacterial efficacy against *S. aureus* and *S. epidermidis* for a prolonged period of at least 5 weeks.

The proposed chlorhexidine diacetate-loaded polyurethane systems could be advantageously adapted, in function of divers practical applications, in different geometries in which a sustained and controlled release of this antiseptic is valuable to prevent or reduce infections.

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