A Current Approach for the Development and Characterization of Antibiotic Loaded Thin Film in Bangladesh

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Abstract

Current developments in (trans) mucosal drug delivery systems have a great impact on the limitations of traditional oral drug delivery for patients. Administration of drug via oral mucosa is a current approach for overcoming several limitations related to the oral dosage forms, such as low bioavailability, enzymatic inactivation and/or drug degradation in gastrointestinal tract, hence showing rapid onset of action. The aim of the study was to develop and characterize a model antibiotic (doxycycline) loaded-thin film for the treatment of a wide range of systemic/non-systemic bacterial and protozoa infections. The bases of each film were prepared using mucoadhesive polymer, cellulose gum/instant release film former, and plasticizer. Optimized films were characterized for uniformity of weight, percentage moisture content and uptake, folding endurance, elongation, swelling index, surface pH, hydration time and in vitro doxycycline permeation studies using modified Franz Diffusion Cells. Concentration of different polymers was tailored by the release rate of doxycycline from the films. In conclusions, the thin films can be an alternative dosage form for the delivery of doxycycline as compared to conventional dosage forms.

Key terms: Thin films; mucosal drug delivery; drug release

Introduction

Oral drug delivery is the most common route for its convenient and easy administration in Bangladesh. But, it has several limitations such as the hepatic first pass effect, enzymatic degradation and inactivation in gastric environment. Another, hypodermic needles are painful, less convenient and expensive for patients (Uddin et al., 2016). However, current drug delivery technologies are facilitated by either (trans) mucosal (Ammar et al., 2016; Bai et al., 2016; Bilbault et al., 2016; Kalia et al., 2016), or transdermal (Uddin et al., 2015) delivery system. The mucosal route of drug administration has some additional advantages, showing the low enzymatic activity, painless delivery that improved patient compliance, easy termination of dosage form,

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option to incorporate permeation enhancer and flexibility in developing fast or controlled release systems for systemic or non-systemic effect (Boateng, Mani and Kianfar, 2013; Boateng and Okeke., 2014 ; Navamanisubramanian et al., 2017). Moreover, it can be beneficial for patients who have complexity in swallowing tablets or capsules. Also, the drug release is characteristically related to the physicochemical properties of drugs in the aqueous medium (Murata, Kofuji and Maida, 2016).

In these circumstances, it was necessary to develop an alternative drug delivery system, in order to overcome these limitations related to the oral drug delivery and injections. In this research work, doxycycline was chosen as a model antibiotic from tetracycline group that showed it activity against many bacterial infections (e.g. acne, urinary tract infections, intestinal infections, eye infections, gonorrhoea, chlamydia, periodontitis and others) (Drugs, 2017). Most importantly, this research work also involves in the development, characterization of doxycycline loaded thin films and the release profile of doxycycline from various amounts of polymer-plasticizer ratios. These will assist to comprehend the relationship between the doxycycline release characteristics, prepared from different polymers.

Materials and Methods

Materials

Doxycycline (DC) (Molecular weight: 444.43 Dalton (Da); ?98.17%), was kindly gifted by Albion Laboratories Ltd. (Chittagong, Bangladesh). Hydroxypropyl methyl cellulose (HPMC) - Mucoadhesive polymer (Molecular weight 1261.4 Da), Sodium carboxy methyl cellulose (NaCMC) - cellulose gum (Molecular weight 262.19 Da), Glycerol (GLY) (Molecular weight 92.0 Da), Calcium chloride, Potassium chloride, Sodium hydroxide and Potassium dihydrogen phosphate were purchased from Merck Ltd. (Damstadt, Germany). Nutrient agar media was purchased from HiMedia Laboratories Ltd. (Mumbai, India). Kollicoat® IR (KOL) - instant release film former (Molecular weight 45,000 Da) was purchased from BASF® (Ludwigshafen, Germany).

Methods

Preparation of blank and doxycycline loaded thin films: Polymers and plasticizer were dissolved in distilled water before the addition of DC. HPMC, NaCMC/KOL and GLY were dissolved slowly into distilled water at room temperature (25°C) and left for an hour in a magnetic stirrer (Huafeng, China) until complete dissolution. The resulting, blank- and DC- loaded formulations were allowed to stand for 24 hours to eliminate all the air bubbles. Each formulation was poured into the petri dishes (90 mm in diameter) and dried in an oven (Genlab Limited, Cheshire, UK) at 40°C for 18-24 hours. The compositions of blank- and DC -loaded formulations are shown in Table 1.

Table 1. Optimized blank- and drug-loaded thin films prepared with different amounts of polymers and plasticizer.

Formulation	Polymers: Plasticizer	Weight ratios	DC
F1 (Blank)	HPMC: NaCMC: GLY	0.9:0.1:1	-
F2 (Blank)	HPMC: NaCMC: GLY	0.8:0.2:1	-
F3 (Blank)	HPMC: NaCMC: GLY	0.7:0.3:1	-
F4 (Blank)	HPMC: KOL: GLY	0.9:0.1:1	-
F5 (Blank)	HPMC: KOL: GLY	0.8:0.2:1	-
F6 (Blank)	HPMC: KOL: GLY	0.7:0.3:1	-
F7 (Drug Loaded)	HPMC: NaCMC: GLY	0.9:0.1:1	4.8
F8 (Drug Loaded)	HPMC: KOL: GLY	0.9:0.1:1	4.8
F9 (Drug Loaded)	HPMC: KOL: GLY	0.8:0.2:1	4.8

Physicochemical characterizations

Blank- and DC -loaded films were cut manually by customised stainless steel cutter, fabricated from sharp aluminium sheet. Individual film was weighed for weight uniformity using digital balance (Shimadzu Co. Limited, Japan). Length & width of the optimised films were measured using digital slide callipers (Mitutoyo, Japan). All experiments were carried out in triplicate to check the reproducibility's.

Moisture content & uptake

Individual weight of each film was taken and held in a desiccator containing calcium chloride granules for 24 hours at room temperature. After 24 hours, films were re-weighed again. The percentage (%) moisture content of each film was determined using following equation (Madhavi et al., 2013):

% Moisture content = [Initial weight - Final weight]/ Final weight × 100.

To calculate the moisture uptake, same protocol was carried out for each blank- or DC- loaded film except holding in saturated solution of potassium chloride (84% relative humidity) for 24 hours. Moisture content and uptake were investigated in triplicate for each formulation. The percentage of moisture uptake was determined from following equation (Madhavi et al., 2013):

% Moisture uptake= [Final weight-Initial weight/Initial weight] × 100.

Surface pH & swelling studies

An agar plate (2% w/v) was prepared for the measurements of surface pH and swelling studies for blank- and DC- loaded films (n=3). A digital pH meter (Hanna Instrument Inc., USA) was employed to study the pH after putting the films in an incubator

maintained at 37±0.2°C. Swelling studies of the films were measured after an hour, following same criteria as pH measurement. The equation involved in calculation of swelling is given below (Nair et al., 2013; Perioli et al., 2004; Wu. Chen and Jin, 2016):
Swelling studies= [Ws - Wd] /Wd × 100; where Wd= Dry weight of thin film, Ws= Weight of film after swelling.

Percent elongation, folding endurance & hydration time
Percent elongation study is necessary to examine the stress that a film can hold. The percent elongation of the films was calculated using the following formula (Madhavi et al., 2013):
Percent Elongation = L x 100/Lo; Where, L= Increase in length of thin film, Lo=Initial length of thin film.
Folding endurance of the films was determined manually for the blank- and DC-loaded films. It was counted when a film folded at a fixed position till it fractures (Boateng, Mani and Kianfar, 2013; Khana,, Agarwal and Ahuja, 1997). Hydration studies require for mimicking or predicting the full wetting time of each film. A hydration study of each film was carried out in 50 ml of phosphate buffer saline (P.B.S) pH 6.5 at a 50 rotation per minute (r.p.m.) till complete dissolution achieved. The buffer solution was prepared using KH2PO4 and NaOH (0.1M) to get a pH of 6.5 simulating salivary conditions (Boateng, Mani and Kianfar, 2013; Shende et al., 2016).

***In vitro* permeation studies**
The *in vitro* permeation study of DC was studied into the dermatomed non-keratinized mucosal tissue (Farm Limited, Chittagong) using modified Franz Diffusion cell (Anton Scientific Limited, India) in P.B.S pH 6.5 at 37°C. Sample (2 ml) was collected at planned intervals (0-60 minutes), from the sampling compartment and analysed at 240 nm using *UV*-spectrophotometer (Boekel & Co., Hamburg, Germany).

Statistical analysis
The data are presented as the mean ± standard deviation (SD) and the results were analyzed via student's t test. Statistical significance is indicated as for $p < 0.05$.

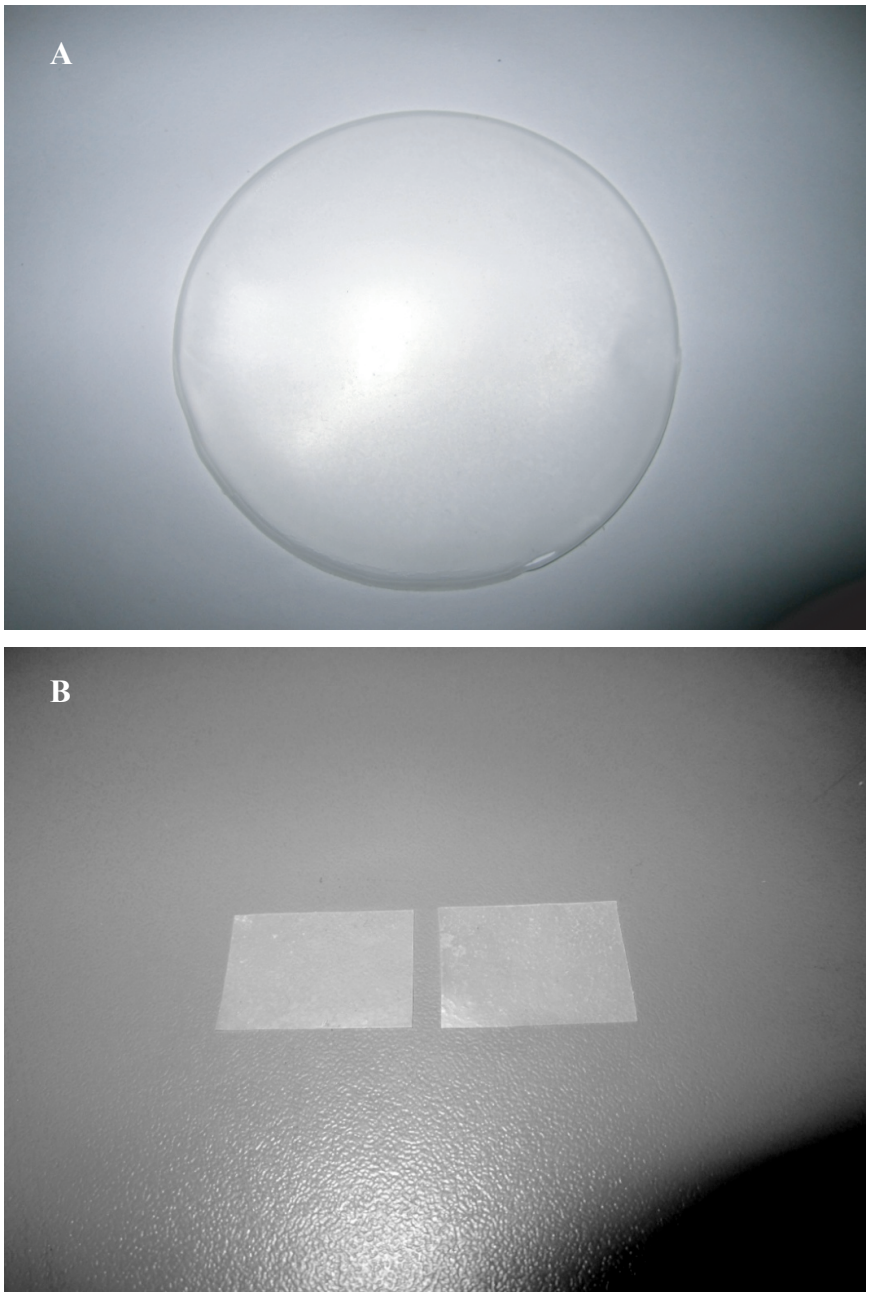

Figure 1. Digital Photographs of doxycycline loaded thin films; (A) Solvent casted film of doxycyline, (B) Sliced thin film.

Table 2. Physicochemical characterizations of oral thin films (Mean±SD).

Formulation	Weight (g)	% Moisture content	% Moisture uptake	Surface pH	% Swelling index	% Elongation	Folding endurance (Number)	Hydration Time (min)
F1	0.11±0.0	19.8±0.5	16.6±2.9	6.7±0.2	100±0.0	7.4±2.5	121.0±2.0	10-15
F2	0.12±0.01	27.1±0.8	13.0±1.8	6.6±0.1	100±0.0	3.9±0.2	85.0±1.0	10-15
F3	0.12±0.0	39.3±2.5	11.2±2.6	6.7±0.2	100±0.0	2.9±0.1	67.0±1.0	10-15
F4	0.12±0.01	20.8±3.9	7.30±1.5	6.6±0.0	100±0.0	7.4±1.4	121.0±4.5	10-15
F5	0.12±0.01	23.7±1.3	3.60±0.8	6.7±0.0	100±0.0	9.8±2.5	112.0±3.0	5-10
F6	0.11±0.01	21.0±4.2	1.30±.2.0	6.6±0.1	100±0.0	14.8±1.7	70.0±2.0	10-15
F7	0.12±0.01	18.1±0.5	17.8±2.8	6.6±0.1	100±0.0	7.4±0.8	121.0±3.0	10-15
F8	0.12±0.01	22.4±0.3	8.10±2.5	6.7±0.1	100±0.0	7.2±1.2	129.0±3.0	10-15
F9	0.12±0.00	25.4±0.7	4.00±1.1	6.7±0.1	100±0.0	8.7±1.5	113.0±4.0	5-10

Results

The aim of the research work was to develop and characterize DC loaded thin film for the treatment of a wide range of bacterial and protozoa infections. Firstly, a solvent evaporation method was used to prepare the thin films. Secondly, this study was carried out on the basis of two factors, namely selection of an appropriate amount of HPMC, NaCMC/KOL and GLY for the development of optimized film and physicochemical characterizations of both blank- and DC- loaded films. As the research involved in the employment of at least two polymers (HPMC and NaCMC/KOL) for each formulation, hydrate polymers that produced the gel solutions with entrapped bubbles. Entrapped bubbles might have some effects on the physicochemical properties and in vitro drug permeation studies. However, all Blank- and DC- loaded films were prepared using HPMC, NaCMC/KOL and GLY.

For all film formulations, GLY (1% w/w) was added to the solution for preparing more flexible and elastic film without any brittleness. The average drying time for the films were 24-48 hours at a temperature not exceeding 40°C. **Figure 1** shows the digital photographs of thin films (27 X 15 mm), prepared during this research work. All films were investigated for the physiochemical characteristics such as weight uniformity, moisture content and uptake, surface pH, swelling studies, elongation, folding endurance and hydration studies. As it can be seen from **Table 2**, weight of each film (0.10-0.13 g) confirmed the reliable and reproducible data though it was cut manually by stainless steel cutter. It was evident from moisture content and uptake studies, hydrophilic drug (DC) and polymers (HPMC/NaCMC/KOL) have a great ability to content (F3>F2>F5>F6>F4>F1 for blank-; F9>F8>F7 for DC -loaded film) and uptake moisture (F1>F2>F3>F4>F5>F6 for blank- ; F7>F8>F9 for DC- loaded film) from the environment. Surface pH of the films was in the range between 6.5-6.8 which is similar to the mucosal environment. The swelling studies of DC loaded films were carried out for an hour in an agar medium (2% w/v) at 37°C in a oven for maintaining the same temperature as human body. In the findings, all of the films were dissolved completely (100%) within an hour for hydrophilic drug and polymers, ability to swell. Plasticizer has a direct influence on the plastic or elongation properties of each thin film. The films showed the practical folding endurance which was not more than 129 (±3.0) in number and no observable fractures. Folding endurance of the films were F4>F1>F5> F2>F6>F3 (blank films) and F8>F7>F9 (DC loaded films). HPMC, NaCMC/KOL and GLY influenced the folding resilience of the film, high the ratios of plasticizer exhibit upper folding endurance. Finally, physicochemical Characterization tests were performed to find out the best films for drug loading and found F1, F4 and F5 suitable for further testing.

To study the release profile of DC from different formulations, DC loaded films were carried out into PBS of pH 6.5 and the cumulative release profile for each film was studied for an hour using modified Franz diffusion cells. DC was selected in order to examine the effect of the polymers-drug combination. **Figure 2** demonstrates the release of the DC where the increase of HPMC ratio, resulted in slower release rates for F7 and F8. The release rates were 68.9% and 80.6% being released after 30 mins for F7 and F8 while faster release was observed for F9 (98.2%). After 60 minutes, all films showed complete release of DC in the simulated mucosal fluid media via buccal tissue. The DC release patterns are considerably faster rate from F9, most probably because of the higher water solubility of DC, HPMC and KOL. Also, KOL is a higher molecular and instant release film forming polymer that might influence in the release of DC. However, DC release rates were significantly lower at the beginning, until the full hydration of film into the buffer medium.

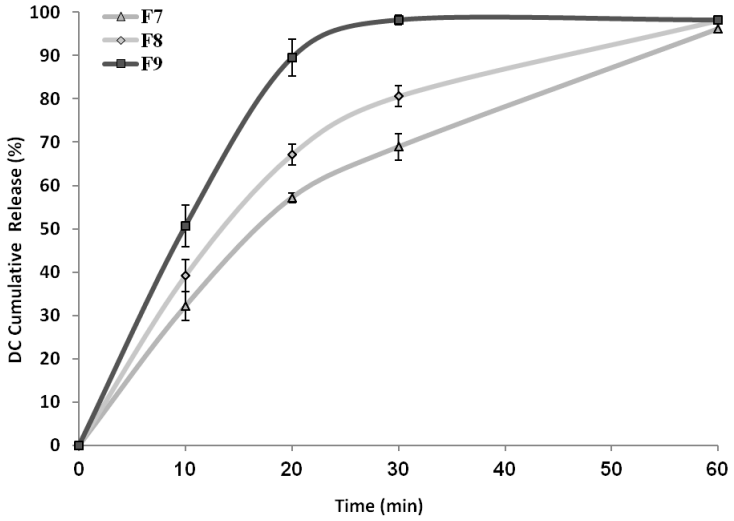

Figure 2. In *vitro* release profile of doxycycline from different film formulations int (F7-F9).

Discussion

The primary objective of this work was to prepare uniformed DC loaded thin films using biocompatible materials approved by the Food and Drug Administration (FDA) with maximum loading of DC. According to the Directorate General of Drug Administration (DGDA), doxycycline tablet or capsule is listed as an essential drug for Bangladesh, launched by around thirty three pharmaceutical companies in Bangladesh (DGDA 2017; BD drugs 2017). Unfortunately, oral drug deliveries lack of bioavailability due to first-pass metabolism (Bilbault, et al., 2016). However, film was used for the relief of discomfort and fear of injection in paediatric and geriatric community (Jiyeon et al., 2016).

KOL (MW: 45 kDa) entrapped air bubbles that might have some effects on the physicochemical properties and in vitro drug release studies. To overcome the technical error for complete hydration without trapped air bubble, a lower temperature with less stirring was used to reduce the air bubble formation (Rosen, 2005). Among all the prepared films, three were selected based on the elongation, folding endurance and hydration time (Table 2). The components used to prepare these thin films were HPMC, NaCMC/KOL and GLY.

This research work consider as a proof of concept and the key purpose was to find out the possibility of administering therapeutically relevant doses of DC using thin films. Furthermore, lower the content of water was probable reason for the brittleness of the film at higher concentration of polymer (Boateng, Mani and Kianfar, 2013).

Plasticizer, such as glycerol was used to decrease film brittleness. However, the physicochemical properties were not affected by drying time of the films. The principal examinations were carried out for the transparency, plasticity and thickness (Boateng et al., 2008). An acidic or alkaline pH may irritate the buccal mucosa and affect the degree of polymer hydration. Therefore, the surface pH of the buccal films was selected to optimise both the mucoadhesion and drug release (Wu, Chen and Jin, 2016). The DC-loaded oral films were dissolved in between 5-15 minutes.

The *in vitro* permeation studies of DC, was investigated with films across buccal tissue using modified Franz diffusion cells revealed that 98.2% ($p < 0.05$) of DC loadings were successfully delivered in 30 minutes. Also, the fresh buffer medium was added at various intervals to maintain sink condition (Kianfar et al., 2012).

Conclusions

In conclusion, thin films were prepared with various ratios of HPMC and NaCMC/KOL with doxycycline and successfully released from the films. The films were uniform, reproducible and accurate in relation to physicochemical characterization and drug release profile. The hydrophilic drug, doxycyline, showed fast release profiles with most of the combinations, released within an hour. This release phenomenon was achieved not only with HPMC but mostly with KOL, a polymer with high solubilising capacity, which increased the drug release rates. Oral thin film is a new technology for delivering a wide range of active pharmaceutical substances compared to conventional approaches and can be further used for encapsulated protein and peptide delivery.

Disclosure: The authors report no conflict of interest.

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