Research Article

Available Online at: www.ijpir.com

International Journal of Pharmacy and Industrial Research

ISSN Print 2231 – 3648
Online 2231 – 3656

Designing buccal mucoadhesive tablets of Doxofylline by using Ethocel, Carbopol 934P and Methocel K15M: A method to improve its bioavailability

Mohd Shoukhatullah Ansari[1], Niranjan Panda[2], Pawan Kumar[3], Prabhat Kumar[4]

[1]Research Scholar, School of Pharmacy & Medical Sciences, Singhania University, Jhunjhunu (Raj.).

[2]PG Department of Pharmaceutics, Anwarul Uloom College of Pharmacy, Hyderabad.

[3]Department of Microbiology, School of Medical Sciences, Singhania University, Jhunjhunu (Raj.)

[4]Department of Biochemistry, School of Pharmacy & Medical Sciences, Singhania University, Jhunjhunu (Raj.)

ABSTRACT

In order to avoid first pass metabolism and increase therapeutic effectiveness, the present studies were aimed to develop a Doxofylline buccal mucoadhesive tablet using mucoadhesive polymers such as Methocel K15M, Carbopol 934P and Ethocel. All the polymers were used alone or in combination as release retarding agent to prolong the drug release as well as to increase mucoadhesive strength. The mucoadhesive buccal tablets were prepared by direct compression method. The dry blend of drug and polymers were evaluated for precompression parameters to ensure flow properties during tablet punching. The drug excipients compatibility was evaluated by FTIR and DSC studies. The formulated mucoadhesive buccal tablets were evaluated for physicochemical parameters such as hardness, thickness uniformity, weight variation, and moisture absorption studies. The prepared buccal tablets were also evaluated for mucoadhesive strength, *in vitro* drug release and ex vivo drug permeation through cellulose acetate membrane. FTIR and DSC results showed no evidence of interaction between the Doxofylline and mucoadhesive polymers used for formulations. *Ex vivo* mucoadhesive strength, and *in vitro* release studies showed that formulation $DBMT_{15}$ containing 20% of Methocel K15M and 10% Ethocel of combination showed satisfactory bioadhesive strength and exhibited optimum drug release (99.25% after 12h). The Stability of Doxofylline mucoadhesive buccal tablets was determined in artificial human saliva and it was found that both Doxofylline and

Author for Correspondence:
Mr. Mohd Shoukhatullah Ansari
Assistant Professor
Department of Pharmaceutics ,
Anwarul Uloom College of Pharmacy,
Newmallepally, Hyderabad-500001,
Telangana, India.

buccal tablets were stable in human saliva. Hence the mucoadhesive polymers such as Methocel K15M, Carbopol 934P and Ethocel in various proportions can be used to prepare mucoadhesive buccal tablets of Doxofylline, that can expected to have prolonged therapeutic effect with enhanced patience compliance by avoiding first pass metabolism.

Keywords: Doxofylline, Mucoadhesion, Methocel K15M, Carbopol 934P, Ethocel, Asthma

INTRODUCTION

Buccal drug delivery system has the potential to fill an unmet need in migraine care by providing direct access to the systemic circulation through the internal jugular vein bypassing the first pass metabolism leading to high bioavailability. Moreover, the buccal cavity is easily accessible for self medication and drug absorption is terminated in case of toxicity by removing the dosage form from the buccal cavity. Buccal drug delivery system utilizes mucoadhesive polymers which become adhere to the buccal mucosa upon hydration and hence act as targeted or controlled/sustained release system. Various mucoadhesive dosage forms suggested for oral drug delivery which include adhesive tablets, adhesive patches, adhesive gels, strip, ointment and discs. Other advantages are non-invasive administration, rapid-onset of action, convenient and easily accessible site, self-administrable, low enzymatic activity, suitability for drugs or excipients that mildly and reversibly, damages or irritates the mucosa, painless administration, easy drug withdrawal, cheap and have superior patient compliance. [1, 2]

Doxofylline, a methylxanthine derivative that works by inhibition of phosphodiesterase IV activities, indicated for asthma and chronic obstructive pulmonary disease. It has recently drawn attention because of its better safety profile and similar efficacy over the most widely prescribed analogue, theophylline, due to decreased affinities towards adenosine A1 and A2 receptors. Doxofylline is chemically designated as 7-(1, 3 dioxolone-2-yl methyl) theophylline. Presence of a dioxolane group in position C-7 differentiates it from theophylline [3]. Doxofylline is an anti-tussive and bronchodilator used for maintenance therapy in patients suffering with asthma and chronic obstructive pulmonary disease (COPD) and is extensively metabolized in liver by demethylation and oxidation to an extent of 80-90% and 48% plasma protein bound. Elimination half life ($t_{½}$) is around 6-7 h and < 4% of an administered dose of Doxofylline is excreted unchanged in the urine. The daily dose is 200-400 mg two to three times in a day. Doxofylline is coming under class III of BCS classification and oral absorption is 62.2%. It is having solubility of 12 mg/ml in water and having pKa 9.87. [4, 5]

Hence, in the present work an attempt was made to formulate mucoadhesive buccal tablet for Doxofylline using different combination of polymers in order to avoid first pass metabolism, for prolonged effect and to obtain greater therapeutic efficacy for improving patient compliance.

MATERIALS AND METHODS

Materials

Doxofylline was procured as a gift sample from Dr. Reddy's laboratories Pvt. Ltd, Hyderabad, India. The mucoadhesive polymer like Methocel K15M, Carbopol 934P and Ethocel were purchased from Indian Drugs, Hyderabad. Pharmatose, PVP K30, Titanium dioxide, Saccharin, Talc and magnesium Stearate were purchased from S.D. fine chemicals Pvt. Ltd' Mumbai, India. All the ingredients were of laboratory grade. The distilled water used in the process of research work was prepared by double distillation process in the laboratory.

Methods

Drug excipients compatibility studies

Drug excipients compatibility studies were done by FTIR and DSC

Fourier Transform Infrared (FTIR) spectroscopy

Fourier transform infrared (FTIR) study was performed to verify any physical or chemical interaction between the pure drug and the

excipients used. The FTIR studies of pure API Doxofylline and the physical mixture that contains all those ingredients with pure API were carried out. It was performed by potassium bromide (*KBr*) pellet method. The samples were triturated with *KBr* and pellet was prepared by setting the pressure to 100 kg/cm^2 for 2 min. The obtained pellet was analyzed in FTIR 8400S, Shimadzu, Japan. The peaks that were obtained for the pure drug, polymers and formulation, characterised for the presence of different functional group and ensured that there was no extra peaks formed which usually indicates formation of new functional group. [6, 7]

Differential Scanning Calorimetric (DSC) analysis

Another method of estimating the physical interaction between drug and polymers used for the formulation of different dosage form is thermal analysis by DSC or TGA techniques. In the present studies the DSC analysis of Doxofylline and the physical mixture that contains all those ingredients with pure API used for preparation buccal mucoadhesive tablets were carried out using a Shimadzu DSC 60, Japan; to evaluate any possible polymer drug thermal interaction. Exactly weighed 5 to 6 mg samples were hermetically sealed in aluminium crucible and heated at constant rate of 10 ^{o}C/min over a temperature range of 40 to 300 ^{o}C. Inert atmosphere was maintained by purging nitrogen gas at a flow rate of 50 ml/min. [7, 8]

Formulation of Doxofylline mucoadhesive tablet matrix tablets (DBMT)

Doxofylline mucoadhesive matrix tablets were formulated by direct compression method. The formulation composition of different batch is shown in **table 1**. All the powders passed through 40 mesh sieve. The required quantity of Doxofylline, various polymers (Methocel K15M, Carbopol 934P and Ethocel), filler (Pharmatose) and binder (PVP K30) were mixed thoroughly by process of trituration. The dry blends were dried at 40^o C for 15 minutes to reduce moisture content so that the final percentage of moisture in powder remained in a range of 2-5 %. Magnesium stearate and talc were finally added as a lubricant and glidant respectively. The dry blends were tested for various pre-compression parameters like bulk density, tapped density, angle of repose, Carr's index, Hausner's ratio etc. The evaluated mixture of powder was directly compressed (10 mm diameter, circular flat faced punches) on a 10 station rotary tablet punching machine (SHAIMAC Technology Pvt. Ltd, India). The total weight of tablet is 500mg and it contains 200 mg of Doxofylline. All the tablets were stored in airtight containers for further study. [7, 8]

Table 1: Formulation of Doxofylline mucoadhesive tablet matrix tablets (DBMT)

Formulations (mg)	$DBMT_9$	$DBMT_{10}$	$DBMT_{11}$	$DBMT_{12}$	$DBMT_{13}$	$DBMT_{14}$	$DBMT_{15}$	$DBMT_{16}$
Doxofylline	200	200	200	200	200	200	200	200
Methocel K15M	50	75	100	125	50	75	100	125
Carbopol 934P	125	100	75	50	-	-	-	-
Ethocel	-	-	-	-	125	100	75	50
Pharmatose	65	65	65	65	65	65	65	65
PVP K30	40	40	40	40	40	40	40	40
Mg Stearate	5	5	5	5	5	5	5	5
Talc	10	10	10	10	10	10	10	10
Titanium dioxide	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Saccharine	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Total	500	500	500	500	500	500	500	500

Evaluation of pre-compression parameters of dry powder blend of all formulations ($DBMT_9$-$DBMT_{16}$)

Angle of Repose (θ)

Angle of repose is indicated as maximum angle possible between the surface of a pile of powder and the horizontal plane. The dry powder blends from different formulations were allowed to flow through the funnel fixed to a stand at definite height (h). The angle of repose was then calculated by measuring the height and radius (r) of the heap of powder formed.

$$\theta = \tan^{-1}\left(\frac{h}{r}\right)$$

According to the specifications the angle of repose value less than 25^0 indicates excellent flow whereas angle "between" 25^0-30^0 indicates good flow. The angle "between" 30^0-40^0 indicates passable flow and angle greater than 40^0 indicates very poor flow. [9]

Bulk density

To determine compressibility index and Hausner's ratios, both the loose bulk density (LBD) and tapped bulk density (TBD) of prepared dry powder blends of all the formulations were determined. The quantity of 2 gm of powder blends from each formulation, previously lightly shaken to break any agglomerates formed; were introduced into a 10 ml measuring cylinder. After the initial volume was observed, the cylinder was allowed to fall under its own weight on to a hard surface form the height of 2.5cm at second interval. The tapings were continued until no further changes in volume were noted. LBD and TBD were calculated using the following formulas. [10]

$$LBD = \frac{weight\ of\ the\ granule}{volume\ of\ the\ packing}$$

$$TBD = \frac{weight\ of\ the\ granule}{tapped\ volume\ of\ the\ packing}$$

Compressibility Index (Carr's index)

Compressibility index (Carr's index) is important parameters to determine the flow properties of powder and granules. Carr's index of prepared dry powder blends were calculated by following formula.

$$Carr's\ index\ (\%) = \frac{TBD - LBD}{TBD} \times 100$$

According to the specification the Carr's index values "between" 5-15 indicates excellent flow where as "between" 12-16 indicates good flow. Values "between" 18-21 indicate fare-passable where as "between" 23-25 indicates poor and "between" 33-38 indicates very poor and greater than 40 indicates extremely poor. [10, 11]

Hausner's ratio

Hausner's ratios are also another parameter to determine the flow properties of powder and granules. The Hausner's ratios of prepared dry powder blends were determined by following formula.

$$Hausner's\ ratio = \frac{TBD}{LBD}$$

According to specifications values less than 1.25 indicate good flow (=20% of Carr's index), where as greater than 1.25 indicates poor flow (=33% of Carr's index). Between 1.25 and 1.5, added glidant normally improves flow. [10, 11]

Evaluation of postcompression parameters of all formulations ($DBMT_9$-$DBMT_{16}$)

Thickness

Ten tablets from each formulation of Doxofylline mucoadhesive sustained release tablets were randomly selected and used for thickness determination. Thickness of each tablets were measured by using digital Vernier Callipers (Mitutoyo dial Thickness Gauge, Mitutoyo, Japan) and the results were expressed as mean values of ten readings, with standard deviations. According to specification tablet thickness should be controlled within a ± 5% variation of standard value. [11, 12]

Tablet Hardness

Hardness of all the formulations under study was measured by using Monsanto hardness tester (Cad Mach). From each formulation the crushing strength of ten tablets with known weights were

recorded in kg/cm^2 and average were calculated and presented with standard deviation. According to specifications of USP hardness values of 4-5 Kg for tablet is considered as acceptable limit. [12]

Friability

Previously weighed ten Doxofylline mucoadhesive sustained release tablets from each batch were taken in Roche friabilator (Roche friabilator, Secor India). After100 revolutions of friabilator tablets were recovered. The tablets were then made free from dust using a soft muslin cloth and the total remaining weight was recorded. Friability was calculated from the following formula.

$$\%F = \frac{(Wi - Wf)}{Wi} \times 100$$

Where W_i and W_f were the initial and final weight of the tablets before and after friability test. For any compressed tablet that the lose less than 0.1 to 0.5 % and maximum upto 1% of the tablet weigh are consider acceptable. [12, 13]

Weight variation test

All formulated Doxofylline mucoadhesive sustained release tablets were evaluated for weight variation as per USP monograph. Twenty tablets were weighed collectively and individually using an electronic balance. The average weight was calculated and percent variation of each tablet was calculated. According to USP monograph, the weight variation tolerance limit for the uncoated tablet having average weight 130mg or less is 10% whereas for average weight between 130-324mg is 7.5% and for average weight more than 324mg is 5%. For the tablet to be accepted, the weight of not more than two tablets deviate from the average weight by not more than 7.5% and no tablet deviates by more than 15%. [13, 14]

Content uniformity

Twenty tablets were taken and triturated to form powder and powder equivalent to one tablet was taken and dissolved in 100 ml of phosphate buffer pH 6.8 and heated at 37 ^{o}C for 15 to 20 min with stirring. The solution was filtered, suitably diluted and the Doxofylline content was measured by using UV Spectrophotometer (Analytical Technologies Ltd. Spectro 2080) at 274nm. Each measurement was carried out in triplicate and the average drug content in each tablet was calculated. [14]

Swelling index study

The extent of swelling was measured in terms of percentage weight gain by the tablet. The swelling index of all formulation was studied. One tablet from each batch was kept in a Petridis containing 2% agar gel plates with the core facing the gel surface and incubated at 37±1 °C. The tablet was removed every two hour interval up to 12 hour and excess water blotted carefully using filter paper. The swollen tablets were re-weighed (W_t). The swelling index (SI) of each tablet was calculated according to the following equation. [15]

$$SI = \frac{(W_t - W_0)}{W_0} \times 100$$

Where W_0 = initial weight, W_t = weight after time t

Measurement of bioadhesive force

Bioadhesive force of the tablets was measured on a modified physical balance. The apparatus consisted of a modified double beam physical balance in which a lighter pan had replaced the right pan and the left pan had been replaced by a glass slide (4 cm length and 2.5 cm width) with plastic hang suspended by Teflon rings and copper wire. The left-hand side of the balance was exactly 5 g heavier than the right side. The height of the total set-up was adjusted to accommodate a glass container of 6.6 cm height. In order to find out the bioadhesion strength first buccal tablet (n = 3) was stacked to the glass slide with the help of the knob, which was situated at the base of the physical balance. Five grams weight from the right pan was then removed. This lowered the glass slide along with the tablet over the membrane with a weight of 5.0 g. This was kept undisturbed for 5 min. Then, the weights on the right-hand side were slowly added in increments of 0.1 g till the tablet just separated from the membrane surface. The excess weight on the right pan, i.e. total weight minus 5g

was taken as a measure of the bio-adhesive strength. By using this weight, bio-adhesive force for all the formulations of Doxofylline buccal mucoadhesive tablets were calculated using following equation

$$N = (W \times g)/1000$$

Where *N* is bio adhesive force, *W* is the weight required for the detachment of two vials in grams, and *g* is the acceleration due to gravity. [16, 17]

In vitro drug release study

The *in vitro* dissolution study was conducted for all the formulations using an eight station USP dissolution rate test apparatus type-II (LABINDIA DS 8000, Mumbai, India.). A total volume of 900 ml of phosphate buffer pH 6.8 was taken as dissolution medium, which was maintain at 37°C ± 0.5°C at 50 rpm. 5ml of aliquots were periodically withdrawn and the same volume was replaced with an equal volume of fresh dissolution medium. Samples were collected at 1 hour intervals and after filtering by Whatmann filter paper, were analyzed spectrophotometrically at 274nm for determination of Doxofylline that were released from mucoadhesive sustained release tablets. [18]

In vitro permeation study

In vitro permeation studies were carried out in a modified Franz's diffusion cells. The medium used for these studies was phosphate buffer pH 6.8, maintained at 37°C ± 0.5°C. Cellulose acetate dialysis membrane was used as a permeation barrier. Samples were collected at each one hour interval upto12 hour and analyzed for drug content with a UV spectrophotometer set at 274nm. The permeation studies for all the formulations were carried out thrice and average were taken. [18, 19]

Ex vivo permeation study of buccal mucoadhesive tablets

Ex vivo permeation study of Doxofylline mucoadhesive buccal tablet was carried out on goat buccal mucosa membrane (as semi permeable membrane) using modified Franz diffusion cell with a diffusion area of 17.35 cm^2 with the acceptor compartment volume capacity of 45 ml and maintained at 37 ± 0.5°C. Fresh goat buccal mucosa was mounted between the donor and receptor compartments. The mucoadhesive tablet was placed into the donor compartments and clamped together. The donor compartment was filled with 1 ml of phosphate buffer pH 6.8. The receptor compartment was filled with phosphate buffer pH 6.8 and the hydrodynamics in the compartment was maintained by stirring with a magnetic bead at uniform slow speed. 5 ml samples were withdrawn at pre-determined time intervals and replaced with 5ml of same fresh buffer. Then the samples were analyzed using an UV spectrophotometer at 274 nm for the amount of Doxofylline absorbed through buccal mucosa membrane. [19, 20]

Characterization of the *in vitro* drug release profile

The rate and mechanism of release of Doxofylline from prepared buccal mucoadhesive tablet were analyzed by fitting the dissolution data into following exponential equations.

Zero order release equation

$$Q = K_0\,t$$

Where *Q* is the amount of drug released at time t and K_0 is the zero order release rate constant.

The first order equation

$$\log(100 - Q) = log100 - K_1\,t$$

Where, K_1 is the first order release rate constant.

The dissolution data was fitted to the Higuchi's equation

$$Q = K_2 t^{1/2}$$

Where, K_2 is the diffusion rate constant.

The dissolution data was also fitted to the Korsmeyer-Peppas equation, which is often used to describe the drug release behaviour from polymeric systems:

$$\log\left(\frac{M_t}{M_\infty}\right) = \log K + n\,logt$$

Where M_t is the amount of drug released at time *t*, M_∞ is the amount of drug release after infinite time, *K* is a release rate constant and *n* is the diffusion exponent indicative of the mechanism of drug release. For matrix tablets, if the exponent $n < 0.5$, then the drug release mechanism is quasi-fickian diffusion (If $n = 0.5$ then fickian diffusion and if the value is $0.5 < n < 1$, then it is anomalous

diffusion coupled with erosion. An exponent value of 1 is indicative of Case-II Transport or typical zero-order and $n > 1$ non-fickian super Case II). The diffusion exponent was based on Korsmeyer-Peppas equation. Hixson-Crowell recognized that area of the particle is proportional to the cubic root of its volume, and derived an equation as follows

$$W_0^{1/3} - W_t^{1/3} = K_s t$$

Where *W*o is the initial amount of drug, *Wt* is the remaining amount of drug in dosage form at time *t*, and K_S is a constant incorporating the surface volume relation. The graphs are plotted as cube root of percent drug remaining versus time. [20, 21]

Stability studies of optimised formulation

The stability studies of optimised formulation of under study were carried out according to ICH guidelines. The optimized formulation was subjected to accelerated stress condition at 40 ^{o}C ± 2 ^{o}C/ 75% ± 5% RH for 90 days. After that period the product was evaluated for friability, hardness, weight variation, thickness, drug content and *in vitro* drug release study. [21]

Stability in human saliva

Stability studies of the buccal tablet were performed for optimized formulation in artificial saliva. The artificial saliva was prepared by using following material listed in **table 2** and filtered through a filter paper. The buccal tablet was placed in separate petri dishes containing 5 ml of artificial saliva and placed in a temperature controlled oven for 9 h at 37 °C ± 0.2 °C at regular intervals (0, 3, 6, and 9 h), the buccal tablet was examined for change in colour, surface area, and integrity. [21, 22]

Table 2: Composition of artificial saliva Materials

Composition	Amount (gm/lit)
Sodium chloride (NaCl)	0.4
Potassium chloride (KCl)	0.4
Calcium chloride($CaCl_2.2H_2O$)	0.8
Sodium di hydrogen phosphate ($NaH_2PO_4.2H_2O$)	0.78
Sodium sulfide ($NaS.9H_2O$)	0.005
Urea	1

RESULTS AND DISCUSSION

By comparing the spectra of Doxofylline and physical mixture used for mucoadhesive tablet under study, the sharp peaks that appear in spectra of Doxofylline at ~ 3110 cm^{-1} due to presence of aliphatic N-H stretching functional group also appears in physical mixture at ~ 2916 cm^{-1} and 3110 cm^{-1}. The characteristic infrared absorption peaks of Doxofylline at ~ 1700 cm^{-1} (C=O stretch), at ~ 1656 cm^{-1} (C=C stretch), at ~ 1547 cm^{-1} (C=N stretch), at ~ 1477 cm^{-1} (C-H bend) and at ~ 1190 cm^{-1} (C-N vibration) were also present in the physical mixture (drug and excipients of mucoadhesive tablet) with no shifting in the major peaks that indicated that there were no interaction occurred between the Doxofylline and excipients used in the preparation of different mucoadhesive tablet formulations. Therefore the drug and excipients are compatible to form stable formulations under study. The FTIR spectra of pure drug Doxofylline and physical mixture used for mucoadhesive tablet under study is shown in **Figure 1**.

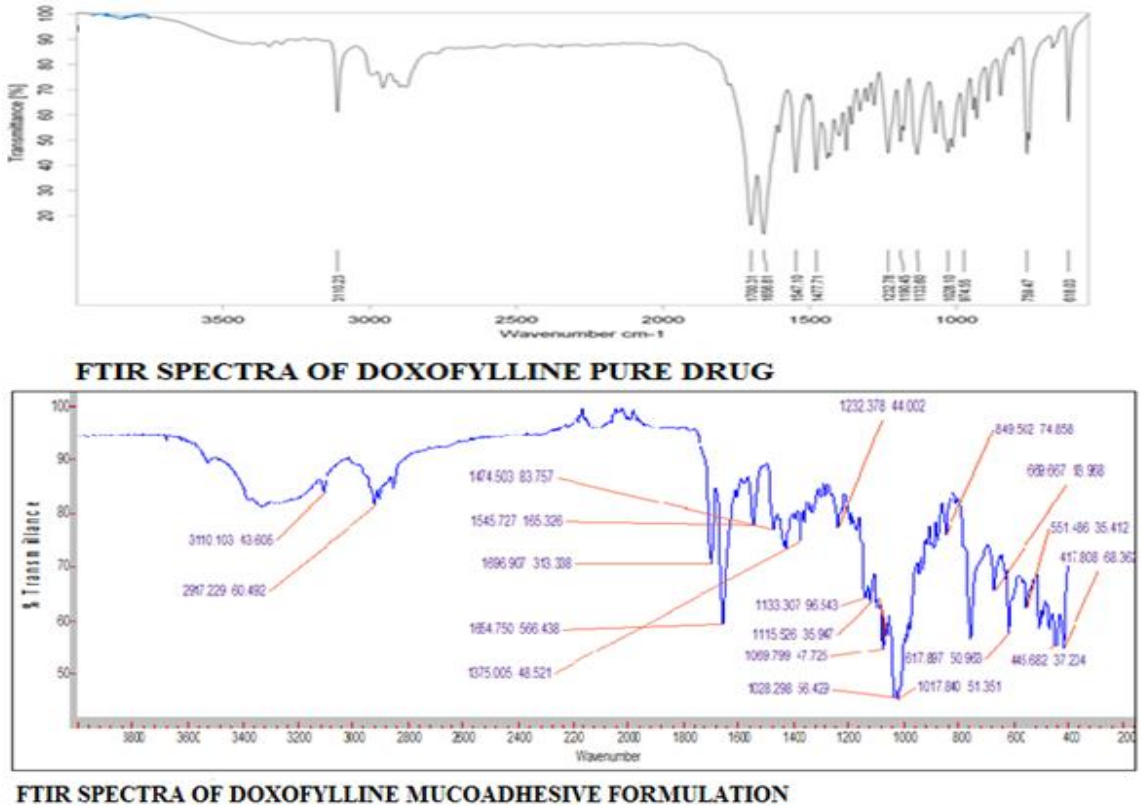


Fig. 1: Compatibility studies through FTIR analysis

From the DSC study, it was observed that the endothermic peak appeared at 146 ^{o}C for pure drug Doxofylline also appeared at 148 ^{o}C in physical mixture used for mucoadhesive tablet respectively. All other endothermic peaks are due to presence of other excipients such as polymers. The DSC thermogram of pure drug and the formulations showed there was no major shifting *i.e* from endothermic to exothermic with appearance of major thermal peaks in the optimised formulation. DSC thermogram of pure drug Doxofylline and physical mixture used for mucoadhesive tablet under study were obtained and shown in **Figure 2.**

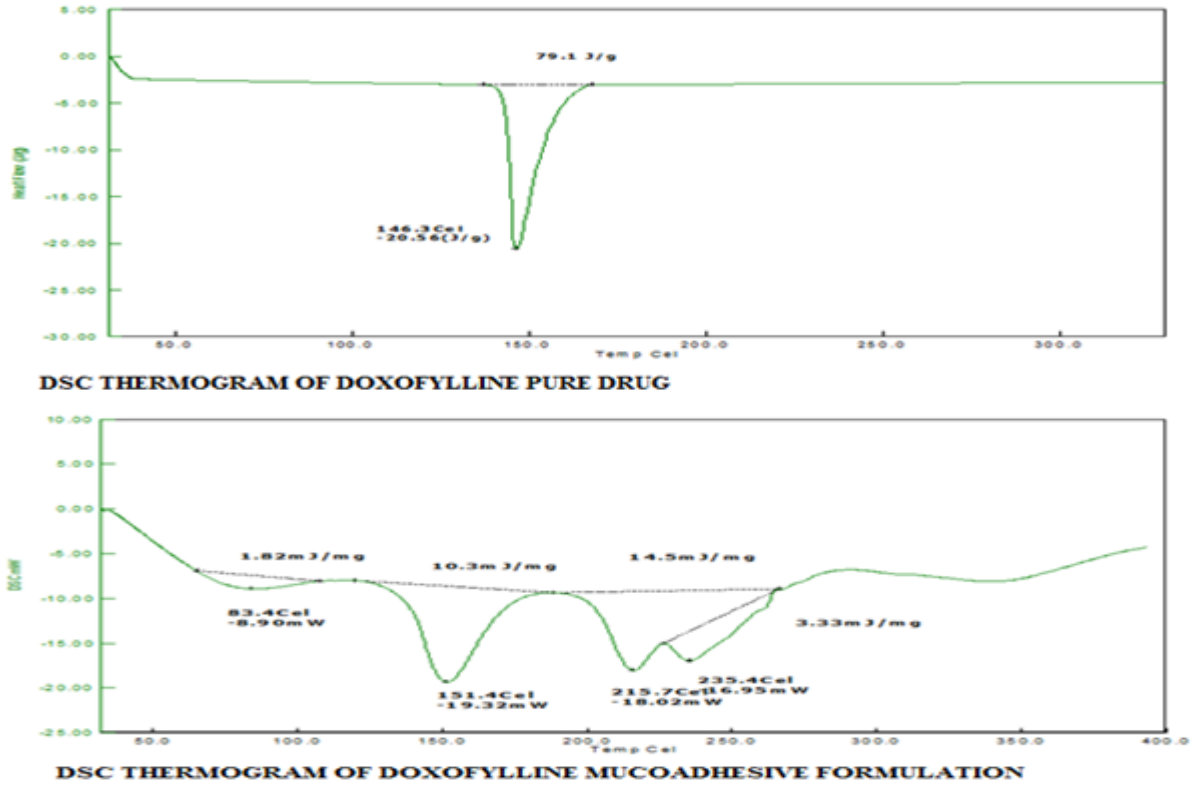


Fig. 2: Compatibility studies through DSC analysis

Evaluations of precompression parameters were usually carried out to ensure the tyre of flow properties of dry powder and granules during tablet punching. The bulk densities of dry powder blends of all formulations were found to be in the range of 0.345±0.08 to 0.394±0.04 g/cm^3 and the tapped densities were found to be in between 0.402±0.07 to 0.449±0.08 g/cm^3. This indicates good packing capacity of powder blends. Bulk density and tapped density measurements found that density of a powder depends on particle packing and that density changes as the powder consolidates.

Values of Carr's index below 16 usually indicate good flowability. Carr's indexes of all the formulations were found "between" 10.19 to 14.25 that indicate excellent flow properties. Formulations $DBMT_9$ and $DBMT_{10}$ having Carr's index more than 14 which indicates presences of non-uniformity of particles.

Hausner's ratio is simple method to evaluate stability of powder column and to estimate flow properties. Low range was observed of Hausner's ratio that indicates good flow ability. In all formulations the Hausner's ratios were found "between" 1.11 to 1.17 that indicates good flow.

Angle of repose is suited for particle > 150µm.Values of angle of repose ≤ 25 generally indicates the free flowing material and angle of ≥ 40 suggest a poor flowing material. The angle of repose is indicative of the flowability of the material. The angle of repose of all formulations fell within the range of 20.40±0.10 to 24.08±0.12 *i.e.* dry powder blends were of good flow properties. The evaluation results of all precompression parameters for the formulation $DBMT_9$ to $DBMT_{16}$ were shown in the **Table 3**.

Table 3: Precompression parameters of dry powder blends of Doxofylline mucoadhesive matrix tablet formulations $DBMT_9$- $DBMT_{16}$

F. No.	Bulk density (gm/cc)	Tapped density (gm/cc)	Angle of repose	Carr's index	Hausner's ratio
$DBMT_9$	0.345±0.08	0.402±0.07	22.21±0.15	14.18	1.16
$DBMT_{10}$	0.361±0.07	0.421±0.09	23.42±0.14	14.25	1.17
$DBMT_{11}$	0.379±0.04	0.430±0.09	21.35±0.12	11.86	1.13
$DBMT_{12}$	0.366±0.05	0.419±0.09	22.46±0.15	12.65	1.14
$DBMT_{13}$	0.364±0.09	0.414±0.10	21.51±0.14	12.08	1.14
$DBMT_{14}$	0.394±0.04	0.449±0.08	24.08±0.12	12.25	1.13
$DBMT_{15}$	0.380±0.07	0.427±0.07	23.25±0.11	11.01	1.12
$DBMT_{16}$	0.388±0.09	0.432±0.09	20.40±0.10	10.19	1.11

All values are expressed as average± SD; (n=3)

All the physical parameters evaluated after compression of Doxofylline mucoadhesive matrix tablets were found to be satisfactory. Typical tablet defects, such as capping, chipping and picking, were not observed. The average thicknesses of the tablets were ranged between 4.18±0.16 mm to 4.31±0.16 mm and all the formulations were within acceptable limits. All the batches showed uniform thickness. Weight variations for different formulations were found to be 4.18±0.24 % to 4.31±0.17 %. The average percentage deviation of all tablet formulations was found within the limit, and hence all formulations passed the test for uniformity of weight as per official requirement. The hardness of all the Doxofylline mucoadhesive matrix tablets formulations were ranged from 5.15±0.4 to 5.28±0.5 kg/cm^2 that were according to the specification. The percentage friability of all the formulations were ranged from 0.58±0.07% to 0.65±0.07% and found within the prescribed limits.

The percentages of drug content of the entire formulations of Doxofylline mucoadhesive matrix tablet ($DBMT_9$ to $DBMT_{16}$) were found "between" 98.45±1.4 to 101.51±1.6 which were within the acceptable limits.

Determination of bioadhesive force is important parameters for mucoadhesive formulation as it decides to what extend the formulation will adhere to the mucosa membrane. Bioadhesive forces were determined for all the formulations ($DBMT_9$ to $DBMT_{16}$). The formulations that contained higher concentration of Ethocel ($DBMT_{13}$) showed more bioadhesive force then other formulations. Formulation $DBMT_{13}$ having 25% of Ethocel had highest bioadhesive force. Next to Ethocel, the formulations that contained higher concentration of METHOCEL K15M showed better bioadhesive force that was noticed in case of $DBMT_{11}$ and $DBMT_{12}$. The physicochemical characterizations of different batches of Doxofylline mucoadhesive tablets are given in **Table 4**.

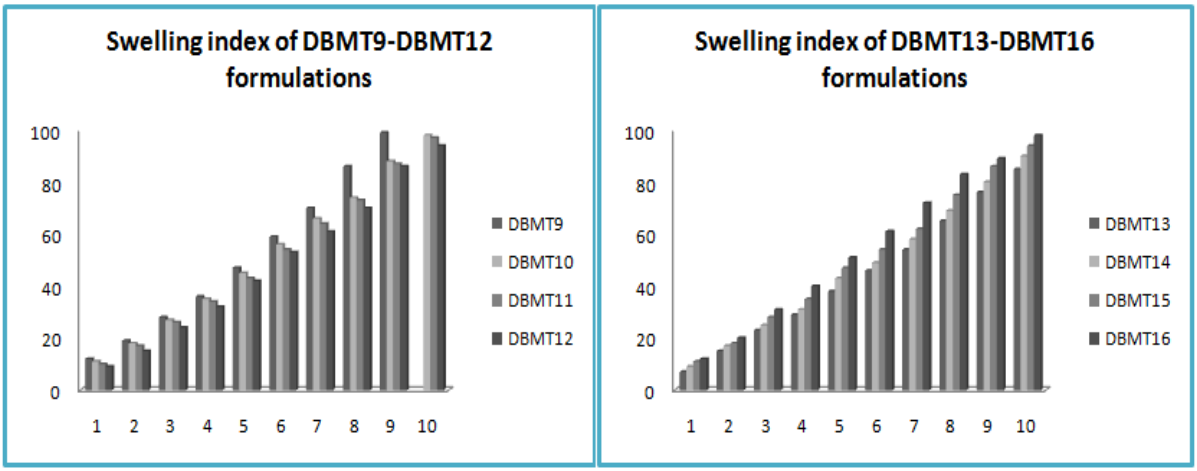
Table 4: Evaluation of post-compression parameters of Doxofylline mucoadhesive matrix tablets formulation $DBMT_9$- $DBMT_{16}$

F. No.	Average hardness (kg/cm^2)	Average Weight Variation (%)	Average friability (% w/w)	Average thickness (mm)	Content uniformity (%)	Bioadhesive strength (N)
$DBMT_9$	5.15±0.4	4.31±0.17	0.65±0.07	4.18±0.16	99.18±1.5	0.412±0.006
$DBMT_{10}$	5.13±0.5	4.18±0.24	0.59±0.05	4.23±0.15	99.15±1.4	0.424±0.004
$DBMT_{11}$	5.18±0.3	4.23±0.26	0.60±0.04	4.19±0.18	101.51±1.6	0.435±0.005
$DBMT_{12}$	5.23±0.6	4.26±0.28	0.60±0.06	4.28±0.14	98.45±1.4	0.452±0.003

$DBMT_{13}$	5.31±0.2	4.19±0.25	0.61±0.05	4.21±0.12	99.48±1.7	0.536±0.006
$DBMT_{14}$	5.25±0.4	4.24±0.31	0.58±0.07	4.27±0.14	99.68±1.5	0.515±0.003
$DBMT_{15}$	5.17±0.2	4.18±0.27	0.60±0.06	4.26±0.15	99.81±1.4	0.497±0.004
$DBMT_{16}$	5.28±0.5	4.25±0.21	0.57±0.05	4.31±0.16	99.72±1.5	0.468±0.005

All values are expressed as average± SD; (n=3)

Swelling study was performed on all the formulations ($DBMT_9$ to $DBMT_{16}$) for 10 hours. The result of swelling index was shown in **Figure 3**. The formulation that contains Methocel K15M and carbopol 934P showed higher swelling indices due to higher hydrophilicity and more water uptake of the polymers. But reverse is observed with the formulations containing higher percentage of Ethocel as it is a hydrophobic polymer. The formulation $DBMT_9$ that contains 25% of carbopol 934P and 10% of Methocel K15M showed higher swelling indices then other formulations but upto 9h, after that material starts deteriorate from surface. The formulation $DBMT_{13}$ that contains 25% of Ethocel and 10% of Methocel K15M showed lower swelling indices but the formulation was intact even after 10h of study. Formulation $DBMT_{13}$ to $DBMT_{16}$, that contain more % of Ethocel had lower swelling index in comparison to other formulation as Ethocel is a hydrophobic polymer.



All values are expressed as mean± SD; (n=3)

Fig. 3: Comparative swelling studies of all the formulations with respect to concentration of polymers used

In order to optimise the *in vitro* drug release along with bioadhesive force, different hydrophilic matrix polymers *viz.*, Methocel K15M, Carbopol 934P and hydrophobic matrix polymer *viz.*, Ethocel were used for 12 different formulations of Doxofylline mucoadhesive matrix tablets. The drug release profiles of different formulations were shown in **Figure 4**. In these studies Methocel K15M was usually used for sustained release effect with bioadhesive strength to some extent. It was observed that using hydrophilic polymer alone caused initial burst release because drug is hydrophilic in nature and maximum release upto 9h to 10h. So one more hydrophobic polymer *i.e* Ethocel was added to reduce the initial burst release and also it had remarkable bioadhesive strength. $DBMT_{15}$ formulation that contained 20% of Methocel K15M and 15% of Ethocel, released the drug upto 12 hour but initial release was very low to elicit therapeutic action. Among all the formulations, $DBMT_{15}$ could be considered as best formulation as the initial release was 10% and

maximum release upto 12h and had remarkable bioadhesive strength that may be adequate criteria for bioadhesive formulation. Formulation containing more % of guar gum showed an initial burst release with maximum release upto 9h and also had lowest bioadhesive strength.

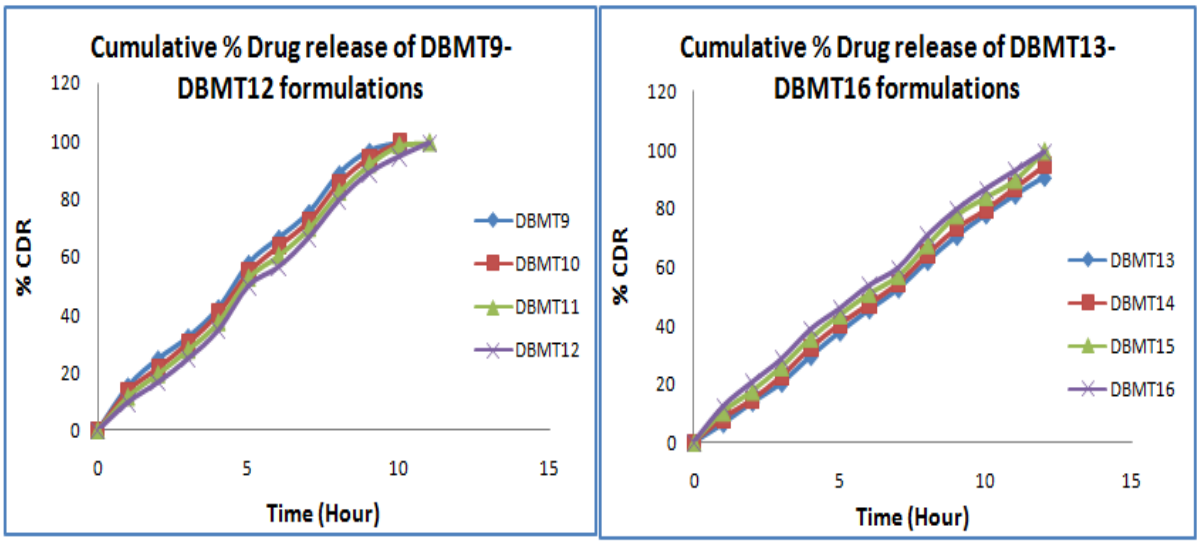


Fig. 4: Comparative dissolution profile of different formulations of Doxofylline mucoadhesive matrix tablet formulations

Ex vivo permeation studies (diffusion studies) were carried out for best formulation ($DBMT_{15}$) using goat buccal mucosa and compared with *in vitro* drug release studies (dissolution studies). From the above studies, the cumulative percentage of drug release for *in vitro* dissolution studies was **99.25** % within 12h whereas it was 86.25% within 12h for *ex vivo* studies. The difference in drug release profiles may be attributed due to low permeability of the drug. Both the release profile were correlated on point to point basis and the correlation co-efficient was found 0.993 which indicates good co-relation level between *in vitro* and *ex vivo* release profile which was shown in **Figure 5**.

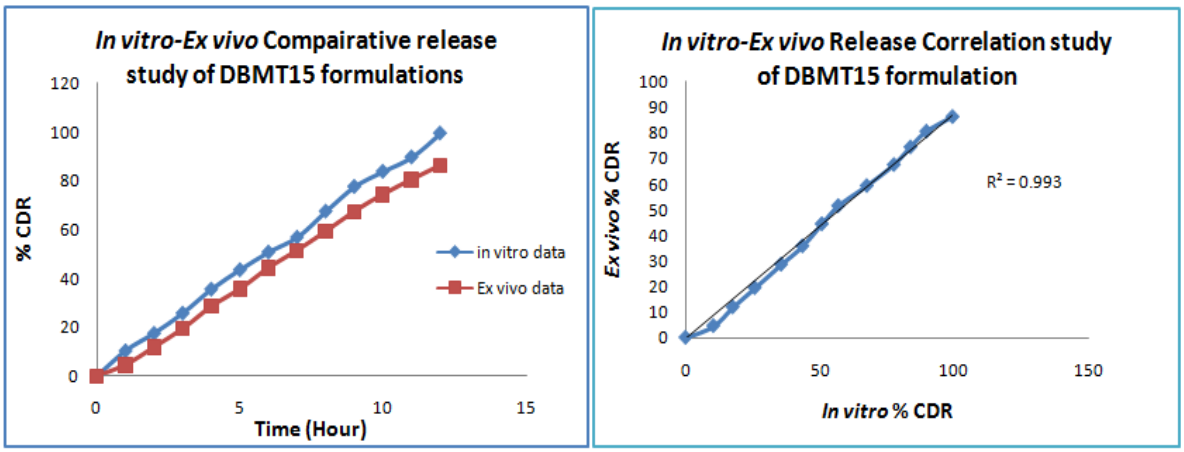


Fig. 5: *In vitro* and *Ex vivo* comparative release profile of best formulation $DBMT_{15}$

The *in vitro* dissolution data of best formulation $DBMT_{15}$ were fitted in different kinetic models viz. zero order, first order, Higuchi, Hixon-Crowell and Korse-Meyer Peppa's and the

graphs were plotted (**Figure 6 & 7**). The zero-order plots were found to be fairly linear as indicated by their highest regression values (0.997). The release exponent 'n' for optimised formulation $DBMT_{15}$ was found to be 0.932 ($0.5 < n < 1$), which appears to indicate a coupling of the diffusion and erosion mechanism so-called anomalous diffusion. So in present study *in vitro* drug release kinetic of best formulation ($DBMT_{15}$) followed zero order release kinetic models and drug release mechanism is anomalous diffusion coupled with erosion. Regression values of *in vitro* release kinetic study of best formulation Doxofylline mucoadhesive matrix tablet ($DBMT_{15}$) is represented in **Table 5**.

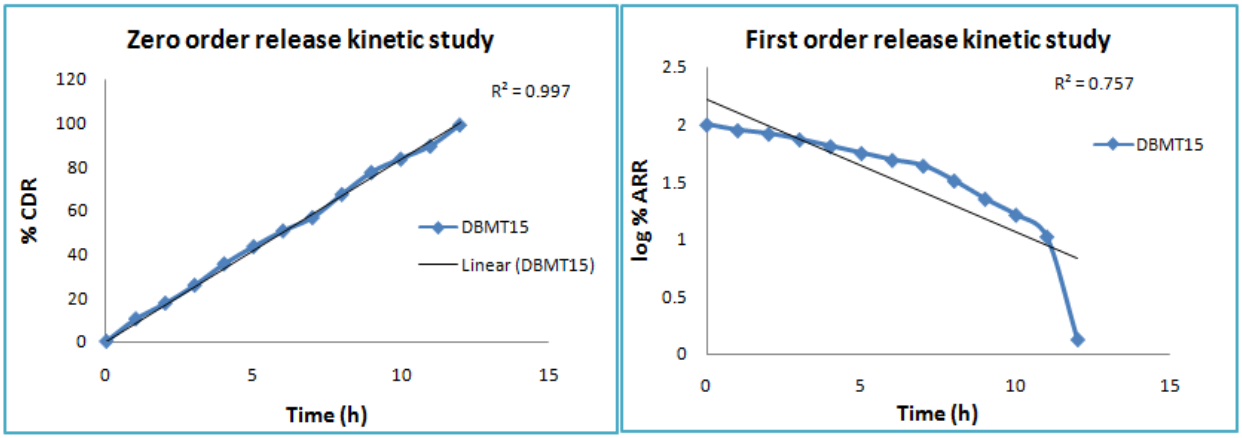


Fig. 6: *In vitro* release kinetic plot of best formulation of Doxofylline mucoadhesive matrix tablet ($DBMT_{15}$)

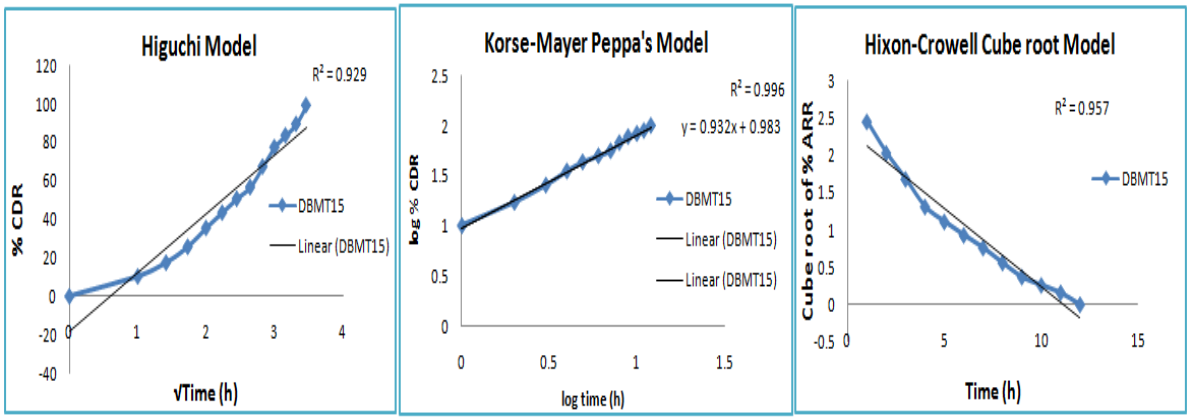


Fig. 7: Mechanism of *in vitro* release plot of best formulation of Doxofylline mucoadhesive matrix tablet ($DBMT_{15}$)

Table 5: Regression values of *in vitro* release kinetic study of best formulation Doxofylline mucoadhesive matrix tablet ($DBMT_{15}$)

Formulation	R^2value of Zero order	R^2 value of 1st order	R^2value of Higuchi model	R^2value of Hixon-Crowell model	R^2value of Peppa's model	'n' value of Peppa's model
$DBMT_{15}$	0.997	0.757	0.929	0.957	0.996	0.932

The optimised formulation ($DBMT_{15}$) was selected for the accelerated stability studies. The Doxofylline mucoadhesive matrix tablets did not show any significant change in physicochemical parameters *i.e* physical appearance, weight variation, hardness, friability, swelling studies, drug content, bioadhesive strength and *in vitro* drug release characteristics. Thus, it was found that the mucoadhesive tablets of Doxofylline ($DBMT_{15}$) were stable under short term storage conditions for at least 3 months. The results of *in vitro* release profile and physicochemical parameters of best formulation at different time interval for accelerated stability conditions were shown in **Figure 8 & Table 6**.

Table 6: Comparative physicochemical characterization of $DBMT_{15}$ at accelerated conditions (40 °C ± 2 °C/ 75% ± 5% RH)

Sl. No.	Physicochemical characteristics	Initial	After 30 days	After 60 days	After 90 days
1	**Physical appearance**	Pale white, circular, concave smooth surface without any cracks	No change	No change	No change
2	**Weight variation**	4.18±0.27	4.23±0.16	4.31±0.34	4.37±0.42
3	**Hardness**	5.17±0.2	5.23±0.5	5.32±0.4	5.42±0.5
4	**Friability**	0.60±0.06	0.63±0.05	0.68±0.07	0.71±0.04
5	**Swelling index**	94 ±1.51	92±1.21	91 ±1.32	90 ±1.39
6	**Drug content**	99.81±1.4	97.45±1.3	95.52±1.4	92.52±1.5
7	**Bioadhesive strength (N)**	0.497±0.004	0.484±0.006	0.446±0.005	0.423±0.007

All values are expressed as mean± SD; (n=3)

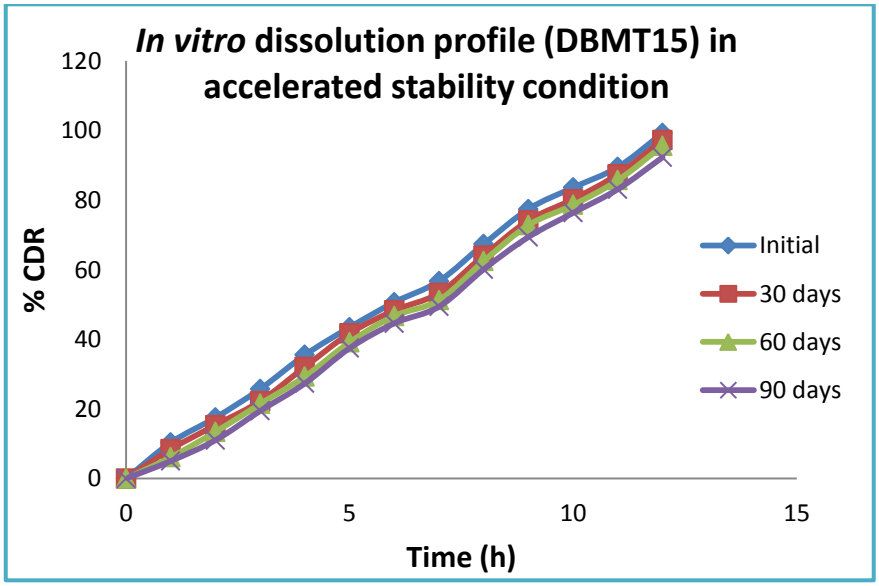


Fig. 8: Comparative *in vitro* dissolution profile of ($DBMT_{15}$) at accelerated conditions (40 °C ± 2 °C/ 75% ± 5% RH)

The stability studies performed in artificial human saliva that was prepared in the laboratory would be more accurate to mimic the stability of the Doxofylline mucoadhesive buccal tablet in oral cavity *in vivo*. Based on the results of *ex vivo* mucoadhesion, *in vitro* release studies, formulation $DBMT_{15}$ was selected for stability study. Stability studies in prepared artificial human saliva showed no change in the colour of Doxofylline buccal tablets, which would have happened if drug was

unstable in human saliva. Results reveal that the buccal tablets are having sufficient stability in the prepared artificial saliva. The thickness and diameter of tablets slightly changed due to swelling of the polymers in prepared artificial saliva but buccal tablets did not collapse till the end of studies confirming that the device strength was sufficient.

CONCLUSION

In the present work Doxofylline buccal mucoadhesive matrix tablet were successfully developed. The major challenge in this work was to study the effect of various low density polymers on *in vitro* release rate of buccal mucoadhesive of Doxofylline with adequate bioadhesive force for prolonging the drug residence time in buccal mucosa. The mucoadhesive strength and *in vitro* drug release effect of different types of low density matrix forming polymers Ethocel, Carbopol 934P and Methocel K15M were studied. FTIR and DSC studies revelled that there is no chemical and thermal interaction between drug and polymers used in the present studies. The hydrophilic polymer like Methocel K15M, Carbopol 934P and hydrophobic polymer like Ethocel, having good bioadhesive nature was successfully examined. Formulation $DBMT_{15}$ that contained 25% of Methocel K15M and 10% Ethocel showed sustained drug release for 12 hour (99.25%) and had adequate bioadhesive strength, emerged as best formulation. Increase in proportion of hydrophilic polymer (Carbopol 934P) caused initial burst release effect and maximum release upto 10 hour achieved. *In vitro* drug release profiles of best formulation were compared with *ex vivo* drug diffusion studies and *in vitro-ex vivo* correlation were established. Kinetic of *in vitro* drug release of optimized formulation $DBMT_{15}$ found to be zero order having drug release mechanism as anomalous diffusion coupled with erosion. The stability studies were carried out in artificial human saliva and the optimised formulation were found to be stable without any remarkable physical changes. Thus from the results of the current study clearly indicate, a promising potential of the Doxofylline buccal mucoadhesive system as an alternative to the conventional dosage form as it enhance bioavailability of the Doxofylline by bypassing the first pass metabolism and by producing sustained release effect for sustainable Asthma. However, further clinical studies are needed to assess the utility of this system for patients suffering from Asthma.

Acknowledgment

The authors are thankful to Aurobindo Pharma Ltd, Hyderabad, India and Dr Reddy's laboratories Ltd., Hyderabad for providing gift samples of drug and polymer. Authors are also thankful to the chairman & principal Anwarul Uloom college of Pharmacy, Hyderabad, Telngana, for permitting to carry out research work.

REFERENCES

[1]. Wong CF, Yuen KH, Peh KK. Formulation and evaluation of controlled release Eudragit buccal patches. Int J Pharm 178, 1999, 11-22.

[2]. Remunnan Lopez C, Portero A, Vila Jato JL, Alonso MJ. Design and evaluation of Chitosan / Ethocel mucoadhesive bilayered devices for buccal drug delivery. J Control Release 55, 1998, 143-52.

[3]. Medline plus A. Service of the U.S National Library of Medicine and the National Institutes of Health. Available from: http://www. medlineplus.gov 2008.

[4]. Panda *et al.* Process optimization, formulation and evaluation of hydrogel {guargum-g-poly(acrylamide)} based doxofylline microbeads, Asian J Pharm Clin Res, 7(3), 2014, 60-65.

[5]. Prasanna RI, Anitha P, Chetty CM. Formulation and evaluation of bucco-adhesive tablets of Doxofylline. Int J. Pharma Investig 1, 2011, 182-91.

[6]. Gavin P. Andrews, Thomas P. Laverty, David S. Jones; Mucoadhesive polymeric platforms for controlled drug delivery. European Journal of Pharmaceutics and Biopharmaceutics. 71, 2009, 505–518.

[7]. S.Velmurugan, P.Srinivas, formulation and in vitro evaluation of Losartan potassium mucoadhesive buccal tablets, Asian J Pharm Clin Res, 6(3), 2013, 125-130.

[8]. Swapnil R. Chaudhari, Amol A.Harsulkar, Design and *in vitro* evaluation of mucoadhesive buccal tablets of carvedilol, Int.J.PharmTech Res. 4(4), 2012.
[9]. Panwar MS, Tanwar YS. Evaluation of stability of Diltiazem hydrochloride floating microspheres at normal and accelerated conditions. J Pharm Biomed Sci. 05(01), 2015, 57-60.
[10]. Patel VM, Prajapati BG, Patel HV, Patel KM. Mucoadhesive bilayer tablets of Propranolol hydrochloride. AAPS PharmSciTech, 8, 2007, E77.
[11]. Patel VM, Prajapati BG, Patel MM. Formulation, evaluation, and comparison of bilayered and multilayered mucoadhesive buccal devices of Propranolol hydrochloride. AAPS PharmSciTech, 8, 2007, 22.
[12]. Shanker G, Kumar CK, Gonugunta CS, Kumar BV, Veerareddy PR. Formulation and evaluation of bio adhesive buccal drug delivery of Tizanidine hydrochloride tablets. AAPS Pharma Sci Tech, 10, 2009, 530-9.
[13]. Gupta A, Garg S, Khar RK. Measurement of bioadhesive strength of mucoadhesive buccal tablets: Design of an *in vitro* assembly. Indian Drugs 30, 1993, 152-5.
[14]. Prajapati ST, Patel PB, Patel CN. Formulation and evaluation of sublingual tablets containing Doxofylline. Int J Pharma Investig, 2, 2012, 162-8.
[15]. Rana Abu-Huwaij, Rana M. Obaidat, Kamal Sweidan, and Yusuf Al-Hiari, Formulation and In Vitro Evaluation of Xanthan Gum or Carbopol 934-Based Mucoadhesive Patches, Loaded with Nicotine, AAPS PharmSciTech, 12(1), 2011.
[16]. Kumria R, Gupta V, Bansal S, Wadhwa J, Nair AB. Oral buccoadhesive films of ondansetron: Development and evaluation. Int J Pharma Investig; 3, 2013, 112-8.
[17]. Vishnu M. Patel, Bhupendra G. Prajapati, and Madhabhai M. Patel, Formulation, Evaluation, and Comparison of Bilayered and Multilayered Mucoadhesive Buccal Devices of Propranolol Hydrochloride, AAPS PharmSciTech 8(1), 2007.
[18]. Dangi AA, Zalodiya PB. Formulation and evaluation of carvedilol melt-in-mouth tablet using mucoadhesive polymer and PEG-6-stearate as hydrophilic waxy binder. Int J Pharma Investig 2, 2012, 183-8.
[19]. Vishnu M. Patel, Bhupendra G. Prajapati, Harsha V. Patel, and Karshanbhi M. Patel, Mucoadhesive Bilayer Tablets of Propranolol Hydrochloride, AAPS PharmSciTech 8(3), 2007.
[20]. Shanker G, Kumar CK, Gonugunta CS, Kumar BV, Veerareddy PR. Formulation and evaluation of bio adhesive buccal drug delivery of tizanidine hydrochloride tablets. AAPS Pharma Sci Tech 10, 2009, 530-9.
[21]. ICH Q1A (R2), Stability Testing Guidelines: Stability Testing of New Drug Substances And Products. The European Agency for the Evaluation of Medicinal Products, CPMP/ICH/2736/99: 2003, 4-20.