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Development of Buccal Patches for Delivery of Darifenacin from Beta-Cyclodextrin Complexes

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ABSTRACT

Drug-cyclodextrin complexes improve aqueous solubility and dissolution rate of poorly water-soluble drugs. Solubilisation followed by buccal delivery of poorly water-soluble drugs can be advantageous for increasing drug absorption. Darifenacin is an antispasmodic used against urinary incontinence and specifically blocks M3 muscarinic acetylcholine receptors in smooth muscle. M3 receptors are mainly located in exocrine glands, smooth muscle and vascular endothelium. The oral absorption of darifenacin is poor owing to its low solubility. It also has poor bioavailability (15-19%) due to a high rate of first-pass metabolism. Complexation with beta-cyclodextrin was carried out to enhance solubility. The best results were obtained by co-grinding in a 1:1 molar ratio of drug: β-cyclodextrin. The solid inclusion complexes were characterized by DSC, X-ray diffractometry and FTIR. Inclusion complexes showed higher dissolution rates than the pure drug. Controlled-release mucoadhesive patches were prepared with two hydroxypropyl methylcellulose (HPMC) polymers, K100M CR and K15. The patches were assessed for surface pH, folding endurance, swelling, mucoadhesive properties, in-vitro residence time, vapor transmission test and in-vitro (cellophane, egg membrane) and ex-vivo (goat buccal mucosa) release. Formulations Ha2 (2%) HPMC K100M CR and Pa4 (4%) HPMC K15 showed good mucoadhesive strength, in-vitro and ex-vivo residence times, with controlled release for 10 hours.

Keywords: Darifenacin. Cyclodextrin. Complexation. Buccal. Patch.

INTRODUCTION

Buccal mucosa is a potential site for the delivery of drugs to the systemic circulation. A drug administered through the buccal mucosa enters directly into systemic circulation, thereby minimizing first-pass hepatic metabolism and gastrointestinal adverse effects. (Jug et al., 2004) Buccal permeation can be improved by using various classes of transmucosal and transdermal penetration enhancers, such as bile salts, surfactants, fatty acids and derivatives, chelating agents and cyclodextrins. (Verma et al., 2011)

Cyclodextrins are capable of forming inclusion complexes with many drugs by taking up a whole drug molecule, or a part of it, into the cavity of the cyclodextrin molecule. Drug-cyclodextrin complexes can improve the clinical usage of drugs by increasing their aqueous solubility, dissolution rate and pharmaceutical availability.

Solubilisation of poorly water-soluble drugs by complexation with cyclodextrins and then delivery via the buccal or sublingual mucosa may be advantageous for increasing drug absorption. Various bioadhesive mucosal dosage forms have been developed, which include adhesive tablets, gels, ointments and patches. Buccal patches are preferred over adhesive tablets in terms of flexibility and patient comfort. Recently developed bioadhesive polymers have received considerable attention as platforms for buccal controlled delivery, on account of their ability to localize the dosage form in specific regions. Various types of polymer can be used in the buccal patches. Hydration of these polymers results in the formation of an outer gel layer that controls drug release. Hydroxypropyl methyl cellulose (HPMC), the non-ionic cellulose ether, is commonly used in the formulation of muco-adhesive patches. (Nafee et al., 2003; Nazila et al., 2005).

Darifenacin is an anti-muscarinic drug for the treatment of urinary incontinence diseases, for instance an overactive bladder in adults who frequently experience symptoms such as an urgent need to urinate or the leakage of urine. It is given as a single daily dose of 7.5mg. According to patient response, dosage may be increased up to 15 mg, once daily, within 2 weeks of starting the therapy (Haab et al., 2004). Darifenacin shows 98% protein binding (primarily alpha$_1$-acid glycoprotein). Its half-life is 13-19 hours. Steady-state plasma levels are achieved on day 6 of dosing. The bioavailability of darifenacin is 15% for a 7.5 mg and 19% for a 15 mg dose. T_{max} is approximately 7 h. Oral administration has shown poor absorption, due to poor water-solubility and high first-pass metabolism. The aim of the present study was to prepare darifenacin beta-cyclodextrin complexes to enhance solubility and develop

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a controlled-release buccal delivery system for better bioavailability.

MATERIALS AND METHODS

Materials

Darifenacin was kindly donated by Microlab Ltd, Bangalore, and beta-cyclodextrin (β-CD) by Gangwal chemicals, Mumbai. HPMC K100CR and HPMC K15 were gifts from Colorcon Asia Pvt Ltd, India, Mumbai. All other materials and solvents used were of analytical reagent grade.

Drug characterization

The melting point of darifenacin was measured by the capillary method. Its solubility was determined in various solvents by UV spectrophotometry. The UV spectrum (Varian Cary 100) was recorded in the range 200-400 nm, in solutions of 10-50 µg/mL darifenacin in distilled water, pH 6.8 buffer and 0.1 N HCl. The wavelength of maximum absorption (λ_{max}) in this range was found and then a calibration (analytical) curve was prepared at that wavelength. The infra-red spectrum was recorded in a Varian 640 FTIR spectrophotometer (Varian, Australia). (Nalluri et al., 2005; Vanshiv et al., 2008).

Phase solubility studies

Phase solubility studies were performed by the method reported by Higuchi and Connors (Higuchi et al., 1965). An excess of darifenacin was added to 20 mL of aqueous solutions containing various concentrations of β-CD (2.5×10^{-3} to 2×10^{-2} M). The suspensions were vigorously shaken at 25 ± 1 °C for 3 days. After equilibrium was attained, samples were filtered through a 0.45 µm Millipore membrane filter and suitably diluted with distilled water. Darifenacin concentration was determined spectrophotometrically at λ_{max}. The apparent 1:1 stability constant, *K*s, was calculated from the phase solubility diagram (plot of apparent drug solubility against β-CD content), by using the equation:

$$\boldsymbol{K}\mathbf{s} = \mathbf{Slope} / \boldsymbol{S}_0(\mathbf{1} - \mathbf{Slope}) \text{ ---------- (1)}$$

where S_0 is the solubility of darifenacin in the absence of β-CD.

Stability study of drug in various aqueous solvents and calibration curve

Darifenacin was dissolved in 0.1N HCl, pH 6.8 buffer and distilled water and sonicated for 10 minutes. After 48 h, samples were analysed by UV spectrophotometry to find the stability of the drug in solution. Analytical curves were prepared in 0.1N HCl, pH 6.8 buffer and distilled water in the range 10-50 µg/mL.

Preparation of solid complexes

Complexes were prepared in the molar ratio of 1:1 darifenacin: β-CD by the following methods:

Physical mixture (PM): Physical mixtures of drug and β-CD were made by mixing equimolar amounts of the two powders (#100) in a pestle and mortar (Nalluri et al., 2005).

Kneading (Kn): Drug and β-CD were ground in a mortar with a small volume of water-methanol solution. The thick slurry was kneaded for 45 min and dried at 40°C. The dried mass was pulverized and sieved through mesh #100 (Nalluri et al., 2005).

Co-evaporation (COE): An aqueous solution of β-CD was added to an alcoholic solution of the drug. The resulting mixture was stirred for 1 h and evaporated at a temp of 45°C until dry. The dried mass was pulverized and sieved through mesh #100 (Nalluri et al., 2005).

Co-grinding (COG): The drug was ground with a minimal quantity of methanol in a glass mortar until it was dissolved. β-CD was added and the suspension was ground rapidly at room temperature until the solvent evaporated (Friedrich et al., 2005).

Freeze–drying (FD): The physical mixture of drug and β-CD was added to 500 mL double-distilled water and stirred for 5 days. The suspension was lyophilized in an ilShin® Freeze Dryer (Ede, Netherlands), and the resulting freeze-dried complex was pulverized and sieved to <38µm (Tsinontides et al., 2004).

Melting (MELT): The drug-β-CD equimolar mixture was melted in sealed ampoules and then cooled slowly at room temperature. The product was stored in desiccators. The solidified product was transferred to a clean mortar, ground and passed through sieves no.16 and 20 (Van et al., 1998).

Spray-drying (SPD): A 1:1 molar mixture of drug and β-CD was dissolved in 250 mL water. The mixture was immediately sprayed for the duration of about an hour, under the following set of conditions: atomizing air-flow rate 400 NL/h, spray nozzle diameter 0.7mm at the atomizing pressure of 2 kg/cm^2 with a feed rate of 4mL/min, inlet temperature 120°C, outlet temperature 90 °C ± 2 °C, vacuum in system 60 mmWc and aspiration rate 40 mBar. After 1 hour, the product was collected, packed, doubly wrapped in aluminium foil and stored in a desiccator (Patil et al., 2010).

Assessment of complexes

Percent yield study

The prepared complexes were weighed and the yield was calculated for each preparation using the following formula:

$$[\%\ \text{Yield} = (a/b) \times 100] \text{ ---------- (2)}$$

where 'a' is the practical weight of complex prepared and 'b' is its theoretical weight (Vasconcelos et al., 2007).

Determination of drug content of complex

Drug: β-CD complex equivalent to 10 mg of drug was stirred with 100 mL methanol for 60 minutes; the solution was filtered and treated as stock solution containing 100 µg/mL drug. This stock solution was diluted to prepare a 10µg/mL solution, whose drug content was determined spectrophotometrically (Shirsand et al., 2009).

Saturation Solubility Studies

Excess quantities of the drug, PM and inclusion complexes were added to 250 mL conical flasks containing 25 mL of double-distilled water. The sealed flasks were shaken for 48hours at room temperature and then left to reach equilibrium for three days. Aliquots were withdrawn through Whatman filter paper and drug content analysed spectrophotometrically (Vanshiv et al., 2008).

Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) has been used to study the solid-state interaction of the drug with β-CD (Vanshiv et al., 2008). Samples of the solid complexes, pure drug, and β-CD were transferred to a flat-bottomed aluminium pan and heated from 25 to 300 °C at a constant rate of 10° C/ min, under purging with nitrogen (50 mL/min), using alumina as a reference standard, in a differential scanning calorimeter (DSC-7, Perkin Elmer).

X-ray diffractometry (XRD)

Powder XRD (pXRD) has been used extensively to study interactions between drugs and β-CD. The diffraction studies were carried out in a powder X-ray diffractometer (STOESTADI-P). The samples were rotated during data collection to reduce orientation effects. PXRD patterns of the solid complex, pure drug, and β-CD were recorded from 2θ = 5 to 50° at 40 kV and 30 mA (Vanshiv et al., 2008).

FTIR spectroscopy

Samples were analyzed by the potassium bromide pellet method in an IR spectrophotometer (Varian, Australia) in the region from 4000 to 400 cm^{-1} (Vanshiv et al., 2008).

Dissolution study of complexes

Dissolution of the complexes of darifenacin and β-CD was studied in phosphate buffer pH 6.8, in a USP type II dissolution apparatus [Make: Electrolab; Model: TDT-08L]. Temperature was maintained at 37±2°C. Dose equivalent amounts of complexes were weighed and enclosed in muslin silk bags. These bags were tied with paddles and dissolution was studied at 60 rpm for 2 hours. (U.S. Pharmacopoeia, 2009).

Stability Studies

Selected complexes were packed in amber-colored bottles, tightly plugged with cotton wool and capped with aluminium. They were stored at 25ºC / 60% RH, 30ºC / 65% RH, and 40ºC / 75% RH for 3 months and then examined for physical changes such as colour and texture, and analysed for drug-cyclodextrin interaction and drug content.

Complex Formulation

Freeze-dried, followed by spray-dried, complexes showed higher saturation solubility and dissolution rates than co-ground complexes. However, in view of the high cost of equipment and processing and low yield of these methods, they are not preferred. For the present study, co-ground complexes were used to develop buccal patches.

Buccal patch preparation

The patches were prepared by the solvent casting method (Noha et al., 2003). Blank patches were prepared first, with various amounts of the polymer HPMC K100CR (Ha) or HPMC K15 (Pa). The plasticizer used was propylene glycol. The contents of the HPMC polymers were varied from 1 to 5% and that of the plasticizer from 20-30% (Tables 1 and 2). The concentrations of polymer and plasticizer were chosen to optimise the plasticity of the films. The samples were packed in aluminium foil and stored in a glass container. For the medicated patches, a calculated amount of drug-β-CD complex (equivalent to 7.5 mg of darifenacin) was incorporated in the above polymer solution, before the addition of plasticizer. The film was then cast in aluminium moulds and dried in an oven. *In-vitro* release was studied for all the trial batches and Ha2 (2%), for the HPMC K100CR patches, and Pa4 (4%), for the HPMC K15 patches, afforded the best profiles of drug release.

Table 1: HPMC K100CR formulations

Ser. No.	Formulation batch	Darifenacin-β-CD complex (mg)	HPMC K100CR (%)	Propylene glycol (%)
1	Ha 1	27.5	1	20
2	Ha 2	27.5	2	20
3	Ha 3	27.5	3	25
4	Ha 4	27.5	4	30
5	Ha 5	27.5	5	30

Table 2: HPMC K15 formulations

Ser. No.	Formulation batch	Darifenacin- β-CD complex (mg)	HPMC K15 (%)	Propylene glycol (%)
1	Pa 1	27.5	1	20
2	Pa 2	27.5	2	20
3	Pa 3	27.5	3	25
4	Pa 4	27.5	4	25
5	Pa 5	27.5	5	30

A ssessment of Buccal Patch

Square buccal patches (1cm x 1cm) were tested as follows:

Patch thickness

Thickness of 5 patches was measured in every batch with a micrometer screw gauge.

Surface pH

Agar plates were prepared by dissolving 2% (w/v) agar in heated isotonic phosphate buffer (IPB) of pH 7.4, with stirring, and pouring the solution into Petri dishes and leaving to gell at room temperature (Noha et al., 2003). Buccal patches were left to swell for 2 h on the surface of these plates. The surface pH was measured by means of a pH paper placed on the surface of the swollen patch. A mean of three readings was recorded.

Folding endurance test

This test was done by repeatedly folding the patch at the same place up to a maximum of 300 times or until it broke (Khanna et al., 1997).

Swelling index

The samples were allowed to swell on the surface of agar plates in an incubator maintained at 37°C. Increase in the weight or diameter was noted after a preset time interval (Thimmasetty et al., 2008). The percent swelling (%S) was calculated by the following equation:

$$\%S = (Wt\text{-}W_0/W_0)*100 \text{ ---------- (3)}$$

where Wt is the weight of the patch after time t and W_0 its initial weight.

***In-Vitro* Bioadhesive Test**

The *in-vitro* bioadhesive test of the buccal patch was done with a chicken pouch as a model mucosal membrane (Parodi et al., 1996). The tissue was taken from a chicken after slaughter and its contents and surface fats were removed. It was stored frozen in simulated artificial saliva solution and allowed to thaw to room temperature before study. A rectangular piece of the tissue was cut and glued with adhesive on the ground surface of two tissue holders made of Plexiglas. One centimetre of the buccal film was then placed between the two tissue surfaces and pressed in contact with each surface, uniform and constant light pressure being applied by the fingers for one minute to facilitate adhesion. The upper tissue holder was allowed to hang on an iron stand with the help of aluminium wire fastened to a hook fixed on the back of the holder. A pre-weighed light-weight polyethylene bag was attached to the hook on the back of the lower tissue holder with aluminium wire. After a pre-load time of one minute, water was added to the polyethylene bag from an intravenous infusion set, at a rate of 2 drops per second, until the lower tissue was detached by the weight of the water added.

The water collected in the bag was weighed and expressed as the tensile stress required to detach the patch, by means of the following equation:

$$\text{Detachment stress (dyne/ cm}^2) = (m*g/A) \text{ ----- (4)}$$

where m is the weight of the water infused at detachment, g the acceleration due to gravity (taken as 980 cm/s^2) and A the area of tissue exposed (cm^2).

Determination of *in-vitro* residence time

The *in-vitro* residence time was determined with a locally modified USP disintegration apparatus, based on the apparatus used by Nakamura (Nakamura et al., 1996). The disintegration medium was composed of 800 mL of pH 6.6 IPB maintained at 37 ± 0.5°C. A 3-cm length of porcine buccal mucosa was glued to the surface of a glass slab. The mucoadhesive patch was hydrated on one surface with 15µL of pH 6.6 IPB and then the hydrated surface was brought into contact with the mucosal membrane. The glass slab was fixed to the apparatus in a vertical position and allowed to move up and down, so as to immerse the patch completely in buffer solution at the lowest point and lift it out at the highest. The time necessary for complete erosion or detachment of each batch of patches from the mucosal surface was recorded.

Water vapour transmission rate (VTR)

Vapour transmission from the patch was determined. A glass bottle (length 5 cm, with narrow mouth of internal diameter 0.8 cm) was filled with 2 g anhydrous calcium chloride and an adhesive (Feviquick®) was spread on its rim. The patch was fixed to the adhesive (across the mouth) and the assembly was placed in a chamber of constant humidity (produced by a saturated solution of ammonium chloride) and temperature (37±2 °C). The weight gains of the bottle assembly after 24 h, 3 days and 1 week were recorded (Nakamura et al., 1996). The experiments were carried out in triplicate and the water VTR was calculated as follows:

VTR = (Mass of moisture transmitted) / (Area x Time) -------- (5)

Content uniformity

Buccal patches were dissolved in 100 mL of pH 6.8 phosphate buffer. The amount of drug was measured spectrophotometrically at λ_{max} of 285 nm.

***In-vitro* drug release**

Drug release was tested in a Keshery-Chien diffusion cell with pH 6.8 phosphate buffer medium in both compartments (Isabel et al., 2007). The cellophane membrane was carefully mounted between the two compartments of the cell of internal diameter 2.1 cm (area 3.46cm^2) and receptor compartment volume 12 mL. Temperature was maintained at 37±2°C. The concentration of drug released into the receptor medium was assayed

spectrophotometrically. Results were obtained for patches with various concentrations of the polymers HPMC K100M CR and HPMC K15. Patches Ha2 and Pa4 were selected for the study of release across an egg membrane. All experiments were conducted on 3 replicates.

Ex-vivo drug release

Tissue preparation: The goat buccal mucosa was obtained immediately after sacrifice from a local slaughter house (Kothrud, Pune, India) and transported to the laboratory in IPB, pH 7.4. The buccal mucosa was rinsed with IPB. The mucosa was removed from the underlying muscular layer by cutting the loose connective fibres with a scalpel. Circular pieces were then punched out. The excised mucosa was immersed in isotonic saline at 60 °C for 1min and the epithelium was then peeled from the connective tissue. Samples were dipped in deionised water, dried on a cellulose filter and frozen at −20 °C until use (no more than 3 weeks).

The *ex-vivo* release study was carried out in the same way as the *in-vitro* release study, with the cellophane membrane replaced by the goat buccal mucosal membrane. The two optimized patches were used for the *ex-vivo* study.

Stability studies and ageing

Plain and drug-loaded patches were packaged in aluminium foil and stored in glass bottles closed with screw caps. These bottles were subjected to accelerated stability testing in stability chambers (Thermolab India) maintained at 25ºC / 60% RH, 30ºC / 65% RH and 40ºC / 75% RH for 3 months and analyzed for their physical changes such as colour and texture, drug-polymer interaction, drug content and diffusion study.

RESULTS

Drug characterization

The melting point of darifenacin was found to be 229-236^{0}C. Darifenacin was freely soluble in chloroform (13.4mg/mL), slightly soluble in methanol (8.8mg/mL) and ethanol (8mg/ml) and practically insoluble in water. IR study of darifenacin showed peaks at 3463cm^{-1} (N-H stretching), 1663cm^{-1} (C=O ester stretch) and 1584cm^{-1} (-C=C aromatic). UV analysis of darifenacin in the above solvents showed an absorption maximum at 285 nm.

PART I

Darifenacin–β-CD complexation

The phase solubility diagram for darifenacin–β-CD is shown in Figure 1. The shape of the line indicates an A_L type system. The apparent stability constant, *K*s, was calculated to be 344.0232 M^{-1}. This result indicates good complexation between darifenacin and β-CD.

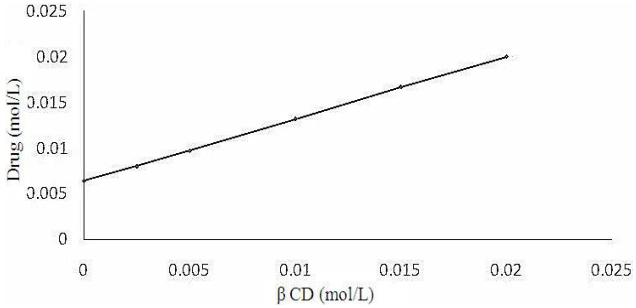

Figure 1: Phase solubility diagram of the Darifenacin- β-CD system.

Gibbs free energy change ($\Delta G_{tr}°$)

In Table 3 it is seen that the Gibbs free energy of solubilisation of the drug by β-CD is negative, indicating a spontaneous process.

Table 3: Gibbs free energy of transfer ($\Delta G_{tr}°$) for solubilization of darifenacin in aqueous solutions of β-CD at 37°C.

Concentration of β-CD (mol/L)	$\Delta G_{tr}°$ (KJ/mol)
0.0025	-0.5529
0.005	-1.03
0.01	-1.793
0.015	-2.376
0.02	-2.823

Stability of drug in various aqueous media and calibration curve

Darifenacin showed neither any shift in the absorption maximum, nor any decrease of absorbance in any of the media, indicating the stability of the drug. Parameters of the analytical equation (y = mx + c) for the drug in these media were: Slope = 0.0050, Intercept = 0.0027, R^2= 0.9997 in distilled water, Slope = 0.0047, Intercept = 0.0027, R^2 = 0.999 in 0.1 N HCl and Slope = 0.0040, Intercept = 0.0028, R^2 = 0.998 in pH 6.8 buffer.

Percent yield

Percent yield was between 46.47 and 87.54%. The lowest yield was in spray drying and the highest in physical mixture complexes. In the case of spray drying, the yield was least because of operational loss or the low efficiency of the instrument.

Drug content of complexes

The drug content of complexes was found to be within the range 88.41-96.14%.

Saturation solubility

Saturation solubility of the pure drug was 3.13 mg/mL and that of the complexes was found to be 8.72-19.64

mg/mL. The increase in solubility of the drug in the co-ground complex was 371.21%, while the maximum was 625.47% for the freeze-dried complex (Table 4).

Table 4: Observed parameters of complexes

Drug: β-CD Complex	% Practical Yield	% Drug Content	Saturation Solubility (mg/mL)	% Increase in solubility
Drug	----------	----------	3.13	----------
Physical mixture	87.54±1.89	90.85±0.983	8.72	277.70±3.74
Kneading	83.31±1.34	94.03±1.08	9.03	287.83±2.39
Co-grinding	85.80±2.03	96.14±1.44	11.86	371.21±5.43
Co-evaporation	76.84±2.45	91.87±1.62	9.101	289.24±3.37
Melting	81.5±3.61	89.23±2.3	8.76	278.98±4.19
Freeze-drying	85.02±2.73	91.30±2.54	19.64	625.47±7.83
Spray drying	46.47±4.58	88.41±3.21	17.18	547.13±4.57

Differential scanning calorimetry (DSC)

As shown in Figure 2 (A), the DSC curve of darifenacin showed an endothermic event (a melting peak), with the onset temperature of 235.02 °C, indicating a crystalline polymorph of the drug. A similar peak, corresponding to darifenacin melting, was also evident in the thermogram of the physical mixture (Figure 2 (C)).

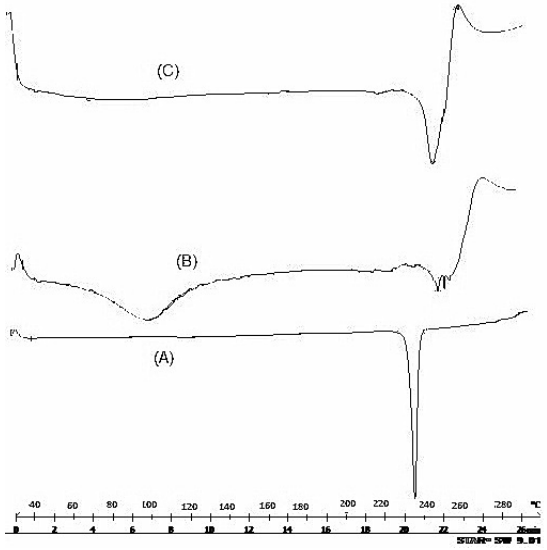

Figure 2: Differential scanning calorimetry (DSC) of darifenacin and β-CD complex.
(A)darifenacin, (B) co-ground complex, (C) physical mixture

X-ray diffractometry

The X-ray diffraction patterns (Figure 3) revealed the crystalline nature of darifenacin, as well as the amorphous state of β-CD. Darifenacin shows major peaks at 2θ = 11.3, 11.4, 11.5, 17.00, 18.2, 20.1, 20.2, 20.3, 25.2, 26.8, 27.3, 27.6, 30.3 and 30.8.

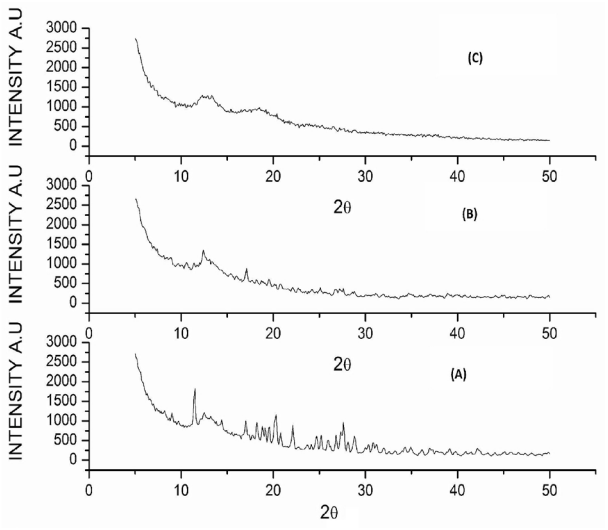

Figure 3: X-ray diffractograms of (A) darifenacin, (B) physical mixture complex and (C) co-ground complex.

FTIR spectra

FTIR spectra are presented in Figure 4. Darifenacin spectra showed a band at 3463 cm^{-1}. It can also be seen that inclusion complexes of darifenacin showed spectra with broader bands at 1663cm^{-1} and 3463 cm^{-1}, suggesting the formation of hydrogen bonds between the carbonyl and N-H groups of darifenacin and the hydroxyl group of the host cavity.

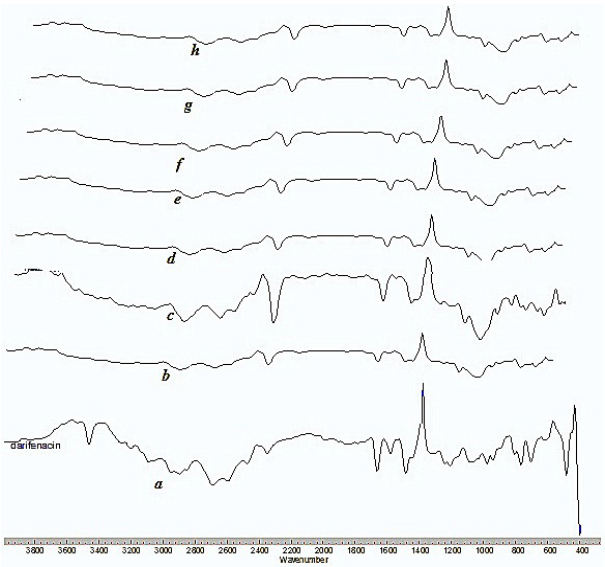

Figure 4: FTIR spectra of darifenacin and complexes. (a) darifenacin, (b) physical mixture, (c) co-ground, (d) co-evaporated, (e) freeze-dried, (f) spray-dried, (g) melted, (h) kneaded.

Dissolution of complexes

In the dissolution test, the co-ground, freeze-dried and spray-dried complexes released above 95% of the drug in two hours. In other complexes, release was less than 90% and, in the pure drug, release was below 40% in 2 hours (Figure 5).

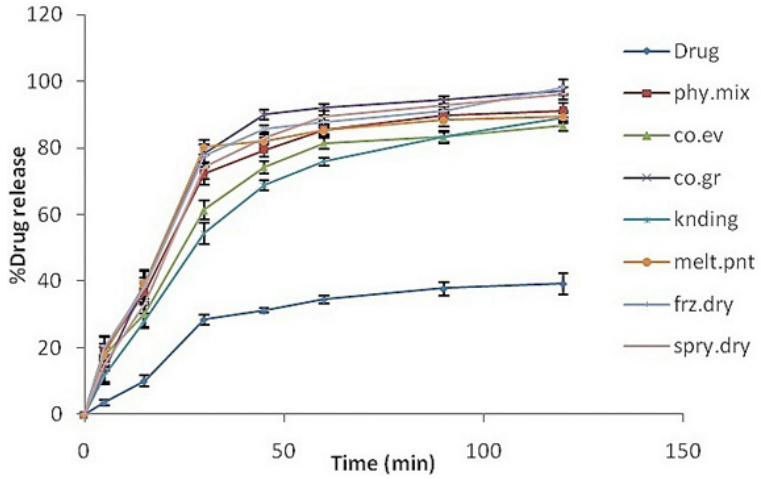

Figure 5: Profile of drug dissolution from various darifenacin- β-CD complexes and the pure drug into pH6.8 buffer

PART II

Assessment of buccoadhesive patches

Buccoadhesive patches of darifenacin alone and its co-ground complex were prepared with HPMC K100CR and HPMC K15.

Thickness uniformity: All the patches had uniform thickness throughout. Average thickness found was about 0.85 mm.

Surface pH: The surface pH of all formulations was in the range 5.7-6.9, near the neutral pH, indicating no mucosal irritation and patient compliance.

Folding endurance: Patches did not show any cracks, even after being folded more than 300 times.

Weight uniformity: The average weight of the patch was about 55.80 mg.

Content uniformity: The results for content uniformity indicated that the drug was uniformly dispersed. Recovery of 88.00 to 94.00 % was possible (Table 5).

Table 5: Assessment of HPMC K100M CR patches

Formulation Code	Thickness (mm)	Weight Uniformity (mg)	Surface pH	Content Uniformity (%)	*In-vitro* drug release(%) (10h)	Swelling index (% weight increase after 3h)
Ha 1	0.63 ± 0.02	48.47 ± 0.59	6.23 ± 0.075	94.97 ± 2.41	89.263 ±1.76	33.37 ± 0.97
Ha 2	0.81 ± 0.04	53.63 ± 0.536	6.73 ± 0.068	97.43 ± 2.07	89.28 ±1.09	35.96 ± 2.96
Ha 3	0.82 ± 0.03	55.86 ± 0.536	6.03 ± 0.091	101.19 ± 3.41	74.236 ±2.32	42.06 ± 1.34
Ha 4	0.86 ± 0.03	58.37 ± 0.642	5.91 ± 0.057	95.22 ± 1.86	63.203 ±2.13	46.99 ± 3.16
Ha 5	1.02 ± 0.07	59.87 ± 0.615	5.98 ± 0.077	93.38 ± 1.42	59.243 ±3.21	48.58 ± 2.09

Swelling studies: The patches had a swelling index of 33.37 ± 0.97- 48.58 ± 2.09 in HPMC K100M CR and 22.7 ± 0.43 - 38.21 ± 2.11 in HPMC K15 patches. The results indicate that the swelling index increased with rising polymer concentration (Tables 5 and 6).

Table 6 : Assessment of HPMC K15 patches

Formulation Code	**Thickness (mm)**	**Weight Uniformity (mg)**	**Surface pH**	**Content Uniformity (%)**	***In-vitro* drug release(%) (10h)**	**Swelling index (% weight increase after 3h)**
Pa 1	0.71 ± 0.04	50.35 ± 0.28	6.33 ± 0.065	96.97 ±2.11	89.19 ±2.48	22.7 ± 0.43
Pa 2	0.79 ± 0.02	55.33 ± 0.36	5.78 ± 0.071	100.83 ±2.09	92.12 ±3.01	26.53 ± 1.15
Pa 3	0.81 ± 0.04	57.41 ± 0.43	6.27 ± 0.061	92.37 ±1.94	85.78 ±3.68	32.11 ± 1.26
Pa 4	0.84 ± 0.04	59.29 ± 0.44	5.87 ± 0.049	94.27 ±2.17	90.67 ±2.54	34.57 ± 1.22
Pa 5	0.92 ± 0.02	58.62 ± 0.31	5.88 ± 0.064	98.29 ±1.93	72.45 ±1.87	38.21 ± 2.11

***In-vitro* Bioadhesive test:** Mucoadhesion involves wetting, interpenetration and mechanical interlocking between mucus and polymer. The strength of mucoadhesion is affected by factors such as molecular mass of polymers, contact time with mucus, swelling of polymer and biological membrane used in study. HPMC K100CR patches (Table 7) showed adhesion force between 0.102 and 0.152N, while HPMC K15 (Table 8) patches had a force of adhesion between 0.096 and 0.120N (Parodi et al., 1996).

Table 7 : Bioadhesive parameters of HPMC K100M CR patches

Formulation Code	Bioadhesive Strength (gm)	Force of Adhesion (N)	Binding Strength ($N\ m^{-2}$)
Ha 1	10.48 ± 0.93	0.102	1027.04
Ha 2	11.56 ± 1.14	0.113	1132.88
Ha 3	11.42 ± 1.37	0.111	1119.16
Ha 4	13.32 ± 1.97	0.130	1305.36
Ha 5	15.56 ± 1.41	0.152	1524.88

Table 8 : Bioadhesive parameters of HPMC K15 patches

Formulation Code	Bioadhesive Strength (gm)	Force of Adhesion (N)	Binding Strength ($N\ m^{-2}$)
Pa 1	9.83 ± 0.64	0.096	963.34
Pa 2	10.13 ± 0.71	0.099	996.74
Pa 3	10.19 ± 0.92	0.099	998.62
Pa 4	11.41± 1.63	0.111	1118.18
Pa 5	12.13 ± 1.71	0.120	1206.38

In- vitro residence time

Observations related to the *in-vitro* residence time, including detachment and erosion, for both plain and medicated patches, indicated good attachment to the mucosal surface without erosion, i.e. 5.7 to 6.8 hours. The HPMC mucoadhesion time was long, because this polymer, although manifesting decisively higher swelling, has a lower affinity for water and hence tends to retain its structure better.

Vapour Transmission rate

Formulations Ha3 and Pa4 (Table 9) showed the highest permeation rates on day seven, while the lowest permeation was found in Ha4 and Pa1.

Table 9: Vapour transmission rate of prepared mucoadhesive buccal patches

Formulation code	Moisture Vapour Transmission ($g.cm^{-2}.h^{-1}$)		
	Day 1	Day 3	Day 7
Ha1	$6.21 \times10^{-3} \pm 0.23 \times10^{-3}$	$3.31 \times10^{-3} \pm 0.26\times10^{-3}$	$0.87 \times10^{-3} \pm 0.48 \times10^{-3}$
Ha2	$8.33 \times10^{-3} \pm 2.46 \times10^{-3}$	$4.05 \times10^{-3} \pm 0.81 \times10^{-3}$	$1.51 \times10^{-3} \pm 0.62\times10^{-3}$
Ha3	$8.53 \times10^{-3} \pm 0.57 \times10^{-3}$	$3.80 \times10^{-3} \pm 0.53 \times10^{-3}$	$1.66 \times10^{-3} \pm 0.07 \times10^{-3}$
Ha4	$9.71 \times10^{-3} \pm 1.82 \times10^{-3}$	$4.78 \times10^{-3} \pm 0.72\times10^{-3}$	$0.82\times10^{-3} \pm 0.72 \times10^{-3}$
Ha5	$8.37 \times10^{-3} \pm 2.19 \times10^{-3}$	$3.77 \times10^{-3} \pm 0.87 \times10^{-3}$	$1.33 \times10^{-3} \pm 0.46\times10^{-3}$
Pa1	$4.87 \times10^{-3} \pm 0.53 \times10^{-3}$	$2.18 \times10^{-3} \pm 0.17\times10^{-3}$	$0.73 \times10^{-3} \pm 0.08 \times10^{-3}$
Pa2	$6.82 \times10^{-3} \pm 3.22 \times10^{-3}$	$2.67 \times10^{-3} \pm 0.81 \times10^{-3}$	$1.31 \times10^{-3} \pm 0.41\times10^{-3}$
Pa3	$7.20 \times10^{-3}\pm 1.33 \times10^{-3}$	$2.82 \times10^{-3}\pm 0.47 \times10^{-3}$	$1.28 \times10^{-3} \pm 0.22 \times10^{-3}$
Pa4	$8.48\times10^{-3} \pm 0.80 \times10^{-3}$	$4.18 \times10^{-3} \pm 0.46 \times10^{-3}$	$1.64 \times10^{-3} \pm 0.45 \times10^{-3}$
Pa5	$8.07 \times10^{-3} \pm 0.93 \times10^{-3}$	$3.91 \times10^{-3} \pm 0.98 \times10^{-3}$	$0.90 \times10^{-3} \pm 0.50 \times10^{-3}$

***In vitro* release:** The data on the release of Darifenacin from all the patches are shown in Figures 6 and 7. Inspection of Figure 6 indicates that the drug release from patch Ha2 (2%) was above 90% in 10 hours. Ha1 also gave 90% release, but that was within 6-7 hours. Formulations Ha3, Ha4 and Ha5 showed less than 75% release in 10 hours. Ha2 was selected for egg membrane and *ex-vivo* studies.

Formulations Ha1-Ha5 contained 1%-5% of the polymer HPMC K100M CR and the β-CD -darifenacin complex, while formulation D was the pure drug.

The diffusion study of the HPMC K15 patches shown in Figure 7 indicates that formulation Pa4 (4%) showed nearly 90% drug release in 10 hours. Formulations Pa1, Pa2, Pa3 showed more than 90% drug release, but this occurred within 6-7 hours, which was not the desired result.

Formulation Pa5 showed less than 73% release in 10 hours. Thus, formulation Pa4 was selected for the *ex-vivo* studies.

Formulations Pa1-Pa5 contained 1%-5% of the polymer HPMC K15 and the β-CD -darifenacin complex, formulation D again containing pure drug.

The diffusion parameters for the HPMC K100M CR formulations (Table 10) and HPMC K15 formulations (Table 11) indicated the best-fit models. (Isabel et al., 2007).

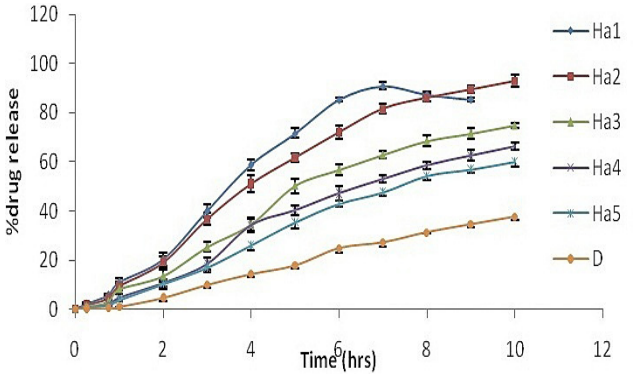

Figure 6: *In-vitro* release profile of various formulations of HPMC K100M CR containing β-CD -darifenacin complex.

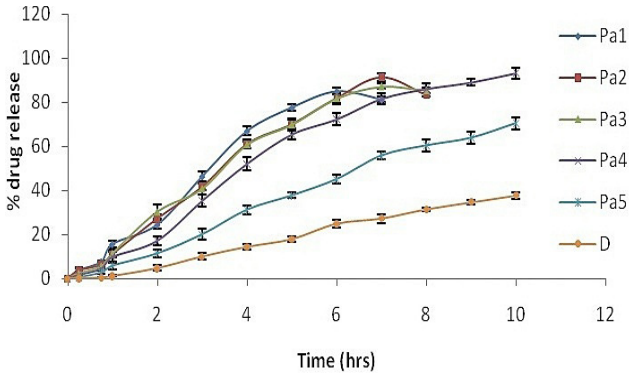

Figure 7 : *In-vitro* release profile of various formulations of HPMC K15 containing β-CD -darifenacin complex.

***Ex-vivo* drug release:** The *ex-vivo* release study with goat buccal membrane showed 72.43% to 77.33% release in 10 hours from the optimized patches (Figure 8) .

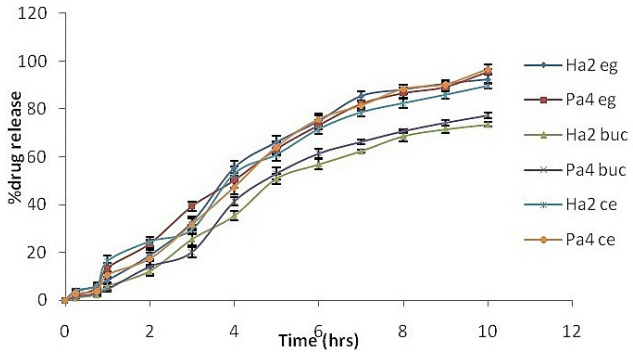

Figure 8: Profiles of drug diffusion through goat buccal membrane (buc), cellophane (ce) and egg membrane (eg).

Table 10: Diffusion parameter of HPMC K100M CR formulations.

Complexes	Best fit model	n	R^2
D	Zero order	1.002	0.9959
Ha1	Peppas	0.9861	0.9144
Ha2	Peppas	0.8764	0.9957
Ha3	Peppas	0.9371	0.9958
Ha4	Zero order	1.013	0.9851
Ha5	Zero order	1.004	0.9938

Table 11: Diffusion parameter of HPMC K15 formulations.

Complexes	Best fit model	n	R^2
D	Zero order	1.014	0.9936
Pa1	Peppas	0.7819	0.9827
Pa2	Peppas	0.8342	0.9827
Pa3	Peppas	0.8782	0.9920
Pa4	Zero order	1.001	0.9959
Pa5	Zero order	1.031	0.9939

The drug diffusion rate through egg membrane was also studied. In Figure 7, this showed similar diffusion characteristics (above 90% release in 10 hours) to cellophane membranes.

Stability and ageing

The data analysis indicates no interaction between the drug and the polymers (Figure 9) and no degradation. Percent drug content was within 95.43-97.86%. Diffusion data for the optimised formulations in the subsequent 3 months were almost the same as the initial *in-vitro* release results.

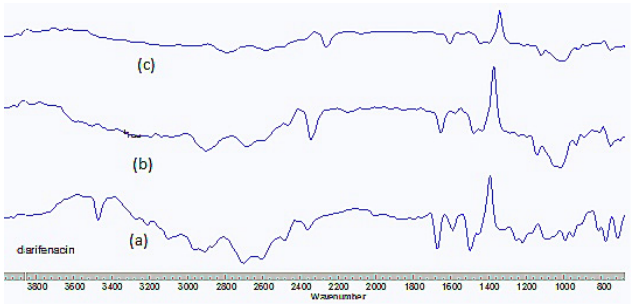

Figure 9**:** FTIR spectra of (a) darifenacin, (b) drug- β-CD complex with HPMC K15, (c) drug- β-CD complex with HPMC K100M CR.

DISCUSSION

The phase solubility study suggested an A_L type curve with 1:1 stoichiometry for the darifenacin– β-CD inclusion complex. This indicates an increase in solubility with increasing concentration of the complexing agent. Gibbs free energy change showed endothermic heats of solution, which indicates an increase in solubility with temperature.

The absence of a DSC signal for the drug in the co-grinding complex indicated the amorphous nature of

the drug in the inclusion complex. Absence of the melt endotherm can account for the enhancement in dissolution rate. In the X-ray diffraction pattern for the physical mixture, the peaks observed were less intense than those of the drug. The diffractogram of the co-ground complex showed no darifenacin crystal signals, demonstrating the conversion of the drug to the amorphous state. The study of all spectra indicated that the degree of crystallinity was decreased by complexation with β-CD. FTIR spectra did not show any evidence of chemical interaction between the drug and β-CD. Co-grinding of darifenacin with β-CD resulted in amorphous products and higher dissolution rates and so was chosen for the formulation of the buccal patch. The stability studies demonstrated that the co-ground complexes were stable over the period of study (3 months).

The *in-vitro* bioadhesive study indicated that increasing the polymer concentration in the patch increased its mucoadhesive strength. The study also indicated that HPMC K100M CR patches have more adhesive strength than HPMC K15 patches. This implies adhesive force depends on the viscosity of the polymers (Parodi et al., 1996). The *ex-vivo* release study showed that 72.43% to 77.33% of the drug was released in 10 hours from optimized patches of HPMC K100M CR and HPMC K15, which may have been less in the case of goat mucosa because of its greater thickness. Drug diffusion rate through the egg membrane was similar to that through cellophane membranes.

The release kinetic analyses for formulations Ha1, Ha2, Ha3, Ha4 and Pa1, Pa2, Pa3 showed values of n be1ow 1, indicating release was predominantly by diffusion and a non-Fickian transport mechanism. The value of n gives an indication of the release mechanism; when $n = 1$, the release rate is independent of time (zero-order) (case II transport), $n = 0.5$ for Fickian diffusion and when $0.5 < n < 1.0$, diffusion and non-Fickian transport are implicated. Lastly, when $n > 1.0$, super case II transport is apparent.

CONCLUSION

Phase-solubility studies revealed A_L type curves, indicating a linear increase in drug solubility with complexant. All the complexes showed better dissolution than pure drug. Darifenacin complexes exhibited a characteristic amorphous pattern. Buccal patches prepared with HPMC K100M CR and HPMC K15 (formulations Ha2 of HPMC K100M CR and Pa4 of HPMC K15) showed above 90% release in 10 hours. Mucoadhesive strength of these patches was sufficient and they also showed good *in-vitro* residence times. Stability study of the drug in these formulations did not show any chemical interaction. *In-vitro* drug release and *ex-vivo* release were also in agreement with the pre-stability release data. Hence, these two formulations could be used for buccal delivery of darifenacin, which would avoid the first-pass effect of the oral route and lead to increased bioavailability of darifenacin.

REFERENCES

Friedrich H, Nada A, Bodmier, R. Solid state and dissolution rate characterization of co-ground mixtures of nifedipine and hydrophilic carriers. Drug Dev Ind Pharm. 2005; 31:719-28, Doi:10.1080/03639040500216097.

Haab F, Stewart L, Dwyer P. Darifenacin, ANM3 selective receptor antagonist, is an effective and well-tolerated once-daily treatment for overactive bladder. Eur Urol. 2004;45: 420–9, Doi:10.1016/j.eururo.2004.01.008.

Higuchi T, Connors K. Phase solubility techniques. Adv Anal Chem Instr. 1965;7:117–212.

Isabel DC, Françoise F, Richard H. Ex vivo evaluation of bioadhesive films for buccal delivery of fentanyl. J Controlled Rel. 2007;122:135–40, Doi:10.1016/j.jconrel.2007.05.017.

Jug M, Becireviclacan, M. Influence of hydroxypropyl-β-cyclodextrin complexation on piroxicam release from buccoadhesive tablets. Eur J Pharm Sci. 2004;21:251–60, Doi:10.1016/j.ejps.2003.10.029.

Khanna R, Agrawal SP, Ahuja A. Preparation and evaluation of buccal films of clotrimazole for oral Candida infections. Ind J Pharm Sci. 1997;59:299-305.

Nafee NA, Ismail FA, Boraie NA, Mortada LM. Mucoadhesive buccal patches of Miconazole Nitrate: In Vitro/In Vivo Performance and Effect of Ageing. Int J Pharm. 2003;264:1–14. Doi:10.1016/S0378-5173(03)00371-5.

Nakamura F, Ohta R, Machida Y, Nagai T. In-vitro and in-vivo nasal mucoadhesion of some water-soluble polymers. Int J Pharm. 1996;134:173–81.

Nalluri B, Chowdary K, Murthy K, Satyanarayana V, Hayman A, Becket G. Inclusion complexation and dissolution properties of nimesulide and meloxicam–hydroxypropyl-b-cyclodextrin binary systems. J Incl Phen Macro Chem. 2005;53:103–10.

Nazila S, Montakarn C, Thomas PJ. The Use of mucoadhesive Polymers in buccal drug delivery. Adv Drug Del Rev. 2005;57:1666–91. doi:10.1016/j.addr.2005.07.003.

Noha AN, Nabila AB, Fatima A, Ismail MM, Lobna MM. Design and characterization of mucoadhesive buccal patches containing cetylpyridium chloride. Acta Pharm. 2003;53:199-212.

Parodi B, Russo E, Caviglioli G, Cafaggi S, Bignardi G. Development and characterization of a buccoadhesive dosage form of oxycodone hydrochloride. Drug Dev Ind Pharm. 1996;22:445–50. Doi: 10.3109/03639049609069353.

Patil J, Pandya N, Marapur S, Shiralashetti S. Influence of method of preparation on physicochemical properties and invitro drug release profile of nimodipine cyclodextrin inclusion complexes: a comparative study. Int J Pharm Sci. 2010;2:71-81.

Shirsand S, Sarasija S, Swamy PV. Formulation design and optimization of fast dissolving clonazeapm tablet. Ind J Pharm Sci. 2009;71;567–72. Doi: 10.4103/0250-474X.58189.

Thimmasetty J, Pandey GS, Satheshbabu PR. Design and in-vivo evaluation of carvedilol buccal mucoadhesive patches. Pak J Pharm Sci. 2008;21(3):241-8.

Tsinontides S, Rajnaik P, Hunke W, Placek J, Reynolds S. Freeze drying-principles and practice for successful scale up to manufacturing. Int J Pharm. 2004;280:1-16. doi:10.1016/j.ijpharm.2004.04.018.

U. S. Pharmacopoeia National Formulary. 2009: 1725-6**.**

Van G, Augustijns P, Blaton N, Kinget R. Physicochemical characterization of solid dispersions of temazepam with polyethylene glycol 6000 and PVP K30. Int J Pharm. 1998;164:67–80.

Vanshiv S, Rao R. Physicochemical characterization and in vitro dissolution of Oxcarbazepine and β-cyclodextrin inclusion complexes. Ind Drug. 2008;45:816-20.

Vasconcelos T, Sarmento B, Costa P. Solid dispersions as strategy to improve oral bioavailability of poor water soluble drugs. Drug Disc Today. 2007;12:1068-75. Doi:10.1016/j.drudis.2007.09.005

Verma S, Kaul M, Rawat A, Saini S. An overview on buccal drug delivery system. Int J Pharm Sci Res. 2011;2(6):1303-21.

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