Formulation and Evaluation of Mucoadhesive Buccal Film Incorporated with Eprosartan Mesylate Nanosuspension

Prabhat Dessai*, Ankita R. Ainkar

PG, Department of Chemistry, Dnyanprassarak Mandal's College and Research Centre, Mapusa – Anjuna Road, Ghateshwar Nagar, Assagao, Khorlim, Mapusa, Goa 403507, India

| **Abstract** | **Original Research Article** |

Mucoadhesive buccal films containing three layers (mucoadhesive layer, Nanosuspension containing layer and backing membrane) were incorporated with Eprosartan mesylate nanosuspension. Formulation and evaluation of nanosuspension incorporated mucoadhesive buccal films of Eprosartan mesylate for bioavailability enhancement by avoiding first-pass metabolism. Buccal route of drug delivery provides the direct access to the systemic circulation through the jugular vein bypassing the first pass hepatic metabolism leading to high bioavailability. Eprosartan mesylate -loaded nanosuspension was prepared by a precipitation–ultrasonication method with varying concentrations of the polymer. nanosuspension have emerged as one of the most promising dosage forms for formulation of water insoluble and sparingly soluble drugs Nanosuspension consist of hydrophobic drug particles in the nano range (100–1000 nm) dispersed in a hydrophilic medium (usually water) which are stabilized with the help of polymers, surfactants or a mixture of both. The low particle radius and enlarged surface area of nanosuspension lead to a consequent improved rate of dissolution that can contribute to increased concentration gradient and thus absorption of low soluble but highly permeable drugs. The formulation showed negative zeta potential of -12.6 mV and was analyzed for size and morphology. Optimized nanosuspension was incorporated into drug gel layer which was sandwiched between a mucoadhesive layer and a backing layer to form tri-layered buccal films. They were evaluated for their physical, mechanical and bioadhesive parameters followed by in vitro and ex-vivo studies. Nanosuspension showed a diameter of around 278-234nm and drug gel layer (62.4% drug loading) was optimized to contain 3% HPMC and 50mg Carbopol934P the mucoadhesive layer, Nanosuspension containing layer and the backing layer respectively. In vitro drug release was 80% and 68.7% in 5 h across egg shell membrane and gout buccal mucosa, respectively. The drug delivery system has been designed as a novel platform for potential buccal delivery of drugs having high first-pass metabolism.

Keywords: Bioavailability, buccal delivery, Eprosartan mesylate, ethyl cellulose, HPMC.

INTRODUCTION

Hypertension is a highly prevalent disorder all over the world requiring prolonged treatment. Usually, therapy is for lifetime. Conventional oral therapies, such as tablets are convenient but for certain drugs that encounter bioavailability problems due to one or the other reasons, a convenient alternative route is much needed. The Angiotensin II type 1 receptor blocker (ARB) Eprosartan is a nonbiphenyl nontetrazole angiotensin II type 1 receptor (AT1) antagonist, which acts to decrease total peripheral resistance. Eprosartan acts at vascular AT1 receptors (postsynaptically) and at presynaptic AT1 receptors, where it inhibits noradrenaline release. Eprosartan, therefore, represents a useful therapeutic option in the management of patients with hypertension, including those with a history of stroke or with co-morbid type 2 diabetes mellitus. Though it is rapidly absorbed after an oral administration, the bioavailability of Eprosartan mesylate is 13% as it undergoes stereo-selective first-pass metabolism and will be eliminated from body through urine (16%) and feces (60%). Eprosartan mesylate is a weak base with pKa value 7.7–7.9 and log PC (partition coefficient) value of 3.967 which indicates sufficient lipophilicity to pass through any biological membrane including buccal membranes [1-5].

Extensive research efforts have recently been focused on placing a drug delivery system in a particular region of the body for maximizing biological drug availability and minimizing dose-dependent side effects. Buccal delivery of drugs provides an attractive alternate to other conventional methods of systemic drug administration, since buccal

mucosa is relatively permeable with rich blood supply and acts as an excellent site for the absorption of drugs. The administration of drugs via buccal route facilitates a direct entry of drug molecules into the systemic circulation, avoiding the first-pass metabolism and drug degradation in the harsh gastrointestinal environment, which are often associated with oral administration. The buccal cavity is easily accessible for self medication, and hence it is safe and well accepted by patients, since buccal patches can be very easily administered and even removed from the application site, terminating the input of drug whenever desired. Moreover, buccal patches provide more flexibility than other drug deliveries [6-15].

In recent years, nanosuspension have emerged as one of the most promising dosage forms for formulation of water insoluble and sparingly soluble drugs .Nanosuspension consist of hydrophobic drug particles in the nano range (100–1000 nm) dispersed in a hydrophilic medium (usually water) which are stabilized with the help of polymers, surfactants or a mixture of both. The low particle radius and enlarged surface area of nanosuspension lead to a consequent improved rate of dissolution that can contribute to increased concentration gradient and thus absorption of low soluble but highly permeable drugs. To exploit this potential benefit of a nanosuspension for buccal delivery, appropriate delivery devices are required to overcome the inherent demerit of low hold time of any liquid formulation such as nanosuspension. Traditional buccal devices including chewing gums release a large part of the dose to buccal fluid which is swallowed leaving a small part of the dose for systemic absorption. Hence, triple layer buccal patch having drug layer sandwiched between a mucoadhesive layer having an absorption promoter and a backing membrane to reduce loss of drug. Into buccal cavity would be an ideal proposition. However, in most of the buccal patches the drug is dispersed within the polymeric matrix, which may result in a growth in particle size. In order to overcome this problem, a micronized drug reservoir–based buccal patch containing Eprosartan mesylate nanosuspension suspended in polymeric gel matrix is proposed in this investigation. The buccal patch developed was evaluated for dosage accuracy, mucoadhesiveness and other film characteristics. In vitro drug permeability and in vivo pharmacokinetics in a rat model were studied to confirm the applicability of the improved buccal patch for controlled drug delivery and eventually for an improvement of bioavailability and therapeutic efficacy [16-21].

MATERIALS AND METHODS

Eprosartan mesylate was a gift sample from Unichem Pharmaceuticals Ltd.Goa; acetone, polyvinyl alcohol (PVA) from S.D fine chem. chloroform, glycerine college laboratory Assagao Goa, and potassium dihydrogen orthophosphate from research lab fine chem. Polyvinyl pyrrolidone (PVP)-K 90, from research lab fine chem. hydroxy propyl methyl cellulose (HPMC) (15 cps) from S.D fine chem. sodium carboxy methyl cellulose (SCMC) from Vikash pharma. Methanol, PEG 400, potassium bromide (KBr), Tween 80 and EC were purchased from LOBA chem.

Methods
Preparation of Eprosartan Mesylate Nanosuspensions

Eprosartan Mesylate nanosuspension was prepared by bottom–up technique adopting anti-solvent precipitation–ultrasonication method. Acetone and water being miscible with each other were selected as solvent and anti-solvent, respectively, and the ratio of solvent to anti-solvent was fixed at 1:20. Eprosartan mesylate was dissolved in acetone to obtain solutions containing 20, 30 and 60 mg/ml of drug. PVA was dissolved in water to obtain anti-solvent solutions of varied concentrations of PVA (0.1%, 0.15%, and 1% w/v). Anti-solvent was cooled to below 3^0C in an ice water bath prior to use. Organic drug solution (2 ml) was further transferred into a beaker containing aqueous PVA solution (20 ml) with continuous stirring using a magnetic stirrer. Drug being insoluble in water immediately precipitated out leading to generation of submicron particles. This precipitated drug suspension was subjected to further size reduction using a probe sonicator. Drug suspension, in an ice bath, was probe sonicated at 90% amplitude for different time intervals (4, 8, 12, 16 and 20 min). The period of ultrasound burst was set to 4 s with a pause of 6 s between two ultrasound bursts. The nanosuspension obtained were concentrated by centrifugation at 16 000 rpm for 40 min in an ultracentrifuge. After centrifugation, Supernatant was replaced with 2ml of 0.2% PVA solution. Solid residue was redispersed using a bath sonicator.

Optimization of concentrations of drug and surfactant

To optimize the concentration of the drug, different formulations were prepared by using varied concentrations of Eprosartan mesylate, i.e. 20, 30, 40 and 60 mg/ml, while keeping the concentration of surfactant and ultrasonication time constant. Similarly, various formulations were prepared using varied concentrations of PVA, i.e. 0.1%, 0.15 and 1% w/v, keeping the drug concentration and ultrasonication time constant. Formulations were evaluated on the basis of size.

Characterization of Eprosartan Mesylate Nanosuspension
Morphology of Carvedilol Particles

The morphology of the particles was investigated using scanning electron microscopy (SEM, ZEISS EVO Series Scanning Electron Microscope EVO 50 S-4800, Carl Zeiss, Germany). SEM was employed to surface microstructure imaging for visualization of shape and morphology of the prepared nanosuspension. The nanosuspension was centrifuged; the supernatant was removed and dried at room temperature. Dried Nanosuspension was placed on a

sample holder with the help of a double sided Carbon tape coated with gold-palladium alloy (150–250A °) with a sputter coater under vacuum and placed in SEM for the analysis.

Entrapment Efficiency

Amount of Eprosartan mesylate entrapped in nanosuspension was estimated by centrifuging the Nanosuspension at 20,000 rpm, decanting supernatant and washing the sediment twice with distilled, deionizer water. Supernatant obtained was assayed for the determination of amount of drug unentrapped by U.V spectrophotometer at 233 nm. Amount of drug entrapped was calculated by subtracting the amount of drug unentrapped from total amount of drug added to prepare nanosuspension. Percentage drug entrapment (% DE) was calculated by the formula mentioned below. Study was done in triplicate and their mean values were reported.

$$\%DE = \frac{\text{total amount of drug adde} - \text{drug present in supernatant}}{\text{total amount of drug}} \times 100$$

Preparation of Eprosartan mesylate nanosuspension incorporated mucoadhesive buccal film

Preparation of nanosuspension incorporated buccal film constituted of three distinct layers, namely, mucoadhesive layer, nanosuspension containing layer and backing membrane. All three layers were prepared by a solvent casting method.

Preparation of Mucoadhesive Layer

The weighed amount of polymer was dispersed in 15 ml distilled water with continuous stirring using a magnetic stirrer and the final volume was adjusted to 20 ml. Two drops of Tween 80 was incorporated into the resulting polymeric solution after levigation with glycerine (15% of dry polymer weight). The solution was kept overnight to form a bubble-free solution and then casted onto glass Petri dishes (10 cm diameter) and kept in hot air oven at 40^0C, for 24 h. The prepared films were packed in aluminum foil and stored in an airtight glass container to maintain the integrity and elasticity.

Choice of mucoadhesive polymer and its concentration plays a pivotal role in uniform film formation. To optimize the right concentration, polymer formulations were prepared by employing different concentrations of HPMC, SCM and PVP-K90 (2%, 3%, 4%, 5% and 6% w/v), Keeping the permeation enhancer (Tween 80) and plasticizer Concentrations constant (Table-1).

Table-1: Optimization of Mucoadhesive Layer

Polymer	Polymer conc % v/v	Permeation enhancer (Tween 80)	Film thickness
HPMC	3%	1 drop	Good film thickness and very smooth
SCMC	2%	1 drop	Thin and has very good flexibility
PVP	4%	1 drop	Good thickness, good uniformity but less flexibility

Preparation of nanosuspension containing layer

Weighed amount of HPMC (15 cps) and carbopol 934P was dispersed as film forming polymers in 20 ml Eprosartan mesylate Nanosuspension with continuous stirring using magnetic stirrer to form a gel. To this resulting gel, 15% PEG 400 w/v was added as a plasticizer and then was casted onto glass Petri dishes (10 cm diameter) and kept in vacuum desiccators for drying up to 24 h. The prepared films were packed in aluminum foil and stored in an airtight glass container to maintain the integrity and elasticity. To optimize the right concentration of polymer and gelling agent, formulations were prepared employing different concentrations of carbopol 934P and 25,30 and 50 mg keeping the HPMC concentration, permeation enhancer, plasticizer concentrations and nanosuspension volume constant as seen in Table-2.

Table-2: Optimization of Nanosuspension containing layer

Formulation	HPMC (15 caps) %	Carbopol 934 P(mg)	Permeation enhancer (Tween 80)	Film characteristics
F1	3%	25	1 drop	Thin, not uniform and cannot take up drug load still retaining the gel matrix.
F2	3%	30	1 drop	Thick, not uniform, brittle
F3	3%	50	1 drop	Thickness suitable, uniform, and flexible and can take up sufficient drug load still retaining the gel matrix.

Preparation of Ethyl Cellulose Backing Layer
Ethanol (5 ml) was added in a beaker containing 10 ml of acetone as solvent. Ethyl cellulose (1 g) was dissolved in the solvent with 0.35 ml of polyethylene glycol as plasticizer. The polymer solution was kept for deaeration and then poured into 9 cm diameter petri dish with an aluminum foil spread over the surface. The solution was kept for controlled evaporation of the solvents at room temperature.

Final composite formulation: The final buccal formulation is a composite of the above mentioned three layers. The layers were joined by spraying chloroform to attach the Nanosuspension containing layer, to the backing membrane on one side and the mucoadhesive layer on the other. The mucoadhesive side was covered with a peel able polyethylene film and the composite cut to 2 cm2 size was kept in vacuum desiccators for 24 h in between two stainless steel stabs. The prepared films were packed in aluminum foil and stored in an airtight glass container to avoid moisture loss thus to maintain the integrity and elasticity.

Polymer Incompatibility Studies
IR study was performed for polymer incompatibility studies with that of Eprosartan mesylate using Fourier transformed infrared spectrophotometry. The KBr disk technique was employed using 1:1 ratio of Eprosartan mesylate and various polymers such as HPMC (15 cps), carbopol 940, PVP K90 and SCMC in spectroscopic grade dried potassium bromide. The study was repeated separately for each polymer blend with Eprosartan mesylate.

Characterization of buccal films
The buccal films were evaluated for their physical, mechanical and bioadhesive parameters followed by in vitro drug release and in vivo studies.

Physical appearance
The films were observed visually for their physical appearance such as color, transparency and texture.

Weight variation
Four films of each batch of formulation were weighed individually and then together by using a digital weighing balance, and average weight and weight variation of the films were calculated.

Thickness
Four films of each combined layer formulation were taken and the thickness of the film was measured using a screw gauge at different points of the plane surface. The average film thickness was calculated.

Folding Endurance
The folding endurance was measured manually. A small strip of film measuring 2 cm^2 of each formulation was taken and folded at the same place till it breaks. The number of times a film could be folded at the same place gave the value of folding endurance. Average of three determinations was calculated.

Surface pH
The surface pH of the film was determined by allowing the film to swell by keeping it in contact with 1ml of phosphate buffer (pH 7.5) for 1 h in a glass Petri dish. The surface pH was noted by bringing a combined glass electrode near the surface of the film for 1 min using a pH meter. The pH was recorded and average of three determinations was calculated.

Drug Content Uniformity
Drug content uniformity was determined by dissolving the three-layered film in 100 ml of an isotonic phosphate buffer (pH 7.5) by homogenization for 2 h with occasional shaking. Aliquot (5 ml) was withdrawn and diluted with isotonic phosphate buffer pH 7.5 up to 20 ml, and the resulting solution was filtered through a 0.45mm what man filter paper. The drug content was then determined spectrophotometrically at 233nm by plotting the calibration curve of Eprosartan mesylate. Average of three determinations was calculated.

Calibration curve of Eprosartan mesylate
A stock solution of Eprosartan mesylate was prepared by dissolving 100 mg of drug in little amount of pH 7.5 phosphate buffer and made up to100 ml with same from stock solution different concentration of Eprosartan mesylate like 2, 4, 6, 8, and 10 ug/ml were prepared by diluting with PH 7.5 phosphate buffer and their absorbance were measured at 233 nm using U.V spectrophotometer. A graph was plotted by plotted by tacking concentration of Eprosartan mesylate (ug/ml) on x-axis and absorbance on y- axis the graph is shown in Figure-1.
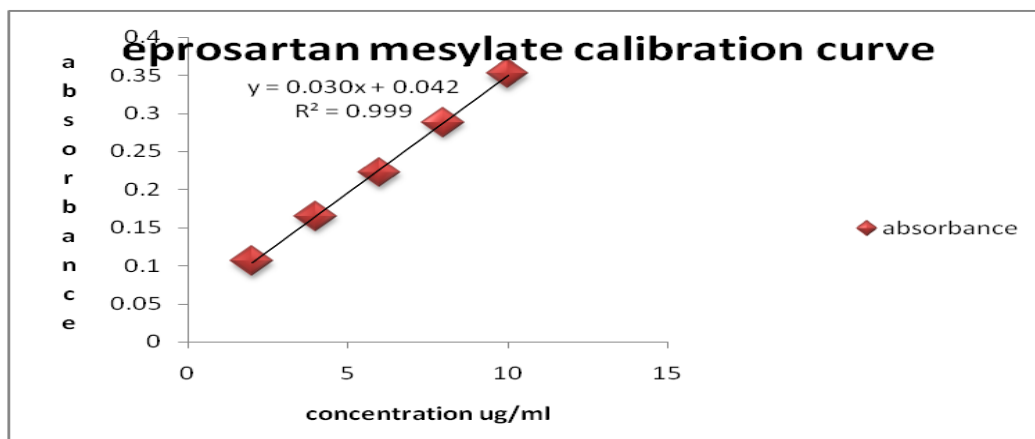
Fig-1: Eprosartan mesylate calibration curve

Swelling Studies

The patch sample of 1.5 cm diameter was weighed and placed porcelain dish containing 15 ml of simulated salivary fluid. At definite time intervals, the patch are removed excess moisture was removed by carefully wiping with absorbent tissue and reweighed. Increase in weight of the film was determined at each time interval until a constant weight was observed. The degree of swelling was calculated using the formula, Where, Wt is weight of the patch at time t and W0 is weight of the patch at time zero.

$$\text{Swelling index} = \frac{W_t - W_o}{W_o}$$

In Vitro Mucoadhesion Test

The mucoadhesive strength of the buccal film was determined using a modified balance method [4]. The strength required to detach the polymeric film from the mucosal surface was applied as a measure of the bioadhesive performance. The goat buccal mucosa was used as the model membrane and Krebs' buffer solution of pH 7.4 was used as the moistening fluid. The fresh goat buccal mucosa obtained from a slaughterhouse was stored in Krebs' buffer pH 7.4 at 4 ^{0}C after collection. A piece (1 cm^2) of cleaned goat buccal mucosa was stuck to the bottom flat surface of the beaker using a suitable glue such that mucosal surface faces upwards. The test films were stuck on to a lower flat side of hanging glass assembly to the left arm of the balance, balanced previously by keeping a 5 g weight on each side. The surface of the mucosa was blotted with the Whatman filter paper and was added with 25 ml of Krebs' buffer solution of pH 7.4. Five grams of weight from the right pan was removed. This lowered the glass assembly along with film over the membrane with a weight of 5 g. This was kept undisturbed for 3 min and then rebalanced by keeping 5 g weight on the right side. Then the weights were slowly added till the film just separated from the membrane surface. The weight added to separate the stuck film from the mucosa was taken as bioadhesive strength. Three films of each formulation were tested and the average of three determinations was used to calculate the mucoadhesive force using the following equation:

$$\text{Mucoadhesive force (N)} = \frac{\text{BIOADHESIVE STRENGHT (g)}}{1000} \times 9.81$$

In Vitro Drug Release Studies

In vitro drug release studies were performed by using egg shell membrane method using a shaking incubator at a rotation speed of 100 rpm. Methanolic phosphate buffer (pH 7.5) (i.e. 90:10 ratio of phosphate buffer 7.5 and methanol, respectively) was used as a dissolution medium. Each egg shell membrane was loaded with buccal film equivalent to 4mg of drug. Volume and temperature of the dissolution medium were 200 ml and 37.0±0.2 ◦C, respectively. At predetermined time interval, samples (2 ml) were withdrawn, replaced with same volume of fresh media, filtered and assayed for drug content at 233 nm against blank using a U.V-V spectrophotometer. Mean results of triplicate measurements and standard deviation were reported.

Ex Vivo Drug Permeation Studies

The ex vivo drug permeation studies of buccal films of Eprosartan mesylate through an excised layer of goat buccal mucosa (washed in isotonic phosphate buffer, pH 7.5 after excising and trimming from the sides) were carried out using the Franz diffusion cell. A ''1 cm'' diameter film of each formulation under study was placed in intimate contact with the excised goat buccal mucosa on the topside. The contents of receptor compartment were filled with 50 ml of pH 7.5 phosphate buffer (with a Teflon bead placed inside) and stirred with a magnetic stirrer and temperature was

maintained at 37±5 ◦C throughout the experiment. The samples were withdrawn at regular intervals, filtered, diluted suitably and then analyzed spectrophotometrically at 233 nm.

Mechanism of Release Kinetics
The mechanism of release was determined by fitting the release data to the various kinetic equations such as zero order, first-order, Higuchi and korsmeyer and peppas model R^2 values were determined by regression formed by the index of linearity.

Stability Studies
The Optimized formulation was subjected to accelerated stability testing. The ageing studies were conducted at 37° and 45° to investigate the effect of temperature on the drug content in formulation. Films were packed in glass Petri dishes lined with aluminum foil and kept in an incubator maintained at 37±0.5° and 45 ±0.5° for one month. Changes in the appearance, drug content of the stored bioadhesive patches were investigated after 7, 14, 21 and 28 days.

RESULTS AND DISCUSSION

Formulation and Optimization of Eprosartan Mesylate Nanosuspension
Nanosuspension was prepared according to the procedure mentioned and further, it was optimized on the basis of particle size of the nanocrystals.

Table-3: optimization of Nanosuspension based on particle size and drug entrapment efficiency

Formulation	Drug Concentration	Stabilizer Conc% w/v	Particle size in nm	Zeta Potential in mv	Drug Entrapement in %
N_1	20mg	0.1%	342nm	-18.57	55.9%
N_2	30mg	0.15%	278nm	-12.6	87.5%
N_3	60mg	1%	455nm	-20	67%

Optimization of Concentrations of Drug and Surfactant
Influence of drug and surfactant concentrations were inversely proportional to each other as the former increases size while the latter decreases. Percentage drug entrapment is another important response parameter that needs to be controlled in addition to size selectivity based on the application of any nanoparticles. Hence, drug and surfactant were taken together for optimization, to balance these two independent parameters to control both particle size and percentage drug entrapment.
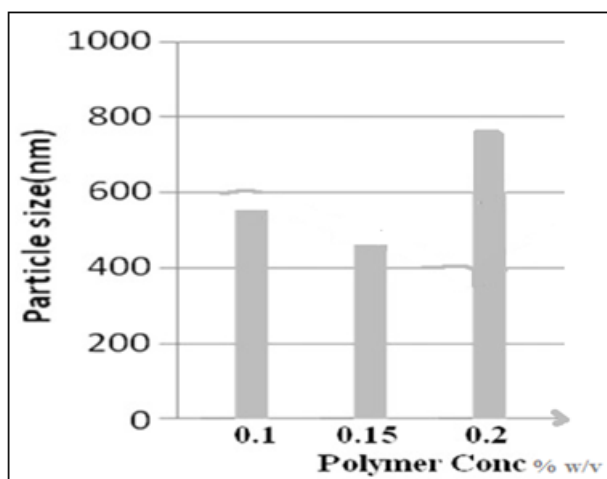
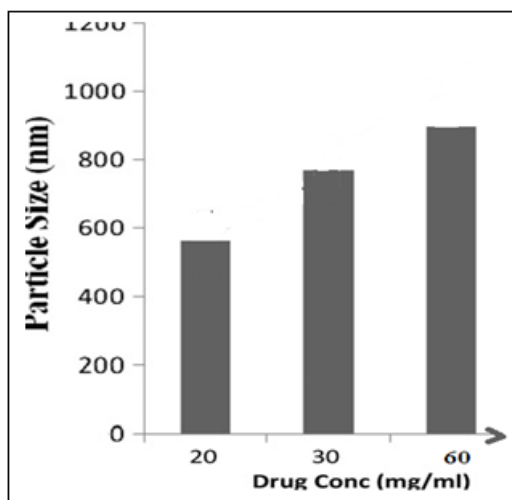**Fig-2: Optimization of surfactant concentration**

Fig-3: optimization of drug amount

The results illustrate consistent increase in the size of precipitated particles with an increase in carvedilol concentration. This can be explained by the theory of crystallization. The process of particle formation includes several steps, namely, particle nucleation, molecular growth and agglomeration or aggregation. The size of the precipitated particle depends on the rate of these processes, which essentially depends on the concentration of the drug and was a precipitating solute in the medium. Here, the concentration of the drug is optimized based on the required particle size and other parameters like entrapment efficiency which would be dealt with in the section ''Characterization of optimized formulation''. Figure 3 represents change in particle size due to an increase in the surfactant (stabilizer) concentration. With increase in stabilizer concentration from 0.1% w/v to 0.15%, the mean particle size reduced dramatically from 278 to 234 nm. However, further increase in the surfactant concentration showed no decrease in the particle size. Hence, 0.15% w/v of the surfactant was optimized in future batches. This indicates the need for the critical concentration of surfactant just to cover the drug surface to reduce the surface free energy (SFE) to avoid agglomeration. Further increase has no effect due to the saturation achieved in surface coverage and the reduction of SFE. The affinity of polymer toward drug controls the polymer adsorption on crystal surface and so the selection of the polymer also determines the critical concentration of polymeric surfactant needed for the process. In the present investigation, carvedilol has carbonyl and amide groups which can form hydrogen bonds. Abundance of hydroxyl groups present in PVA provides a high degree of hydrogen bonding resulting in the adsorption of polymer on hydrophobic particle surface which provided enough steric repulsion between crystals, and hence prevented the agglomeration.

Characterization of optimized formulation

In vitro characterization included parameters such as morphology, size and particle charge.

Morphology

Optimized nanosuspension formulation was visualized under a SEM for surface morphology. SEM photographs revealed that the Eprosartan mesylate particles in nanosuspension were flaky in shape. SEM images are shown in Figure-4.
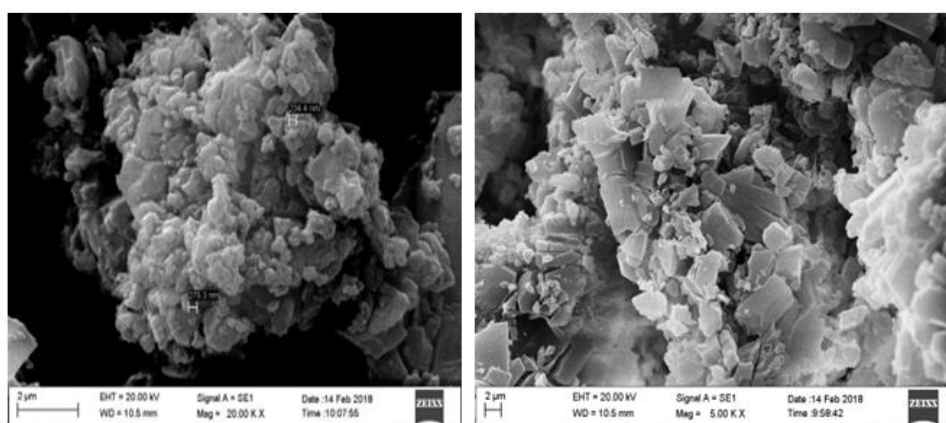

Fig-4: SEM images of Eprosartan mesylate

Surface Charge on Particle

Determination of zeta potential of a nanosuspension gives an idea about the physical stability of nanosuspension. The drug Eprosartan mesylate is neutral in character but exhibits high negative potential due to the medium (PVA) in which it is suspended. Zeta potential value turned out to be ±17.10, yet the stability of formulated nanosuspension was quite high because of the presence of interfacial film of polymers on the drug particles which generally exhibit irreversible adsorption.

Preparation of Nanosuspension Incorporated

Mucoadhesive buccal film of Eprosartan mesylate. The drug Eprosartan mesylate was prepared as nanosuspension to improve its dissolution rate and to modulate its release behavior from buccal patches. Particles in nanosuspension formed were of 278 -234 nm size and were expected to improve buccal absorption and bioavailability. Nanosuspension was incorporated along with a permeation enhancer in the nanosuspension layer and was sandwiched between mucoadhesive buccal film and the backing membrane. In total, the buccal formulations prepared constitute of three distinct layers, which were easily prepared by the solvent casting method and optimized for their polymer concentration.

Optimization of polymer concentration in mucoadhesive layer

Polymer selection and optimization of its concentration was conducted by measuring the thickness and uniformity of the films. The mucoadhesive layer not only helps in adhesion to the buccal mucosa, its role is equally important in controlling the penetration of the drug from the drug gel layer to the mucosal surface for absorption. The result of the optimization study showed that HPMC (3%), SCMC (2%) and PVP-K90 (4%) are suitable as mucoadhesive layers. Out of these batches, F2 containing HPMC (3%) was found to be best in terms of thickness, uniformity of thickness, smoothness and flexibility and hence was selected as the optimized formulation.

Optimization of Polymer Concentrations in Nanosuspension Containing Layer

The nanosuspension layer was prepared by using a mucoadhesive film-forming polymer (HPMC 15 cps) and a gel forming agent Carbopol 934P to form a smooth, uniform film having high flexibility. The film containing 3% w/v of HPMC 15 cps and 50 mg of Carbopol 934P was best on the basis of uniformity, smoothness and flexibility. The films containing polymer concentrations lower than 3% were not uniform and the ones having higher than this were not flexible and uniform.

Buccal Formulation: Preparation and Characterization

Three batches of mucoadhesive buccal formulations (H1 to H3) were prepared using optimized mucoadhesive films with optimized drug Nanosuspension Incorporated gel (N_2) and the backing membrane. They were joined and compressed to form the buccal formulation. They were securely joined due to the mucoadhesive property of the sandwiched layer (drug gel layer) prepared using HPMC and Carbopol 934P, which impart adhesivity and accommodated sufficient nanocrystals of Eprosartan mesylate . Formulation was cut into pieces of 2 cm^2 for the convenience of usage.

Polymer Incompatibility Studies

Compatibility studies were performed using an IR spectrophotometer. The IR spectra of pure Eprosartan mesylate, HPMC, Carbopol and their physical mixture are given in Figure-5. The characteristic peaks of these pure compounds are found in the physical mixture of the drug and the polymers indicating the absence of any interaction between the drug and these polymers

Characterization of Mucoadhesive Buccal Film Formulation
Physical Characterization

The mucoadhesive buccal firm formulations have been evaluated for various physical characteristics and are tabulated in Table-4. The mucoadhesive buccal film formulations were opaque with smooth surface structure. Weight of the optimized batch of films was found to be in the range of 29.53±0.27 mg to 38.39±0.65 mg. Folding endurance test indicated no crack even after 300 times folding, revealing satisfactory flexibility of the films. The surface pH of films ranged from 7.361 to 3.56 (Table-4). These values were found to be around the saliva pH (range 5.6 to 7.4) confirming the compatibility of the films with buccal mucosa and no risk of mucosal damage or irritation on usage.

Drug Content Uniformity

The average Eprosartan mesylate content in mucoadhesive buccal films was estimated to be 4.12 mg per cm^2. The drug content uniformity values were between 91.05 % and 83.69% of the theoretical values (Table-4). The observed results of content uniformity indicated that the drug was uniformly distributed throughout the film.

Swelling Studies

The swelling of the films were observed in pH 7.5 phosphate buffer solution. The comparative swelling in different formulations was in the order of H2>H1>H3. Swelling was more pronounced in films H2 and H1 that contain HPMC and SCMC due to the presence of more hydroxyl group in SCMC molecules. The percentage swelling of H3 was reduced considerably due to PVP K-90.

In vitro mucoadhesion strength

Mucoadhesive strength was determined for all the formulations using the modified balance method [4]. As shown, the bioadhesive strength of formulations H1 and H2 which contained HPMC and SCMC showed higher value than that of PVP K-9O (H3). It was also observed that an increase in polymer concentration showed an increase in bioadhesive strength as shown in Table-5.

In Vitro Release Studies

The in vitro drug release of the nanosuspension incorporated buccal film was carried out in methanolic PBS pH 7.5 for all the formulations for 540 min. The percentage releases of drug from all formulations from H1 to H3 were 80%, 67.9%, 78.6% at the end of 540 min as shown in Figure 5. About 45%−47% of the drug release was observed from all formulations only at the end of 240 min. Among various formulations, H1 released maximum amount of drug within 540 min. The order of retardation time for different films was as follows: H1>H3>H2. The reason might be due to water permeability characteristics of the film prepared out of different polymers, such as HPMC (H1), PVP-K90 (H3) SCMC (H2). Lower the water permeability coefficient the greater the sustained drug release characteristics observed. Swelling of polymers may be an additional factor which controlled the release rate.

Ex Vivo Permeation Studies

The ex vivo permeation study of selected buccal formulation (H1) was conducted excised goat buccal mucosa. The cumulative amount of Eprosartan mesylate permeated through the buccal epithelium was maximum 62.6% of drug in 5h as shown in Figure-6. The results indicated that Eprosartan mesylate could permeate rapidly across the mucosal membrane probably due to high lipophilicity of the drug. The cumulative amount of Eprosartan mesylate penetrated through the membrane was rapid in the first hour followed by a steady rate for 5 h. Overall penetration was about 60% in 6 h indicating that the performance of the formulation was quite convincing.

Mechanism of Release Kinetics

The in vitro release and ex vivo permeation profiles of the formulation H1 were subjected to a kinetic analysis for fitting into various models, such as zero-order, first-order, Higuchi equation to ascertain the drug release and permeation mechanism of selected formulation. In both the cases, the plots were found linear in case of Higuchi and korsmeyer and peppas model release kinetics with r^2 values nearer to 1 (r^2= 0.989 and 0.985, respectively). In the case of ex vivo permeation profile, exponent values (n) suggested the drug released and permeated by the diffusion mechanism following super case-II transport.

Stability Studies

The Optimized formulation when subjected to accelerated stability testing. It was observed that no Changes in the appearance, drug content and surface pH of the stored bioadhesive patches even after 28 days. Results given in Table-6.
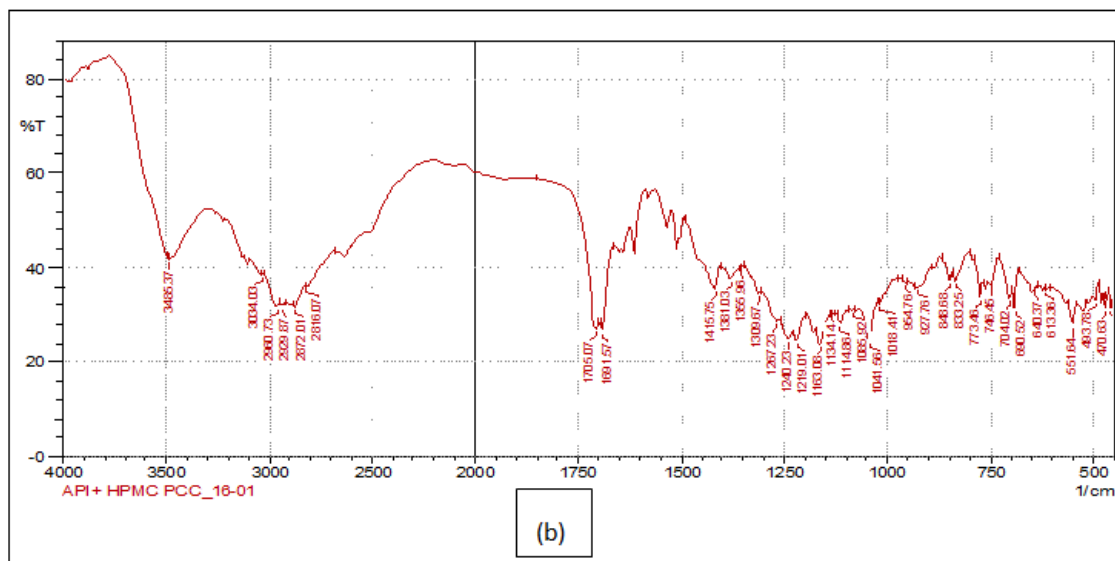

(a)
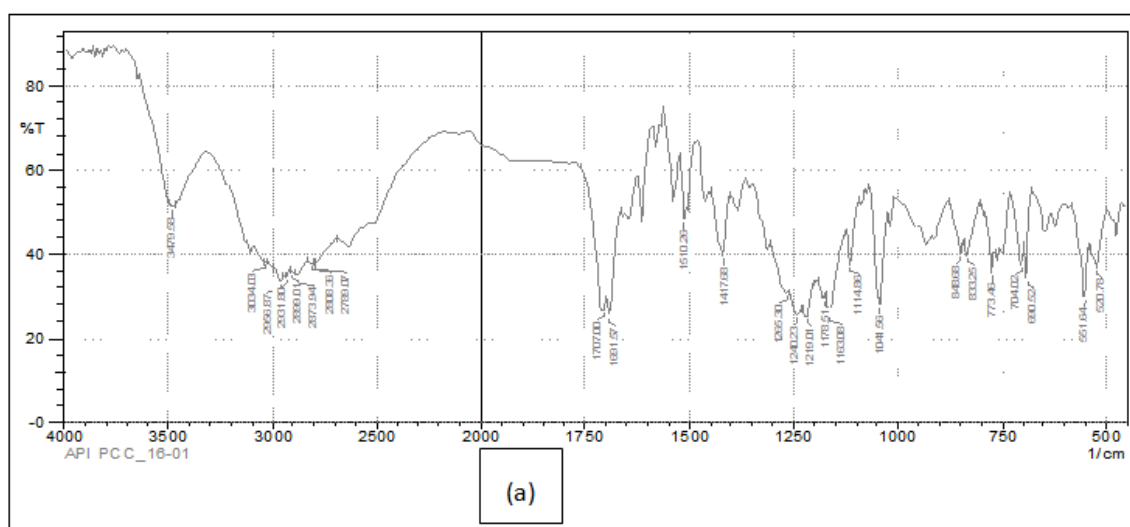
Fig-5: FTIR Spectrum of (a) Eprosartan mesylate (b) Formulation (HPMC: Carbopol: Eprosartan mesylate)

Table-4: Characterization of buccal films

Formulation code	Weight uniformity (mg)	Thickness (mm)	Swelling %	Content uniformity %	Surface pH	Folding endurance
H$_1$(HPMC)	30.16	0.45	52.2%	91.05%	7.361	>300
H$_2$(SCMC)	29.53	0.35	83%	87.41%	3.56	>300
H$_3$(PVP)	38.390	0.63	22.9%	83.69%	5.45	>300

Table-5: In-vitro mucoadhesion strength

Formulation	Mucoadhesive strength (gm)	Force of adhesion
H1(HPMC)	13.5	0.13
H2(SCMC)	11.5	0.11
H3(PVP)	12.5	0.12

Table-6: Results of stability studies

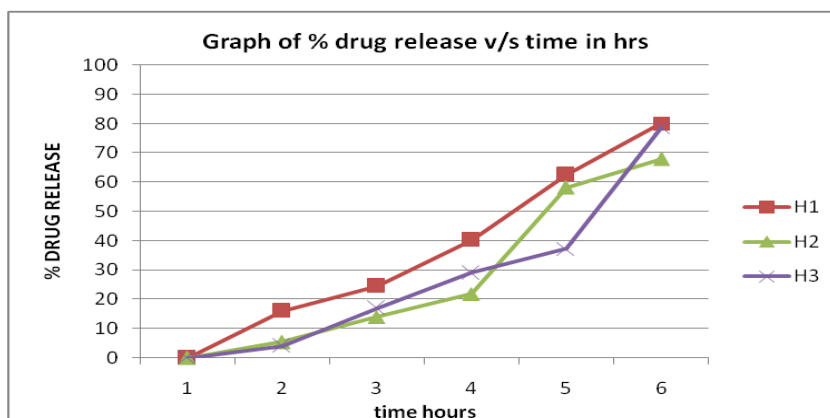| Day	Appearance	Drug content in mg	Surface pH
7	Opaque and smooth surface	91.05%	7.361
14	Opaque and smooth surface	90.98%	7.245
21	Opaque and smooth surface	91.05%	7.245
28	Opaque and smooth surface	91.05%	7.233

Fig-5: In vitro release profile of Eprosartan Mesylate nanosuspension incorporated buccal film
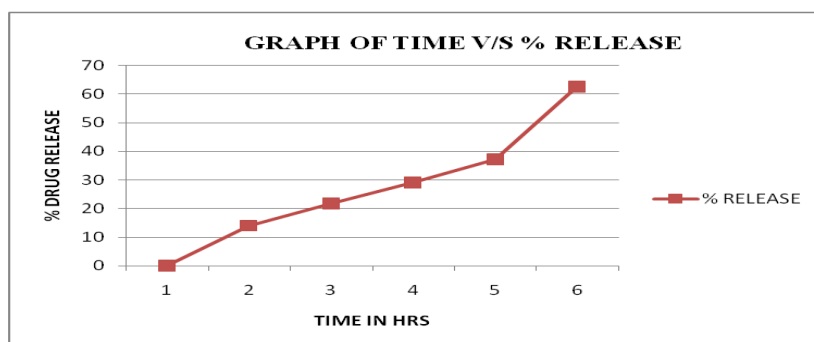
Fig-6: Ex vivo release profile of Eprosartan Mesylate nanosuspension incorporated buccal film

CONCLUSION

The present study has investigated the merits of incorporation of Eprosartan mesylate nanosuspension into hydrophilic films to prepare a suitable formulation for buccal drug delivery. Eprosartan mesylate formed a stable nanosuspension in the presence of PVA having appropriate size, size distribution and surface charge. The nanosuspension incorporation in mucoadhesive films showed increased release rate due to increased drug surface area. In vitro study and ex – vivo study of mucoadhesive patch showed excellent improvement in bioavailability. From the experimental findings, it may be concluded that the prepared nanosuspension incorporated mucoadhesive buccal films are capable of surmounting the shortcomings of oral administration of Eprosartan mesylate, such as high dosing frequency, low oral bioavailability and patient incompliance

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