Enhanced Bioavailability of Nimodipine from Bioadhesive Buccal Bilayered Patches in Human Volunteers

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ABSTRACT

Aims: The objective of the present study was to develop a bioadhesive bilayered buccal patch of Nimodipine (15 mg) using Eudragit Rs 100 as secondary layer and a primary layer with Hydroxy propyl methyl cellulose and Hydroxy propyl cellulose JF.

Methodology: Bilayered buccal patches were prepared by solvent casting technique. The absence of physiochemical interactions between NMDP and the polymer were investigated by differential scanning calorimetry (DSC). Bilayered buccal patches of NMDP were evaluated for *in vitro* drug permeation through porcine buccal membrane, *in vitro* drug release, moisture absorption, surface pH, mechanical properties and *in vitro* bioadhesion.

Results: The results indicated that suitable bioadhesive bilayered buccal patches with desired permeability could be prepared. The bioavailability study was performed in healthy humans in a crossover experimental design. Bioavailability studies revealed that nimodipine possessed good buccal absorption. The relative bioavailability of the optimized buccal patch was found to be 205% in comparison to 30 mg marketed oral tablet. The formulation CC3 showed 68.84 ± 1.4 % release and 46.85 ± 5.1% of drug permeated through porcine buccal membrane in 4 hr. A good correlation was seen between percentage *in vitro* release the extent of bioavailability for nimodine buccal patch.

Conclusion: An improvement of bioavailability was obtained by buccal route to the extent of 2.05 times higher than that of oral route for NMDP. Hence, the development of a bioadhesive bilayered buccal patch for NMDP might be a promising one, as the necessary dose of drug could be decreased, resulting less side effects. Good *ex vivo* - *in vivo* correlation was obtained for NMDP.

Keywords: Bilayered buccal patches, Nimodipine, Bioadhesion, Mechanical properties, Bioavailbility, *In vitro*- *In vivo* correlation

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1. INTRODUCTION

Buccal drug delivery provides an attractive alternative to the oral route of drug administration, particularly in overcoming deficiencies associated with the oral route. Buccal mucosa has an excellent accessibility, an expanse of smooth muscle and relatively immobile mucosa, hence suitable for administration of retentive dosage forms. The direct entry of the drug into the systemic circulation avoids first-pass hepatic metabolism leading to increase in bioavailability [1-4]. Other advantages such as low enzymatic activity, painless administration, easy drug withdrawal, facility to include permeation enhancers/enzyme inhibitors or pH modifiers in the formulation and versatility in designing as multidirectional or unidirectional release systems for local or systemic actions [3]. Various mucoadhesive formulations were suggested for buccal delivery that included buccal patches [5, 6] adhesive tablets [7, 8] and adhesive gels [9]. However, buccal films are preferred to adhesive tablets in terms of flexibility and comfort [10].

Nimodipine (NMDP), a classical BCS II drug, is a dihydropyridine calcium channel blocker originally developed for the treatment of high blood pressure [1,2]. It is not frequently used for this indication, but has shown good results in preventing a major complication of subarachnoid hemorrhage (a form of cerebral hemorrhage) termed vasospasm. In humans, it is administered primarily orally and reaches peak plasma concentrations within one and a half hours. It was reported to be rapidly absorbed after oral administration, resulting in extensive first pass metabolism leading to poor bioavailability (13%). Nimodipine has low dose (30mg), molecular weight (418.4), extensive first pass effect and lipophilic nature (log P, 3.05); need for long term treatment and repetitive dosing. These qualities make this drug an interesting candidate for buccal administration.

The objective of this study was to develop nimodipine bioadhesive buccal bilayered patches for human applications. Initial trials were done by using monolayer patches with different polymers such as hydroxypropyl methyl-cellulose E15, hydroxyl propyl cellulose (HPC JF), polyethylene oxide (PEO) and polyvinyl pyrrolidine (PVP K 30). Drug diffusion from mono-layer patches was not suitable. In order to prevent diffuse of drug from the surface of the patch, mucoadhesive bilayered buccal patches were developed and evaluated for *in vitro* and *in vivo* performance.

2. Materials and Methods

2.1. Materials

NMDP and Eudragit RL100 were generously provided by Dr Reddy's Laboratories, (India). Hydroxy propyl methyl cellulose (Methocel E15) was gifted by Colorcon Asia (Mumbai) and hydroxypropyl cellulose (HPC JF) was gifted by Hercules Inc, USA. Mucin (Crude Type II) was procured from Sigma-Aldrich (Germany) and Dulbecco's buffer and Phenol red were purchased from Himedia (India). High-performance liquid chromatography (HPLC) solvents, (methanol and acetonitrile) were purchased from Merck., India. All other reagents and chemicals used were of analytical grade.

2.2. Drug- polymer interaction study

Differential scanning calorimetric (DSC) studies were used to evaluate any possible drug interaction between NMDP and polymeric materials of the patches. DSC analysis was carried out utilizing a DSC (Mettler- Toledo). The samples size used was 3-5mg and heated from 20 to 450°C at a ramp rate of 40°C/min under nitrogen purge at a flow rate of 20 mL/min.

2.3. Ex vivo permeation of drug through porcine buccal membrane

Porcine buccal mucosa was used because it better resembles human buccal mucosa with regard to lipid barrier composition, permeability, thickness and histology [11]. Porcine buccal tissue from domestic pigs was obtained from local slaughterhouse and used within 2 hours of slaughter. The tissue was stored in Krebs buffer at 4°C after collection. The epithelium was separated from the underlying connective tissue by surgical technique and the delipidized membrane was allowed to equilibrate for approximately one hour in receptor buffer to regain the lost elasticity.

2.4. *In vivo* drug permeation studies in human beings

Buccal absorption test was performed for NMDP solution in 8 healthy male volunteers aged between 24 and 29 years and weighing in between 60 to 75 kg. The human ethical committee of the University College of Pharmaceutical Sciences, Kakatiya University, India, approved the protocol. This method used phenol red, a non absorbable marker for determining saliva volumes. Phenol red was lost neither by absorption nor by swallowing [12, 13]. Before the test, volunteers were asked to moisten their mouth with 20 mL of buffer solution. Twenty mL of phosphate buffer saline (pH 6.6), alcohol and propylene glycol (42:15:43) containing 4 mg NMDP and phenol red (20 µg mL^{-1}) was given to volunteers and were asked to swirl the solution about 60 swirlings per min. The samples of 1 mL were collected from the floor of the mouth at 2, 4, 6, 8, 10, 12, 14, and 16 min using a micropipette. While collecting the samples, volunteers were asked to stop swirling momentarily. After the last sample was collected, all the solution was expelled into beaker. Volunteers were asked to rinse their mouth twice with 20 mL of PBS pH 6.6 and the washings were pooled with the original sample. Volume was noted and the quantity of NMDP present in the samples was estimated by high performance liquid chromatography (HPLC). Phenol red was estimated colorimetrically by making the solution alkaline with sodium hydroxide.

2.5. Estimation of drug content by HPLC

Analysis of samples was performed with a Shimadzu HPLC system equipped with LC-10AT pump, UV-Vis spectrophotometric detector (SPD-10A) and C18 column (Phenomenex; 250 × 4.6 mm; 5 µm) at temperature 45°C. The mobile phase used was a mixture of acetonitrile: water: triethylamine (60:40:0.5). A flow rate of 1 mL min^{-1} was maintained and the detection wavelength was 240 nm. A calibration curve was plotted for NMDP in the range of 5–500 ng mL^{-1}. A linear relationship was observed between the concentration of NMDP and the peak area of NMDP with a correlation coefficient ($r2$ = 0.990). The required studies were carried out to estimate the precision and accuracy of the HPLC method. Sample preparation briefly involved the filtration through 0.45 µm membrane filter, diluted with mobile phase and 20 µL was spiked into column.

2.6. Preparation of bilayered mucoadhesive buccal patches

Bilayered buccal patches were prepared using solvent casting technique with HPMC E15 AND HPC JF as primary polymeric layer, Eudragit RL 100 as secondary layer and propylene glycol as plasticizer. The primary polymer was added to 25 mL of solvent mixture (dichloromethane and methanol, 1:1) and allowed to stand and swell for 4h. Propylene glycol and NMDP were dissolved in 5 mL of solvent mixture and added to the polymeric solution. The resulting solution was kept aside for 2 h to remove entrapped air, transferred to a petri plate, and dried at room temperature. The secondary polymeric solution was prepared by dissolving Eudragit RL 100 and 240 µL of propylene glycol in 10 mL of solvent mixture and poured on the primary layer and allowed for drying at room temperature. The developed patches were removed carefully, cut to size and stored in a desiccator. The composition of the patches is shown in Table 1. Patches were tested for Weight variation, thickness and content uniformity.

Table 1. Formulation ingredients of NMDP bilayered buccal patches

Formulation Codes	NMDP (mg)	Primary layer HPMC E 15 (gm)	Primary layer HPC (gm)	Secondary layer Eudragit RL 100 (mg)
CC1	408	2	-	100
CC2	408	2.5	-	100
CC3	408	3	-	100
CC4	408	3.5	-	100
CD1	408	-	2	100
CD2	408	-	2.5	100
CD3	408	-	3	100
CD4	408	-	3.5	100

2.7. Evaluation of buccal bilayered patches

The weight of the patches was determined using a digital balance (Shimadzu Japan) and thickness with a digital screw gauge (Mitatyo, Japan).

2.7.1. *In vitro drug* release studies

The drug release from bilayered buccal patches was studied using USP type II dissolution test apparatus (Electrolab TDT-08L). Patches were designed to release drug from one side only; therefore, an adhesive impermeable polyester backing layer was placed on the other side of patch. The assembly for release studies was prepared by sandwiching the patch between dialysis membrane 50 KD (Hi Media, Mumbai, India). A piece of glass slide was placed as support to prevent the assembly from floating. The dialysis tubing with tablet inside was secured from both ends using dialysis closure clips and placed in the dissolution apparatus. The dissolution medium was 500 mL having 0.5% Sodium lauryl sulphate (SLS) at 25 rpm and temperature was maintained at 37°± 0.5 C. Samples of 5 mL were collected at predetermined time intervals and analyzed by spectrophotometer at 240 nm.

2.8. Moisture absorption studies

Moisture absorption studies were performed in accordance with the procedure reported earlier [14]. In brief, 5% w/v agar in distilled water, was heated and in hot condition was transferred to Petri plates and allowed to solidify. Then 6 patches from each formulation were weighed and placed over the surface of the agar and left for 2 hr at 37° C and the patches was reweighed . The percentage of moisture absorbed was calculated using the following formula:

% Moisture absorbed = [(Final weight -Initial weight)/Initial weight] X100

2.9. Surface pH study

A combined glass electrode was used for this purpose. The patches were allowed to swell by keeping them in contact with 1 mL of distilled water (pH 6.5 ± 0.1) for 2 h at room temperature, and pH was determined by bringing the electrode in contact with the surface of the patches, allowing it to equilibriate for 1 minute [15].

2.10. Measurement of mechanical properties

Mechanical properties of the patches were evaluated using a microprocessor based advanced force gauge having a motorized test stand (Ultra Test, Mecmesin, West Sussex, UK) and a 25 kg load cell. Strips from the patch with dimensions of 60 x 10 mm and no visual defects were cut and positioned between two clamps separated by a distance of 3 cm. Clamps were designed to secure the patch without crushing it. During test, lower clamp was held stationary and the strips were pulled apart by the upper clamp moving at a rate of 2.0 mm/sec until the strip broke [16]. The force and elongation of film at the point when the strip broke were recorded. The tensile strength (TS) and elongation at break (E/B) values were calculated using the following formula:

$$\text{TS}\ (\text{Kg.mm}^{-2}) = \frac{\text{Force at break (Kg)}}{\text{Initial cross sectional area of the sample (mm}^2)} ---$$

$$\text{E/B}(\%\text{mm}^{-2}) = \frac{\text{Increase in length (mm)}}{\text{Original length (mm) x Cross sectional area (mm}^2)} \text{x } 100$$

2.11. *In vitro* bioadhesion measurement

The adhesive binding of the patches containing NMDP to porcine buccal mucosa was studied in triplicate with the same equipment as the one used for measurement of mechanical properties except that a load cell of 5 kg was used for this study. In this test, porcine buccal membrane was secured tightly to a circular stainless steel adaptor and the buccal patch to be tested was adhered to another cylindrical stainless steel adaptor similar in diameter using a cyanoacrylate adhesive. During test, 100 µL of 1% w/v mucin solution was spread over the surface of the buccal mucosa and the patch was immediately brought into contact. A force of 0.5 N was applied for 180 sec to enhance the contact of the patch with the mucosa. At the end of the contact time, upper support was withdrawn at a speed of 0.5 mm sec-^{1} until the patch was completely detached from the mucosa [17]. The work of adhesion was determined from the area under force-distance curve while the peak detachment force was the maximum force required to detach the patch from the mucosa.

2.12. *In vitro* permeation of NMDP through porcine buccal membrane from buccal Patch

In vitro permeation of NMDP from buccal patches for the selected formulation (CC3) through porcine buccal membrane was studied. Buccal membrane was isolated as described in tissue preparation section. The membrane was mounted over a Franz diffusion cell whose internal diameter is 2.1 cm. The buccal patch was sandwiched between the buccal mucosa and the dialysis membrane, so as to secure the patch tightly from getting dislodged from the buccal membrane. The entire set up was placed over magnetic stirrer and temperature was maintained at 37° C. Samples of 1 mL were collected at predetermined time points from receptor compartment and replaced with an equal volume of fresh solution, and analyzed by HPLC.

2.13. Bioavailability study

The study protocol was reviewed and approved by the institutional human ethical committee (file no. UCPSc/BA/2011-2) University College of Pharmaceutical Sciences, Kakatiya University, Warangal, India. *In vivo* bioavailability study was conducted in eight healthy male volunteers. Randomized cross over design was employed. The bioavailability of optimized bioadhesive buccal patch was compared with marketed tablet (Nimotab). The volunteers participated in the study were non-alcoholic and had no medication for two weeks prior to the study. Volunteers were allowed free access to food and water, until the night prior to dosing and were fasted for 10 h. Randomized cross over design was followed; Volunteers were divided into two groups, each group consisting of four volunteers. To one group, marketed tablet (Nimotab 20mg) was administered and bioadhesive buccal patch to another group in first phase. In second phase vice versa was followed and was conducted after 2 weeks of wash out period. Blood samples (5 mL) were collected at preset time intervals of 0.5, 1, 1.5, 2, 3, 4, 8, 12 and 24 for patch as well for marketed product. The maximum plasma concentration of nimodipine (C_{max}) and the time to reach C_{max} (t_{max}) were read directly from the plasma concentration versus time data. The area under curve (AUC) was calculated using the linear trapezoidal rule up to the last data point. The elimination rate constant (k) was the slope of the terminal four points in plasma concentration–time curve, and the half life of the preparation ($t_{1/2}$) was calculated by 0.693/k. All values were expressed as their mean ± S.D. (standard deviation).The relative bioavailability values F was calculated using the following formula:

$$F = AUC_{test} / AUC_{reference} \times 100\%$$

2.14. Analysis of serum samples by HPLC method

The quantitative determination of nimodipine in human serum was carried out by HPLC method. To 0.5mL of serum, 200 µL of nifedipine solution (2 µg/mL) was added as internal standard and vortexed for 2 minutes on a cyclomixer. To this 0.3 mL of 1% sodium hydroxide solution was added and vortexed for 3 minutes. Then 5mL of dichloromethane was added and vortexed for 5 minutes followed by centrifugation at 3500 rpm for 10 minutes. The organic layer was separated and subjected to evaporation in a Vacuum oven. The residue was reconstituted with 100 µL of mobile phase and 20 µL of this solution was spiked on to the HPLC Column. The retention time of NFDP and NMDP were 3.6 and 6.4 min respectively and the total runtime was for 8 min.

2.15. Stability of buccal patch

Stability studies of buccal patches were performed for optimized formulation (CC3) in normal human saliva which was collected from humans (aged 22–26) and filtered through Whatman (0.2 µm) membrane filter. Buccal patches were placed in separate petri dishes containing 5 mL of human saliva and placed in a temperature-controlled oven (BioTechnics, India) for 6 h at 37±0.2°C. At regular time intervals (0, 2, 4, and 6 h), the buccal patches were examined for change in color, surface area, and integrity [18]. The experiments were repeated in triplicate (n=3) in a similar manner. Drug content was determined by approprate dilution of human saliva in phosphate buffer pH 6.8 and analyzed by spectrophotometer at 240 nm [19].

3. RESULTS AND DISCUSSION

3.1. DSC Study

DSC analysis of NMDP, HPMC and physical mixture are shown in the Fig.1. NMDP exhibited a sharp endothermic a melting peak with an onset temperature of 130.42^{0}C (ΔH =59.62 J/g).The thermal behavior of HPMC exhibited no such phenomenon in any of the temperature intervals. The appearance of a peak corresponding to the melting of NMDP was also evident in the thermogram of the physical mixture. The results revealed a negligible change in the melting point of NMDP in the presence of polymeric materials.

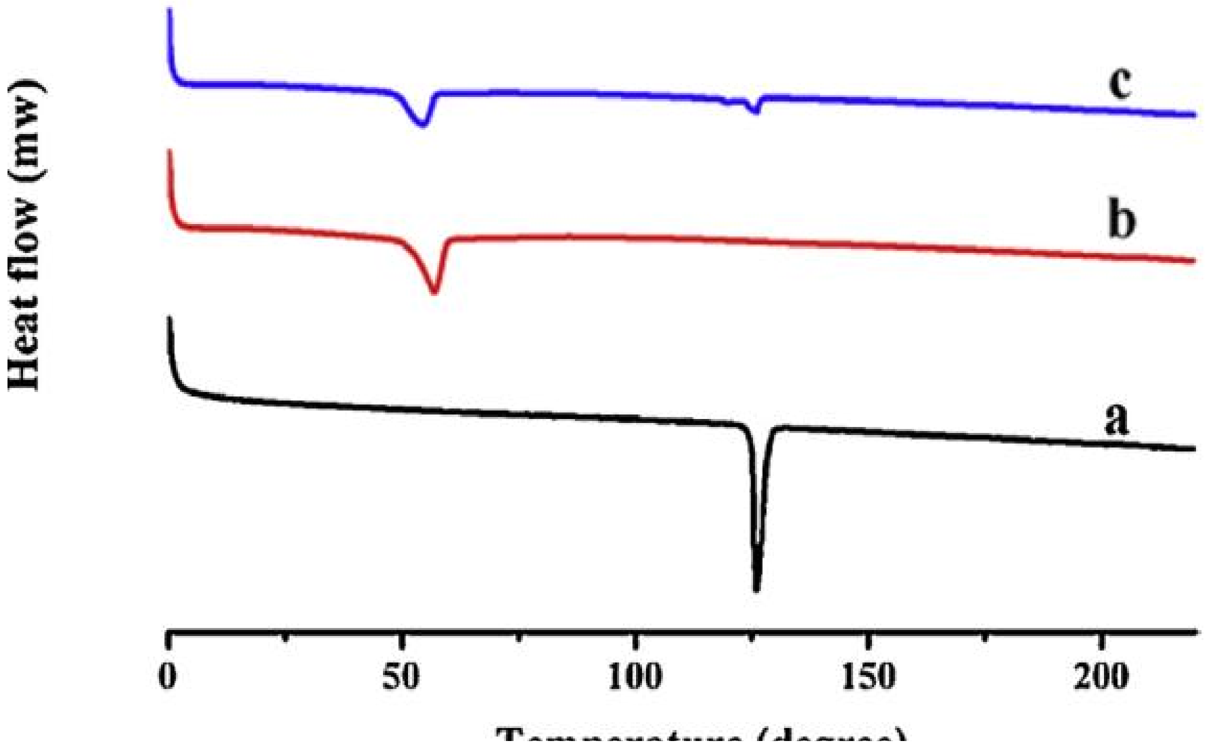

Fig. 1. DSC thermograms of (A) NMDP, (B) HMPC E15 and (C) Physical mixture

3.2. Drug permeation studies of NMDP through porcine buccal membrane

The cumulative amount of NMDP permeated in 4h was found to be 62.21± 6.7 µg/mL and the flux was calculated to be 0.154 µg/hr.cm^2 was presented in Fig. 2.The penetration of drug through the porcine buccal epithelium was found to be rapid up to 1 hour followed by a slow penetration in the next 3 hours. The permeated drug was determined by using the calibration curve plotted with HPLC. The tissue was isolated successfully because no detectable level of phenol red (marker compound) was found in the receiver compartment, whereas NMDP could penetrate freely.

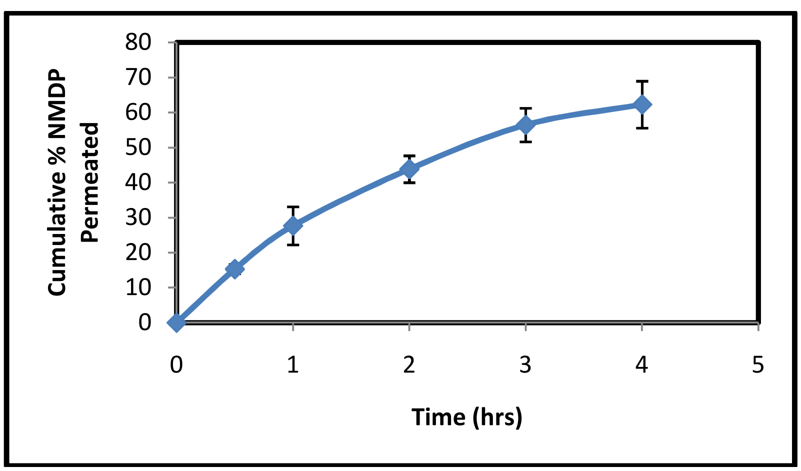

Fig. 2. *In vitro* permeation of NMDP solution through porcine buccal mucosa (mean ± S.D., n = 3)

3.3. Buccal absorption study

The results of buccal absorption study revealed that NMDP could penetrate through the oral cavity. Calculations were performed and results are presented in Fig.3. It was observed that about 42.28 % of the drug was absorbed through the buccal membrane in 16 min. The drug was absorbed at a rapid rate till first 2 min and then onwards the drug absorption was at a uniform rate (Fig.3). However the total amount of phenol red present in 8 collected samples was found to be the same when compared to the initial collected samples of phenol red (400 µg) in solution. This indicated that the volunteers did not swallow the solution. The volunteers reported numbness in the mouth for about 12 to 18 minutes after the test. Hence, there is scope for the development of a buccal patch for NMDP.

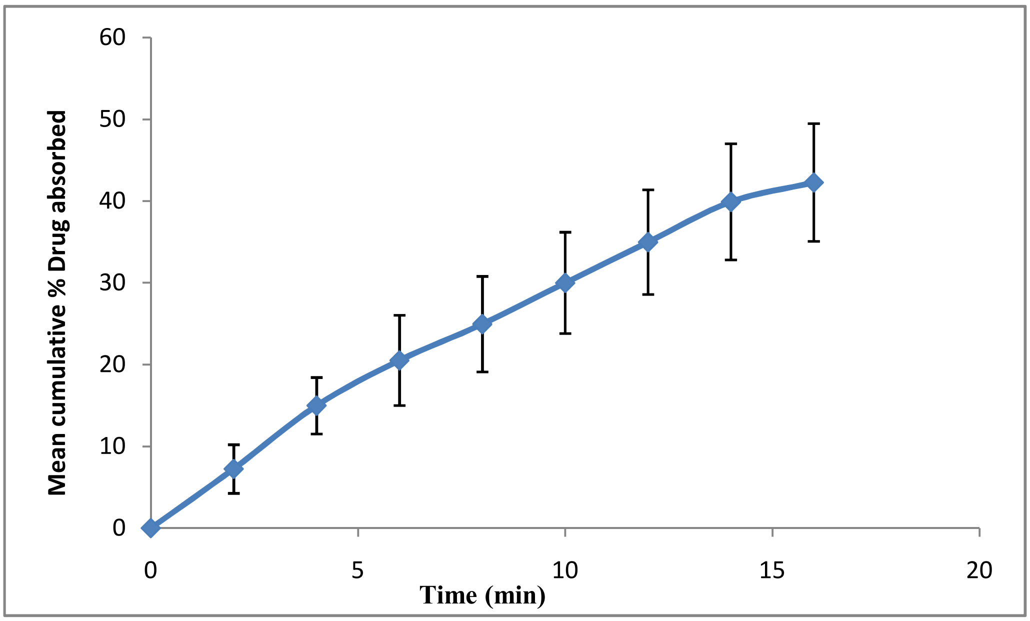

Fig.3. ***In vivo*** **permeation (buccal absorption) study of NMDP in healthy human volunteers mean ± S.D. (n=8)**

3.4. Mass, thickness and drug content determination

The prepared bilayered patches were smooth in appearance, uniform in thickness, mass and drug content, and showed no visible cracks. The mass of the patches ranged from 80 ± 2 to 84 ± 1 mg and the thickness ranged from 494 ± 10 to 580 ± 14µm (Table 2). The drug content in the buccal patches ranged from 88.2 ± 1.2 to 96.3 ± 0.3 %, indicating the favorable drug loading and patches uniformity with respect to drug content.

Table 2. Physicochemical parameters of bilayered buccal patches of NMDP

Parameter / Formulation code	Mass[a] (mg)	Thickness[a] (μm)	Drug Content[a] (%)	Surface pH [a]	Mean% Moisture Absorbed[a]
CC1	80 ± 2	520 ± 10	88.2 ± 1.2	6.6 ± 0.3	136.4 ± 2.2
CC2	82 ± 2	540 ± 15	90.6 ± 0.6	6.2 ± 0.2	124.9 ± 3.2
CC3	84 ± 1	560 ± 12	94.3 ± 0.4	6.4 ± 0.2	112.2 ± 2.4
CC4	83 ± 1	580 ± 14	96.3 ± 0.3	6.8± 0.3	102.8 ± 2.2
CD1	80 ± 2	494 ± 10	88.2 ± 1.2	5.8 ± 0.3	136.4 ± 2.2
CD2	82 ± 2	510 ± 15	90.6 ± 0.6	6.0 ± 0.2	146.9 ± 3.2
CD3	84 ± 1	525 ± 12	92.3 ± 0.4	6.4 ± 0.2	154.2 ± 2.6
CD4	83 ± 1	540 ± 14	94.3 ± 0.3	6.2± 0.3	166.8 ± 2.4

[a] Mean ± SD, *n* = 3

3.5. *In vitro* drug release studies

The drug release profiles of NMDP from buccal patch are shown in Fig. 4. It was clear from the plots that the drug release was governed by polymer content. No lag time was observed as the patch was directly exposed to the dissolution medium. An increase in the polymer content was associated with decrease in drug release rates. The drug release profiles by a model function was attempted using zero order and first order; kinetic pattern using Korsmeyer et al (20,21,22). $Mt/Má=K.t^n$,where Mt/Má is the fractional release of drug, Mt is the amount released at time t, Má is the total amount of drug contained in the patches, t is the release time, K is the kinetic constant and n is the release exponent indicative of the operating release mechanism.

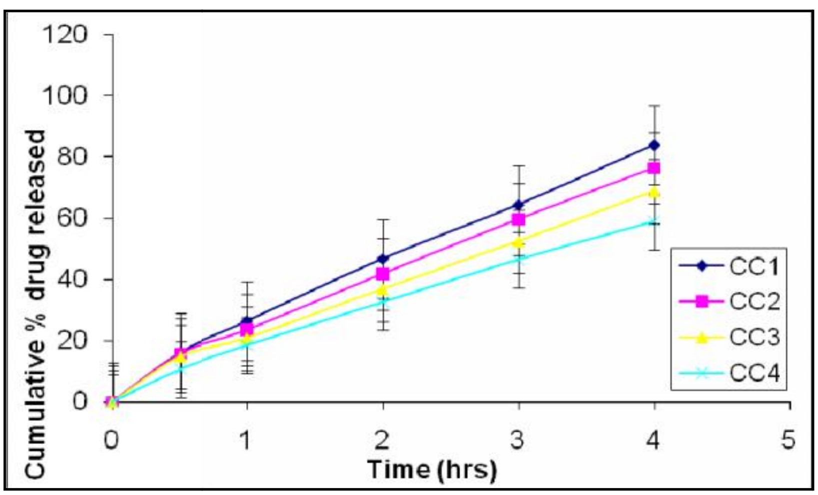

Fig.4. *In vitro* drug release profiles of all the formulations values represented as Mean ± SD. (n=3)

Formulation CC1 showed maximum cumulative drug release at 4hrs among the formulations. The drug release ranged from 58.95% (CC4) to 83.99 % (CC1). However, the difference among the formulations (CC1, CC2, CC3 and CC4) was statistically significant. All the formulations followed Higuchi model release kinetics, as evident from the correlation coefficients of the formulations. CC1, CC2 and CC4 formulations showed fickian release pattern as it was evident from release exponent (n<0.5) except CC3. The formulation CC3 showed non-fickian type of release pattern and Higuchi model as it was evident from release exponent (n>0.51) Table 3.

Table 3. Estimated values of NMDP release exponent (n) and correlation coefficient (R^2) *f*rom bilayered buccal patches for all the formulations

Formulation Code / Release kinetics	CC1	CC2	CC3	CC4	CD1	CD2	CD3	CD4
Zero Order	0.99	0.99	0.98	0.99	0.98	0.99	0.99	0.99
First Order	0.94	0.95	0.97	0.93	0.94	0.98	0.92	0.82
Higuchi	0.95	0.95	0.94	0.95	0.93	0.9	0.91	0.89
Peppas	0.711	0.662	0.521	0.585	0.585	0.511	0.329	0.316
n value	0.5	0.5	0.6	0.5	0.5	0.5	0.6	0.6

Increasing the amount of the polymer in the patches produced the water-swollen gel like state that could substantially reduce the penetration of the dissolution medium into the patches and so the drug release was delayed. The Eudragit -RL 100 layer minimized the diffusion of the drug molecules from the patches. In addition, Eudragit layer could control the release of the drug from the patches. This was evident from the release studies of the monolayer patches where the drug release was rapid. Therefore, a rate controlling membrane could be used to control the release. Formulation CD1 showed maximum drug release among the formulations. The drug release ranged from 50.98 (CD4) to 74.98 % (CD1). However, the difference among the formulations (CD1, CD2, CD3 and CD4) was statistically insignificant. All the formulations followed Higuchi model release kinetics, as evident from the correlation coefficients of the formulations. CD1, and CD2 formulations showed fickian release pattern as it was evident from release exponent (n<0.5) except CD3 and CD4.

3.6. Moisture absorption studies of NMDP bilayered patches

Moisture absorption studies evaluated the integrity of the formulation upon exposure to moisture. The results of moisture absorption studies, mass, thickness, drug content and surface pH are presented in Table.3. Results showed that there are differences in moisture absorption with CC1 to CC4 and CD1 to CD4. The percentage moisture absorbed ranged from about 136.4 to 102.8 % w/w for CC1 to CC4 formulations and 136.4 to 166.8 % w/w for CD1 to CD4 formulations. When the patches were placed without backing membrane complete swelling followed by erosion was observed indicating that the drug release mechanism involved swelling of the polymer initially, followed by drug release from the swollen matrix by diffusion.

3.7. Surface pH studies of NMDP bilayered patches

The surface pH of the patches was determined in order to investigate the possibility of any irritation or side effects, *in vivo*. Since, an acidic or alkaline pH may cause irritation to the buccal mucosa, it was attempted to keep the surface pH as close to neutral as possible (Table 2). The surface pH of all the patches was ranged from 5.8 ± 0.3 to 6.8 ± 0.3 and was near or above 6 and hence, these patches could be expected, not to cause any irritation in the buccal cavity. The pH of buccal membrane and the patches were having a pH nearer to this value.

3.8. Mechanical properties of films

An ideal buccal film, apart from good bioadhesive strength, should be flexible, elastic, and strong enough to withstand breakage due to stress caused during its residence in the mouth. The tensile strength (TS) and elongation at break (E/B) shows the strength and elasticity of the film. A soft and weak polymer is characterized by a low TS and E/B; a hard and brittle polymer is defined by a moderate TS, and low E/B; a soft and tough polymer is characterized by a moderate TS and a high E/B; whereas a hard and tough polymer is characterized by high TS and E/B. An ideal buccal film should have a relatively high TS and E/B. The results of the mechanical properties, i.e., TS and E/B, are presented in Table 4. TS and E/B increased with the increase in polymer content in the formulations CC1 to CC4. Maximum TS was exhibited by CC4 (12.07 ± 2.8 $kg.mm^{-2}$) which was statistically significant different ($p<0.05$) compared to CC1 (5.46 ± 1.0 $kg.mm^{-2}$). The optimized formulation CC3 showed 9.69 ± 2.1 $Kg.mm^{-2}$ and 27.4 ± 3.2 % mm^2 of TS and E/B respectively. Maximum E/B was seen with CC4 (36.6 ± 3.0 % mm^2) and the least was observed with CC1 (17.2 ± 3.2 % mm^2). In the CD series TS increased with the increase in polymer content in the formulations CD1 to CD4. Maximum TS was exhibited by CD4 (14.07 ± 2.6 Kg/mm^2) and minimum for CD1 (2.46 ± 1.0 Kg/mm^2). E/B was found to decrease from CD1 to CD 4 with increase in polymer concentration. Maximum E/B was found for CD1 (36.3 ± 3.2% mm^2) and the least was for CD4 (12.6 ± 3.0% mm^2).

Table.4. *In vivo* residence time, mechanical and bioadhesive parameters of bilayered buccal patches of NMDP (HPMC) values represent Mean ± SD (n = 3)

Parameter / Formulation code	I.R[1] (min)	T.S[2] (Kg/mm^2)	E/B[3] (% mm^2)	P.F[4] (N)	W.A[5] (mJ)
CC1	185 ± 20	5.46 ± 1.0	17.2 ± 3.2	1.42 ± 0.04	0.41 ± 0.01
CC2	218 ± 16	7.48 ± 1.2	24.2 ± 2.2	1.84 ± 0.06	0.81 ± 0.01
CC3	240 ± 22	9.69 ± 2.1	27.4 ± 3.2	2.68 ± 0.08	1.12 ± 0.02
CC4	256 ± 20	12.07 ± 2.8	36.6 ± 3.0	3.32 ± 0.12	2.18 ± 0.02
CD1	185 ± 20	2.46 ± 1.0	36.3 ± 3.2	2.12 ± 0.04	0.62 ± 0.01
CD2	218 ± 16	7.08 ± 1.4	22.2 ± 2.2	2.84 ± 0.06	1.21 ± 0.02
CD3	240 ± 22	9.48 ± 2.2	16.4 ± 3.2	3.48 ± 0.08	1.08 ± 0.02
CD4	256 ± 20	14.07 ± 2.6	12.6 ± 3.0	4.32 ± 0.12	2.68 ± 0.03

[1]**I.R:** *In vivo* Residence Time, [2]**T.S:** Tensile strength, [3]**E/B:** Elongation at a break,
[4] **P.F:** Peak detachment force, [5]**W.A:** Work of adhesion

3.9. *In vitro* bioadhesion studies

In vitro bioadhesion measurements are performed routinely for mucoadhesive dosage forms, and the most commonly used technique for evaluation of buccal patches is the measurement of adhesive strength. Work of adhesion, calculated from area under the force distance-curve, is a measure of work that must be done to remove a patch or film from the tissue. Peak detachment force is the maximum applied force at which the patch detaches from tissue. The peak detachment force and work of adhesion for all formulations is shown in Table 4 and for the optimized formulation (CC3) it was calculated as 2.68 ± 0.08 N and 1.12 ± 0.02 mJ respectively. The work of adhesion and peak detachment force values increased with increase in the polymer concentration in the formulation. However, differences could exist due to change in the polymer type or composition of the film.

3.10. *In vitro* permeation of NMDP through porcine buccal membrane from bilayered buccal patch

Formulation CC3 was selected for the *in vitro* permeation studies due to its superior drug release properties in terms of percentage drug released, its capacity to retain the structure in moisture absorption studies, and bioadhesion studies *in vitro.* The results indicated that the drug permeation was slow and about 46.85± 5.1% of NMDP could permeate through the buccal membrane with a flux of 0.124 $\mu g/cm^2/hr$ in 4 hours. The required flux calculated for NMDP (0.134 $\mu g/cm^2/hr$) was closely obtained with formulation CC3 (0.124 $\mu g/cm^2/hr$). In order to reach the required flux, the patch area was to be increased slightly. The results of drug permeation revealed that NMDP was released from the formulation and permeated through porcine buccal membrane and hence could possibly permeate through the human buccal membrane.

3.11. Selection of the formulation for bioavailability studies

Formulations CC3 was selected for the bioavailability studies because of its good drug release properties in terms of percentage drug permeated (42.21 % in four hours), its capacity to retain the structure in moisture absorption studies and bioadhesion studies *in vitro* and *in vivo*. Bioadhesion values both *in vivo* and *in vitro* revealed that CC3 could be suitably used for bioadhesive buccal delivery. The bioavailability study was conducted with 30 mg IR tablet as standard and 15 mg patch (CC3) as test.

3.12. *In vivo* bioavailability study in humans and evaluation of PK parameters

All the volunteers tolerated the treatments well and there were no cases of adverse affects during the study period. In the study 30mg of NMDP tablet was compared with 15mg of NMDP patch. Jindal scientic sigmastat statistical software was used for statistical analysis.There was no statistically significant difference in pharmacokinetic parameters, C_{max}, T_{max}, $T_{1/2}$, $AUC_{0-\infty}$, AUC_{0-24} and Cl. The pharmacokinetic parameters C_{max} decreased from 25.85 ± 5.8 to 21.17 ± 4.6 ng / mL, T_{max} increased from 1.68 ± 0.59 to 3.25 ±0.46 hrs, AUC_{0-n} increased from 233.06 ± 71.7 to 252.55 ± 56.3 ng.hr/mL. AUC_{total} increased from 346.33 ± 96.6 to 354.75 ± 67.6, $T_{½}$ decreased from 15.49 ± 3.6 to 13.05 ± 1.1 hrs and Cl decreased from 0.091±0.03 to 0.082 ± 0.01 in the patch. The results suggested that the NMDP was absorbed well from the buccal tissue and circumvented the first pass metabolism and thereby increased the NMDP concentration in serum. From the results it was clear that patches containing half dose (15mg) could be used instead of tablets having 30mg dose (Fig.5). The relative bioavailability of the optimized buccal patch was found to be 205% by considering 30mg marketed oral tablet as a standard if proportionate changes are made to the marketed product dose.

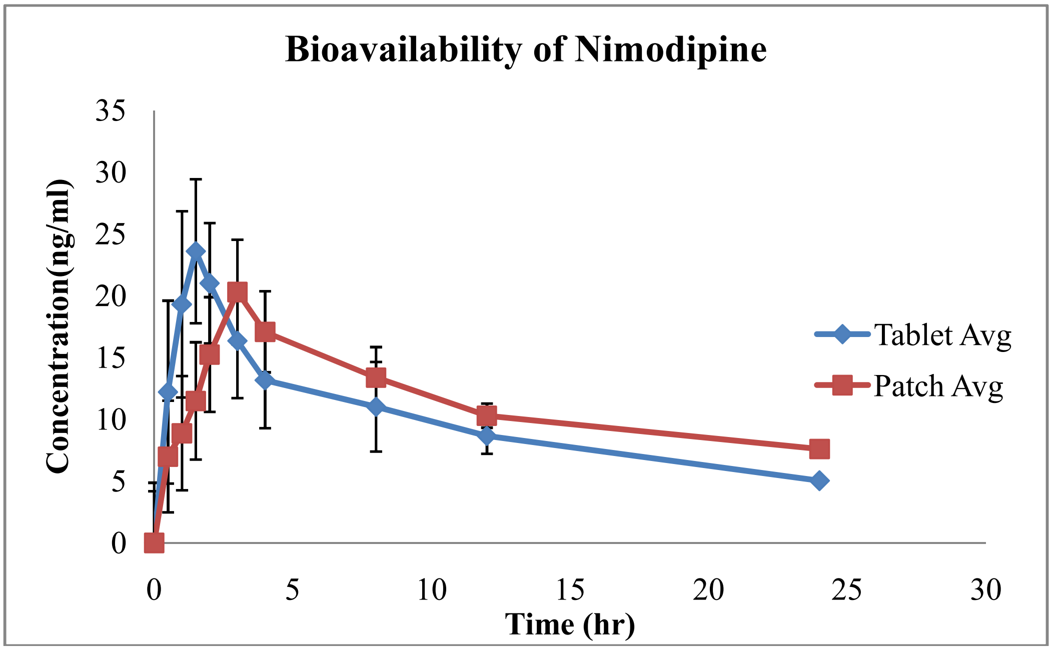

Fig.5. Serum concentration and time profiles of NMDP in tablets and patches

3.13. *In vitro – in vivo* correlation of NMDP between AUC and % released *in vitro*

In vitro - in vivo correlation between the cumulative % of drug released *in vitro* and AUC is presented in Figure 6. The figure shows a biphasic curve pattern, which could be clearly distinguished as two regions. Each region had shown a good correlation coefficient R^2 = 0.8008 and R^2 = 1. This may be due to the fact that, the drug was released from the formulation which got partitioned into buccal membrane and absorbed in to the systemic circulation. The initial lag phase in the curve was attributed to the dissolution of drug and building up of flux at the buccal membrane. The flux results in rapid absorption of NMDP into systemic circulation and resulted as second part of the curve Fig.6).

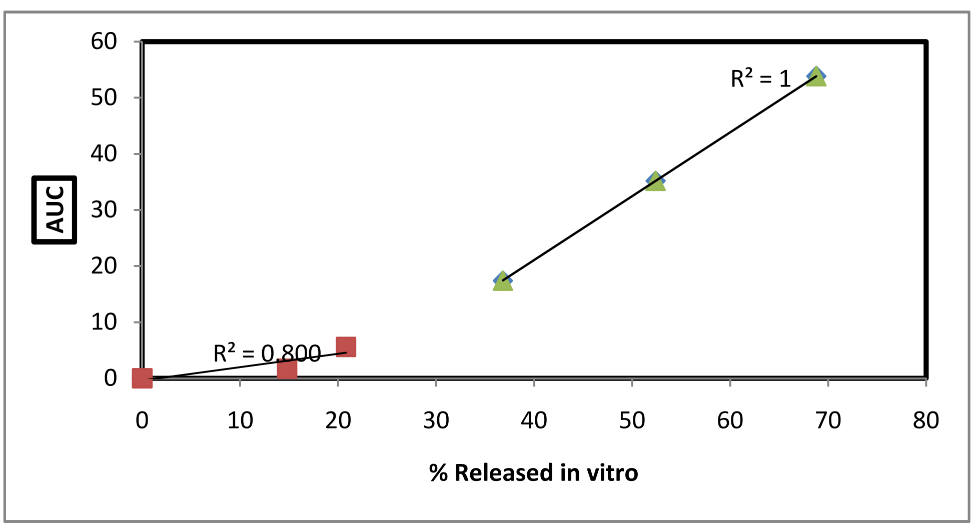

Fig.6 *In vitro – in vivo* correlation of NMDP between AUC_{0-n} and % released *in vitro*

3.14. Stability study of NMDP bilayered patch

The stability of the optimized formulation (CC3) was investigated as per ICH guidelines. The formulation was stored at a temperature 40 ± 0.5^{0}C and 75 ± 5% RH for 3 months. The results of the stability studies revealed that there was no significant change in release, drug content and *ex vivo* permeation through porcine buccal membrane (Table.4.43). Only a 4.2% of change (lesser content than initial drug content) was observed. As the change is less than 5% in the formulation stability of the bilayered buccal formulations could be expected to have the required stability.

Table 5. Stability study of the optimized formulation (CC3) for three months

Parameter / Duration	Drug content [a](mg)	% drug released	Cumulative % drug permeated
Initial	9.90 ± 0.08	65.9 ± 1.89	46.4 ± 2.87
1 Month	9.84 ± 0.08	64.4 ± 3.29	44.2 ± 1.49
2 Months	9.80 ± 0.16	62.6 ± 2.34	42.8 ± 1.88
3 Months	9.58 ± 0.18	60.2 ± 1.22	40.2 ± 1.42

Mean ± SD, *n* = 3.

4. CONCLUSION

Nimodipine bilayer buccal patches were developed and based on the results, it was concluded that polymers selected were suitable for the development of bilayered mucoadhesive matrix type buccal patches. Bilayered formulations containing drug: polymers at a ratio of 1:8 showed reasonable bioadhesion measured in terms of peak detachment force and work of adhesion values and also exhibited satisfactory *in vivo* residence time in the buccal cavity. The optimized buccal patch CC3 contained hydroxyl propyl methyl cellulose E15 was selected based on the buccal absorption, *in vitro* release, moisture absorption, bioadhesion, *in vivo* residence time and stability studies. Results of bioavailability study showed improved permeation of NMDP from bilayered buccal patch when compared with oral tablet. An improvement of bioavailability was obtained by buccal route to the extent of 2.05 times higher than that of oral route for NMDP. Hence, the development of a bioadhesive bilayered buccal patch for NMDP might be a promising one, as the necessary dose of drug could be decreased, resulting less side effects. Good *ex vivo* - *in vivo* correlation was obtained for NMDP.

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