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Utilizing Natural Mucoadhesive Polymers for Development of Nanoparticle Based Cilnidipine Buccal Films

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Abstract

The less oral bioavailability of Cilnidipine is primarily due to low solubility leads to reduced therapeutic efficacy. The study aimed to develop mucoadhesive buccal films to increase the bioavailability resulting in improved efficacy of this poorly soluble drug trough the buccal mucosa for the treatment of hypertension. Buccal films of Cilnidipine nanoparticles were developed by using different concentration of polymers such as HPMS K4M, HPMC K15M and Carbopol 934 by solvent casting technique. The developed films were characterized for the surface texture, thickness, surface pH, folding endurance, swelling index, content uniformity, mucoadhesive strength, moisture loss, moisture absorption, drug release, ex-vivo drug permeation and stability studies. F9 formulation was optimized as best formulation based on the physico chemical parameters, in vitro dissolution and ex-vivo permeation studies. The result indicates that the drug releases from the buccal films are slow and prolonged over the period of 12 h. There was no significance changes were observed during the stability studies indicates that, the prepared buccal films are stable. Hence, mucoadhesive buccal films can be used as an alternative particularly in the bed time with improved absorption of the dug through buccal mucosa, resulting in better bioavailability and therapeutic efficacy.

Keywords: *Mucoadhsive drug delivery systems, Buccal films, Cilnidipine, in-vitro dissolution, ex-vivo permeation study*

1. Introduction

Therapeutic agents administered through the oral route is one of the most convenient route because of ease of administration, low cost and preferred of drug delivery to systemic circulation which leads to high patient compliance. But, drugs in conventional dosage forms administered through oral route have limitations because, they unable to restrain and localize systems at GIT[1]. Hence, change is required for the better absorption of drugs and also for the better systemic drug delivery[2].

The term mucoadhesion refers to adhesive interactions with any biological membrane and substances originating biologically[3]. Administration of medication through the mucosal buccal membranes lining the cheeks and gums delivers the drug via buccal mucosa provides an alternative strategy to deliver the drug to systemic circulation[4]. Aadvantages of buccal drug delivery systems are directly delivered the drug to systemic circulation, avoiding degradation by gastro-intestinal enzymes, avoids first pass metabolism, rapid drug transport, drug bioavailability can be increased because of rich blood supply and permeability of buccal mucosa[5], self-medication is possible, in case of toxicity dosage form can be removed, frequency of drug administration can be reduced due to prolonged drug delivery and improves patient compliance[6].

Hypertension is the cardiovascular disease; nearly 1 billion individuals worldwide are affected by hypertension[7]. It refers to prolonged and persistent elevation of blood pressure above the normal range (80/120Hg). Hypertension has no symptoms, but it may result in problems like stroke, heart attack and kidney damage. Risk factors of hypertension are age, weight and size, alcohol, smoking tobacco, sodium salt intake, gender, family history, sedentary life style and stress[8].

Cilnidipine is a new generation of CCB and dual blocker of L-type Ca2+ channels in vascular smooth muscle and N-type Ca2+ channels in sympathetic nerve terminals that supply blood[9]. It inhibits the Ca+ influx in both in vessel and in the nerve. Hence, it causes vasodilation and inhibits the releases of norepinephrine's, which decrease the heart rate & also decreases cardiac contraction in heart[10, 11]. It was reported that Cilnidipine has low bioavailability determined to be approximately 13%. This low bioavailability is due to its low aqueous solubility. Hence, efforts were made in order to find an innovative formulation that can significantly improve the bioavailability of this drug[12]. Therefore, the current study aims to develop mucoadhesive buccal films to modulate the rate of drug release and improve the bioavailability of Cilnidipine.

2. Materials And Methods

Materials

Cilnidipine was provided free sample by Micro labs, Bangalore, India. HPMC K4M, HPMC 15KM, Carbapol 934 were acquired from Balaji Drugs. Analytical grade chemicals and solvents were used.

Development of buccal films of cilnidipine nanoparticles in QbD framework

Quality Target Product Profile (QTPP): The design criteria for the product development essentially provided by the QTPP. Based on the functional attributes of mucoadhesive buccal films and also based on literature review (table 1).

Table 1: QTPP for Cilnidipine buccal film formulations

QTPP Elements	Target	Justification
Type of Dosage	Mucoadhesive drug delivery	Improvement of bioavailability
Dosage form	Films	Administration easy
Strength of Dosage	10 mg	Target dose
Route of administration	Buccal	Convenient route
Stability	In accordance with terms of ICH Q stability studies	To evaluate drugs and formulation excipients' degradation patterns.

Critical Quality Attributes (CQAs): Product attributes defining the QTPP for mucoadhesive drug delivery systems includes swelling index and % drug release. CQAs of mucoadhesive buccal films with proper justification have been illustrated (table 2).

Table 2: CQAs of Cilnidipine buccal film formulations and their justification

Quality attributes of the product		Target	CQA	Justification
Physical attributes	Color	Suitable to the patient	No	The products effectiveness and safety are not directly correlated with its physical characteristics.
	Odor			
	Appearance			
Drug release at 12h (%)		90.003 to 99.93	Yes	Has direct correlation with bioavailability
Swelling Index (%)		63.33 to 90.20	Yes	Has direct correlation with bioavailability

Mixture design: Development and optimization of buccal films was done by using a DoE method called Mixture design. For the development, Extreme Vertices Mixture Design[13] is used, where the factors are the mixture components are subjected to constraints such as low and high level for each factor and the components or the factors expressed as fractions which sum to one or 100 %[14]. For the development of buccal films, the independent variables factored in the EVMD design were HPMC K4M, HPMC K15M and Carbopol 934 (table 3).

Table 3: Composition and limits

Factors	Role	Low Values	High Values
HPMC K4M (mg)	Mucoadhesive polymer	30	90
HPMC K15M (mg)	Mucoadhesive polymer	30	90
Carbopol 934 (mg)	Mucoadhesive polymer	30	90

The dependent variables in the design are drug release at 12 hr (%) and swelling study (table 4).

Table 4: Responses

Responses	Objectives	Lower Limit	Upper Limit
Drug release at 12hr (%)	Maximize	90.003	99.93
Swelling index	Maximize	63.33	90.20

Least Squares Fit: % CDR (12h) & Swelling Index

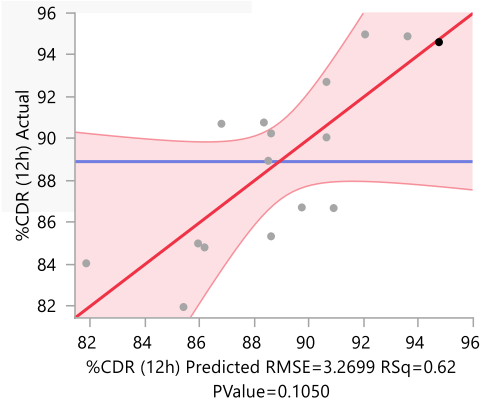

Fig. 1a %CDR (12h)

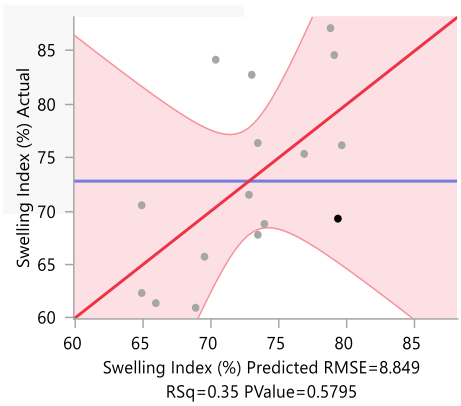

Fig. 1b Swelling Index

Least Squares Fit: Prediction Profiler % CDR (12h) & Swelling Index

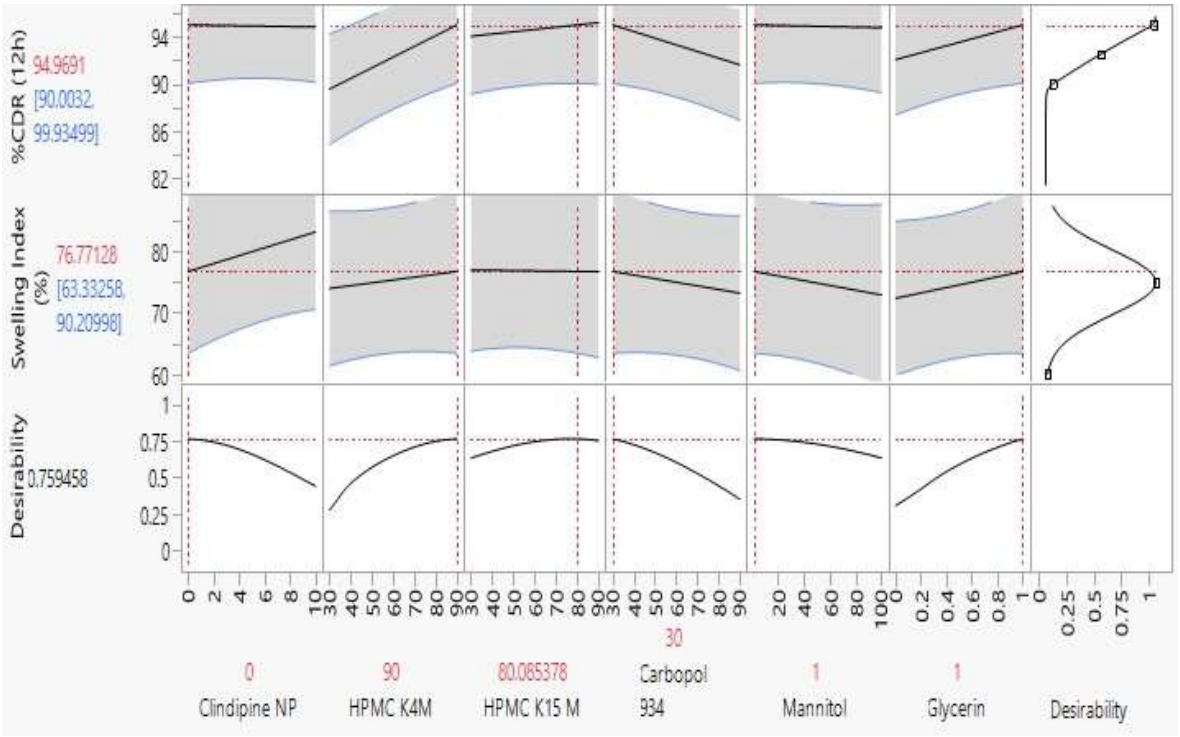

Fig. 2. Least Squares Fit: Prediction Profiler % CDR (12h) & Swelling Index

Preparation of buccal films by solvent casting method

Solvent casting method was employed to prepare the buccal films by using mucoadhesive polymers such as HPMC K4M, HPMC K15M and Carbopol 934. Polymers were weighed based on the batch formula and dissolved in 15 ml water and kept aside for swelling of polymers for 15 min. After swelling, it was stirred on the magnetic stirrer for 1h to form bubble free and clear solution and finally the volume was made up to 20 ml with water. Cilnidipine nanoparticles with mannitol was dissolved with ethanol 5 ml in another beaker and stirred for 4 h. The polymeric solution, drug solution and glycerin were mixed in a magnetic stirrer to get homogeneous casting solutions. The casting solutions poured in to the pre lubricated glass petri plate and kept for drying in hot air oven at 40°C for 12 h. The dried buccal films were removed from petri plate and packed in the aluminium foil and stored in the airtight container[15].

Preparation of backing membrane

Ethyl cellulose was weighed and well mixed with 15 ml of chloroform with a magnetic stirrer. PEG 400 (1%) was mixed and set aside to get un-bubbled and clear solution. This mixer was casted on to petri plate and to remove the chloroform completely, it was placed in the vacuum desiccators for up to 24 hours. In the aluminum foil, the dried films were packed[14].

Preparation of the final composite

Chloroform was sprayed on to the one side of the buccal film and another side of the backing membrane to adhere each other. The prepared films dried to remove the chloroform and cut in to 2 x 2 $cm^2$and packed in the aluminum foil and packed in the glass container for further studies[14].

Characterization of buccal films

Appearance

Appearance of the prepared buccal films was done for visible imperfection and contact analysis was done on surface texture[16].

Surface texture

Pressing lightly the films with a finger in the corners and the center, the surface roughness of the films was assessed[18].

Thickness

Thickness of the mucoadhesive buccal films was measured at five separate locations including four corners and one in the middle and average thickness of films was calculated. A digital micrometer screw gauge was used to measure thickness[21].

Folding endurance

The buccal films were repeatedly folded at the same place till the film breaks. The numbers of times folded without breaking give the value of folding endurance[20].

Swelling index

Initial weight of the films were noted and kept it in petri plate having 5ml of distilled water to swell for 30min. After that the films were then taken, dried with blotting paper and reweighed and percentage swelling index was calculated employing the formula[21].

Swelling index (%) = Final Weight - Initial Weight / Initial Weight x100

Mucoadhesive strength

It was determined by using an analytical balance, film was attached to the glass slide by using one drop of water and placed one side of the analytical balance and weighing pan was attached on another side. Weight was gradually increased until glass slide separated from the film. The weight needed to separate the film from the glass slide was noted[21].

Surface pH

Distilled water (1ml) was placed in the petri plate and the prepared films were exposed to it for 15 minutes for swelling. The surface pH of the film was measured by using pH meter by placing the electrode in contact with the film surface and allowing it to equilibrate for 1 minute and the surface pH was noted[15].

Moisture loss

Pre weighed films were put in a desiccator with anhydrous calcium chloride for three days. Films were taken out from desiccator and reweighed and percentage of moisture loss was estimated[20].

Moisture loss (%) = Final Weight – Initial Weight / Initial Weight x100

Moisture absorption

The films were pre weighed and put in a desiccator with saturated ammonium chloride for three days and the relative humidity was maintained with 79.5% RH. The moisture uptake was

determined when the films were taken and again weighed[20].

Moisture uptake (%) = Final Weight – Initial Weight /Initial Weight x100

Content uniformity

Randomly three films were selected from each formulation and films were cut in to small pieces and dissolved in phosphate buffer pH 6.8 in the 100 ml volumetric flask. Using buffer solution, the volume was adjusted upto the mark after the solution had been sonicated for 30 min. The prepared solutions were filtered using 0.45mm Whatman filter paper. From the filtrate 1 ml of the solution was taken and diluted to 10 ml with same solution and analyzed[22].

In-vitro dissolution studies

Utilizing a USP dissolution equipment type II (Paddle), the studies were conducted. A dissolution media volume of 900 ml was kept at a temperature of 37 ± 0.5°C while the paddle was rotated at a rate of 50 rpm. The film had one side that was adhesively connected to a glass disk, and that film was then placed in the bottom of the dissolution tank so that it remained on the upper side of the disk. At certain intervals, samples were taken out of the vessel and replaced with the equal volume of fresh buffer medium. The collected samples were filtered using 0.45mm Whatman filter paper with the necessary dilutions, and a UV-Visible spectrophotometer was used to measure them at 240nm[22].

Ex-vivo diffusion studies

To the Franz diffusion cell phosphate buffer pH 6.8 was poured into the receptor compartment until it was full. In between the donor and receptor compartments, fresh sheep mucosa was placed and then the buccal film was hydrated with buffer solution and in contact with the mucosal membrane. The temperature of the diffusion cell assembly was maintained at 37±1°C, and a magnetic stirrer was used to stir it. Samples were taken out at predetermined intervals of time and analyzed spectrophotometrically at 240nm[20].

Accelerated stability studies

Formulation F9 was optimized based on the physico chemical parameters, *in-vitro* drug release and *ex-vivo* diffusion patterns and subjected to stability studies. The films were wrapped with aluminium foil and kept at temperature of 40 ± 2°C and 75±5 % RH. After one-, three- and six-month period, studies were done on things like drug content, *in-vitro* drug release, and folding durability[22].

3. Results and Discussion

Buccal films of Cilnidipine nanoparticles using different polymers in different ratios were developed. The prepared films were examined for a number of physiochemical factors and the influence of different polymers in different ratios on drug release was investigated (table 7). After evaluating the films surface textures, it was discovered that they were smooth. The thickness of formulated buccal films was measured and it varies from 0.545 ± 0.07 to 0.905 ± 0.04 mm. All of the formulations' folding endurance values fall between 314 ± 0.21to 475 ± 0.45 and it reveals that the prepared films are having good strength and flexible with good film properties. The values were in range of 6.11 ± 0.011to 6.98 ± 0.015 for different formulations i.e., within the range of buccal mucosa. Hence, it will not produce any local irritation to the mucosa. % moisture absorption of all the batches was in ranges of 4 ± 0.01 % to 7 ± 0.02 %.

Table 7 Evaluation Parameters

Formulation Code	Thickness (mm)	Folding endurance	Surface pH	% Moisture absorption
BTF1	0.545±0.07	314±0.21	6.87±0.01	4±0.04
BTF2	0.665±0.02	343±0.38	6.25±0.013	5±0.02
BTF3	0.730±0.03	397±0.74	6.11±0.011	7±0.01
BTF4	0.550±0.01	322±0.57	6.48±0.011	5±0.01
BTF5	0.675±0.06	394±0.31	6.33±0.01	5±0.03
BTF6	0810±0.03	449±0.46	6.17±0.002	7±0.02
BTF7	0.570±0.01	334±0.71	6.98±0.015	4±0.01
BTF8	0.710±0.02	403±0.92	6.65±0.013	4±0.03
BTF9	0.905±0.04	475±0.45	6.21±0.011	5±0.02

All the formulations showed that the moisture loss (2.14 ± 0.05 % to 4.41 ± 0.04) from the films was within acceptable bounds is proof that they were stable against microbial development. The mucoadhesive strength of the films was between 10.5 ± 0.05 g to 17.7 ± 0.04 g, that the findings indicate that strength improves as polymer concentration does. Swelling index results were between 31 ± 0.034 % to 45 ± 0.023 %. For all of the formulations, the estimated percentage of drug content ranged from 94.72 ± 0.11% to 99.34 ± 0.15%. The results are tabulated in table 8.

Table 8 Evaluation Parameters

Formulation code	Moisture loss (%)	Mucoadhesive strength (g)	Swelling index (%)	Drug content (%)
BTF1	2.37±0.03	10.5±0.05	77±0.031	97.54 ± 0.14
BTF2	3.12±0.07	12.3±0.04	71±0.018	96.13 ± 0.18
BTF3	4.41±0.04	15.1±0.07	75±0.023	94.72 ± 0.11
BTF4	2.25±0.06	10.9±0.01	81±0.034	95.07 ± 0.23
BTF5	2.77±0.09	13.5±0.12	82±0.016	99.34 ± 0.15
BTF6	4.15±0.04	15.7±0.05	79±0.043	97.50 ± 0.17
BTF7	2.14±0.05	11.2±0.06	83±0.034	95.25 ± 0.22
BTF8	2.98±0.01	12.7±0.08	91±0.016	96.68 ± 0.25
BTF9	3.85±0.08	17.7±0.04	90±0.043	98.21 ± 0.21

In-vitro drug release test findings (fig.3) show that the drug release was influenced by the kind and concentration of polymers, and that as polymer concentration increased, the drug release decreased.

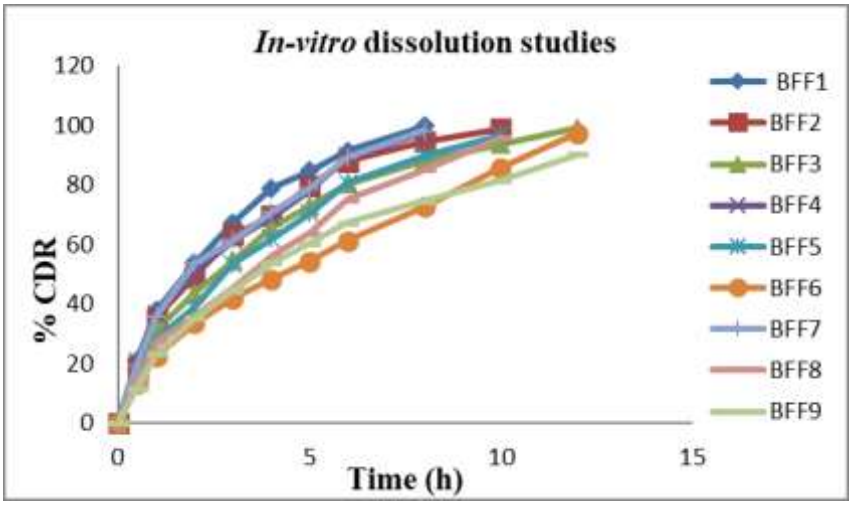

Fig. 3. Comparative *in-vitro* drug release

The *in-vitro* drug release data was fitted into a number of kinetics models, including Zero order, First order, Higuchi, and Korsemeyer peppas (fig.4A, 4B, 4C, 4D). Higuchi model plots were found linear with r^2 values (0.993) nearer to 1and the value of "n" was determined to be 0.614 (table 9). The formulation F9 follows diffusion-controlled mechanism and non Fickian release

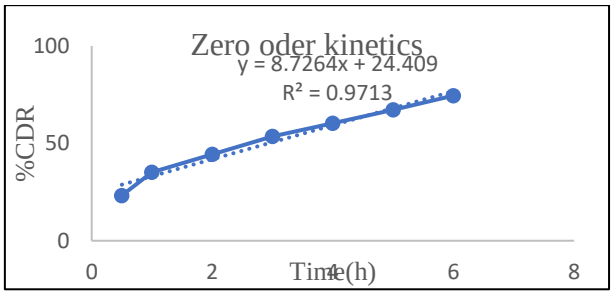

Fig. 4 a). Zero order kinetics

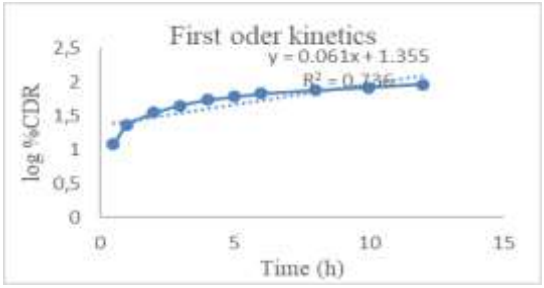

Fig. 4 b). First order kinetics

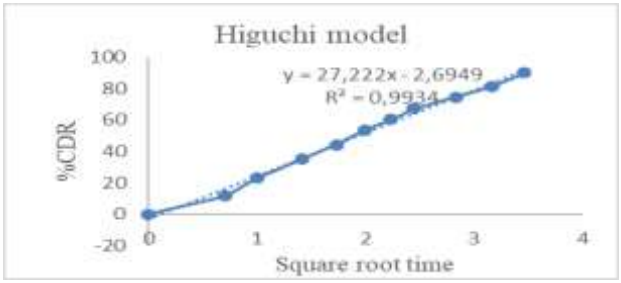

Fig.4 c). Higuchi kinetic plot

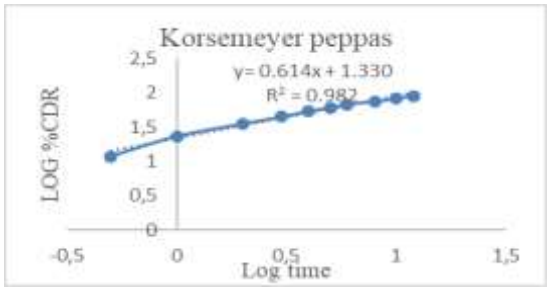

Fig. 4 d). Korsemeyer peppas plot

Table 9: Drug release kinetics

Formulation Code	Kinetic drug release				Mechanism of release			
	Zero order		First order		Higuchi		Korsemeyer peppas	
	r^2	n	r^2	n	r^2	n	r^2	n
BFF9	0.971	8.726	0.736	0.061	0.993	27.22	0.982	0.614

Ex-vivo permeation studies were done to understand the permeation of drug through buccal mucosa. The findings show that an increase in polymer concentration was linked to decrease in the rate drug permeation, due to formation of water-swollen gel-like state could slow down medium penetration into the film, which would subsequently delay the release of the drug (fig.5).

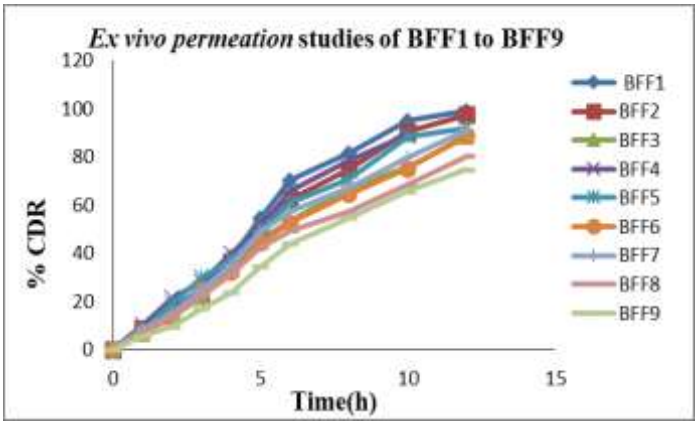

Fig.5. % Drug permeation profile

Formulation F9 was packed in aluminum packaging and kept in stability chamber at the temperature of 40 ±2°C and humidity of 75± 5%. Folding endurance tests, drug content analyses, and *in-vitro* drug releases were conducted after one, three, and six months. Results from stability studies suggest that the formulation F9 was stable over a period of 6 months at 40° ± 5°C and 75% RH, with no appreciable changes in the folding endurance, drug content and drug release.

4. Conclusion

The objective of the study was to develop buccal films containing cilnidipine nanoparticles to increase the drug's bioavailability. Buccal films were prepared utilizing the solvent casting method with a variety of mucoadhesive film polymers, including HPMC K4M, HPMC K15, and Carbapol 934, each with different concentration. Evaluation parameters such as texture, thickness, folding endurance, surface pH, % moisture absorption, % moisture loss indicated that the prepared buccal films were well

within the specified standards. The swelling index was correlated with the polymer concentration, and all the buccal films displayed strong mucoadhesion and significant adhesion forces. The mucoadhesive strength was increased by the addition of increased polymers concentration. Drug content values ensuring uniformity of drug in all the formulated buccal films. *In-vitro* drug release and *ex-vivo* permeation studies shows that the drug release from the film can prolonged up to 12 h. Based on the physico chemical parameters, dissolution and permeation studies formulation F9 was optimized as best formulation and was subjected to kinetics studies and it follows the diffusion controlled and non Fickian release mechanism. Stability studies reveal that no significance changes, indicates that the buccal films are stable. Hence, it can be concluded that mucoadhesive films of Cilnidipine nanoparticles can be successfully prepared by using mocoadhesive polymers to increase its absorption through buccal mucosa for the better treatment of hypertension.

Declaration of Conflict

Authors declare that no financial interests that could have appeared to influence the work reported in this paper.

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