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Formulation and *Invitro* evaluation of Buccoadhesive tablets of Furosemide

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Abstract

The objective of this study was to develop effective mucoadhesive buccal bilayered tablets of a drug containing bioadhesive layer and drug free backing layer, expected to release the drug in unidirection for extended period of time. Tablets of Furosemide were prepared by direct compression method using bioadhesive polymers like Carbopol 941NF, 971P, Methocel K4M, Methocel K15M and combination of NaCMC, Carbopol 971P in different ratios with backing layer of Cyanoacrylate adhesive tape. Buccal tablets were evaluated by different methods for parameters such as thickness, hardness, weight uniformity, content uniformity, swelling index, surface pH, *ex vivo* bioadhesive strength, *ex vivo* residence time, *in vitro* drug release, *ex vivo* drug permeation, stability studies in human saliva, *in vivo* mucoadhesive performance studies. Bioadhesion strength was increased with increase in the concentration of carbopol and HPMC formulations. The tablets were evaluated for *in vitro* release in pH 6.6 phosphate buffer for 6 hr in standard dissolution apparatus. Burst release was observed in formulation with carbomers when compared to HPMC grades, but in combination (i.e. NaCMC with Carbopol-971P) improves the release and permeation rate when compared with carbopol-971P individually. In order to determine the mode of release, the data was subjected to Zero order, first order, Higuchi, Korsmeyer and Peppas diffusion model. The optimized formula (Fa_2) followed fickian release mechanism with Peppas diffusion kinetics. Drug, HPMCK4M in the ratio of 1:1 could be used to design effective and stable buccoadhesive tablets of Furosemide.

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INTRODUCTION

Buccal delivery of drugs provides an attractive alternative to the oral route of drug administration, particularly in overcoming deficiencies associated with the latter mode of dosing [1]. Problems such as first pass metabolism and drug degradation in the GIT environment can be circumvented by administering the drug via buccal route. Moreover,

the oral cavity is easily accessible for self medication and be promptly terminated in case of toxicity by removing the dosage form from buccal cavity. It is also possible to administer drugs to patients who cannot be dosed orally via this route [2, 3].

In the, oral cavity the delivery of drugs are classified into three categories: 1.Sublingual delivery, which is systemic delivery of drugs through the mucosal membranes lining the floor of the mouth; 2.buccal delivery it is the drug administration through mucosal membranes lining the cheeks (buccal mucosa); and 3. Local delivery it is the drug delivery into the oral cavity [4,5]. Among these routes, buccal delivery is suitable for administration of retentive dosage forms because of an excellent accessibility, an expanse of smooth muscle and immobile mucosa. The other advantages of buccal drug delivery include: low enzymatic activity, suitable for drugs or excipients that mildly and reversibly damage or irritate the mucosa, painless drug administration, easy drug withdrawal, possible to include the permeation enhancer/enzyme inhibitor or pH modifier in the formulation. A suitable buccal drug delivery system should be flexible and should possess good bioadhesive properties, so that it can be retained in the oral cavity for the desired duration. In addition, it should release the drug in a controlled and predictable manner to elicit the required therapeutic response [6-11]. Buccal drug delivery system utilized bioadhesive polymers which will adhere to the buccal mucosa upon hydration and hence act as targeted or controlled release system. [12]

Furosemide, a widely used "high-ceiling" loop diuretic drug, is indicated for congestive heart failure, chronic renal failure, and hepatic cirrhosis. Furosemide is absorbed mostly in the stomach and upper small intestine, possibly due to its weak acidic properties (pKa3.93), Furosemide is rapidly but incompletely absorbed following oral administration and undergoes first pass metabolism resulting in a narrow absorption window, leads to its low bioavailability (43-50 %). The biological half life of Furosemide is (1-2 hrs).The physicochemical properties of Furosemide, its low half-life and molecular weight (330.7g/mol) make it suitable candidate for administration by buccal route. Hence the present study is aimed to prepare and evaluate buccal tablets of Furosemide using various bioadhesive polymers, in order to overcome bioavailability related problems, to reduce dose dependent side effects and frequency of administration. Such a dosage form would be retained for prolonged periods of time in the oral cavity and release the drug in a sustained manner, thus providing the drug continuously to its absorption sites in a controlled manner, extending the absorption phase and increasing the magnitude of the drug effect. [13] Hence, in the present work an attempt was made to formulate mucoadhesive buccal tablet for Furosemide using different mixtures of polymers in order to avoid extensive first pass metabolism, degradation in the stomach and prolonged effect.

MATERIALS AND METHODS:

Materials:

Furosemide was purchased from Rachana laboratories, Hyderabad, India. HPMCK4, K15, were purchased from International Specialty Products (Isp), Hyderabad, India. Carbopol-941NF, 971P and Perlitol-SD200, was purchased from Dr. Reddy's Laboratories, Hyderabad, India. Sodium Stearyl Fumerate(SSF) was purchased from Vilin Biomed Ltd, Roorkee, India. All other reagents used were of analytical grade.

Methods:

Bilayered buccal tablets were prepared by a direct compression method, before going to direct compression all the ingredients were screened through sieve no.100. FUROSEMIDE was mixed manually with different ratios of HPMC K4M, HPMC

K15M, Carbopol 971P & NaCMC as mucoadhesive polymers and Perlitol S.D 200 as diluent for 10 min. The blend was mixed with sodium stearyl fumerate (SSF) for 3-5 min and then compressed into tablets by the direct compression method using 8mm flat faced punches. The tablets were compressed using a Cadmach rotary tablet machine. The mass of the tablets was determined using digital balance. Composition of the prepared bioadhesive buccal tablet formulations of Furosemide were given in Table 1.

Table 1: Composition of Furosemide buccal tablets

Formulation Code	Ratio (Drug:Polymer)	API (drug) (mg)	Polymer (mg)					Filler (mg) perlitolSD 200	SSF (mg)
			HPMCK4M	HPMCK15M	Carbool 941NF	Carbopol 971P	NaCMC + Carbopol 971P		
Fa_1	1:0.5	20	10	-	-	-	-	88	2
Fa_2	1:1	20	20	-	-	-	-	78	2
Fa_3	1:2	20	40	-	-	-	-	58	2
Fa_4	1:3	20	60	-	-	-	-	38	2
Fa_5	1:4	20	80	-	-	-	-	18	2
Fb_1	1:0.5	20	-	10	-	-	-	88	2
Fb_2	1:1	20	-	20	-	-	-	78	2
Fb_3	1:2	20	-	40	-	-	-	58	2
Fc_1	1:0.5	20	-	-	10	-	-	88	2
Fc_2	1:1	20	-	-	20	-	-	78	2
Fc_3	1:1.5	20	-	-	30	-	-	68	2
Fc_4	1:2	20	-	-	40	-	-	58	2
Fc_5	1:2.5	20	-	-	50	-	-	48	2
Fd_1	1:0.25	20	-	-	-	5	-	93	2
Fd_2	1:0.5	20	-	-	-	10	-	88	2
Fd_3	1:1	20	-	-	-	20	-	78	2
Fd_4	1:1.5	20	-	-	-	30	-	68	2
Fd_5	1:2	20	-	-	-	40	-	58	2
Fe_1	1:0.7:0.3	20	-	-	-	-	14+6	78	2
Fe_2	1:0.8:0.2	20	-	-	-	-	16+4	78	2
Fe_3	1:0.9:0.1	20	-	-	-	-	18+2	78	2

Fa- Indicates the formulation containing HPMCK4M
Fb- Indicates the formulation containing HPMCK15M
Fc- Indicates the formulation containing carbopol-941NF
Fd- Indicates the formulation containing carbopol-971P
Fe- Indicates the formulation containing NaCMC + carbopol-971P
SSF- Sodium Stearyl Fumerate

Evaluation of buccal tablets of Furosemide

Physical Evaluation:

According to the methods mentioned in monograph of Furosemide the thickness, weight variation, hardness of formulations F_{a1} to F_{e3} were studied using digital micrometer, electronic balance, Pfizer hardness tester, respectively.

Content uniformity (Assay):

Ten tablets were weighed and grounded in a mortar with pestle to get fine powder; powder equivalent to the mass of one tablet was dissolved in methanol & sonicated for 30 min and filtered through whatman's filter paper. The drug content was analyzed spectrophotometrically at 277 nm using an UV spectrophotometer.

In vitro drug release of buccal tablets

The United States Pharmacopeia (USP) XXIII rotating paddle method was used to study the drug release from the buccal tablets. The dissolution medium consisted of 500ml of phosphate buffer pH 6.6. The release was performed at 37°C ± 0.5°C, with a rotation speed of 50 rpm [14]. The backing layer of

buccal tablet was attached to the glass slide with instant adhesive (cyanoacrylate adhesive). The slide was placed in to the bottom of the dissolution vessel. Samples (5 ml) were withdrawn at predetermined time intervals and replaced with fresh medium. The samples were filtered through filter paper and analyzed after appropriate dilution by UV spectrophotometer at 277 nm.

Swelling Index:

Buccal tablets were weighed individually (designated as W_1) and placed separately in Petri dishes containing 15 ml of phosphate buffer (pH 6.6) solution. At regular intervals (0.5, 1, 2, 3, 4, 5 and 6hr), the buccal tablets were removed from the Petri dishes and excess surface water was removed carefully using the filter paper. The swollen tablets were then reweighed (W_2) .This experiment was performed in triplicate. The swelling index (water uptake) calculated according to the following Eq. [15]

%Swelling index= $(W_2-W_1)/W_1$ X 100

W_1---initial weight of tablet,

W_2--- weight *of swollen* tablet

***E x vivo* permeation of buccal tablets:**

Ex vivo permeation study of buccal tablets through the porcine buccal mucosa was performed using Franz- diffusion cell at 37°C ± 0.2°C and 50rpm. The tissue was stored in Krebs buffer at 4°C upon collection. After the buccal membrane was equilibrated for 30 min with Krebs buffer solution, the membrane was placed between both the chambers, the receiver chamber was filled with fresh pH 7.4 buffer solutions [16] .The buccal tablet was placed in donor chamber and suspended with 1ml of buffer solution (pH 6.6) [17] Aliquots (5 ml) were collected at predetermined time intervals and filtered through a filter paper, and the amount of drug permeated was then determined by measuring the absorbance at 277 nm using a UV spectrophotometer. The medium which was pre warmed at 37°C was then replaced into the receiver chamber. The experiments were performed in triplicate (n = 3) and mean value was used to calculate the flux (J), permeability coefficient (P).

$$J = \frac{(dQ/dt)}{\Delta CA} \qquad P = \frac{(dQ/dt)}{A}$$

Where J is Flux ($mg.hrs^{-1}cm^{-2}$); P is permeability coefficient (cm/h); dQ/dt is the slope obtained from the steady state portion of the curve; ΔC, the concentration difference across the mucosa and A the area of diffusion (cm^2).

Measurement of bioadhesion strength [18]

The bioadhesion strength of the tablets was measured using the Ultra test equipped with a 5 kg load cell. The fresh porcine buccal mucosa obtained from the slaughterhouse. The mucosa was secured tightly to a circular stainless steel adaptor (diameter 2.2 cm). A backup membrane was placed over the buccal tablet to be tested and fixed with the help of cyanoacrylate adhesive to the cylindrical stainless steel of similar diameter. The entire setup was mounted onto the platform of a motorized test stand. During measurement 100mcl of 1% mucin solution was used to moisten the porcine buccal membrane, the upper support was lowered at a speed of 0.5mm/s until contact was made with the tissue at the predetermined force of 0.5N for contact time of 180s. At the end of the contact time, the upper support was withdrawn at a speed of 0.5mm/s to detach the membrane from the tablet. Two parameters, namely the work of adhesion and peak detachment force were calculated using the data plot software package of the instrument, which are used to study the buccal adhesiveness of tablets.

Moisture absorption study [19]

Agar (5% w/v) was dissolved in hot water, transferred into Petri plates and allowed to solidify. Six buccal tablets from each formulation were placed in vacuum over night prior to the study to remove moisture if any and weighed initially, laminated on one side with water impermeable backing membrane. They were taken &placed on the surface of the agar and incubated at 37°C for 4 hr. Then the tablets

removed and reweighed and the percentage moisture absorption was calculated using the following formula. % Moisture

$$\text{Absorption} = \frac{\text{Final weight} - \text{Initial weight} \times 100}{\text{Initial weight}}$$

Surface pH Study:

The bioadhesive tablet was allowed to swell by keeping it in contact with 1 ml of distilled water for 2 hr at room temperature. The pH was measured by bringing the pH-meter electrode, in contact with the surface of the tablet and allowing it to equilibrate for 1 min [20]

Stability of buccal tablets:

Stability studies of buccal tablets were performed for optimized formulation. The human saliva was collected from humans and filtered. Buccal tablets were placed in separate Petri dishes containing 5 ml of human saliva and placed in a temperature-controlled oven for 8 hr at 37°C ± 0.2°C. At regular time intervals (0, 2, 4 and 6 hr), the buccal tablets were examined for change in color, surface area and integrity. The experiment was repeated triplicate.

***In vivo* mucoadhesive performance of tablets:**

In vivo studies were performed by applying tablets on five healthy volunteer (aged 23-28 years) gums to assess the residence time, the organoleptic characteristics, the fragment loss, the salivary level variation, and the possible production of irritation or pain. Food was prohibited from 0.5 hr before the study until its conclusion, after 0.5hr of application water was provided as needed. [21]

RESULTS AND DISCUSSION

Physicochemical properties:

The hardness of prepared buccal tablets was found to be in the range of 3.3 Kg/cm² to 4.2Kg/cm² .The thickness was found to be 2.26 mm to 2.6 mm and complied with the theoretical value (2.6mm).The friability of all tablets was less than 1% i.e., in the range of 0 .07 – 0.46 %. The percentage deviation from mean weights of all the formulations of tablets was found to be within the prescribed limits. The low values in standard deviation indicates uniform drug content in all the formulations prepared as observed from table given table 2:

Table 2: Physico-chemical parameters of Furosemide buccal tablets.

Formulation code	Thickness (mm)	Weight Variation(mg)	Friability (%)	Hardness (Kg/cm²)	%Drug content
Fa_1	2.2±0.16	119.3±1.7	0.09	3.1±0.14	101.5
Fa_2	2.3±0.24	118.6±1.2	0.17	3.1±0.29	94.3
Fa_3	2.37±0.17	120±0.8	0.08	3.2±0.49	95.2
Fa_4	2.33±0.17	120±2.1	0.07	3.4±0.18	91.5
Fa_5	2.4±0.21	119.3±1.2	0.24	3.9±0.2	102.5
Fb_1	2.37±0.18	120.6±1.7	0.31	3.4±0.17	97.5
Fb_2	2.3±0.14	119.6±2.4	0.42	3.5±0.28	89.5
Fb_3	2.37±0.14	120.6±1.7	0.08	3.9±0.24	101.5
Fc_1	2.30±0.08	118.3±1.2	0.08	3.3±0.12	109.5
Fc_2	2.57±0.04	118.3±1.2	0.42	3.4±0.38	111.5
Fc_3	2.30±0.21	120±2.4	0.08	3.8±0.17	107.5
Fc_4	2.3±0.24	118.6±1.2	0.46	4.3±0.56	111.5
Fc_5	2.3±0.04	118.3±1.2	0.12	4.7±0.37	94.2
Fd_1	2.2±0.18	119±1.6	0.42	3.5±0.49	100.1
Fd_2	2.3±0.17	119.1±0.8	0.08	3.7±0.41	92.5
Fd_3	2.3±0.14	121.3±1.2	0.06	3.9±0.2	97.5
Fd_4	2.33±0.12	120.6±2.4	0.12	4.1±0.18	94.3
Fd_5	2.3±0.17	119±1.4	0.25	4.5±0.41	98.2
Fe_1	2.4±0.14	120±2.4	0.24	3.43±0.24	90.3
Fe_2	2.3±0.08	119.3±2.05	0.28	3.47±0.17	92.1
Fe_3	2.3±0.18	119±2.16	0.42	3.57±0.12	86.5

Each value represents the mean ±SD (*n* =3).

***In vitro* drug release of buccal tablets:**

In vitro drug release studies revealed that the release of furosemide from different formulations varied according to the type and ratios of the matrix forming polymers. Burst release was observed in formulations with carbopol than HPMC and combinated formulation. The buccal tablets contained lower concentrations either HPMCK4M, K15 and carbopol-941NF, 971P and NaCMC + coarbopol-971P in Fa, Fb, Fc, Fd and Fe series respectively, tended to release the drug in shorter time periods. While the release slowed down as the concentration of gelling polymer increased, thus confirming the dominant role of the swellable hydrophilic polymer in the release of furosemide from buccal tablets. Formulation Fa_2 (96.6 ± 0.25) composed of 1:1 (drug: HPMCK4M) ratio; Fb_2 (94.14 ± 0.142) 1:1 (drug: HPMCK15) ratio; Fc_4 (85.15±0.240) 1:2 (drug: carbopol-941NF) ratio; Fd_1(80.30±0.34%) composed of 1:0.25 (drug:carbopol-971P) and Fe_3 (92.56±0.35%) 1:0.9:0.1 (drug:NaCMC:carbopol-971P) ratio showed maximum release among their respective series. The results were shown in the in the Table 3. & Figure 1.

Table 3: *In vitro* cumulative percentage drug release profile of selected (better release) furosemide formulations.

Time(hr)	Fa_2	Fb_2	Fc_4	Fd_1	Fe_3
0	0	0	0	0	0
0.5	37.37±0.33	28.25±0.12	19.28±0.26	26.14±0.13	26.87±0.34
1	44.79±0.25	31.94±0.06	24.32±0.22	28.22±0.08	29.86±0.23
1.5	58.88±0.14	57.38±0.34	32.81±0.21	41.70±0.12	33.68±0.30
2	69.39±0.35	58.51±0.03	66.21±0.07	46.67±0.38	37.68±0.07
3	88.78±0.22	79.79±0.03	76.20±0.07	52.49±0.05	54.76±0.33
4	92.88±0.14	93.06±0.06	79.37±0.19	66.56±0.09	63.56±0.12
6	96.6±0.25	94.14±0.14	89.15±0.24	80.30±0.34	92.56±0.33

Each value represents the mean ± SD (n=3)

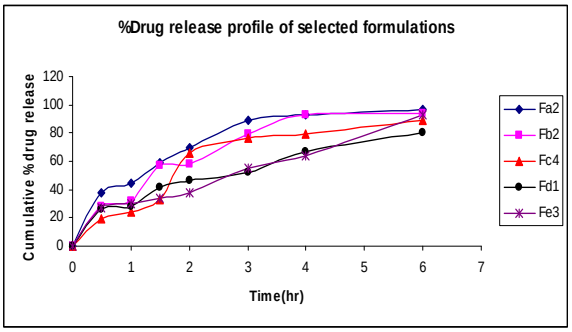

Fig 1: % Drug release profile of selected formulation.

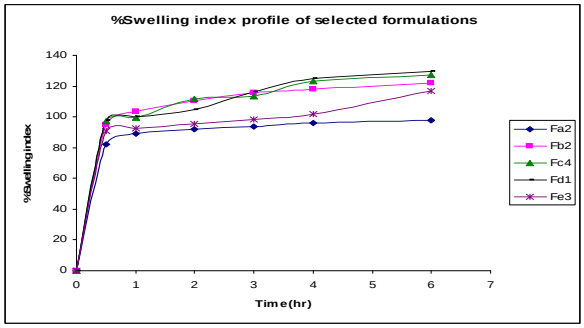

Fig 2: % swelling index profile of selected formulations

Swelling Studies of buccal tablets:

In formulations containing HPMC K15M, Fb_2 (selected optimized formulation) -shows swelling index of 121.8; the formulations containing HPMC K4M ,Fa_2 show high swelling index of 98.6; the formulations containing carbopol-941NF(Fc_4) and 971P(Fd_1) show maximum swelling index i.e. 127.2, 129.8 respectively. The formulation containing carbopol shows higher swelling index values than combination and HPMC containing formulation (table.4 and fig 2. shows SI values).

Time (hr)	Fa_2	Fb_2	Fc_4	Fd_1	Fe_3
0	0	0	0	0	0
0.5	82	95	97.1	98.01	90.8
1	89	103.5	99.5	100.01	92.3
2	92	110.3	111.6	104.6	95.6
3	94	115.5	113.5	116.1	98.3
4	96	117.8	123.3	125.1	101.8
6	98	121.8	127.2	129.8	117.1

Table 4: % swelling index profile of selected Furosemide formulations

***Ex vivo* permeation of buccal tablets:**

Based on the *in vitro* drug release studies, Fa_2, Fb_2, Fc_4, Fd_1 and Fe_3 were selected for the *ex vivo* permeation study. The flux, permeation coefficient and cumulative percent drug permeated from formulation Fa_2 & Fe_3 were found to be 1.002408 mg.hrs^{-1}cm^2, 0.0565cm/h and 70.48±0.005% ; 1.002489, 0.0596 and 80.36±0.009 respectively. The drug permeation was slow and steady in case of both Fa_2 and Fe_3. Burst permeation was observed with carbopol formulations due to its high viscous nature. The values of cumulative amount of drug permeated and cumulative percent drug permeated were given in the table 5. The values of flux, permeability coefficient were given in Table 6 and Comparison of cumulative percent drug permeated from different selected formulations was given in figure 3.

Time (hr)	Drug solution		Fa_2		Fb_2	
	Cum. amt drug per[a] (mg)	Cum % drug per[b]	Cum amt drug per[a] (mg)	Cum % drug per[b]	Cum amt drug per[a] (mg)	Cum % drug per[b]
0	0	0	0	0	0	0
0.5	0.59±0.005	14.67±0.005	0.46±0.005	2.45±0.005	0.29±0.005	1.64±0.005
1	0.79±0.005	19.73±0.005	1.13±0.005	6.02±0.004	0.65±0.007	3.71±0.009
2	1.33±0.009	33.43±0.009	3.23±0.005	17.18±0.005	0.72±0.003	4.04±0.005
3	1.59±0.005.	39.70±0.005	5.15±0.006	27.39±0.009	1.82±0.007	10.14±0.009
4	1.65±0.005	41.25±0.005	6.99±0.012	37.18±0.112	2.45±0.009	13.72±0.009
6	1.69±0.005	42.25±0.005	10.87±0.009	57.79±0.009	3.38±0.005	18.88±0.005
8	1.71±0.009	42.39±0.009	13.25±0.005	70.48±0.005	6.69±0.009	37.39±0.009

Time(hr)	Fc_4		Fd_1		Fe_3	
	Cum amt drug per[a] (mg)	Cum % drug per[b]	Cum amt drug per[a] (mg)	Cum % drug per[b]	Cum amt drug per[a] (mg)	Cum % drug per[b]
0	0	0	0	0	0	0
0.5	0.27±0.005	1.44±0.005	0.03±0.005	0.14±0.005	0.22±0.009	5.34±0.009
1	0.58±0.009	3.01±0.009	0.08±0.009	0.41±0.009	0.53±0.014	12.95±0.14
2	0.73±0.005	3.88±0.005	0.21±0.009	0.88±0.004	1.25±0.005	31.17±0.05
3	0.88±0.005	4.67±0.005	0.23±0.009	1.24±0.009	1.73±0.094	45.07±0.94
4	0.95±0.005	4.98±0.009	0.24±0.005	1.72±0.004	2.21±0.008	55.60±0.08
6	1.12±0.005	5.98±0.005	0.27±0.009	5.8±0.009	3.2±0.009	70.23±0.09
8	2.03±0.047	10.62±0.047	0.28±0.005	6.9±0.005	4.12±0.009	80.36±0.09

Each value represents the mean ±SD (n =3).

Table 5: *Ex vivo* drug permeation profiles of drug solution and selected Furosemide formulations. [a]Cum amt drug per- Cumulative amount of drug permeated. [b]Cum % drug per- Cumulative percentage drug permeated.

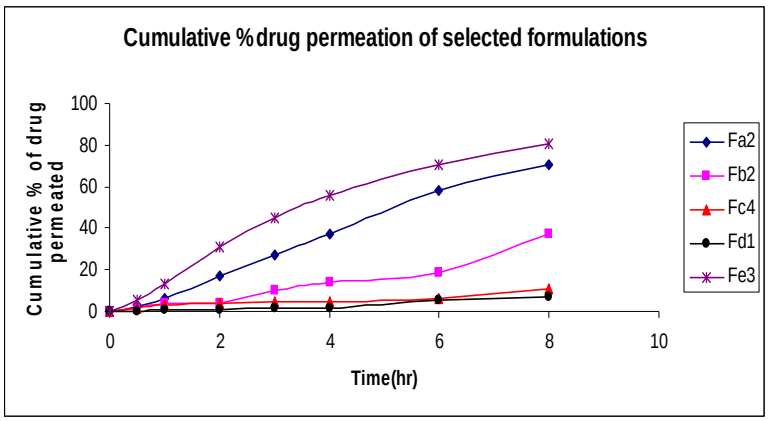

Fig 3. Cumulative % drug permeation of selected Furosemide formulations

Table 6: Flux and Permeability coefficient values of drug solution and selected formulations.

Formulation code	Flux(mg.hrs^{-1}cm^{-2})	permeability coefficient(cm/h)
Drug solution	0.145 7	0.0559
Fa_2	1.0024	0.0565
Fb_2	1.0021	0.0555
Fc_5	1.0019	0.0541
Fd_1	1.0012	0.0453
Fe_3	1.0025	0.0596

Measurement of bioadhesion strength:

This evaluation test was conducted for selected formulations (Fa_2, Fb_2, Fc_4, Fd_1, and Fe3); there is a gradual increase in bioadhesion strength from Fa_2 to Fd_1. The order of bioadhesion was NaCMC+Carbopol971p<HPMCK4M<HPMCK15M< Carbopl941NF<Carbopol971p. Buccal tablets formulated with carbopol and HMCK15M showed stronger mucoadhesion than HPMCK4M and combinated formulations. Very strong bioadhesion could damage the epithelial lining of the buccal mucosa. Optimized tablet (Fa_2) showed 34.02±0.193 g of bioadhesion strength. Results are shown in table7 and fig 4.

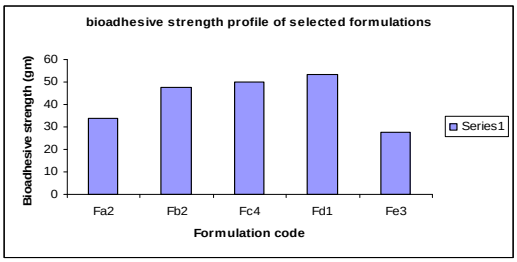

Fig 4: Bioadhesion strength profile of selected formulations

Moisture Absorption:

These studies give an indication of the relative moisture absorption capacities of polymers and whether the formulations maintained their integrity after its absorption. The order of increasing moisture absorption was HPMCK4M<HPMCK15M<Carbopol941NF<NaCMC +Carbopol971P<Carbopol971P, as shown in Table 7.

Table 7: The bioadhesive strength, residence time, moisture absorption and surface pH values of selected Furosemide tablets.

Formulation code	Bio adhesion Strength (gm)	*Ex vivo* residence time(hr)	Surface pH	% Moisture absorbance
Fa_2	34.02±0.193	5.34±0.12	5.57±0.30	43.76±0.25
Fb_2	47.62±0.615	5.52±0.09	5.63±0.38	47.55±0.36
Fc_4	50.08±0.808	6.26±0.6	5.83±0.33	56.75±0.19
Fd_1	53.19±0.870	6.34±0.6	5.47±0.73	64.66±0.34
Fe_3	27.78±0.376	4.12±0.47	6.20±0.36	78.52±0.08

Each value represents the mean ±SD (n =3).

Solubility and Surface P^H study:

Solubility of furosemide in the pH 6.6 and pH 7.4 was found to be 8.96 mg/ml, 11.16 mg/ml respectively. Surface pH of the optimized formulation Fa_2, Fe_3 was found to be 5.57 ± 0.3, 6.20 ± 0.36 respectively. This pH is near to the neutral, so the formulation does not cause any irritation on the mucosa. Surface pH values for all the formulations shown in Table 7.

Release kinetics and mechanism:

In-vitro drug release data of Fa_2 and Fe_3 were fitted to zero order, first order, Higuchi and Korsmeyer-

Peppas equations to ascertain the pattern of drug release (Table 8). In case of Fe_3 formulation the r^2 value indicated that the highest r^2 (0.9852) value was found for first order. According to n value it is between 0.5-1, so it follows non-fickian diffusion with first order release.

Table 8: Release kinetics and mechanism of optimized formulations

Formulation code	Mathematical models(Kinetics)				
	Zero order	First order	Higuchi	Peppas model	
Fa_2	r^2	r^2	r^2	n	r^2
	0.9059	0.879	0.9059	0.129	0.9802
Fe_3	0.9775	09852	0.9077	0.5027	0.8911

Stability of buccal tablets:

Stability study was conducted only for optimized formulations (Fa_2 and Fe_3). There was no change in the color and integrity of the tablets with HPMCK4M (Fa_2 formulation), but slight changes observed in case of integrity of the Fe_3 formulation after 3rd hr. The data obtained from the study presented in Table 9 &9.1. From the stability results, it was known that formulation Fa_2 has stability in human saliva.

Sampling interval(hrs)	Change in color	Change in surface area(cm2)	Change in integrity
0	NO	NO	NO
2	NO	0.5	NO
4	NO	1.32	NO
6	NO	2	NO
8	NO	2.5	NO

Table 9: Stability profile of optimized formulation Fa_2 in human saliva

Sampling interval(hrs)	Change in color	Change in surface area(cm2)	Change in integrity
0	NO	NO	NO
2	NO	0.8	NO
4	NO	1.56	YES
6	NO	2.2	YES
8	NO	2.8	YES

Table 9.1: Stability profile of optimized formulation Fe_3 in human saliva

***In vivo* mucoadhesive performance of tablets:**

This study was conducted for optimized formulation (Fa_2). In bioadhesive buccal drug delivery comfort ability of system in oral cavity is very important. The result of five healthy human volunteers to each subjective parameter was calculated and shown in Table 10. From the human volunteer studies of optimized formula (Fa_2), it was observed that the bitter taste was found at 6hr, due to higher swelling of the mucoadhesive polymers. The higher swelling was responsible for the increasing thickness of the buccal tablet this leads to improve the bi-directional (radial) release of drug. This is negligible during initial hours. This bi-directional release increases the amount of drug into the mouth, which is responsible for bitter taste.

Table 10: Response of healthy human male (23-28 years) volunteers to various subjective parameters for optimized formulation (Fa_2)

Sl No.	Criteria	Volunteer's response (%)
1	Irritation	
	a)None	100
	b)Slight	
	c)Moderate	
	d)Severe	
2	Taste	
	a)Normal	60
	b)Slightly	20
	c)Very unpleasant	20
	d)Pleasant	
3	Comfort	
	a)Very comfortable	
	b)Comfortable	80
	c)Slightly uncomfortable	20
	d)Moderately uncomfortable	
	e)Severely uncomfortable	
4	Dryness of mouth	
	a)None	80
	b)Slight	20
	c)Moderate	
	d)Severe	
5	Salivary secretion	
	a)None	20
	b)Slight	60
	c)Moderate	20
	d)Severe	
6	Heaviness at the place of attachment	
	a)None	90
	b)Slight	10
	c)Moderate	
	d)Severe	
7	Dislodgement of the system during study	
	a)No	100
	b)Yes	

CONCLUSION

Development of bioadhesive buccal drug delivery furosemide tablets is one of the alternative routes of administration to avoid first pass effect and provides prolonged release. When compared to individual formulations Fa_2 composed of 1:1 (drug: HPMCK4M) formulation showed the complimentary physical properties with sustained buccal delivery of furosemide. In combination Fe_3 composed of 1:0.9:0.1 (drug: NaCMC: carbopol-971P) formulation also showed complementary physical properties, *invitro* drug release, cumulative percent drug permeation. But, from the stability and bioadhesive strength point of view Fa_2 formulation showed better results that meet all the criteria required than Fe_3 formulation. The surface pH of the optimized formulation Fa_2 was found to be 5.57 ± 0.30. This pH is near to the neutral therefore, it was inferred that neutral pH of the formulation does not cause any irritation on the mucosa. From a study on healthy human volunteers Fa_2 formulation was revealed that all subjective parameters and mucoadhesion behavior was found to be satisfactory. Therefore, bioadhesive buccal delivery of furosemide may be good way to bypass the first pass metabolism and the formulation containing drug and polymer in 1:1 ratio was found to be an optimized formulation. Formulation Fa_2 (96.6 ± 0.25) composed of 1:1 (drug: HPMCK4M) ratio; Fb_2 (94.14 ± 0.142) 1:1 (drug: HPMCK15) ratio; Fc_4 (85.15±0.240) 1:2 (drug: carbopol-941NF) ratio; Fd_1 (80.30±0.34%) composed of 1:0.25 (drug: carbopol-971P) and Fe_3 (92.56±0.35%) 1:0.9:0.1 (Drug: NaCMC: Carbopol-971P) ratio showed maximum release among their respective studies.

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