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Original Article

FORMULATION DESIGN AND EVALUATION OF MUCOADHESIVE BUCCAL TABLETS OF NITROGLYCERIN

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ABSTRACT

Objective: The main objective of present investigation is to prolong drug action and enhance bioavailability.

Methods: The tablets were prepared using Carbopol 934 in varying concentration with secondary polymers like HPMC K4M, HPMC K15M and sodium alginate by direct compression method. The compatibility studies like TLC and FTIR spectroscopy were carried out. The tablets were evaluated for hardness, thickness, weight variation, friability and drug content and concluded that all these parameters were in acceptable range of pharmacopoeial specification. The tablets were furtherevaluated for their mucoadhesive characteristics such as surface pH, swelling index, *ex vivo* residence time, mucoadhesive strength, *ex vivo* permeation,*in vitro* drug release and also for the effect of Carbopol concentration on mucoadhesive parameters.

Results: The surface pH of the tablet was 6.48 to 6.75 which fall in the range of salivary pH and all the tablet of batch C containing sodium alginate as secondary polymer showed *ex vivo* residence time of 10 to 12.30 hrs indicated good mucoadhesive capacity of tablet. The buccal tablets showed good swelling up to 8 hrs maintaining the integrity of polymers. The formulation (C3) showed better control of drug release and able to release entire amount of drug in 12 hrs than the other formulations. All the formulations of batch C & A except A1 followed zero order kinetics and all other formulations of batch B and A1 formulation followed Hixson-Crowell model.The 'n' value of all the formulations was found to be more than 0.89 indicating that the drug release followed Super case II transport type of release mechanism due to the erosion of the polymer. All the tablets showed good mucoadhesive strength in the range of 21.87 to 26.26.*Ex vivo*permeation studies of the optimized formulation C3 revealed that percent drug permeated through sheep buccal mucosa was 38.294 % for 8 hrs. The slopes of the basic *in vitro* data suggests that drug permeates across the membrane but slowly as the mucosa offers barrier to the transportation of the drug.

Conclusion: Hence Carbopol 934 and Sodium alginate polymers can be used to prepare mucoadhesive buccal tablets of nitroglycerin having prolonged therapeutic effect with enhanced bioavailability.

Keywords: Nitroglycerin, Swelling index, *In vitro* drug release. Mucoadhesion, *Ex vivo* permeation.

INTRODUCTION

Buccal drug delivery [1-3] has gained significant attention and momentum since it offers remarkable advantages. Over past few decades, buccal route for systemic drug delivery using mucoadhesive polymers to significantly improve the performance of many drugs has been of profound interest. Administration of compounds via the mucosa of the oral cavity avoids pre-systemic metabolism in the gastrointestinal (GI) tract and hepatic first pass elimination. In addition, the buccal mucosa is a well-vascularised tissue and is easily accessible for both application and removal of a delivery device. It's having facility to include permeation enhancer/enzyme inhibitor or pH-modifier in the formulation and versatility in designing as multidirectional or unidirectional release systems for local or systemic action, etc. Buccal drug delivery systems is a promising area for continued research with the aim of systemic delivery of orally inefficient drugs as well as a feasible and attractive alternative for non-invasive delivery of potent peptide and protein drug molecules.

Nitroglycerin[4-5]is a vasodilator used in treatment of angina. It is well absorbed from the oral cavity and has low molecular weight, which favours its administration through buccal route.

The objective of this research was to formulate and evaluate mucoadhesive buccal tablet containing nitroglycerin to prolong therapeutic effect, increase bioavailability of the drug and reduce the dosing frequency.In the present investigation mucoadhesive polymers [6-7] like Carbopol 934p, HPMC K4M, HPMC K15M and Sodium alginate were used to prepare mucoadhesive buccal tablets by employing direct compression technique. The prepared buccal tablets are subjected to evaluated for physicochemical properties and release characteristics

MATERIALS AND METHODS

Nitroglycerin was obtained as gift sample from Shasun pharmaceuticals Ltd, Pondicherry. Carbopol 934, Sodium alginate, Lactose and Talc were purchased from S.D. Fine – Chem. Ltd, Mumbai. HPMC K4M and HPMC K15M were purchased from Colorcon Asia Pvt. Ltd, Goa. The chemical reagents used were of analytical grade.

Drug – Excipient compatibility studies

Drug – Excipient compatibility study by Thin Layer Chromatography

In this method drug and excipients in 1:2 ratio were mixed and analyzed for compatibility by using TLC after one, two, three and four weeks. 10µl of reference and test solutions were applied as spots on the dry activated plate. The solvent system was allowed to run up to desired height; the plates were removed and allowed to dry. The dry plates were then exposed to iodine vapors in a chamber to observe the spots. The plates were then removed and the R_f values calculated. The details of chromatographic conditions were shown in Table no. 1.

Drug – Excipient compatibility study by Fourier Transform Infrared Spectroscopy (FTIR)

The pure drug and optimized formulations were subjected for FTIR analysis. The samples were scanned over a range of 4000-400 cm^{-1} using Fourier transformer infrared spectrophotometer. Spectra's were analyzed for drug polymer interactions.

Formulation of mucoadhesive buccal tablets

Direct compression[8] method was employed to prepare buccal tablets of Nitro-glycerine using Carbopol 934p, HPMC K15M, HPMC K4M and Sodium alginate as polymers. Carbopol 934 is used as primary polymer due to its good mucoadhesive property. All the ingredients including drug, polymer and excipients were weighed accurately according to the batch formula and were passed through sieve no 40 to get uniform particle size.

The drug and all the ingredients except lubricants were taken on a butter paper with the help of a stainless steel spatula and the ingredients were mixed in the order of ascending weights and blended for 10 min in an inflated polyethylene pouch. After uniform mixing of ingredients, lubricant was added and again mixed for 2 min. The prepared blend of each formulation was compressed by using 8mm punch on a tablet punching machine. The formulation details are shown in Table no 2.

Table 1: Details of Chromatographic Conditions

Stationary phase	Thin plates of Silica gel G having thickness of 0.25cm and activated at 110ºC for 30min prior to use
Mobile phase	Chloroform: Methanol (9:1)
Separation technique	Ascending
Reference solution	5mg of Nitroglycerin was shaken with 5ml of mobile phase, decanted and used for spotting
Test solution	Drug-Excipient mixture equivalent to 5mg of Nitroglycerin was shaken with 5ml of mobile phase, decanted and used for spotting.

Table 2: Composition of Nitroglycerin buccal tablets

S. No.	Ingredients	Formulations								
		Batch A*			Batch B**			Batch C***		
		A1	A2	A3	B1	B2	B3	C1	C2	C3
1.	Nitroglycerin	10	10	10	10	10	10	10	10	10
2.	Carbopol 934p	25	30	40	35	40	45	35	40	45
3.	HPMC K4M	45	40	30	--	--	--	--	--	--
4.	HPMC K15M	--	--	--	35	30	25	--	--	--
5.	Sodium alginate	--	--	--	--	--	--	35	30	25
6.	Lactose	18	18	18	18	18	18	18	18	18
7.	Talc	2	2	2	2	2	2	2	2	2

*HPMC K4M, **HPMC K15M and ***Sodium alginate used as a secondary polymer.

Evaluation of mucoadhesive buccal tablets

Evaluation was performed to assess the physicochemical properties and release characteristics of the developed formulations.

Pre-compression parameters [9]

Angle of Repose

Angle of repose is defined as the maximum angle possible between the surface of the pile of the powder and the horizontal plane. The flow characteristics of different microcapsules were studied by measuring the angle of repose employing fixed funnel method. The angle of repose was calculated by using the following formula.

$$\text{Tan}\theta = \frac{\text{Height of the pile}}{\text{radius of the base of the pile}}$$

where θ = tan-1 (h / r) θ = angle of repose.

Bulk Density & Tapped Density

Bulk density and tapped density were measured by using 10 ml of graduated cylinder. The pre weighed sample was placed in a cylinder; its initial volume was recorded (bulk volume) and subjected to tapings for 100 times.

Then the final volume (tapped volume) was noted down. Bulk density and tapped density were calculated from the following formula.

$$\text{Bulk Density} = \frac{\text{mass of microparticles}}{\text{bulk volume}}$$

$$\text{Tapped Density} = \frac{\text{mass of microparticles}}{\text{tapped volume}}$$

Carr's Index s

Compressibility index (CI) or Carr's index value of microparticles was computed according to the following equation:

$$\text{Carr's Index (\%)} = \frac{\text{Tapped density - Bulk density}}{\text{Tapped density}} \text{X}\,100$$

Hausner's Ratio

Hausner ratio of microspheres was determined by comparing the tapped density to the bulk density using the equation:

$$\text{Hausner's Ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Post compression parameters [10]

Tablet thickness and diameter

Thickness and diameter of tablets were important for uniformity of tablet size. Thickness and diameter were measured using Vernier calipers.

Hardness

This test is used to check the hardness of a tablet which may undergo chipping or breakage during storage, transportation and handling. In this five tablets were selected at random and the hardness of each tablet was measured with Monsanto hardness tester. The hardness is usually measured in terms of kg/cm^2.

Uniformity of weight

This test is performed to maintain the uniformity of weight of each tablet which should be in the prescribed range, this is done by sampling and weighing 20 tablets at random and average weight is calculated.

Friability test

The friability of tablets was determined using Roche friabilator. It is expressed in percentage (%). Ten tablets were initially weighed ($W_{initial}$) and transferred into friabilator. The friabilator was operated at 25rpm for 4 min or run up to 100 revolutions. The

tablets were weighed again (W_{final}). The % friability was then calculated by –

$$F = \frac{W_{initial} - W_{final}}{W_{initial}} \times 100$$

Content uniformity

Tablet containing 10mg of drug is dissolved in 100ml of pH 6.4 saline phosphate buffer taken in volumetric flask. The drug is allowed to dissolve in the solvent. The solution was filtered, 1ml of filtrate was taken in 100ml volumetric flask and diluted to mark with pH 6.4 saline phosphate buffer and analyzed spectroscopically at 210nm. The concentration of Nitroglycerin in mg/ml was obtained by using standard calibration curve of the drug. Drug content studies were carried out in triplicate for each formulation batch.

Swelling studies [11]

Buccal tablets from each batch were individually weighed (W1) and placed separately in each petri dish with 15 ml of phosphate buffer (pH 6.4 saline phosphate buffer). At time intervals of 1, 2, 3, 4, 5, 6, 7 and 8, the tablet was removed from each Petri dish and excess surface water from the tablet was wiped out carefully with filter paper. Each swollen tablet was reweighed (W2) and the swelling index (SI) was calculated using the following formula

$$\text{Swelling index} = \frac{W_2 - W_1}{W_1} \times 100$$

Where W_1 = Initial weight of the tablet,

W_2 = Weight of the tablet after specific time interval.

Mucoadhesion strength [12]

Mucoadhesion strength of the tablet was measured on a modified physical balance (fig.6) employing the method as described by Gupta *et al* using sheep buccal mucosa as model mucosal membrane. Fresh sheep buccal mucosa was obtained from a local slaughter house and was used within 2 hrs of slaughtering. The mucosal membrane was washed with distilled water and then with phosphate buffer pH 6.8. A double beam physical balance was taken and to the left arm of balance a thick thread of suitable length was hanged and to the bottom side of thread a glass stopper with uniform surface was tied. The buccal mucosa was tied tightly with mucosal side upward using thread over the base of inverted 50 ml glass beaker which was placed in a 500 ml beaker filled with phosphate buffer pH 6.8 kept at 37° C such that the buffer reaches the surface of mucosal membrane and keeps it moist.

The buccal tablet was then stuck to glass stopper from one side membrane using an adhesive (Feviquick). The two sides of the balance were made equal before the study, by keeping a weight on the right hand pan. A weight of 5 g was removed from the right hand pan, which lowered the glass stopper along with the tablet over the mucosal membrane with a weight of 5 g. The balance was kept in this position for 3 min. Then, the weights were increased on the right pan until tablet just separated from mucosal membrane. The excess weight on the right pan i.e. total weight minus 5gm was taken as a measure of the mucoadhesive strength.

The mean value of three trials was taken for each set of formulations. After each measurement, the tissue was gently and thoroughly washed with phosphate buffer and left for 5 minutes before placing a new tablet to get appropriate results for the formulation. After calculating mucoadhesion strength the force of adhesion and bond strength parameters were calculated from following equations as;

Force of Adhesion (N) = Mucoadhesive strength × 9.8 / 1000

Bond Strength (N/m^2) = Force of Adhesion / Surface Area.

Surface pH study [13]

The surface pH of the buccal tablets was determined in order to investigate the possibility of any side effects *in-vivo*. As the acidic or alkaline pH may cause irritation to the buccal mucosa, the pH was maintained to neutral as closely as possible. A combined glass electrode was used for this purpose. The tablet was allowed to swell by keeping it in contact with 1 ml of distilled water (pH 6.4 ± 0.05) for 2 hrs at room temperature. The pH was measured bringing the electrode in contact with the surface of the tablet and allowing it to equilibrate for 1 min.

***Ex vivo* drug permeation [14]**

Ex vivo drug permeation through the sheep buccal mucosa was performed using modified Franz diffusion cell at 37±0.5°C. The freshly cut sheep buccal mucosa after removing underlying fat and loose tissues and washing with phosphate buffer pH 6.4 and distilled water was mounted between the donor and receptor compartments. The receptor compartment (16 ml capacity) was filled with phosphate buffer pH 7.4, and the buccal mucosa was allowed to stabilize for 30 min by hydrodynamics in the receptor compartment by stirring on a magnetic stirrer at 50 rpm and was maintained for the entire study. A 1 ml aliquot was withdrawn at predetermined time intervals and replaced with fresh medium. The aliquots were analyzed after appropriate dilution by UV spectrophotometer (Systronics 117) at 210 nm.

***Ex vivo*mucoadhesion time [15]**

The mucoadhesive performance of the buccal tablets was evaluated using Fresh sheep buccal mucosa obtained from local slaughter house. The *ex vivo* mucoadhesion time was performed after application of the buccal tablet on freshly cut sheep buccal mucosa. The fresh sheep buccal mucosa was tied on the glass slide and a buccal tablet was wetted with 1 drop of phosphate buffer pH 6.8 and pasted to the sheep buccal mucosa by applying a light force with a fingertip for 30 sec. The glass slide was then put in the beaker, which was filled with 200 ml of the phosphate buffer pH 6.8 and was kept at 37 ± 1°C. After 2 min, a 50 rpm stirring rate was applied to simulate the buccal cavity environment and tablet adhesion was monitored for 12 hrs. The time for the tablet to detach from the sheep buccal mucosa was recorded as the mucoadhesion time.

***In vitro* dissolution studies [16]**

In vitro release studies were carried out using USP dissolution testing apparatus II (paddle type). The dissolution medium consisted of 500 ml of saline phosphate buffer (pH 6.4). The release was performed at 37ºC ±.5ºC, with a rotation speed of 50 rpm. Samples (5ml) were withdrawn at predetermined time intervals (1, 2, 3 up to 8 hrs) and volume was replaced with the fresh medium. The samples were filtered through Whatman filter paper and analyzed after appropriate dilution by UV spectrophotometer at 210nm. The experiments for different Batches A, B and C formulations were conducted in triplicate and average values were recorded. The release kinetics such as zero order, first order, Higuchi and Hixson- Crowell were determined.

Drug release kinetics [17-18]

To analyze the mechanism of release and release rate kinetics of the dosage form, the data obtained were fitted into Zero order, First order, Higuchi matrix, Peppas and Hixson-Crowell model using PCP-DISSO-v3 software. Based on the r-value, the best-fit model was selected. To study the release kinetics, data obtained from *in vitro* drug release studies were plotted as log cumulative percentage drug release *versus* log time.

Stability studies

In the present study, stability studies were carried out at 40°C and 75% RH for a specific time period up to 4 weeks for optimized formulation. For stability study, the tablets were sealed in aluminium packaging coated inside with polyethylene. These sample containers were placed in desiccator maintained at 75% RH. The samples were analyzed for the following parameters like surface pH, hardness and drug content.

RESULTS AND DISCUSSION

Drug – Excipient compatibility study by TLC

This study confirmed no interaction between the drug and excipients i.e., the physical appearance of drug-excipient mixture, R_f valueand λ_{max} of drug observed for 4 weeks were found to be almost similar with the initial observations. Hence, it can be concluded that the drug Nitroglycerin was found to be compatible with the excipients used in the designed formulation. The observations of drug-excipient compatibility study were tabulated in Table 3 and 4.

Table 3: Observations of Drug-Excipient study by TLC

Ingredients	Observations at 0 day		
	Appearance	R_f	λ_{max} (nm)
Drug	White	0.315	210
Drug ± HPMC K4M	White	0.312	210
Drug ± HPMC K15M	White	0.314	210
Drug ± Carbopol 934p	White	0.313	210
Drug ± Sodium alginate	White creamy	0.316	210
Drug ± Lactose	White	0.315	210

Table 4: Observations of Drug-Excipient study by TLC at 1st, 2nd, 3rd and 4th week.

Ingredients	1st week			2nd week			3rd week			4th week		
	A	R_f	λ_{max}	A	R_f	λ_{max}	A	R_f	λ_{max}	A	R_f	λ_{max}
Drug	NC	0.315	NC	NC	0.316	NC	NC	0.315	NC	NC	0.312	NC
Drug + HPMC K4M	NC	0.314	NC	NC	0.314	NC	NC	0.312	NC	NC	0.315	NC
Drug + HPMC K 15M	NC	0.311	NC	NC	0.312	NC	NC	0.311	NC	NC	0.316	NC
Drug + Carbopol 934p	NC	0.316	NC	NC	0.313	NC	NC	0.316	NC	NC	0.317	NC
Drug + Sodium alginate	NC	0.312	NC	NC	0.316	NC	NC	0.315	NC	NC	0.311	NC
Drug + Lactose	NC	0.314	NC	NC	0.317	NC	NC	0.314	NC	NC	0.313	NC

A = Appearance, NC = No change.

Drug – Excipient compatibility study by FTIR study:

Based on the FTIR interpretation results, all the major drug peaks were identified when compared with the physical mixture of drug and polymer, which ensures that there was no any chemical interaction between them. The major sharp and significant peaks (functional groups) of the drug and drug and polymer mixture are shown in Table No.5 and the spectra are shown in Figure No 1, 2, 3, and 4.

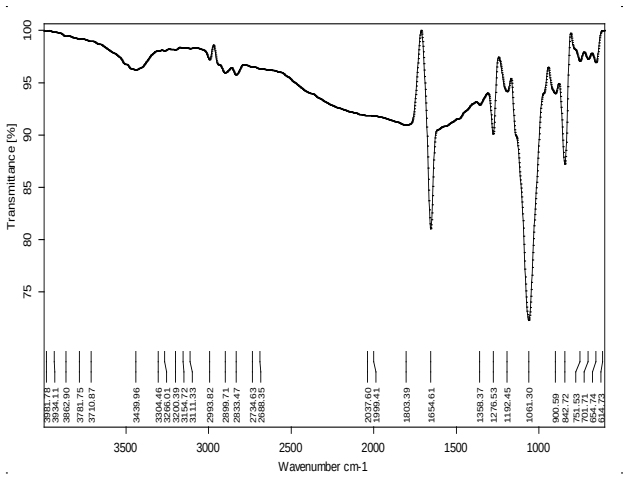

Fig. 1: FTIR spectra of pure Nitroglycerin.

Pre-compression parameters: The angle of repose values were found to be in the range from 23.37º to 27.15º. This indicates good flow property of the blend.The values of bulk density were found to be in the range from 0.255gm/cm^3 to 0.317gm/cm^3 and tapped density from 0.312gm/cm^3 to 0.380gm/cm^3. Compressibility index value ranges between 15.06% to 19.44% indicating that the powder blend have the required flow property for direct compression.The values of Hausner's ratio were found to be in range 1.177 to 1.24. The details of pre-compression parameters are tabulated in Table No. 6.

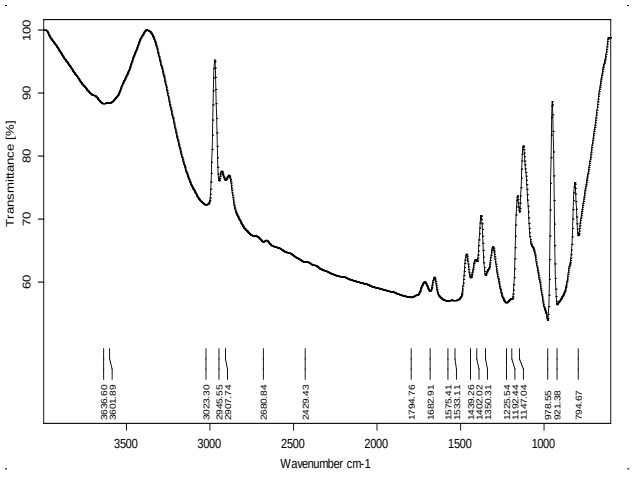

Fig. 2: FTIR spectra of A batch.

Post-compression parameters: Tablets mean thickness were almost uniform in all the formulations and were found to be in the range of 1.57mm to 1.61mm.

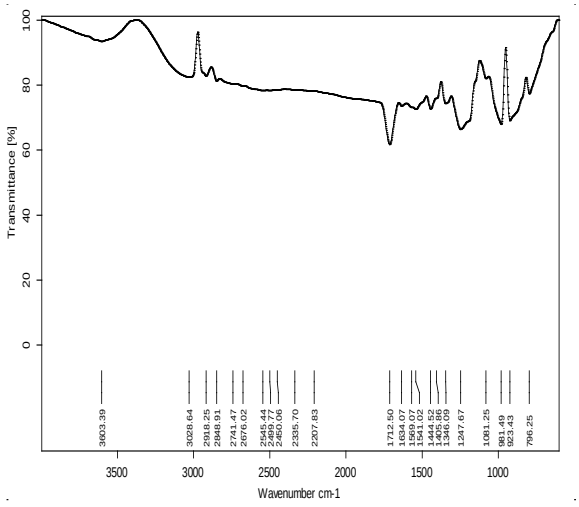

Fig. 3: FTIR spectra of B batch

The measured hardness of tablets of each batch ranged between 4.5kg/cm² to 5.2kg/cm². This ensures good handling characteristics of all batches.All the tablets passed weight variation test as the % weight variation was within the pharmacopoeial limits of ±10% of the weight. The weights of all the tablets were found to be uniform with low standard deviation.

The % friability was less than 1% in all formulations ensuring that the tablets were mechanically stable.The percentage of drug content was in the range of 96.13% to 99.67% and found to be within acceptable limits. The details of post compression parameters are shown in Table No. 7.

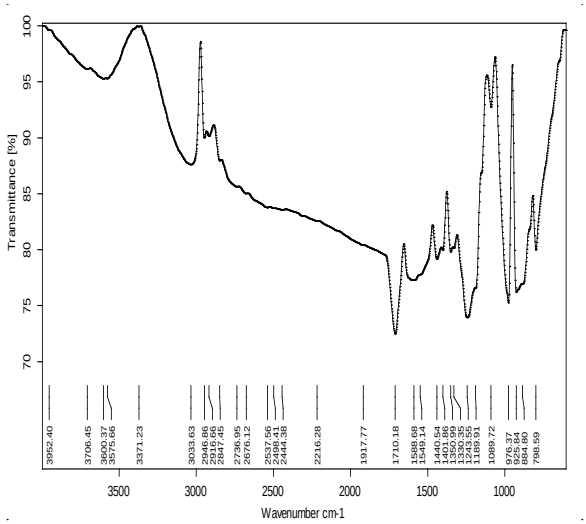

Fig. 4: FTIR spectra of C batch

Table 5: FTIR Spectral Interpretation of pure drug and formulations

S. No.	Ingredients	Functional groups			
		C-O(Str)	N=O (Str)	C-H (Str)	CH (Df)
1.	Drug	1276.53	1654.61	2899.77	1358.37
2.	Drug +HPMC K4M	1233.91	1661.37	2865.66	1378.30
3.	Drug + HPMC K15M	1274.27	1655.37	2834.56	1337.84
4.	Drug + Carbopol 934p	1270.05	1656.60	2836.45	1376.22
5.	Drug + Sodium Alginate	1274.46	1658.23	2853.30	1350.50
6.	Drug + Lactose	1275.87	1654.63	2898.56	1352.56
7.	Drug + All excipients(A)	1225.54	1682.91	2907.74	1350.31
8.	Drug + All excipients(B)	1247.67	1634.07	2848.91	1346.09
9.	Drug + All excipients(C)	1243.55	1654.62	2847.45	1350.99

Table 6:Pre-compression parameters of powder blend

Code	Angle of repose (θ)	Bulk density g/cm³	Tapped density g/cm³	%compressibility index	Hausner's ratio
A1	24.65±0.020º	0.266±0.028	0.326±0.026	18.40±0.021	1.225
A2	25.76±0.021º	0.261±0.023	0.324±0.030	19.44±0.030	1.241
A3	24.98±0.015º	0.255±0.011	0.312±0.028	18.26±0.027	1.223
B1	25.19±0.020º	0.278±0.025	0.340±0.032	18.32±0.036	1.223
B2	27.15±0.038º	0.270±0.026	0.335±0.036	19.40±0.019	1.240
B3	26.85±0.018º	0.276±0.015	0.333±0.016	17.11±0.026	1.206
C1	26.64±0.038º	0.317±0.026	0.380±0.022	16.57±0.019	1.198
C2	23.37±0.038º	0.310±0.016	0.369±0.015	15.98±0.023	1.190
C3	24.75±0.038º	0.310±0.029	0.365±0.026	15.06±0.013	1.177

Table 7: Post-compression parameters of Nitroglycerin buccal tablets

Code	Weight variation	Hardness (kg/cm²)	Thickness (mm)	Friability (%)	Drug content (%)
A1	0.101±0.005	4.55±0.05	1.58±0.02	0.74±0.04	98.72
A2	0.102±0.006	4.79±0.04	1.59±0.04	0.72±0.06	97.78
A3	0.100±0.005	4.82±0.03	1.60±0.05	0.45±0.02	97.07
B1	0.102±0.004	4.90±0.06	1.59±0.03	0.56±0.04	96.13
B2	0.100±0.005	5.10±0.05	1.61±0.04	0.61±0.07	99.67
B3	0.101±0.005	5.15±0.05	1.59±0.04	0.76±0.04	97.59

C1	0.102±0.005	4.85±0.05	1.60±0.04	0.65±0.04	98.15
C2	0.100±0.005	4.95±0.05	1.57±0.04	0.63±0.04	96.85
C3	0.101±0.005	4.97±0.05	1.58±0.04	0.68±0.04	97.78

Swelling studies

The swelling of all the tablets was increased as the time proceeds because the polymer gradually absorbs water due to hydrophilicity of the polymer. The outermost hydrophilic polymer layer hydrates/swells first and as the hydrated layer progressively dissolves or disperse, the hydration swelling process will continuous towards new expose surfaces thus maintaining the integrity of dosage form. The swelling index was 182.844 to 193.654 for the formulation Batch A which contains Carbopol 934 as primary polymer and HPMC K4M as secondary polymer. The swelling index was 203.119 to 213.356 for the formulation Batch B which contains Carbopol 934 as primary polymer and HPMC K15M as secondary polymer. The swelling indices of the tablets with Carbopol 934 P and HPMC increased with increasing amounts of Carbopol 934 P. It was observed that when tablet came in contact with aqueous medium, wetting occurred first at the lower surface of tablet and then progressed to whole. The rate of spreading of water was dependent on the ratio of two polymers used. The swelling was getting affected in the formulations containing secondary polymer along with Carbopol as a primary polymer. The highest swelling was 283.458 to 299.851 for formulations Batch C which contains sodium alginate as secondary polymer because sodium alginate is more water soluble and rapidly get hydrated. The swelling index was affected by the increasing the concentration of Carbopol 934 as represented in fig 5.

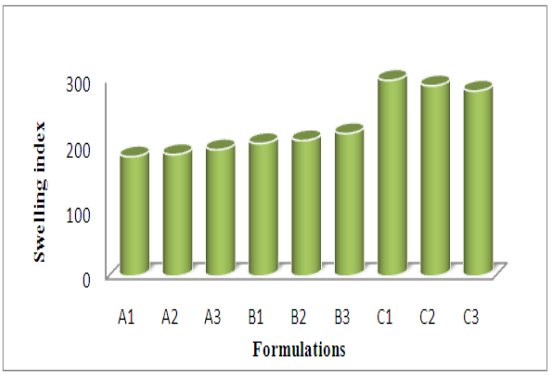

Fig. 5: Swelling study profiles of all three Batches A, B and C formulations.

Mucoadhesive strength

The tablets with the HPMC K4M have bioadhesive strength in between the 22.33 gm to 26.26 gm. The tablets with the HPMC K15M have bioadhesive strength in between the 23.68 gm to 25.59 gm. The tablets with the Sodium alginate have bioadhesive strength 21.87 gm to 24.89 gm. Carbopol 934 is selected as primary polymer because of its good mucoadhesive property. Results indicate that by increasing concentration of Carbopol 934, the mucoadhesive strength increases. The results are illustrated in Figure 6.

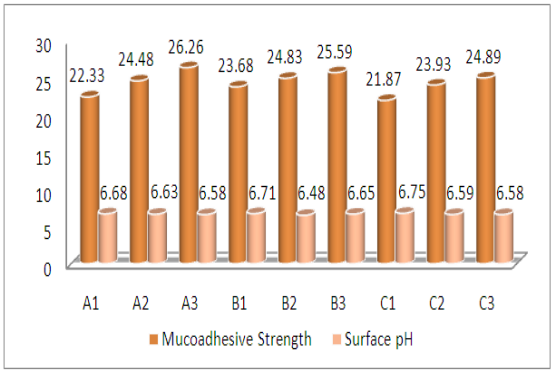

Fig. 6: Mucoadhesive strength and Surface pH profile of all three batches A, B and C formulations

Surface pH

The maximum surface pH of all the formulation was found to be 6.75 and the minimum was 6.48. The results reveal that all the formulations provide an acceptable pH in the range of 5.5 to 7.0 (salivary pH). Hence, they may not produce any local irritation to the mucosal. The results are illustrated in Figure 6.

***Ex vivo* permeation studies:**

The optimized formulation C3 which showed 75.39% of *in vitro* drug release in 8 hrs is selected for performing *ex vivo* permeation studies by using sheep buccal mucosa. The results are shown in Table 8.

Table 8: Permeation study profile of C3 formulation

S. No.	Time in hrs	Cumulative amount released ± S.D
1	1	2.800 ±0.007
2	2	5.760 ±0.008
3	3	11.354 ±0.014
4	4	15.702 ±0.014
5	5	20.785 ±0.029
6	6	26.626 ±0.029
7	7	32.376 ±0.024
8	8	38.297 ±0.023

***Ex vivo* mucoadhesive time**

The Batch C formulations which are showing controlled drug release for 8 hrs are selected for performing mucoadhesive residence time. The *ex-vivo* mucoadhesion time was examined after application of the buccal tablet on sheep buccal mucosa. The result showed in Table No. 9, revealed that the mean adhesion time was promising in the batch Cformulations containing Carbopol 934 and sodium alginate. This may be due to the flexibility of Carbopol 934, which easily diffuses and interpenetrates into the mucin and get entangled with that of mucin. The mucoadhesive time on sheep buccal mucosa ranged from 10.46±0.040 to 12.26±0.010 hours.

Table 9: *Ex vivo* Residence time of Batch C formulations

Formulation	Mucoadhesive time
C1	10.46±0.040
C2	11.58±0.032
C3	12.30±0.010

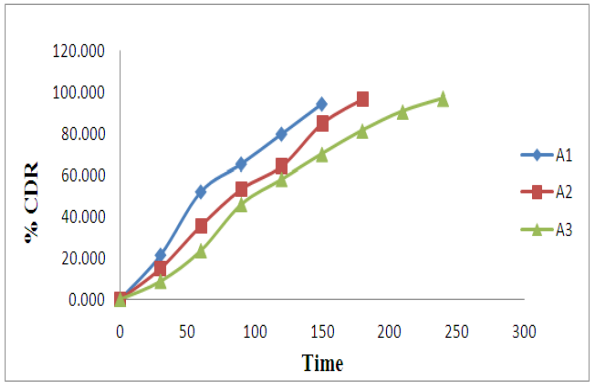

Fig. 7: *In vitro* dissolution study of Batch-A formulations

In vitro release study

The *in vitro* release of Nitroglycerin was mainly affected by drug polymer ratio, nature and amount of polymer and the dissolution medium. The buccal tablets of Batch A containing Nitroglycerin with primary polymer Carbopol 934 and secondary polymer HPMC K4M showed initially a rapid burst release of the drug 94.31%, 96.78% and 97.03% for 2½, 3 and 4hrs respectively. The buccal tablets of Batch B containing HPMC K15M as secondary polymer showed drug release of 96.90%, 90.21% and 85.74% for 5hrs.

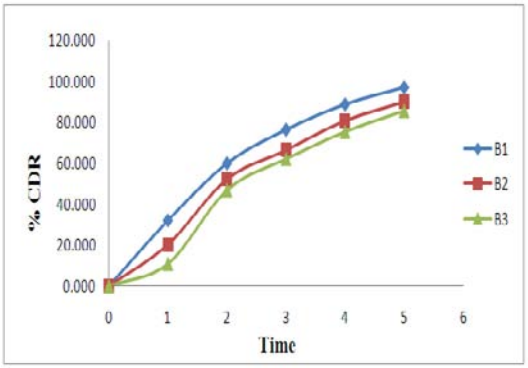

Fig. 8: *In vitro* dissolution study of Batch-B formulations.

The buccal tablets of Batch C containing sodium alginate as secondary polymer showed drug release of 91.31%, 86.55% and 75.39% for 8 hrs. Among the secondary polymers used, sodium alginate showed extended drug release up to 8 hrs.

Formulation C3 which is showing 75.39% drug release in 8 hrs is selected as optimized formulation. The increase in concentration of Carbopol in all tablet formulations significantly affects the release of drug. The *in vitro* release profiles are shown in Fig No. 7, 8 and 9.

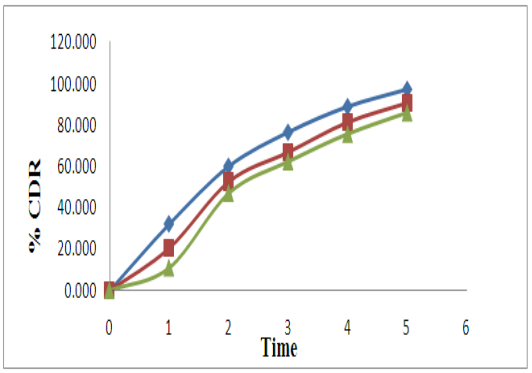

Fig. 9: *In vitro* dissolution study of Batch-C formulations

Drug release kinetics

The results of dissolution data were fitted to various kinetic equations to analyze the release mechanism. All the formulations of batch C & A except A1 followed zero order kinetics and all other formulations of batch B and A1 formulation followed Hixson-Crowell model.

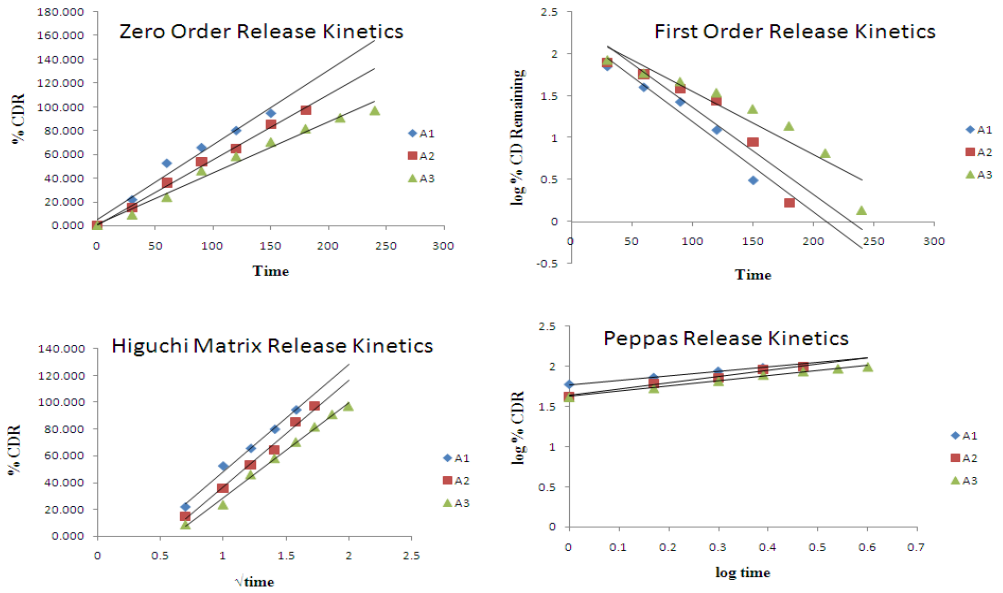

Fig. 10: release kinetic plots for formulation A

The 'n' value of all the formulations was found to be more than 0.89 indicating that the drug release followed Super case II transport type of release mechanism due to the erosion of the polymer.

But the formulation B1 showed the 'n' value 0.69 indicating that the drug release occurred via non-fickian diffusion. The kinetic plots obtained of respective batches are shown in Fig 10, 11 and 12.

Stability studies

Stability studies were carried out for optimized formulation C3 at 40^{0}C/75% RH for 30 days. The results of stability studies revealed no change in Surface pH, hardness, in-vitro drug release and drug content indicating the formulation was stable. The values are tabulated in Table11.

Table11: Stability study of optimized formulation C3.

Time in weeks	Formulation C3			
	Surface pH	Hardness (Kg/cm^2)	*In vitro* release(%CDR)	Drug content(%)
0	6.58	4.95	75.39	99.72
1	6.62	4.95	74.56	99.55
2	6.58	4.93	77.63	99.21
3	6.60	4.92	75.74	98.96
4	6.62	4.92	73.87	98.71

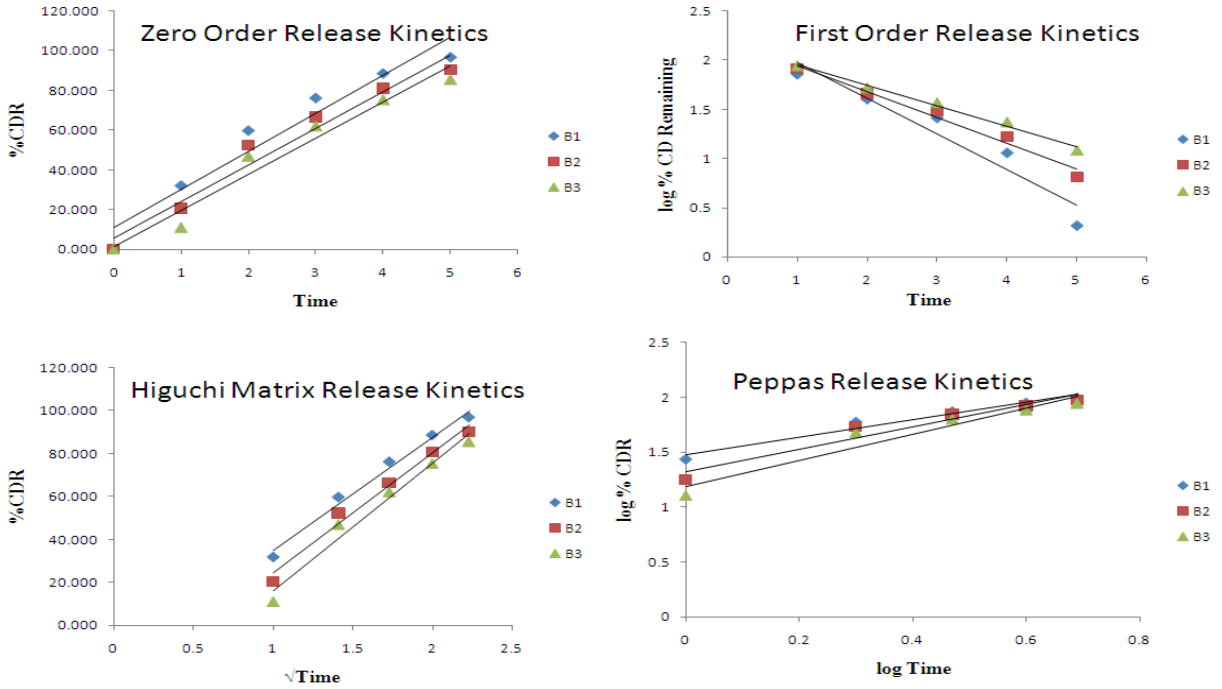

Fig. 11: release kinetic plots for formulation B.

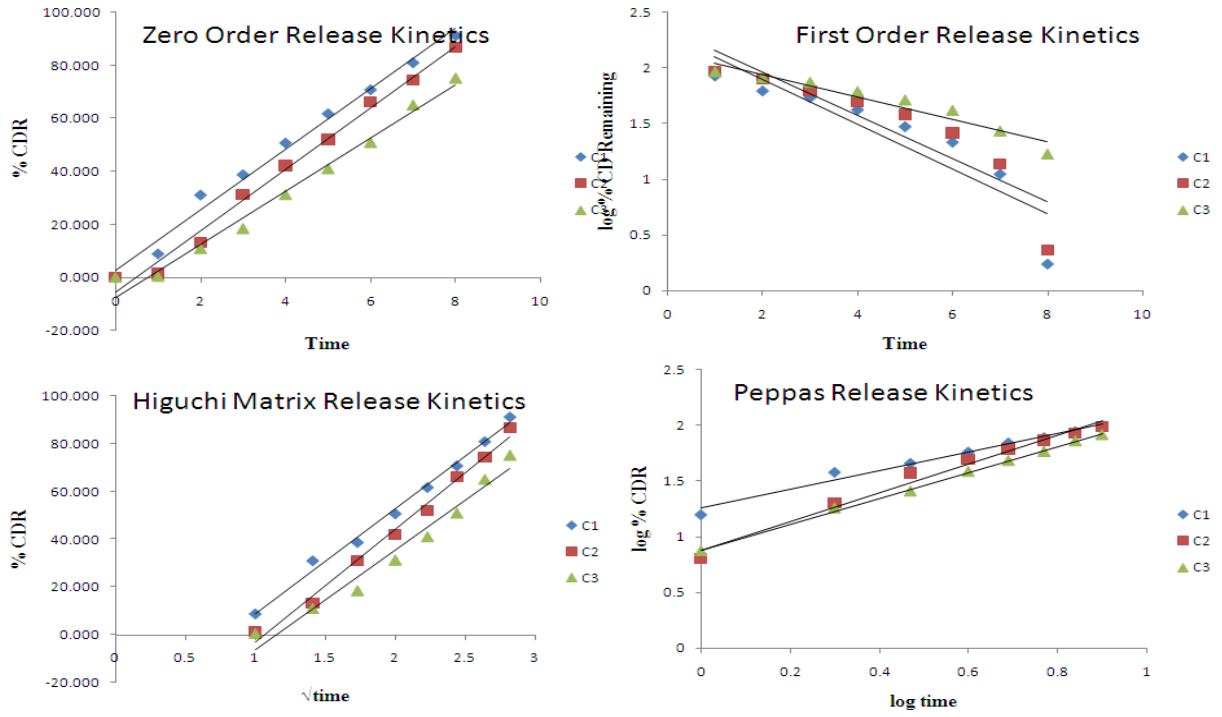

Fig. 12: release kinetic plots for formulation C

CONCLUSION

The mucoadhesive buccal tablets of Nitroglycerin could be prepared using Carbopol 934 as primary polymer in combination of secondary polymers like HPMC K4M, HPMC K15M and sodium alginate by direct compression method. All the prepared tablets were in acceptable range of weight variation, hardness, thickness, friability and drug content as per pharmacopoeial specification. The increase in concentration of Carbopol can increase hardness.The surface pH of prepared buccal tablets was in the range of salivary pH, suggested that prepared tablets could be used without risk of mucosal irritation.

The buccal tablets showed good swelling up to 8 hrs in pH 6.4 saline phosphate buffer maintaining the integrity of formulation which is required for bioadhesion. The increase in Carbopol concentration significantly increased the swelling. Buccal tablets containing sodium alginate as secondary polymer showed better swelling index.The *in vitro* release of Nitroglycerin was extended 4-5 hrs, if Carbopol used in combination with secondary polymers like HPMC K4M and HPMC K15M. While the tablets contained Carbopol along with sodium alginate could be used to prepared prolonged released buccal tablet.

The *in vitro* release obeyed zero order kinetics with mechanism of release was erosion followed by non-fickian diffusion due to more hydrophilic nature of polymer. The increase in concentration of Carbopol significantly affects the *in vitro* release of Nitroglycerin. Although all formulations of Batch C exhibited satisfactory drug release, formulation C3 is considered as optimized formulation as it is able to release complete drug for 12 hrs.All the tablets of Batch C prepared using sodium alginate as secondary polymer showed good mucoadhesive residence time of 10.46±0.040 to 12.26±0.010 hrs indicated good adhesive capacity of polymers used. All the tablets showed good mucoadhesive strength of 21.87 to 26.26 with high force of adhesion. The mucoadhesive strength was increased by increasing the concentration of Carbopol 934 as it is having good mucoadhesive property.*In vitro* diffusion studies of the optimized formulation C3 revealed that percent drug permeated through sheep buccal mucosa was 38.294 % for 8 hrs. The slopes of the basic *in vitro* data suggests that drug permeates across the membrane but slowly as the mucosa offers barrier to the transportation of the drug. Stability studies were conducted according to ICH guidelines region IV at 40^0C / 75 % RH indicates that there is no decrease in drug content, surface pH or no significant difference between the means of profiles without stress and with stress at $p<0.05$ observed for a period of 4 weeks. Based on the results obtained so far, it was concluded that the objectives of the investigation was fulfilled. Hence, the mucoadhesive buccal tablets of Nitroglycerin can be prepared with enhanced bioavailability and prolonged therapeutic effect for the prevention of angina attacks. The study conducted so far reveals a promising result suggesting scope for pharmacodynamic and pharmacokinetic evaluation.

CONFLICT OF INTERESTS

Declared None

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