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Preparation and Evaluation of Muco-adhesive Buccal Tablets of Hydralazine Hydrochloride

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Multilayered muco-adhesive buccal tablets of hydralazine hydrochloride were developed using polymers, which as Carbopol- 934 and carboxymethylcellulose. Release enhancers such as citric acid, D-mannitol and PEG-4000 and techniques like lyophilization were utilised for optimization of the tablet core. A millipore membrane filter (0.22 μm) was used satisfactorily to replace animal membrane for drug permeation studies across the membrane.

THE interest in novel routes of drug administration results from their ability to enhance the bioavailability of drugs impaired by a narrow absorption window in the gastrointestinal tract. Optimal therapy requires efficient delivery of therapeutically effective dosage to tissue or organ that needs treatment. The need is not only of reproducibility and predictability of drug release kinetics but also of patient compliance[1]. Drug delivery via the buccal mucosa using bioadhesive dosage forms offers such a novel route of drug administration. The route has successfully been tried for the systemic delivery of a number of drug candidates[2-6].

The present study was an attempt to develop a muco-adhesive buccal delivery system for hydralazine hydrocholoride (HLZ. HCl), a hypotensive agent. The drug is well absorbed through the gastrointestinal tract but is subjected to extensive first pass metabolism[7]. So, the dose required to produce effective therapeutic serum concentration is relatively high. Since buccal route bypasses first pass metabolism, the dose of HLZ.HCl may be reduced to almost 20% of the oral dose. This in turn would reduce the dose dependent side effects which are a major limiting factor to its benefits.

The various physico-chemical properties of HLZ.HCl such as low molecular weight (196.64), optimum solubility characteristics, suitable half life (2 to 4 h), dissociation constant (7.1), small dose requirement and absence of objectionable taste and odour make it a suitable candidate for buccal administration[7,8].

EXPERIMENTAL

HLZ.HCl was a gift sample from Sarabhai Chemicals Ltd. Carbopol- 934 (CP-934) and hydroxypropylcellulose-M (HPC-M) were obtained as gift samples from Ranbaxy Labs and carboxymethylcellulose (CMC) from Unichem Labs. Citric acid (E-Merck), D-mannitol (BDH) and PEG-4000 (S.D. Fine Chemicals) were obtained from commercial sources. Other solvents and materials used in the study were of reagent grade. Millipore membrane filters of various pore sizes were obtained from Millipore, India.

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Preparation of buccal tablets

The ingredients (HLZ.HCl and polymers) were weighed accurately and mixed by trituration in a glass pestle and mortar. The mix (90 mg) was then compressed using a 9.6 mm diameter die in a hydraulic press (Riken Saiki Co. Ltd., Japan) at a pressure of 200 kg/cm^2 for 30 sec. The tablets were 0.87 to 0.93 mm thick, depending upon the polymer combination used.

For multilayered tablets, the ingredients (100 mg) of the core were compressed using a 9.6 mm die punch set at a pressure of 50 kg/cm^2 for 10 sec. The core was then removed and placed in the centre of a 13.6 mm die and the ingredients (100 mg) of cap layer poured over it and recompressed at 100 kg/cm^2 for 15 sec. The upper punch was removed and the mixed ingredients (50 mg) of the backing layer were then added over the cap and compressed at a pressure of 50 kg/cm^2 for 5 sec.

Lyophilization of polymers

The polymers were taken in a definite ratio (selected by release studies) and dissolved in sufficient quantity of distilled water with stirring. The gel so obtained was prefreezed and then lyophilized at -70° and 10 to 50 atmosphere for 48 h. The dried solid matrix was then crushed, passed through sieve number 100 and stored in a dessicator till further use.

Water absorption study

This was done on 1% agar gel plates[9]. The tablets were placed with the core facing the gel surface and incubated for 6 h at 37°. The tablets were weighed before and after standing on the agar plate and examined for any physical change.

In vitro dissolution study

Dissolution rate of the drug from buccal tablets was studied using a modified USP dissolution rate test apparatus. The modification consisted of an internal compartment made up of a 150 ml glass beaker (i.d. 40 mm) into which was placed a teflon cylinder (40 mm diameter, 20 mm height) having a cavity (13 mm diameter, 4mm depth) on one side. The tablet was inserted into the cavity of teflon cylinder so that the core faced the dissolution medium (100 ml isotonic phosphate buffer (IPB) pH 6.6 at 37°). A stirrer was lowered so that it remained at least 1 cm above the tablet surface and stirring was done at 50 rpm. Samples (5 ml) were withdrawn and replaced by fresh dissolution medium every half an hour for 6 h. Filtered samples were then diluted suitably and absorbance was read at the 262 nm. Preliminary studies indicated that the polymers used in the study did not interfere with the estimation of the drug at this wavelength.

In situ diffusion studies

In situ studies of the diffusion of HLZ.HCl from buccal tablets were carried out using the Franz diffusion cell. Fresh bovine cheek pouch membrane was attached to the top of lower compartment. The buccal tablet with core facing the membrane was covered with a glass cap from three sides and lowered in the donor compartment so that the tablet got stuck on the mucous membrane. The receptor compartment was filled with IPB pH 7.4 and contained a magnetic needle. The assembly was maintained at 37° and stirred magenetically. Samples were withdrawn at 30 min. intervals for 6 h and analyzed as for the dissolution samples.

Millipore membrane filters of 8.0, 1.0, 0.45 and 0.22 μm pore size were also tried for permeation studies instead of mucosal membrane in an attempt to evaluate the suitability of using membrane filters for transmucosal permeation studies. Studies were performed initially on C-4 formulations and finally on optimised formulation also.

In vivo studies of optimized tablets

In vivo studies of the optimized tablets was carried out on healthy, male, white rabbits, weighing

Table 1 : Effect of Polymer Ratio on the Drug Release from Various Muco-adhesive Tablets

Composition	Formula Code														
	A-1	A-2	A-3	A-4	A-5	B-1	B-2	B-3	B-4	B-5	C-1	C-2	C-3	C-4	C-5
HLZ.HCl (mg)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
CP-934 (mg)	40	26.7	53.3	20	16	40	26.7	53.3	20	16	40	26.7	53.0	20	16
HPMC-K4M (mg)	40	53.7	26.7	60	64	–	–	–	–	–	–	–	–	–	–
PVP-K30 (mg)	–	–	–	–	–	40	53.3	26.7	60	64	–	–	–	–	–
CMC (mg)	–	–	–	–	–	–	–	–	–	–	40	53.3	26.7	60	64
Polymer ratio	1:1	1:2	2:1	1:3	1:4	1:1	1:2	2:1	1:3	1:4	1:1	1:2	2:1	1:3	1:4
Cumulative % drug release* (at 6 hrs.)	24.9	39.3	22.1	43.9	34.1	21.2	38.2	25.6	41.3	49.1	26.3	57.5	37.5	60.4	55.8

* Average of three determinations

one to two kg. The rabbits were fasted overnight and divided into four groups of two rabbits each. Dose of drug was selected on the basis of surface area ratio of rabbits[10]. The first group (A) was given an oral dose of HLZ.HCl with the help of plastic tube. Buccal tablet was placed in the cheek pouch of group (B) with the help of spatula. The mount of the rabbits were tied tightly with a cloth to prevent swallowing of dosage form. The blood samples were withdrawn from the marginal ear vein, using xylene for vein dilation. The blood was collected into heparinized tubes and was centrifuged at 1000 rpm for 20 min to separate plasma. To 0.5 ml of freshly prepared plasma was added 1 ml of IPB (pH 7.4) and 6 ml of methanol. This was centrifuged at 850 rpm and to 2 ml of supernatant was added, 1ml of a mixture of buffer and methanol (65:35 v/v). This was filtered through a 0.45 μm filter and 20 μl of this was injected into the liquid chromatographic column using the loop injector. A standard curve of pure drug was prepared by HPLC in the concentration range of 0.1-2.5 μg/ml.

The blood samples were analyzed by reverse phase HPLC with detection by ultraviolet spectroscopy[11]. The technique utilised a Waters stainless steel column (30 cm x 3.9 mm) packed with μ Bondapak C. The eluting solution used was 35% of methanol in 0.1 M potassium phosphate buffer (pH 7.4) containing 10 mM EDTA. The solvent flow rate was maintained at 1.5 ml/min. Cumulative drug release was obtained after stipulated time intervals.

RESULTS AND DISCUSSION

The polymers for the preparation of HLZ.HCl buccal tablets were selected on the basis of bioadhesive property, non-toxicity, non-irritancy, stability and compatibility with the drug. CP-934 was combined with either HPMC-K4M, PVP-K30 or CMC. Table 1 gives the cumulative percentage drug release of the buccal tablets prepared using varying ratios of different polymers. Formulation C-4 (containing CP-934 and CMC in the ratio of 1:3) was selected for further study as it had the maximum drug release in 6 h.

Effect of addition of Citric acid

Citric acid was added in a concentration from 2 to 10% in order to enhance the release of drug from

Table 2 : Composition of Optimized Formulation

Composition	Weight in mg of		
	Core	Cap	Backing layer
HLZ.HCl	10.00	–	–
Freezed dried polymer mix of CP-934 :CMC (1:3)	20.00	–	–
CP-934	13.50	66.75	–
CMC	40.50	–	–
Citric acid	6.00	–	–
D-mannitol	8.00	–	–
PEG-4000	2.00	–	–
HPC-M	–	33.25	–
Microcrystalline cellulose	–	–	44.98
Sodium saccharin	–	–	5.00
Tartrazine	–	–	0.02

formulation C-4. The release was enhanced which could be attributed to a decrease in the micro-environment pH of the basic drug (HLZ.HCl) by the addition of citric acid. A concentration of 6% w/w was found to be optimum for maximum drug release.

Effect of addition of D-mannitol

D-mannitol has been used to accelerate the release of drug from polymer matricess[12]. It has a sweet taste, a negative heat of solution and dissolution enhancing properties[13] and hence may be regarded as a suitable excipient for buccal tablets. Maximum release of 78.5% was obtained on addition of 8% D- mannitol to C-4 matrix.

Effect of addition of PEG 4000

Addition of 2% PEG 4000 was found to increase the release of drug to 81.6% from C-4 matrix in 6 h. A further increase in concentration of PEG caused the tablet to erode before 6 h. This is in agreement with earlier studies where PEG has been utilized for development of muco-adhesive erodible buccal tablets[14,15]. Hence, 2% PEG-4000 was added to the optimized formulation.

Effect of lyophilization of polymer blend

Use of lyophilized polymer blend of CP-934 and CMC (1:3) upto a concentration of 20% caused an increase in the release of drug from the polymer matrix. However, a further increase in the percentage of lyophilized polymer in the formulation slowed down the release rate.

Development of Optimized formulation

Based on the above studies, a multilayered optimized formulation (Table 2) was developed. The core consisted of the drug, citric acid, D-mannitol, PEG 4000 and a physical mixture alongwith a freeze dried polymer blend of CP-934 and CMC. The cap layer consisted of CP-934 and HPC (2:1) and surrounded the core on three sides so as to allow the release of drug to take place only from the side of core sticking to buccal mucosa. This layer would also aid in the adhesion of tablet to the buccal mucosa

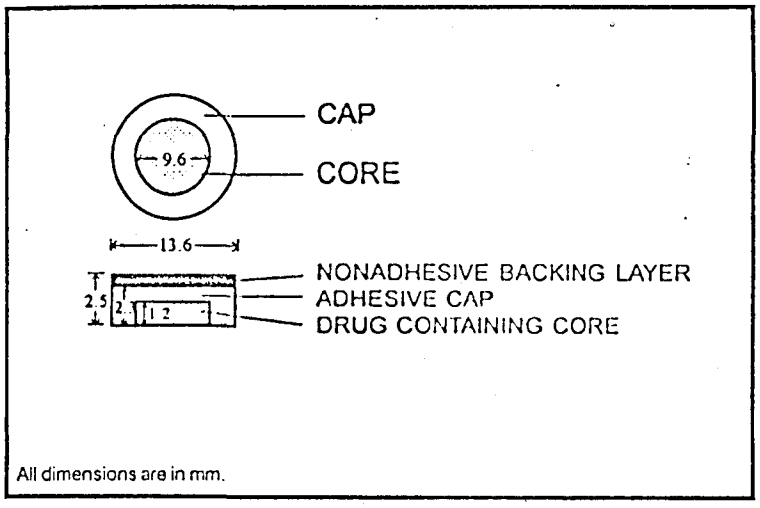

Fig. 1 : Scheme illustration of buccal tablet

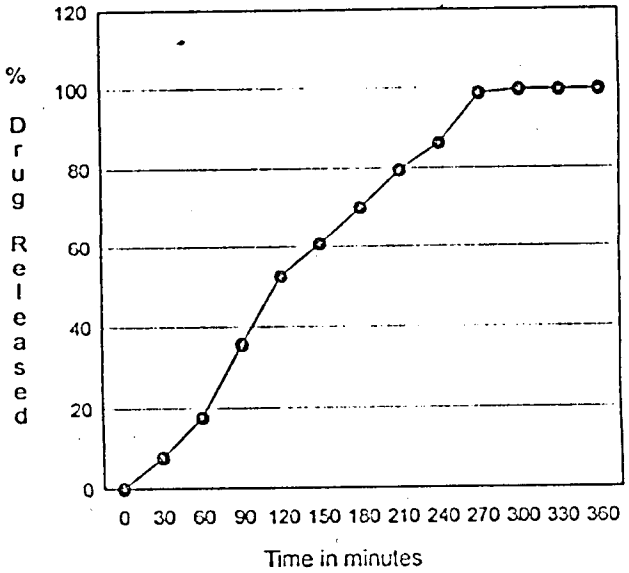

Fig. 2 : *In vitro* dissolution rate study of optimized formulation

for 6 h. The backing layer consisting of microcrystalline cellulose covered the cap layer at the top and overcame the problem of stickiness of the cap layer. It also contained sweetner (sodium saccharin), flavour (lemon oil) and colour (tartrazine) so as to impart an aesthetic appeal to the formulation. Fig. 1 gives a schematic illustration of the buccal tablet.

The tablets were found to be satisfactory when evaluated for weight variation, friability and drug content uniformity. The results of the water absorption study indicated that the tablets did not show any appreciable change in their shape and form during the 6 h, they were kept on agar plate. Swelling of the surface in contact with 1% agar gel was noted and the optimized tablet had a 84.3% gain in weight due to absorbed water after 6 h.

In vitro and in situ drug release studies

The optimized formulation exhibited an *in vitro* release of 99.7% (Fig. 2) in 6 h during the dissolution study. When the formulation was subjected to *in situ* diffusion studied using a diffusion cell, a drug permeation of 23.8% was obtained in 6 h across freshly obtained bovine cheek pouch. The correlation between *in vitro* release rate and permeation across the bovine membrane was found to be positive with a correlation coefficient of 0.955.

Analysis of the release rate and permeation rate data showed that the coefficient of variation of zero order rate constant was lower than the corresponding first order values thereby suggesting that the drug release from the optimized formulation may be following zero order pattern.

Comparison of bovine cheek pouch membrane with synthetic membrane

Fig. 3 shows the drug from formulation C-4 permeated through bovine cheek pouch (J-1), millipore filters with pore size 8.0 μm (J-2), 1.0 μm (J-3), 0.45 μm (J-4) and 0.22 μm (J-5). It was seen that permeation obtained from the animal membrane was very close to millipore filter of 0.22 μm. Fig 4 shows the diffusion of drug from the optimized buccal tablet across bovine cheek pouch and 0.22 μm millipore membrane. It appears that the diffusion across the synthetic membrane follows a pattern similar to the animal membrane although the permeation is more (32.5% as compared to 23.8% in 6 h) across the millipore membrane.

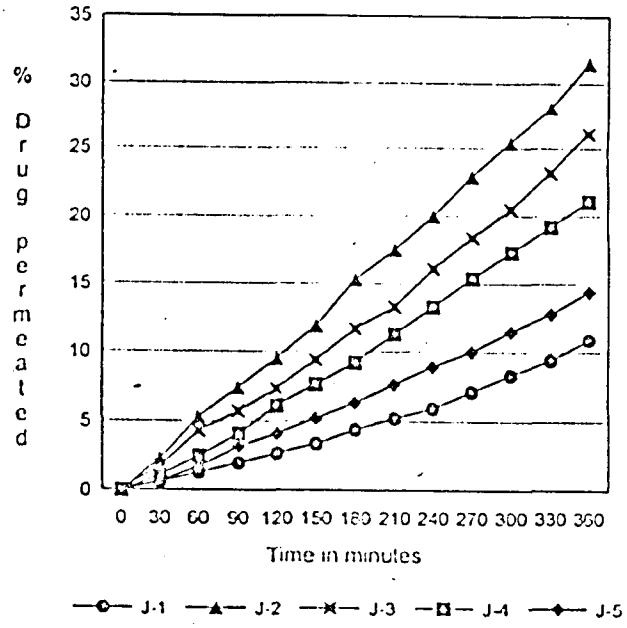

Fig. 3 : Comparison of drug permeation across different types of membranes

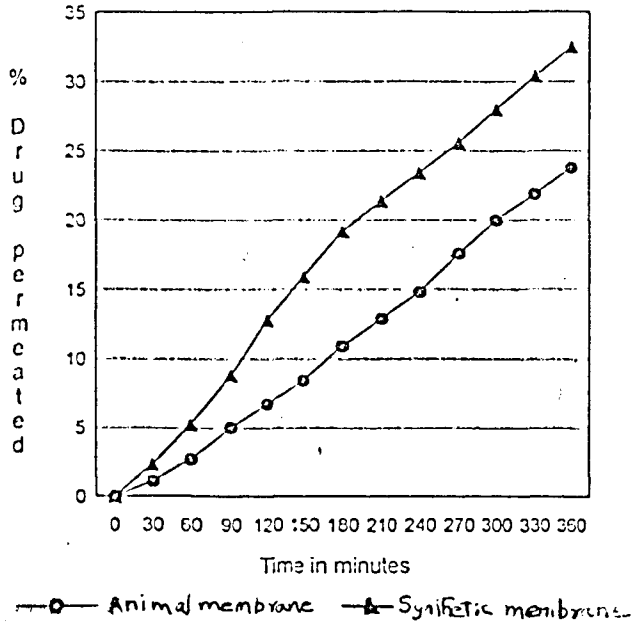

Fig. 4: Comparison of drug permeation across animal and synthetic membrane

In vitro evaluation of the optimized formulation

In vivo evaluation of the optimized formulation in rabbits showed that the drug permeated well across the buccal mucosa. Concentration in blood using equivalent doses of HLZ.HCl via buccal route was found to be the same after 4 h compared to the oral route (Fig. 5).

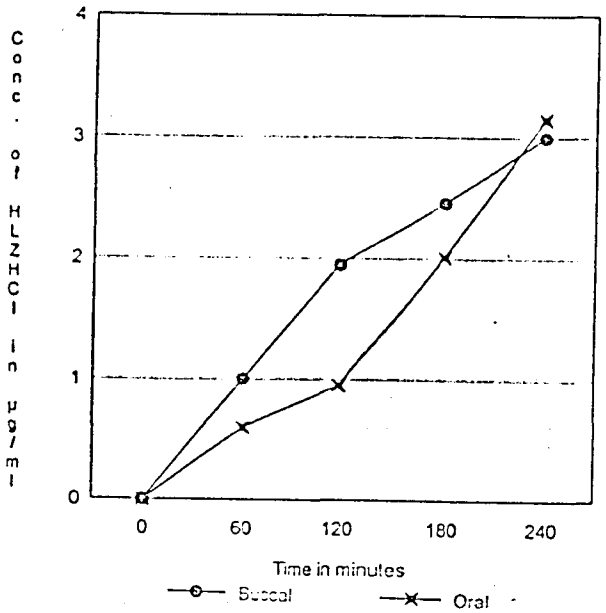

Fig. 5 : *In vitro* release of Hydralazine HCl by oral and buccal route, in rabbits

In conclusion. buccal formulation in the form of multilayered muco-adhesive tablets of HLZ.HCl was developed to a satisfactory level with respect to drug release and bioadhesive strength. Citric acid, D-mannitol and PEG-4000 were found to increase the release of drug from the polymer matrix. A nearly 100% release *in vitro* was obtained using a lyophilized blend of polymers. *In vivo* permeation of drug from buccal tablets in rabbits was also found to be satisfactory.

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