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In vitro and *in vivo* evaluation of locust bean gum and chitosan combination as a carrier for buccal drug delivery

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The object of the study was to evaluate locust bean gum and chitosan in ratios of 2 : 3; 3 : 2 and 4 : 1 (F1, F2 and F3) as a mucoadhesive component in buccal tablets and to compare the bioavailability of a propranolol hydrochloride buccal tablet with the oral tablet in healthy human volunteers. Propranolol hydrochloride buccal tablets containing various weight ratios of locust bean gum and chitosan were prepared and coated with 5% w/v ethyl cellulose on one face, and oral tablets containing 10 mg propranolol hydrochloride alone were formulated using a direct compression technique. The strength of mucoadhesion of the tablets was quantified based on the tensile force required to break the adhesive bond between a model membrane (porcine buccal mucosa) and the test polymer. The forces of detachment for the mucoadhesive buccal tablets were 14.61 ± 0.14, 13.21 ± 0.13 and 11.71 ± 0.12. An *in vitro* study was carried out in pH 6.8 phosphate buffer and the cumulative percentage release of propranolol measured at 10 min intervals for 600 min was found to be 98.31 ± 0.10, 92.24 ± 0.41 and 90.18 ± 0.76 respectively. A bioavailability study was conducted with the prepared formulation in 16 healthy human volunteers to determine the plasma concentration of propranolol at 0, 1, 2, 3, 4, 6, 8, 10 and 12 h. The bioavailability (AUC_{0-t^*} ng · h/ml) of the buccal propranolol hydrochloride tablets (F1, F2 and F3) and oral tablet (F4) was found to be 2244.18 ± 210, 3580.69 ± 460, 3889.19 ± 290 and 1732 ± 96 ng · hr/ml respectively. The study indicates that locust bean gum and chitosan in a weight ratio of 2 : 3 (F1) not only releases the drug unidirectionally from the dosage form, but also gives buccal tablets which are sufficiently mucoadhesive for clinical applications.

1. Introduction

In recent years, there has been increasing interest in the use of bioadhesive polymers to control the systemic or local delivery of biologically active agents (Lenaerts and Gumy 1990). Several studies have been made using chitosal and chitosan derivatives with other polymucoadhesive components in modern drug delivery e.g. chitosan has been investigated as a mucoadhesive polymer and as a permeation enhancer for drug delivery *in vitro* and at mucosal epithelia (Borchard et al. 2001). Mucoadhesive patches containing miconazole nitrate using anionic (SCMC), cationic (chitosan) and non-ionic (PVA, HEC, HPMC) polymers showed satisfactory mucoadhesive characteristics (Nafee et al. 2003). Buccal bioadhesive systems appear attractive because they avoid significant limitations of traditional routes of drug administration such as poor absorption, enzymatic degradation and first-pass metabolism. A variety of drug substances have been administered by the buccal route. Examples include peptides like TRH (thyrotropin releasing hormone), calcitonin (Heiber et al. 1994), buserelin (Hoogstraate et al. 1996) and oxytocin (Li et al. 1997); analgesics such as morphine (Hoskin et al. 1989) and vasodilators such as nitroglycerin (Dellborg et al. 1991). Oral mucosal dosage forms have been investigated for the systemic administration of insulin (Ishida et al. 1981) and for the local delivery of lidocaine (Ishida et al. 1982). Buccal delivery necessitates the use of mucoadhesive polymers as these dosage forms should ideally adhere to the mucosa and withstand salivation, tongue movement and swallowing for a significant period of time. Examples of mucoadhesive polymers include sodium carboxy methyl cellulose, carbopol 934, hydroxyl propyl cellulose, hydroxyl propyl methyl cellulose, acacia, gelatin etc.

Locust bean gum is a neutral polysaccharide having a molecular weight of 310000 derived from the endosperm of the seed of *Ceratonia siliqua* Linne (Fam: Leguminosae). Locust bean gum contains about 88% D-galacto-D-mannoglycan, 4% pentan, 6% protein, 1% cellulose and 1% ash. Chitosan is a deacetylated chitin (poly (*N*)-deacetylglucosamine), obtained industrially by hydrolyzing the aminoacetyl groups of chitin from crabs or shrimps in aqueous alkaline solution (Vijaya Raghavan et al. 2002).

Table 1: Composition of mucoadhesive layer of buccal tablet

Formulation	Composition (mg)			Microcrystalline cellulose	Magnesium stearate
	Propranolol HCl	Locust bean gum	Chitosan		
F1 (2 : 3)	10	20	30	89	1
F2 (3 : 2)	10	30	20	89	1
F3 (4 : 1)	10	40	10	89	1

The present investigation was aimed at using the inexpensive, natural and abundantly available locust bean gum and chitosan as a mucoadhesive component in buccal tablets and to quantify the plasma concentrations of propranolol hydrochloride following administration of mucoadhesive buccal tablets and oral tablets in human volunteers and subsequently estimate its bioavailability. In addition the bioadhesive strength as reflected by the force of detachment of these buccal tablets was quantified by an *in vitro* study using freshly excised pig buccal membrane as a model biological interface. The overall goal of the present study was not to determine whether a conventional drug substance could be administered via the buccal route but rather to demonstrate the utility of a new, previously untested natural polymer to serve as a mucoadhesive tablet excipient.

2. Investigations, results and discussion

Table 1 shows the composition of the buccal tablets. The microcrystalline cellulose is added to the formulation as a direct compression adjuvant, since the locust bean gum and chitosan do not produce sufficient hardness. Tablet hardness varied between 4.4 and 4.9 kg/cm^2 and friability ranged between 0.5 and 0.7%. Tablet weight varied between 148.4 and 151.6 mg and the assay content of propranolol hydrochloride varied between 98.4 and 99.66%. Thus all the parameters of the compressed tablets were practically within the control limits.

Curves showing the mean value of the force of detachment of the propranolol HCl buccal tablets containing various weight ratios of locust bean gum and chitosan after application to excised pig buccal membrane are shown in Fig. 1. It may be noted that the mean values of the force of detachment increased with time until they reached a plateau. The mean values of the force of detachment were generally greater for formulation F1 containing a 2 : 3 weight ratio of locust bean gum and chitosan and the bioadhesive strength decreased with decrease in the quantity of chitosan.

The swelling index for the various formulations is shown in Table 2. The profiles indicate the uptake of water into the tablet matrix producing an increase in weight. Formulation F1 containing a 2 : 3 weight ratio of locust bean gum and chitosan takes up water over the first hour. The higher locust bean gum content of formulations F2 and F3 showed a slower initial water uptake, taking longer to become fully hydrated. After 1 h formulation F1 displayed loss of weight due to tablet disintegration. Higher concentrations of locust bean gum gave a greater hydration capacity. The capacity of the formulation to take up water is an important intrinsic parameter of the polymeric system in view of the release of the drug at the mucosal surface. A remarkable increase in swelling properties was observed in the case of chitosan miconazole nitrate patches, producing a sustained pattern of drug release. In our study formulations F2 and F3 which contained a higher amount of locust bean gum were found to absorb more than the other formulation, F1, and exhibited an n value characteristic of a non-Fickian release mechanism involving a combination of diffusion and chain relaxation. These results suggest that formulation F3 containing a 4 : 1 weight ratio of locust bean gum and chitosan is suitable for a hydrophilic swellable matrix to achieve controlled drug release.

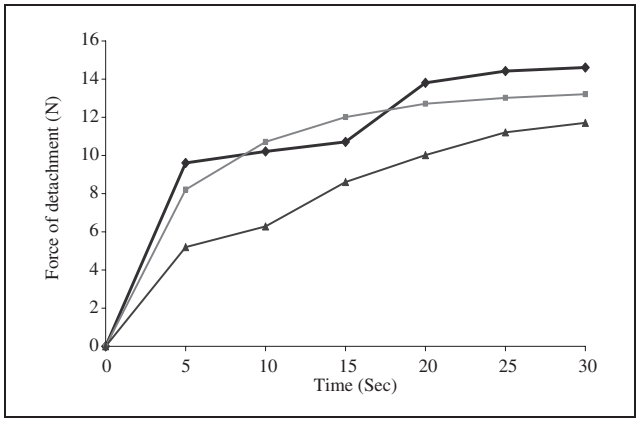

Fig. 1: The force of detachment from pig buccal membrane for directly compressed buccal tablets containing 2 : 3, 3 : 2 and 4 : 1 weight ratios of locust bean gum and chitosan. All data points represent mean value ± standard deviation of three experiments
F1 Locust bean gum/chitosan 2 : 3
F2 Locust bean gum/chitosan 3 : 2
F3 Locust bean gum/chitosan 4 : 1

An acidic or alkaline pH may cause irritation to the buccal mucosa. The surface pH of the tablets was determined in order to investigate the possibility of any side effects *in vivo*. In case of chitosan miconazole nitrate patches the surface pH was found to be in the range of 5–6 (Nafee et al. 2003). The surface pH of all the locust bean gum and chitosan formulations was found to be within ±1.5 units of neutral pH (range from 6.3–6.7) and hence these formulations would not produce any irritation in the buccal cavity (Table 3).

Drug release profiles from propranolol HCl tablets prepared with containing various weight ratios of 2 : 3, 3 : 2 and 4 : 1 locust bean gum and chitosan are shown in Fig. 2. Propanolol HCl was released more rapidly from F1 compared with F2 and F3. Thus, an increased concentration of locust bean gum decreased the release of propanolol HCl. In case of chitosan containing miconazole nitrate patches sustained release of the drug was observed. The minimum release rate was observed in a chitosan system containing 5% w/v PVP, where only 2.7% of the micona-

Table 2: Index of swelling in water of prepared buccal tablets containing 2 : 3, 3 : 2 and 4 : 1 weight ratios of locust bean gum and chitosan

Formulation	Swelling index				
	0.5 Hr	1 Hr	2 Hr	3 Hr	4 Hr
F1	0.416 ± 0.06	0.512 ± 0.03	0.490 ± 0.02	0.210 ± 0.01	0.121 ± 0.03
F2	0.442 ± 0.04	0.616 ± 0.03	0.646 ± 0.04	0.692 ± 0.08	0.70 ± 0.07
F3	0.481 ± 0.01	0.712 ± 0.03	0.791 ± 0.04	0.810 ± 0.03	0.820 ± 0.01

Table 3: Surface pH of buccal tablets containing 2 : 3, 3 : 2 and 4 : 1 weight ratios of locust bean gum and chitosan

Formulation	Surface pH
F1	6.3 ± 0.04
F2	6.4 ± 0.01
F3	6.7 ± 0.08

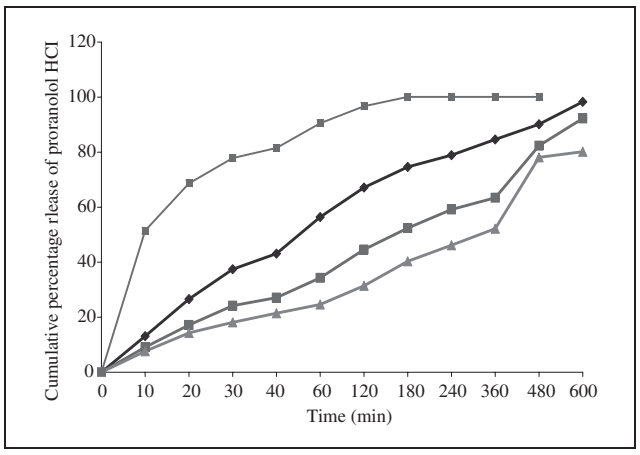

Fig. 2: Cumulative mean (± sd) percentage release of propranolol HCl compressed buccal tablets containing 2 : 3, 3 : 2 and 4 : 1 weight ratios of locust bean gum/chitosan and oral tablets in phosphate buffer pH 6.8
F1 Locust bean gum/chitosan 2 : 3
F2 Locust bean gum/chitosan 3 : 2
F3 Locust bean gum/chitosan 4 : 1
F4 Oral tablet

zole nitrate was released in the first hour, slowly progressing to 30.74% after 5 h (Nafee et al. 2003).

The data obtained from studies of dissolution kinetics were analysed using PCP Disso V2.08 software.

Dissolution profiles for locust bean gum and chitosan containing formulations in Fig. 2 demonstrate the rapid release of propanolol HCl from F1 containing a 2 : 3 weight ratios of locust bean gum and chitosan as a result of tablet erosion and disintegration. Formulations F2 and F3 containing 3 : 2 and 4 : 1 weight ratios of locust bean gum and chitosan show slower propranolol HCl release due to a combination of swelling and erosion in the matrix.

The values of n obtained for formulations F2 and F3 were 0.5364 and 0.5393 respectively, indicating non-Fickian release kinetics, which is indicative of drug release mechanisms involving a combination of both diffusion and chain relaxation, but F1 released 67% of the drug within 2 h. Thus formulation F1 did not follow any of these release characteristics. The kinetic release constant, K, decreased

Table 4: Linear correlation coefficient (r), determination coefficient (r^2), kinetic release constants (K), and diffusion exponents (n) after fitting release data to simple power law (Log Mt/M ∝ Vs Log t)

Formulation	r	r^2	K (h^{-n})	n^a
F1	0.9666	0.9831	1.7062	0.3901
F2	0.9932	0.9965	1.0326	0.5364
F3	0.9910	0.9954	0.8477	0.5393

n^a = diffusion release exponent, indicative of the release mechanism: n = 0.5 for Fickian diffusion mechanism: n = 1 for zero order release (case II transport); n lies between 0.5 and 1.0 (0.5 < n < l) for non-Fickian (anomalous) release and n > 1 for super case II transport

Table 5: Time (h) for 50% and 90% nifedipine release from prepared buccal tablets containing locust bean gum and chitosan

Formulation	$T_{50\%}$	$T_{90\%}$
F1	0.92	7.55
F2	2.55	9.45
F3	5.5	10

with an increase in the amount of locust bean gum (shown in Table 4). This may be attributed to the fact that with an increase in polymer concentration, the viscosity of the gel layer around the tablet tends to limit further release of the active ingredient.

The times for 50% ($T_{50\%}$) and 90% ($T_{90\%}$) release of propranolol HCl from the prepared buccal tablets were estimated by linear regression of log (Mt/M∞) vs. log (t) for different formulations and are shown in Table 5. For F1, F2 and F3, the $T_{50\%}$ values were 0.92, 2.55 and 5.5 respectively. These results clearly indicate the increased half life ($T_{50\%}$) of propranolol HCl release from the prepared tablets obtained by increasing the concentration of locust bean gum.

The mean plasma profiles of propranolol HCl from the prepared buccal tablets in comparison with formulated oral tablets are shown in Fig. 3. The relevant pharmacokinetic parameters are listed in Table 6. The plasma profiles exhibited a higher C_{max} with a faster decline for buccal tablet F1 but a lower C_{max} and more sustained levels for buccal tablets F2 and F3.

Levonorgestrel with a carbomer and chitosan mucoadhesive agents administered nasally in rats was found to be superior for maintaining effective drug concentration over an extended period of time compared with the presently available orally administered form. Mucoadhesive agents (chitosan and carbomer) in the nasal formulations were found to produce a three-fold increase in drug bioavailability. Bioavailability was improved from 29.9% to 101.7% and 99.4% respectively, for chitosan (0.5%) and carbopol 934p (0.5%) containing formulations and the plasma half life was significantly improved from 7 h to 55.7 h and 52.9 h respectively (Shahiwala et al. 2004).

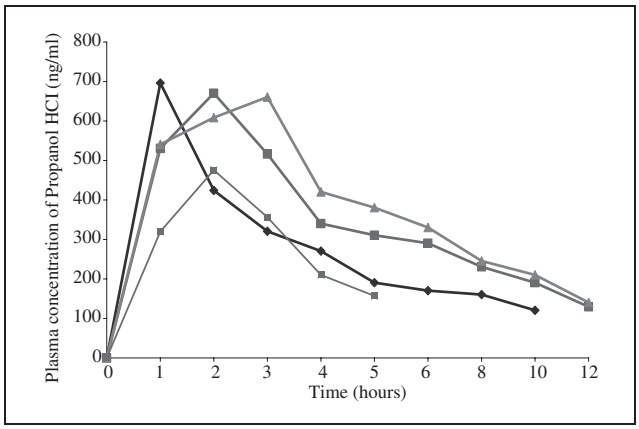

Fig. 3: Mean (± sd) plasma profile of propranolol HCl in health human volunteers from directly compressed buccal tablets containing 2 : 3, 3 : 2 and 4 : 1 weight ratios of locust bean gum/chitosan and oral tablets
F1 Locust bean gum/chitosan 2 : 3
F2 Locust bean gum/chitosan 3 : 2
F3 Locust bean gum/chitosan 4 : 1
F4 Oral tablet

Table 6: Pharmacokinetic parameters of propranolol hydrochloride directly compressed buccal tablets from *in vivo* studies in healthy human volunteers

Parameters	F1	F2	F3	F4 (0ral)
C_{max} (ng/ml)	696.2 ± 68	670.30 ± 121	660.46 ± 18	475.61 ± 12.4
T_{max} (h)	1 ± 0.0	2 ± 0.0	3 ± 0.0	2 ± 0.0
Kel (h)	0.112 ± 10.07	0.0955 ± 0.02	0.0759 ± 0.01	0.293 ± 0.013
$T_{1/2}$ (h)	6.18 ± 0.71	7.25 ± 0.28	9.13 ± 0.47	2.36 ± 0.40
AUC_{0-t^*} (ng · h/ml)	2244.18 ± 210	3580.69 ± 460	3889.19 ± 290	1732 ± 96
$AUC_{0-\infty}$ (ng · h/ml)	3321.05 ± 180	4934.72 ± 310	5738.72 ± 460	2091 ± 8.88
$AUMC_{0-t^*}$ (ng · h^2/ml)	9533.19 ± 290	17775.99 ± 680	20103.29 ± 890	4068.4 ± 61
MRT	9.01 ± 0.89	9.77 ± 0.42	11.66 ± 0.42	3.56 ± 0.35

Table 7: One-way analysis of variance of the in vivo characteristics of buccal tablets containing locust bean gum/chitosan and oral tablets

Formulation	Pharmacokinetic parameter	
	C_{max}	AUC_{O-t^*}
F1	696.20 ± 35.49*	2244.18 ± 44.17*
F2	670.30 ± 23.06*	3580.69 ± 191.51*
F3	660.46 ± 4.32*	3889.19 ± 63.47*
F4 oral	475.61 ± 20.82	1732.61 ± 21.40

Values are mean ± SEM (n = 16 in each group)
Statistical analysis performed using Instat (Graph Pad) one-way analysis of variance (ANOVA)
* $P < 0.01$ Vs oral tablets

It will be observed that by increasing the locust bean gum in formulations F2 and F3 C_{max} was decreased and T_{max} was increased (Table 6). This could be attributed to a slower *in vitro* release of the drug from the increased polymer concentration.

For F1, F2 and F3 the mean C_{max} values were 696 ± 68, 670.3 ± 21 and 660.5 ± 18 and the mean T_{max} values were 1 ± 0.0, 2 ± 0.0, 3 ± 0.0 respectively (Table 6). The higher C_{max} and lower T_{max} value for the formulation F1 is due to faster release of the drug from the polymer. The area under the curve (AUC) for formulations F1, F2 and F3 was found to be 2244.2 ± 210, 3580.7 ± 460 and 3889.2 ± 290. respectively. The highest $AUC_{0-\infty}$ value for tablets prepared with a 4 : 1 weight ratio of locust bean gum and chitosan is due to slow release of the drug by the polymer. All the formulated buccal tablets showed a higher AUC than the formulated oral tablets. This could be attributed to the avoidance of first pass metabolism by the buccal dosage form. The mean residence time (MRT) for the various formulations increased from 9.0 ± 0.9 to 11.7 ± 0.4, with an increasing concentration of locust bean gum.

Table 7 presents a statistical analysis of the pharmacokinetic parameters obtained. C_{max} and $AUC_{0\text{-}t^*}$ were significantly ($P < 0.01$) affected by the type and composition of the buccal tablets, which could be attributed to differences in the *in vitro* release of the drug.

3. Experimental

3.1. Materials

Propranolol hydrochloride was obtained from Unichem Laboratories Ltd. Mumbai, India. Locust bean gum was obtained from Fluka Biochemica, Switzerland. Chitosan was obtained from Central Institute of Fisheries Technology, Kochi, India. Microcrystalline cellulose and ethyl cellulose were obtained from Loba Chemie Pvt. Ltd., India. Magnesium stearate was obtained from SD Fine Chem. Ltd. Mumbai. India.

3.2. Preparation of bilayered buccal tablets

3.2.1. Preparation of mucoadhesive layer

The mucoadhesive layer was prepared by using the drug and various natural polymers (Reumanan-Lopez et al. 1998; Ahmed et al. 1995; Javed 1999). The composition of the different formulations is given in Table 1. The various components of each formula were weighed, mixed and passed through a mesh (250 μm) to ensure complete mixing. The average weight of about 150 mg was separately weighed out and compressed using a 13 mm diameter die on an infrared hydraulic pellet press (Kimaya Engineers, India) using a force of 8 t for 60 s. The prepared adhesive tablets were 13.32 mm in diameter.

3.2.2. Formation of backing layer to the mucoadhesive layer

The backing layer was made up of ethyl cellulose (Senal et al. 1998). The solution was prepared by dissolving 5% w/v of ethyl cellulose in chloroform. The prepared solution was sprayed on to one surface of the mucoadhesive layer leaving the other side free. Then it was air dried at room temperature. The double layered structure design was expected to provide unidirectional drug delivery to the mucosa. It avoids loss of drug due to wash out of saliva and the swelling profile of the buccal tablet can be changed dramatically by the amount of backing material and those changes could alter drug release profile.

3.3. Evaluation of tablets

Ten tablets from each batch were evaluated for uniformity of weight and drug content. Six tablets from each batch were examined for friability using a Roche-type friabilator. (Tropical Equipment Pvt. Ltd., Mumbai, India) and hardness using a Monsanto-type hardness tester (Campbell, Mumbai, India).

3.4. Swelling study

The swelling index of the tablets (Fergany and Hussain 2003) was evaluated for six tablets of each formulation. These were weighed and placed separately in a pre-weighed basket made of stainless steel mesh. The total weight was recorded (W_1). This basket was placed in a plastic vessel containing 4 ml of demineralized water, and placed in an incubator at 37 °C. At 0.5, 1, 2, 3 and 4 h, excess water was carefully removed, and the swollen tablets were weighed (W_2). The swelling index was determined from the formula.

$$\text{Swelling index} = (W_2 - W_1)/W_1 \quad (1)$$

3.5. Surface pH of the tablet

The surface pH of the tablet (Bottenberg et al. 1991) was determined to investigate the effect of pH on bioadhesion and possible side effects of the tablet *in vivo*. This was determined by allowing the tablet to swell in 1.0 ml. of demineralized water (pH 6.3 ± 0.06) for 2 h. A combined glass pH electrode was brought into contact with the swollen tablet and pH was measured after 1 min. equilibration.

3.6. Bioadhesion studies

Satisfactory bioadhesion (Choy Fun Wong et al. 1999) is essential for the successful application of a buccal bioadhesive drug delivery system. It involves the strength of attachment of the dosage form to the biological tissue. Several techniques for *in vitro* determination of bioadhesion have been reported, which included tensile testing (Park and Robinson 1987), shear stress testing (Smart et al. 1984), an adhesion weight method (Smart and Kellaway 1982), a fluorescent probe method (Park and Robinson 1984), flow channel techniques (Mikos and Peppas 1986), and a colloidal gold staining method (Park 1989). In our study the polymers were evaluated using a $TA.XT_2$ (Stable Micro System, Haslemere, Surrey, U.K.) texture

analyzer (Choy et al. 1999) with porcine buccal mucosa (Chen and Hwang 1992) as a model tissue under simulated buccal conditions.

3.7. In vitro drug release studies

Release of propranolol HCl from the buccal tablets (Wen-Gang Chen et al. 1992) was studied in 250 ml of pH 6.8 phosphate buffer (Kreuser et al. 1972; Ferguson and Fort 1974) using an USP XXII/XXII dissolution rate test apparatus, with a paddle rotating at 75 rpm and at $37 \pm 0.5\,^{\circ}C$. A specially designed glass cylinder closed at one end and open at the other end was placed inside the dissolution apparatus to allow the tablets to dissolve from a fixed place without any movement (since the tablet should release the drug from a fixed area in the buccal region). Samples were withdrawn through a filter (0.45 μm) at intervals and were assayed at 290 nm for propranolol hydrochloride using a Jasco V 530 1400 UV visible double beam spectrophotometer. The drug release experiments were conducted for concurrent results.

3.8. Drug release kinetics

To examine the release mechanism of propranolol HCl from the prepared bioadhesive tablets (Peppas and Korsmeyer 1986), the results were analysed according to the following Eq. (2).

$$\frac{Mt}{M\alpha} = Kt^{n} \qquad (2)$$

where $Mt/M\alpha$ is the fraction of the drug released at time t, K is the kinetic constant incorporating structural and geometrical characteristics of the drug/polymer system (device) and n is the diffusion exponent that characterizes the mechanism of drug release. For non-Fickian release, n falls between 0.5 and 1.0 ($0.5 < n < 1.0$), while in the case of Fickian diffusion $n = 0.5$, for zero order release (case II transport) $n = 1$, and for supercase II transport, $n > 1$. The values of n as estimated by linear regression of log $Mt/M\infty$ vs. log (t) of the different formulations are shown in Table 4.

3.9. In vivo bioavailability study

3.9.1. Protocol

Each study was carried out in 16 healthy male volunteers of 20–23 years of age and 55–70 kg weight. A complete crossover design was employed in which each subject received the test product and the reference product. Their liver and kidney functions were assessed to be normal by clinical and standard biochemical investigation. None of the subjects used alcohol or tobacco or had taken any medication for a week prior to the study. The purpose of the study was fully explained and each volunteer had given his written consent. The study was approved by the ethical committee of the institution.

Volunteers were fasted overnight and zero hour blood samples were collected early in the morning from each volunteer. For oral administration one tablet containing the drug (10 mg propranolol HCl) was administered at 8 h together with 200 ml of water. The mouth was rinsed with an additional 100 ml of water which was also swallowed. Food was withheld for a period of 2 h. The samples of blood were collected at various time intervals. The blood samples obtained were immediately centrifuged and the plasma was separated and stored at $-20\,^{\circ}C$ for analysis. For buccal administration, a buccal tablet was placed in the buccal cavity while the subjects were in a sitting position. Samples of blood (5 ml) were collected at various time intervals. The blood samples obtained were immediately centrifuged and the plasma was separated and stored at $-20\,^{\circ}C$ until analysis.

3.9.2. Estimation of propranolol HCl in plasma

The frozen samples (Trivedi et al. 1986) were thawed at room temperature and 1 ml was pipetted into a clean borosilicate, graduated centrifuge tube. 6 ml of methanol was added and vortexed for 1 min and then centrifuged at 5000 rpm for 15 min. One ml of the supernatant was then diluted with 1.0 ml of distilled deionized water. The fluorescence of the samples was observed at λ_{emi} 340 nm and λ_{exi} 317 nm. The plasma concentration of the drug was calculated from a standard plot.

3.9.3. Data analysis

Data were generated by assuming first order absorption and a one compartment model with first order elimination (Gibaldi et al. 1982). Maximum plasma concentration (C_{max}), time required to reach maximum concentration (T_{max}), elimination rate constant (K_{el}), biological half life ($t_{1/2}$), area under the plasma concentration time curve from 0–t h (AUC_{0-t}) and from 0–α ($AUC_{0-\alpha}$), area under first moment curve from 0–t ($AUMC_{0-t}$) and from 0–α ($AUC_{0-\alpha}$) and mean residence time (MRT) were determined from the data of drug concentrations in plasma following buccal administration of 10 mg propranolol prepared with different concentrations of locust bean gum and chitosan.

3.10. Statistical analysis

The results obtained for *in vivo* studies were subjected to statistical analysis using the computer program Instat (Graph Pad) for one way analysis of variance ($p < 0.01$) followed by Dunnett's test.

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