RESEARCH ARTICLE

In vitro and *In vivo* Evaluation of Chitosan/ Karaya Gum Interpolymer Complex Based Mucoadhesive Buccal Films of Tramadol HCl

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Abstract

Aim: The main objective of the study was to assess the potential application of Chitosan/Karaya gum interpolymer complex to develop the novel rate controlled buccal mucoadhesive drug delivery system. Developed drug delivery system is capable to administer opioid analgesic drug tramadol HCl directly into systemic circulation via buccal mucosa. The developed drug delivery system would be more effective in terms of improving bioavailability, quick onset of action and reduction in dose related side effects of drugs which are suffering from hepatic first pass metabolism. **Materials and Methods:** Chitosan and karaya gum were selected for the preparation of interpolymer complex. Resulted interpolymer complex was characterized by Fourier-transform infrared and scanning electron microscopy. Further, the swelling behaviour of interpolymer complex was studied compare to karaya gum alone. Mucoadhesive bilayered buccal films were prepared from Interpolymer complexes by solvent casting method. An impermeable ethyl cellulose backing layer was placed to ensure unidirectional drug release from filmes. The resulted mucoadhesive buccal films were evaluated for swelling index, folding endurance, bioadhesive strength, *ex vivo* diffusion of the drug, *in vitro* dissolution and *in vivo* absorption of the drug. An opioid analgesic Tramadol HCl was used to study the nature of drug release from buccal films. A software aided optimization was carried out to understand the effect of formulation factors like amount of inter polymer complex and plasticizer at three different levels on three major properties of films like swelling index, tensile strength and *in vitro* dissolution. **Result and Discussion:** It has been observed that all resultant buccal films prepared by using inter polymer complex between Karaya gum and chitosan have shown good structural integrity, good mucoadhesive strength, satisfactory drug release and are capable to improve the *in vivo* absorption as well as plasma residence time.

Key words: Bioadhesive strength, Chitosan, interpolymer complex, karaya gum, mucoadhesive films, tramadol HCl

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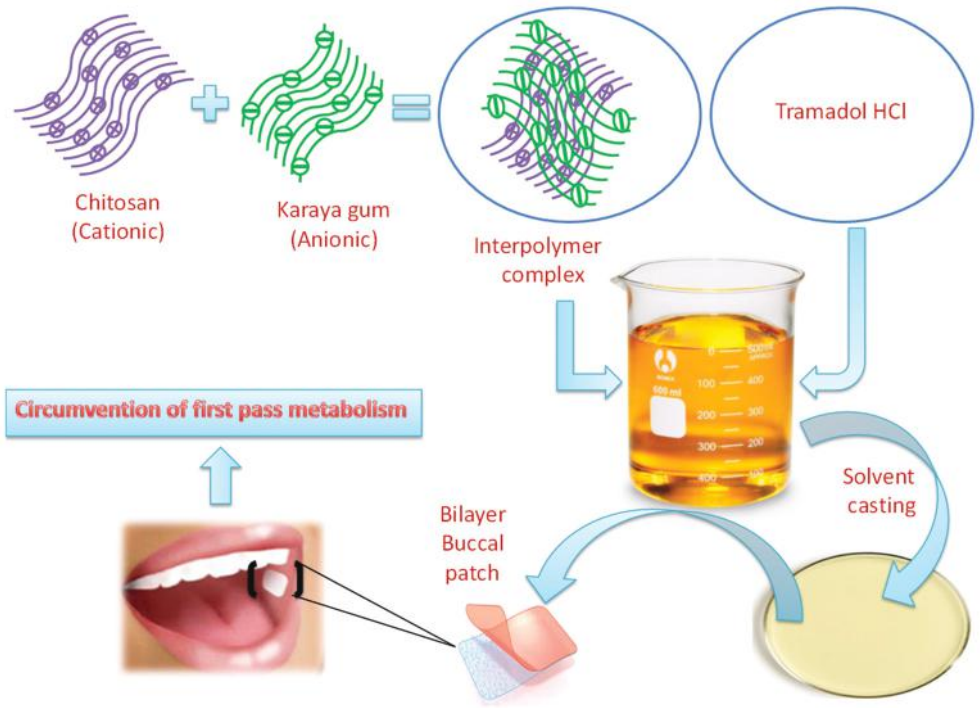
Graphical Abstract



INTRODUCTION

The oral mucosal route has been an attractive option for the administration of many drugs directly into systemic circulation for many researchers. Factors like rich vascularization and good permeability or buccal mucosa make this route more attractive in comparison to oral and parenteral routes. Absorption of many drugs delivered orally is adversely affected by hepatic metabolism. Also, it has been observed that the enzymatic degradation and the destructive effect of acidic environment in the gastric region lead to the low oral bioavailability.[1,2] The use of mucoadhesive polymers in the formulation of buccal mucosal dosage forms increases the retention time of dosage at the site of application. In addition to that buccal mucosal dosage forms do not interfere with speech or mastication unlike sublingual route and thus ensures good patient compliance. In comparison to parenteral drug delivery system, buccal mucosal drug delivery system can administer the drug into systemic circulation in a completely pain-free manner. Other advantages of buccal mucosal drug delivery include self-medication and termination of therapy in case of adverse effects.[3]

It has been very challenging to utilize natural gums and mucilages in the formulation development though they are biocompatible and do not produce any toxicities. The high degree of hydrophilicity of natural gums and mucilage makes them unsuitable for being utilised in the development of rate controlled drug delivery systems.[4] This problem can be solved by preparing interpolymer complex of biopolymers with synthetic or semisynthetic polymers. Interpolymer complexes are prepared by the physical linkage between two oppositely charged electrolytes. In case of the development of buccal mucosal drug delivery system mainly chitosan is selected as it has mucoadhesive properties.[5] Chitosan is cationic in nature and thus in order to make interpolymer complex, an anionic gum or mucilage is selected. By making interpolymer complex of chitosan and anionic gums and mucilage the solubility of biopolymers can be controlled. Further, these interpolymer complexes can be utilized to develop a variety of rate controlled drug delivery systems i.e., polymeric microparticles, floating drug delivery systems, gastro retentive drug delivery and colon targeted drug delivery systems etc.[6]

Tramadol HCl falls under the category of centrally acting synthetic opioid analgesic. It binds to specific opioid receptors. Being a non-selective pure agonist it has an affinity for mu, delta and kappa opioid receptors. Among three receptors drug has a higher affinity for the mu receptor. The drug is freely soluble in water and has good absorption capacity after oral administration. Tramadol HCl has a systemic bioavailability of 68% after oral administration. It is a centrally acting analgesic mainly used for the management of chronic pain. It is mainly prescribed by physician a first line drug treatment for producing pain relief in orthopaedic injuries. The drug has half life of about 5.5 h and the usual oral dosage regimen is 50–100 mg every 4–6 h. The maximum dose of the drug is 400 mg/day.[7]

Table 1: Formulation chart

Ingredients	Formulations and quantity								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Tramadol hydrochloride (mg)	50	50	50	50	50	50	50	50	50
Inter polymer complex (mg) (60:40)	25	50	100	50	100	25	100	25	50
Glycerin (% w/v)	2	4	6	2	4	6	2	4	6
Sodium dihydrocholate (% w/v)	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
EDTA disodium salt (% w/v)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Ethyl cellulose (1% w/v)	Backing layer on F1-F9 formulations								

EDTA: Ethylenediaminetetraacetic acid

Table 2: Result of physico-chemical evaluation of Chitosan/karaya gum inter polymer complex based buccal films

Code	Thickness (mm)	Weight uniformity (mg)	Swelling index	Surface pH	Folding endurance
F1	0.24±0.03	155.21±7.45	45.08±0.3	6.74±0.5	318±06
F2	0.27±0.04	254.53±6.83	51.23±0.5	6.73±0.7	305±04
F3	0.23±0.03	423.27±6.11	49.88±0.5	6.69±0.6	258±07
F4	0.28±0.05	169.70±6.32	49.47±0.4	7.08±0.5	260±06
F5	0.32±0.04	473.58±7.53	48.81±0.6	7.15±0.6	267±08
F6	0.36±0.03	311.40±6.83	46.03±0.3	7.12±0.6	269±07
F7	0.39±0.03	331.45±6.47	58.49±0.6	7.13±0.7	273±05
F8	0.38±0.04	112.34±7.41	58.35±0.5	7.07±0.5	326±06
F9	0.40±0.03	331.89±6.72	56.56±0.6	7.11±0.6	268±07

Table 3: Result of drug content evaluation of Chitosan/karaya gum inter polymer complex based buccal films

Formulation	% drug content
F1	97.15±1.13
F2	96.86±1.64
F3	98.65±1.37
F4	92.39±1.24
F5	96.91±2.26
F6	97.29±2.45
F7	94.22±1.97
F8	96.18±2.53
F9	94.23±1.48

MATERIALS AND METHODS

Materials

Tramadol hydrochloride was obtained as a gift sample from Karnataka Antibiotics and Pharmaceuticals Ltd. Bengaluru. *Karaya gum* was received as gift sample from Nutriroma Hyderabad. Chitosan was purchased from Pure Chem Pvt. Ltd. Ankleshwar. All other chemicals were purchased from Merck Ltd. Mumbai.

Experimental design

Optimization of buccal films was done using a 3^2 randomized full factorial design. This method includes evaluation of two factors individually at three levels shown in Table 5. Different codes such as −1, 0 and +1 were given to lower, medium and higher levels of both variables. Two independent variables were the amount of interpolymer complex in specific ratio of drug (X_1) and the concentration of plasticizer (X_2)

Preparation of a buccal mucoadhesive film of tramadol HCl

All mucoadhesive buccal films were prepared by solvent casting method. The compositions of all formulations are shown in Table 1. Chitosan was dissolved in 60 ml 5 M acetic acid to produce 2.5% chitosan solution. To this solution, 10 ml 5 M ammonium solution was added. The drug was dissolved in 40 ml 2.5% karaya gum solution with constant stirring for 15 min using mechanical stirrer. Glycerin was used as a plasticizer. The resulting solution was sonicated for 45 min to remove air bubbles. Post sonication the solution was poured into a petri dish having the diameter of 8 cm and was dried in vacuum oven at 55°C for 24 h. The backing layer of 1% ethylcellulose was placed by pouring solution over medicated buccal film and dried in vacuum oven at 55°C for

Table 4: Result of *in vitro* drug release study of Chitosan/karaya gum inter polymer complex based buccal films

Time (h)	% Drug release								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	10.92±0.8	12.88±0.7	15.52±0.7	16.93±0.6	17.12±0.7	15.74±0.8	19.67±0.5	18.69±0.7	19.39±0.6
2	15.98±0.5	18.89±0.4	25.16±0.5	25.38±0.5	24.62±0.4	23.25±0.7	35.84±0.4	28.82±0.4	31.32±0.7
3	32.83±0.4	36.11±0.8	28.87±0.8	35.58±0.3	33.95±0.5	33.67±0.8	44.39±0.7	39.32±0.9	36.62±0.9
4	49.82±0.7	45.16±0.6	44.18±0.4	46.78±0.7	42.13±0.3	39.26±0.6	52.54±0.6	50.44±0.7	45.43±0.4
5	56.53±0.4	54.22±0.7	54.37±0.6	57.32±0.6	53.19±0.6	47.24±0.8	63.24±0.7	65.29±0.5	52.81±0.4
6	69.25±0.3	65.12±0.5	59.87±0.7	66.88±0.4	61.88±0.9	62.67±0.4	68.34±0.6	75.58±0.7	59.84±0.8
7	71.73±0.8	69.87±0.4	71.86±0.7	77.19±0.8	77.35±0.8	82.38±0.8	77.68±0.8	80.21±0.8	79.77±0.9
8	86.52±0.7	85.56±0.5	87.66±0.9	89.62±0.3	90.55±0.5	96.32±0.7	91.46±0.6	94.38±0.5	93.49±0.5

Table 5: Translation of coded levels in actual units

Variable levels	Low (−1)	Medium (0)	High (+1)
IPC:Drug (X_1)	0.5:1	1:1	2:1
% Plasticizer glycerin (X_2)	2	4	6

Table 6: Effect of variables on selected responses

Batch no.	Variable levels in coded form		Tensile strength (kg/mm²) Y_1	Bioadhesive force (N) Y_2	Drug release (%) Y_3
	X_1	X_2			
F1	−1	−1	11.66±0.23	0.48±0.12	86.52±0.7
F2	−1	0	14.15±0.28	0.55±0.14	85.56±0.5
F3	−1	+1	14.72±0.30	0.62±0.09	87.66±0.9
F4	0	−1	12.84±0.25	0.87±0.10	89.62±0.3
F5	0	0	15.78±0.25	0.93±0.13	90.55±0.5
F6	0	+1	17.14±0.22	1.02±0.14	96.32±0.7
F7	+1	−1	15.35±0.25	1.21±0.10	91.46±0.6
F8	+1	0	17.13±0.28	1.34±0.14	94.38±0.5
F9	+1	+1	19.23±0.21	1.34±0.13	93.49±0.5

4 h. Dried films were cut into 1.5 cm² films containing 50 mg Tramadol HCl.[8]

Characterization of buccal films

Thickness and weight

The thickness of the film was measured using micrometre screw gauge. For each formulation, three films were randomly selected with surface area of 1.5 cm². The weight of individual films was noted down using analytical balance. Later the average weight of buccal film was calculated.

Swelling studies

Pre-weighed films (designated as w1) were placed separately in Petri plate having phosphate buffer 6.8 pH. At regular intervals (5, 10, 15, 20, 25, 30, 35, 40, 60 min), films were removed from the Petri plate. Excess water was removed carefully using filter paper. The swollen films were reweighed (w^2). The following formula was used to calculate % swelling index.

$$\% \text{ Swelling index} = (W_2 - W_1)/W_1 \times 100 \quad (1)$$

Measurement of surface pH

The surface pH study was performed by selecting 3 films randomly. Digital pH meter was used to find out pH. The pH electrode was placed in close contact with the wetted film surface and the pH was recorded for each film.

Folding endurance

The folding endurance was determined to check the flexibility of films. All selected films were folded repeatedly at the same place until the film was completely broken. The action was

Table 7: Chitosan/Karaya gum inter polymer complex based buccal films

Formulation	Zero order	First order	Korsmeyer-Peppas		Higuchi model
F6	r^2	r^2	r^2	N	r^2
	0.96	0.98	0.95	0.86	0.82

Table 8: Pharmacokinetic parameters after *in vivo* study

Formulation	Pharmacokinetic parameters		
	C_{max} (ng/ml)	T_{max} (min)	$AUC_{0-\infty}$ (ng-h/ml)
Oral solution	15.48±2.48	30±5.50	33.49±1.75
FX	19.67±3.51	240±20.75	115.37±5.78

Table 9: Chitosan/Karaya gum inter polymer complex based buccal films

Evaluation parameter	Formulation F6	
	Before stability study	After stability study
Mass uniformity (mg)	311.40±6.83	309.19±5.27
Drug content (%)	97.29±2.45	96.38±1.96
Thickness (mm)	0.36±0.03	0.37±0.05
Folding endurance	269±07	271±09
Surface pH	7.12±0.6	7.15±0.7
Swelling index (%)	46.03±0.3	47.24±0.7
Mucoadhesive strength (N)	1.02±0.14	1.04±0.21
Ex-vivo permeation study (%)	90.44±1.87	91.32±0.8
In-vitro drug release (%)	96.32±0.7	95.87±0.2

repeated until films broke or were folded for 300 times which ever is less.[9]

Tensile strength

Texture analyzer (CT-3/10,000, Brookfield, USA) equipped with a 10 kg load cell was used to check tensile strength of the formulation. Films from all formulations were randomly selected and were fixed between the two clamps of probe TA-DGA and for a hold time of 60 s. The lower clamp was held stationary, and films were pulled apart by the upper clamp. The film was pulled at a speed of 2.0 mm/s to a distance of 6 mm with trigger load 0.05 N. The force of the film at the point when the film broke was recorded.

Texture- Pro CT V1.3 Build 14 Software was used for data collection and calculations. The tensile strength break value was calculated using the formula:

Tensile strength (kg/mm^2) = Force at break/initial cross sectional area (2)

In vitro bioadhesion force

Texture analyzer (CT-3/100, Brookfield, USA) equipped with a 100 g load cell was used to determine the bioadhesion force of buccal filmes. The porcine buccal mucosa was used as the model membrane for the measurement of buccal mucosa. The mucosal membrane was isolated by removing the underlying connective tissue. The mucosal membrane was washed thoroughly with phosphate buffer (pH 6.8). Then, the membrane was fixed between two circular discs which were at lower Perspex support. The upper circular disc had a cavity of 12.7 mm diameter through which the mucosal membrane was exposed to the probe. The discs were lowered into the jacketed glass container filled with phosphate buffer (pH 6.8) which was maintained at 37 ± 1°C. The test was started once the membrane was equilibrated at 37 ± 1°C for 30 min. The buccal film was firmly tight with the help of thread on the lower side of the probe. The probe and circular cavity were aligned in such a way that film comes into direct contact with the exposed surface of the mucosal membrane. The exposed area of the buccal film was moistened with phosphate buffer pH 6.8 before test starts. The probe was lowered at a speed of 0.5 mm/s to contact the tissue with load, 90 g and with contact time 120 s. It was removed at the speed of 2 mm/s.

Texture-Pro CT V1.3 Build 14 software was used for data collection and processing. The adhesive force and adhesiveness were found out to evaluate the bioadhesive strength of the film.[10] Bioadhesion force (N) was calculated using the formula:

Bioadhesion force (N) = (Bioadhesive strength/1000)×9.81 (3)

Ex vivo diffusion study

The *ex-vivo* diffusion study was carried out by using Franz diffusion cell. The Chick buccal mucosa was procured freshly from local slaughter shop and washed thoroughly with PSS. The membrane was washed and rinsed with phosphate buffer pH 6.8 during the study. The chick buccal mucosa was attached to the receiver compartment filled with phosphate buffer 6.8 pH. Upon the mucosa, the film was placed and the whole assembly was maintained at 37 ± 1°C and the medium stirred at 100 rpm. An aliquot of sample (1 ml) was taken at suitable time intervals from the receiver compartments using sampling port and equal volume was replaced with fresh 6.8 pH phosphate buffer. Samples were analyzed by ultraviolet (UV)- spectrophotometer.[11]

In vitro dissolution test

In order to check the release rate and pattern of the drug from buccal films in vitro dissolution study was done. Films were selected randomly from all formulations. The amount of drug release from the prepared buccal film was determined by using the dissolution test apparatus USP Type-II. The dissolution medium contained 500 ml of phosphate buffer pH 6.8 maintained at a temperature of 37 ± 0.5 °C with the paddle rotation speed of 50 rpm. The film was fixed on the glass slide using glue or thread. At predetermined time intervals 1 ml of sample was collected till 8 h. The amount of drug was determined by a UV spectrophotometer at 271 nm.[12]

In vivo buccal permeation studies of tramadol HCl from mucoadhesive buccal films in rabbits

On the basis of *in-vitro* dissolution data and the mucoadhesive strength, the formulation F6 was selected for the *in vivo* study as it gives maximum % cumulative drug release after 8 h. Buccal films were prepared to have 50 mg of tramadol HCl in each individual buccal film. A backing layer of 1% ethyl cellulose was placed to buccal films in order to ensure the unidirectional release of drug during the study. All necessary permissions were taken from the institutional animal ethical committee.

New Zealand white rabbits having a weight of 2.5–3 kg of either sex were selected for the study. Animals were anaesthetized before applying films to the buccal mucosa. Animals were fasted for overnight prior to the actual test by giving ad libitum water. All animals were kept in separate cages for acclimatization period of 1 week before carrying actual test. Rabbits were divided into three groups, each group having four animals. Rabbits were reweighed and an intramuscular injection of ketamine HCl (40 mg/kg) and xylazine (10 mg/kg) was given in order to anaesthetize them. All rabbits remained anaesthetized for 6 h without a sign of respiratory depression. Once the anaesthesia started showing effect buccal filmes containing 50 mg of tramadol HCl were moistened with simulated saliva juice of pH 6.75 and applied to the mucosa of buccal cavity of one group of rabbits. To the second group of rabbits, an aqueous solution containing 50 mg of tramadol HCl was given orally with the help of infant feeding tube. The third group was treated by using placebo films. At the time interval of 30 min initially and then after 1 h, 1 ml of blood was removed by using 22 gauge needles through a butterfly cannula from the marginal ear vein of rabbits. The last blood sample was collected at the end of 8 h. These blood samples were centrifuged at 10,000 rpm for 10 min in order to separate the plasma and immediately stored at −20°C, until analysis. At the end of the study, all buccal films were removed and analyzed for the remaining drug content.[13-15]

Quantification of tramadol HCl from rabbit blood plasma

The quantification of tramadol HCl in rabbit blood plasma was done by an high-performance liquid chromatography (HPLC) method coupled with fluorescence detection proposed by Curticapean *et al*. In this method an RP 18 column with C18 pre column was used with elution gradient over 26 min. The mobile phase selected was 0.1% formic acid:acetonitrile (20:80). Liquid liquid extraction was used with the mixture of ethyl acetate and sotalol. During the analysis the flow rate was maintained at 1 ml/min, column temperature was kept 22°C. The fluorescence detectors were set at λ_{ex} = 280 nm and λ_{em} = 310 nm wavelengths.[16,17]

RESULTS AND DISCUSSION

Preparation of buccal films

The formulation chart was prepared by using 3^2 full factorial design. Total 9 formulations were prepared each

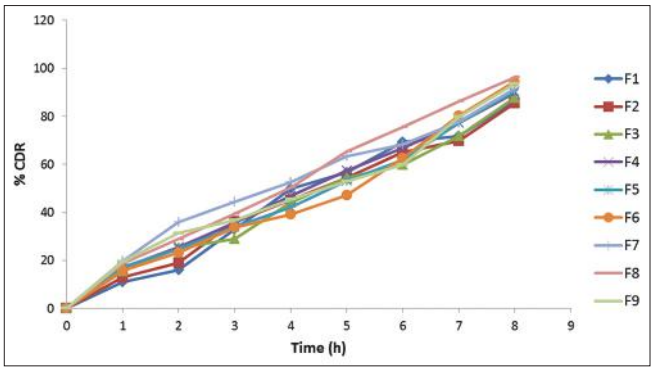


Figure 1: Graph of % CDR versus time of buccal films based on Chitosan/Karaya gum interpolymer complex

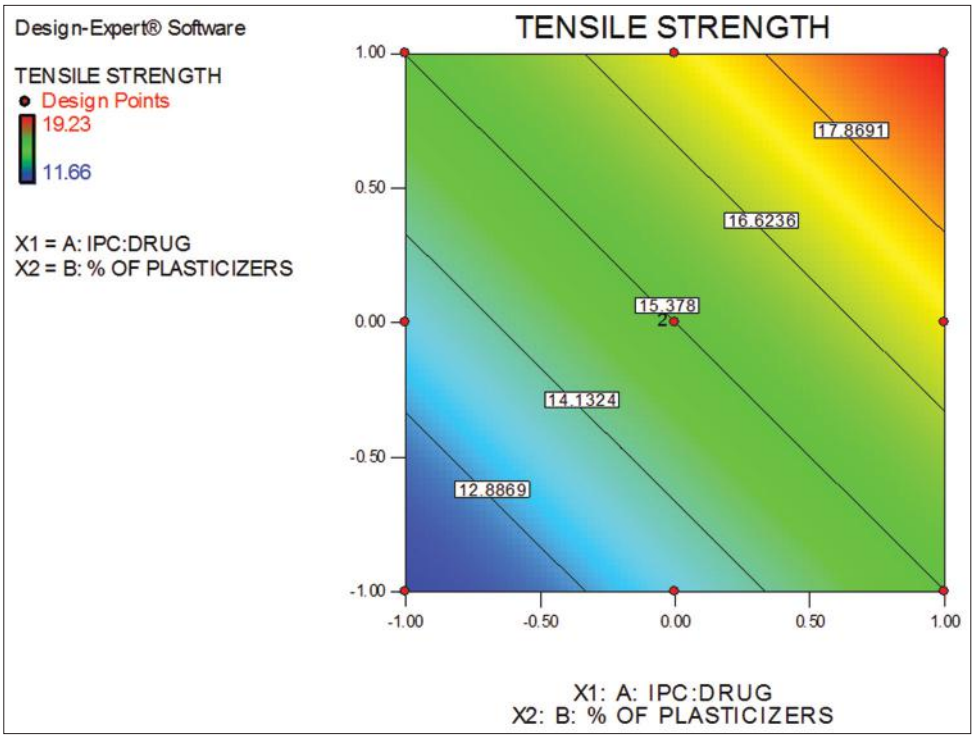


Figure 2: 2D contour plot of effect of formulation variables on tensile strength of Chitosan/Karaya gum inter polymer complex based buccal films

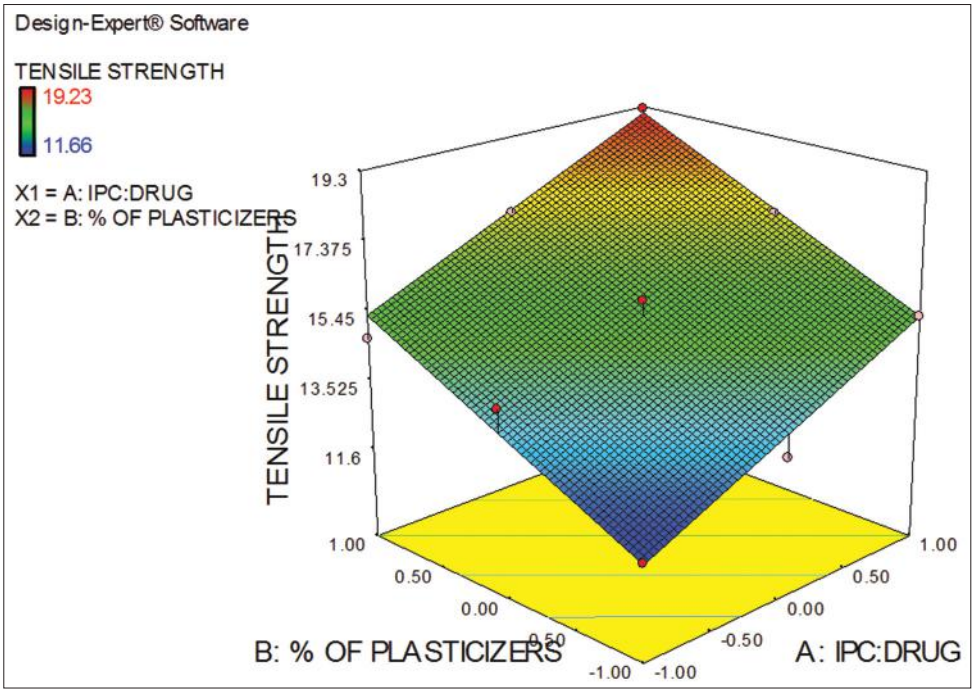


Figure 3: 3D surface plot of effect of formulation variables on tensile strength of Chitosan/Karaya gum inter polymer complex based buccal films

containing 50 mg of Tramadol HCl. The interpolymer complex of chitosan and karaya gum was used as the rate controlling polymer. To impart the flexibility of buccal films glycerin was used as a plasticizer. Two buccal permeation enhancers i.e., sodium dihydrocholate and ethylenediaminetetraacetic acid disodium salt were used. To prevent the drug loss from the other side of the buccal films a water impermeable backing layer of 1% W/V was placed. All films were prepared by solvent casting method.

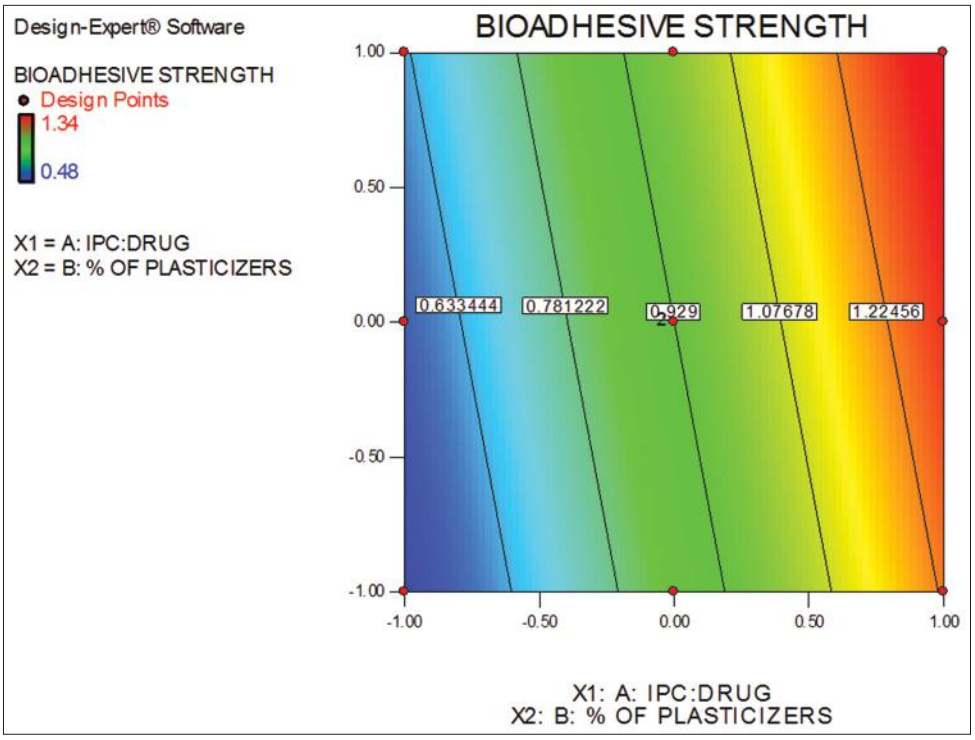


Figure 4: 2D contour plot of effect of formulation variables on bioadhesive strength of Chitosan/Karaya gum inter polymer complex based buccal films

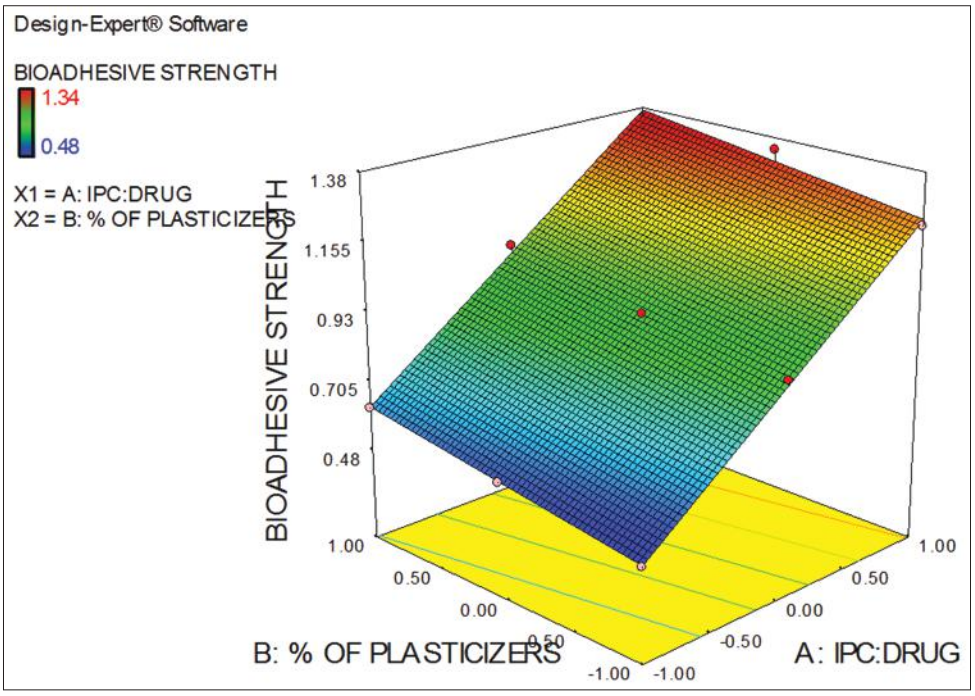


Figure 5: 3D Surface plot of effect of formulation variables on bioadhesive strength of Chitosan/Karaya gum inter polymer complex based buccal films

Characterization of buccal films

Films from all 9 formulations were randomly selected and evaluated for various physicochemical parameters (Table 2). The thickness of films was checked by using micrometer screw gauge. The thickness was found to be in the range of 0.23 ± 0.03 to 0.40 ± 0.03 mm. This indicates that the solvent casting was done properly. Values of the weight variation test were in the range of 155.21 ± 7.45–473.58 ± 7.53 mg. This variation was due to the change in the amount of chitosan/karaya gum

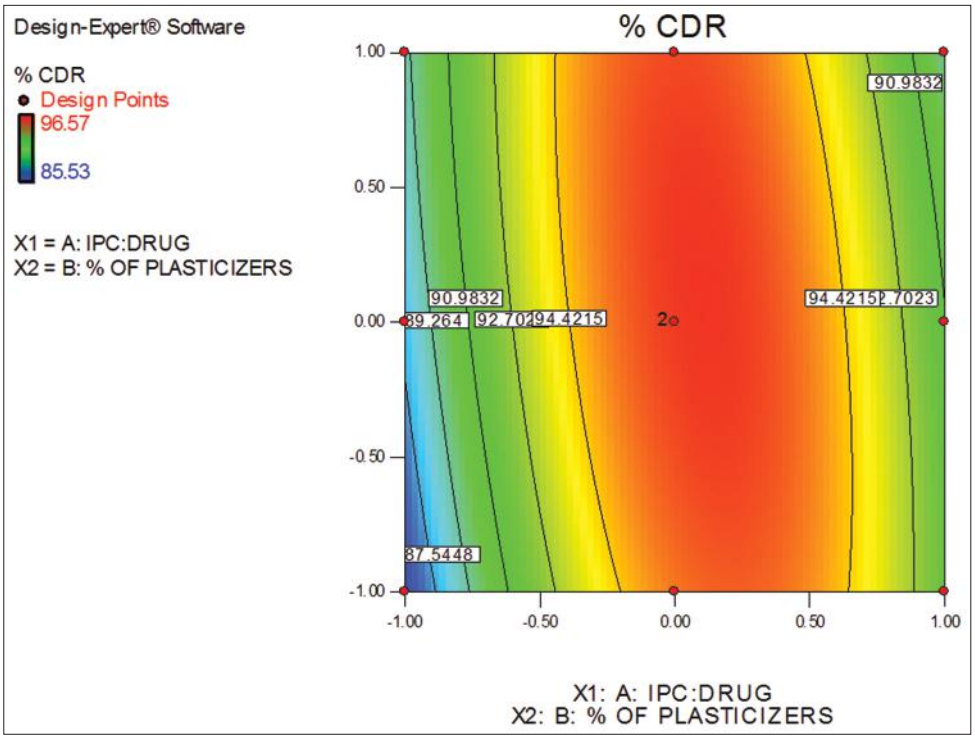


Figure 6: 2D contour plot of effect of formulation variables on % CDR of Chitosan/Karaya gum inter polymer complex based buccal films

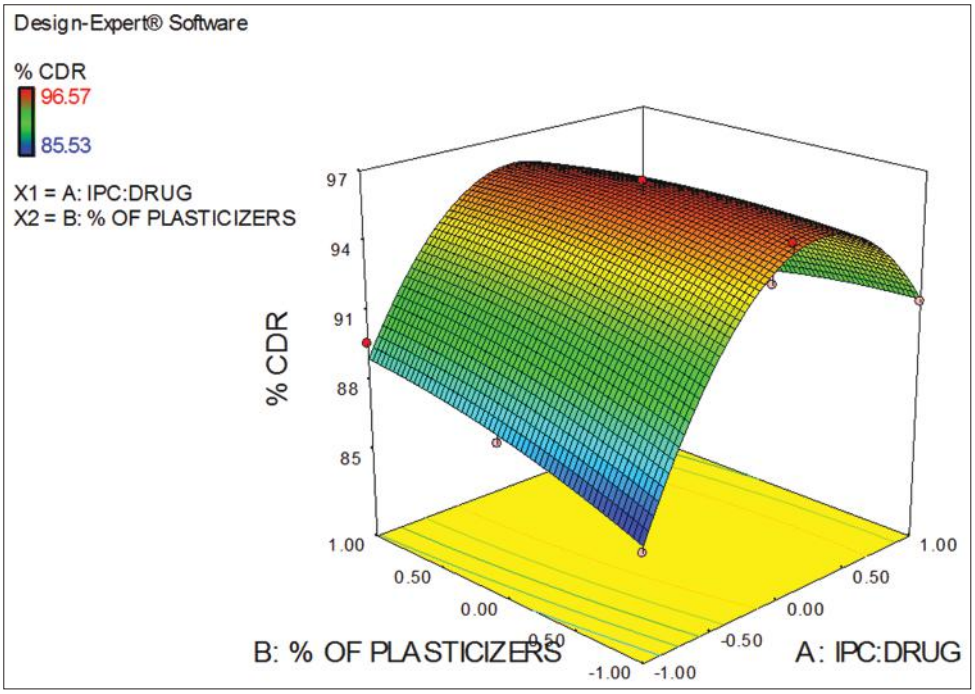


Figure 7: 3D Surface plot of effect of formulation variables on % CDR of Chitosan/Karaya gum inter polymer complex based buccal films

interpolymer complex. All values were found to be within the acceptable range and that further ensures dose uniformity.

The swelling index values of buccal films from all nine formulations were found to be in the range of 45.08 ± 0.3–58.49 ± 0.6%. The swelling index is critical as the drug release from buccal films is governed by the swelling. The swelling index was observed to increase with the increase in the amount of chitosan/karaya gum interpolymer complex.

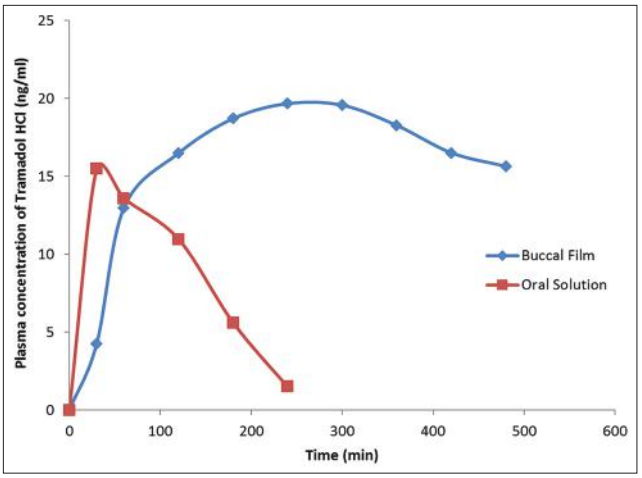


Figure 8: Plot of mean plasma drug concentration–time profile of tramadol HCl from buccal film and oral solution

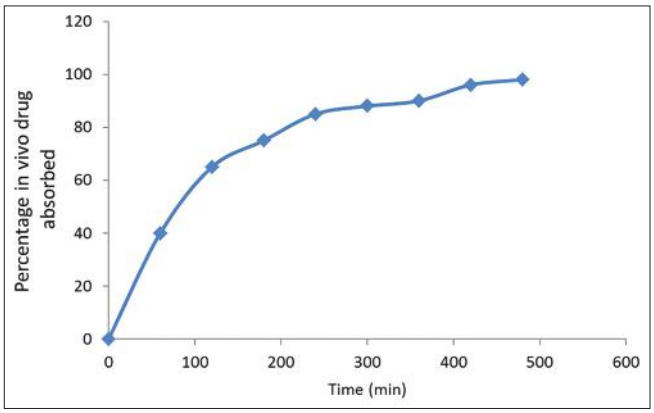


Figure 9: Plot of percentage *in vivo* drug absorbed versus time

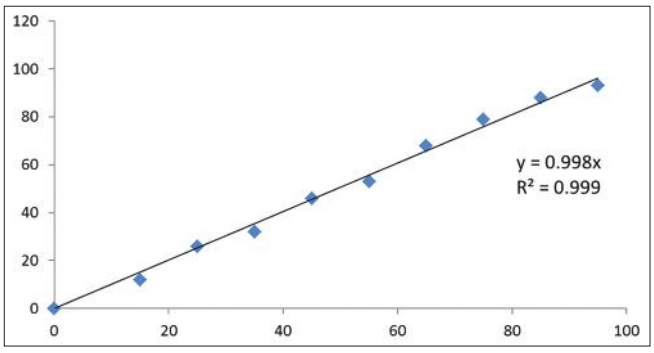


Figure 10: *In vitro* - *in vivo* correlation of percentage drug released and percentage drug absorbed

The surface pH values for buccal films of all 9 formulations were found to be in the range of 6.69 ± 0.6–7.15 ± 0.6. These values are close to the buccal pH i.e., 6.8. These results indicate that buccal films from all 9 formulations may not cause any irritation to the buccal mucosa. In addition to that at this pH the sialic acid present in the mucus remains in an ionized form, this is essential for the good mucoadhesive properties.

The folding endurance values for buccal films of all 9 formulations were in the range of 258 ± 07–326 ± 06. This indicates that all buccal films from different formulations have sufficient flexibility and they may remain intact at the site of application during the course of treatment.

Drug content analysis

The drug content evaluation was done to check the uniform distribution of the drug in individual buccal films (Table 3). Values of drug content evaluation were in the range of 94.23 ± 1.48–97.15 ± 1.13%. This indicates that the drug is uniformly distributed in all individual buccal films belong to different formulations. This may ensure the dose uniformity during the treatment.

In vitro dissolution test

The *in vitro* dissolution test was done to check the drug releasing pattern from buccal films (Table 4). The result shows that maximum cumulative drug release i.e., 96.32 ± 0.7% was observed in the case of formulation F6 after 8 h. Whereas the minimum cumulative drug release i.e., 85.56 ± 0.5% was observed in the case of formulation F2 (Figure 1). The data obtained after the *in vitro* drug release study was further utilized for kinetic model treatment to check the exact drug release mechanism from buccal films.

Optimization of buccal films

The optimization of buccal film formulation was done by using 3^2 full factorial design. Three different levels of two variables i.e., amount of interpolymer complex and concentration of glycerin was selected to check the effect on three different responses i.e., tensile strength, bioadhesive force and in vitro drug release (Table 6). For individual responses 2D contour plot, 3D surface plot and overlay plots were prepared after applying various models for optimization (Figures 2-10).

Study of drug release kinetics from buccal films

Various kinetic models were applied on data obtained after in vitro drug release study (Table 7). The value of n in case of Korsmeyer-Peppas model was found to be 0.86 along with the r^2 of 0.96, 0.98 and 0.95. The value of regression coefficient of various models applied indicates that the release of drug from buccal films does not follow one particular mechanism but it is the combination of diffusion and swelling controlled drug release. This type of mechanism is also termed as anomalous non fickian drug release. The release of drug from buccal films depends on both the rate of diffusion of mucus inside the polymeric film and the rate of swelling of polymeric films.[19]

In vivo drug release study of buccal films

During the entire study, all buccal filmes of tramadol HCl were remained intact and adhered well to the rabbit buccal mucosa. No signs of mucosal irritation or discomfort were observed in any animal during the course of study. The HPLC method selected for the quantification of tramadol HCl in rabbit blood plasma was found to be adequately sensitive and suitable for the post study analysis. By using the calibration curve the plasma drug concentrations were determined for individual rabbit and the mean plasma drug concentrations were calculated along with standard deviations for each group. The data obtained was used to plot the drug plasma concentration vs time profile. Pharmacokinetic parameters such as Cmax, Tmax, and AUC0-∞ were determined using model-independent methods, with nonlinear least-squares regression analysis using the software i.e., WinNonlin® and Pharsight from the plasma drug concentration time profiles of each individual rabbit (Table 8). Cmax was the peak plasma drug concentration, Tmax was the time required to reach peak plasma drug concentration, and AUC was the area under the curve.

Statistical analysis of data from rabbit plasma drug concentrations

The one way ANOVA method was used to carry out statistical analysis of plasma drug concentration data obtained after study. Results of the statistical analysis showed that the difference in data obtained from groups that received the oral solution and buccal film were statistically significant with respect to C_{max}, T_{max}, and AUC. It has been reported in various literature that the tramadol HCl suffers from low oral bioavailability due to extensive liver metabolism. This study shows that the AUC and values of C_{max} of Tramadol HCl was found to be significantly greater ($P < 0.005$) from the buccal film, as compared to those from the oral solution containing the same dose of the drug. This confirmed that the bioavailability of this drug could be improved by the buccal administration.

The T_{max} values from the buccal film were significantly greater ($P < 0.005$) as compared to those from the oral solution indicating the slower release of the drug from filmes, thereby providing prolonged effects. Therefore these formulations could be considered suitable for sustained release of the drug.

In vitro – in vivo correlation

As per the BCS classification, tramadol HCl belongs to Class 1. When Tramadol HCl is put into rate retarding drug delivery system it behaves like BCS Class 2 which means low solubility and high permeability. Hence Level A *in vitro in vivo* correlation was done on data obtained. Level A correlation is a point-to-point relationship between the *in vitro* dissolution and *in vivo* absorption rates of a drug from the dosage form. A graph of percentage *in vivo* drug absorbed versus percentage of *in vitro* drug released was prepared to find out the correlation coefficient. The percentage of the drug absorbed was determined using the Wagner Nelson method by the deconvolution of the plasma level data, using the following equation.[18]

$$FA = [(C_t + k_e AUC_{0-t})/k_e AUC_{0-\infty}] \times 100$$

Where FA is the fraction of drug absorbed, C_t is the plasma drug concentration at time t, k_e is the overall elimination rate constant obtained by the least squares regression analysis of the terminal phase of the first order plot, AUC_{0-t} and $AUC_{0-\infty}$ are areas under the curve between time zero and time t and between time zero and infinity, respectively. The values thus obtained were correlated with the *in vitro* percentage of the drug released at the same time intervals. Good *in vitro–in vivo* correlation was obtained for the buccal film formulation of tramadol HCl.

Stability study

Buccal films from best formulation i.e., F6 were kept in a stability chamber and the accelerated stability study was carried out as per ICH guidelines (Table 9). Buccal films were removed after the prescribed time as per ICH guidelines and reevaluated. It was observed that results of all evaluation parameters were showing minor changes from the results of original evaluation parameters before accelerated stability study. From the study, it was observed that when films were exposed to high humidity and elevated temperature with the use of proper packaging material there was no significant change. This means all buccal films remained stable when exposed to high humidity and temperature and formulations may remain stable when properly packed by using suitable packaging materials with proper labeling instructions regarding the storage and the usage.

CONCLUSION

With the emergence of green chemistry and special focus on patient safety it is an essential job of a formulation scientists and pharmaceutical industries to develop nature based biopolymers and other excipients. These excipents are completely safe for the internal consumption by the patients and they are biocompatible as well as biodegradable. At the end of the study, it can be concluded that the interpolymer complex between chitosan and karaya gum can be effectively used to develop mucoadhesive buccal drug delivery system. All the evaluation parameters were good and buccal mucoadhesive films had successfully released drug during dissolution test and *in vivo* animal study. Developed dosage form will help to achieve good bioavailability in patients with less amount of drug as this dosage form will directly deliver

drug into systemic circulation by avoiding hepatic first pass metabolism. This will be helpful in achieving good therapeutic results with reduced risk of dose related side effects. Also the patient compliance will increase significantly as this method of drug administration into systemic circulation is non invasive and pain free drug delivery. Patients can easily terminate the therapy by removing buccal films in case of any unwanted side effects.

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