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Buccal Gel Of Verapamil HCl Based On Fenugreek Mucilage And Xanthan Gum: In-Vitro Evaluation

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ABSTRACT

As a site for drug delivery the oral cavity offers advantages over the conventional gastrointestinal route and the parenteral and other alternative routes of drug administration. It provides direct entry into the systemic circulation thereby avoiding the hepatic first pass effect. Verapamil HCl belongs to a drug group of calcium channel antagonists. The oral absorption of the drug from these forms is 90% but its bioavailability approaches only 10–20%, due to a extensive first-pass effect. Here attempt is made to extract mucilage and use as gelling and mucoadhesive agent. The yield of natural mucoadhesive fenugreek extract was 28-29 percent. Fenugreek mucilage shows synergistic effect with xanthan gum and provide higher viscosity. Fenugreek is used with xanthan gum in the selected ratio of 2.5:1. Different parameters were evaluated like % yield of fenugreek, viscosity, gel strength, mucoadhesive study, in-vitro diffusion study, ex-vivo permeation study and differential scanning calorimetry. The mucosal permeation of drug from the formulation was evaluated using Franz diffusion cell, goat buccal mucosa as semi-permeable membrane. The amount of the drug released was determined by evaluating drug diffused through the membrane by using UV- spectrophotometry. The verapamil HCl release was sustained up to 6 hrs by optimizing concentration of fenugreek and xanthan gum.

Keywords: Fenugreek mucilage, Verapamil HCl, Buccal, Mucoadhesion, Xanthan gum

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INTRODUCTION

The oral mucosa has a rich blood supply. Drugs are absorbed from the oral cavity through the oral mucosa, and transported through the deep lingual or facial vein, internal jugular vein, and braciocephalic vein into the systemic circulation. Lymphatic uptake of drug also occurs but is less common. Following buccal or sublingual administration the drug gains direct entry into the systemic circulation thereby avoiding the hepatic first-pass effect. Contact with the digestive fluids of the upper gastrointestinal tract is avoided. In addition the rate of drug absorption is not influenced by food or gastric emptying rate[1].

Verapamil belongs to a drug group of calcium channel antagonists. It is currently employed in the treatment of hypertension, arrhythmia and angina pectoris. The oral absorption of the drug from these forms is 90% but its bioavailability approaches only 10–20%, due to extensive first-pass effect mainly in the liver. The buccal mucosa may be a more favorable site of absorption of Verapamil than the digestive tract[2,3]. Non-keratinized and strongly supplied with blood buccal mucosa, with a dense capillary vessel network, it constitutes a relatively large drug absorption area. Drug can thus reach the systemic circulation directly through capillary vessels, bypassing the first-pass metabolism in the intestines and liver or avoiding inactivation in the stomach[4]. That in turn contributes to higher bioavailability parameters after administration of a smaller dose of the drug than in conventional tablets[5].

Trigonella Foenum-graceum, commonly known as Fenugreek, is an herbaceous plant of the leguminous family and is native to Western Asia, from where it has spread widely over Europe, the Mediterranean, and the rest of Asia. It is one of the oldest cultivated plants and has found wide applications as a food, a food additive, and as a traditional medicine in every region where it has been cultivated. It is used with xanthan gum as a viscosity enhancing agent[6,7].

The aim of the present work is to isolate natural mucoadhesive polymer from fenugreek with high yield and to develop buccal mucoadhesive gel of verapamil HCl by using fenugreek mucilage and xanthan gum as a mucoadhesive polymer. Different parameters like % yield of fenugreek, viscosity, gel strength, mucoadhesive study, in-vitro diffusion study, ex-vivo permeation study were evaluated and drug release was studied upto 6 hrs.

MATERIALS AND METHODS

Verapamil HCl received as a gift sample from Zydus research center, Fenugreek from local market, Xanthan gum received from Loba chemicals (Mumbai, India) and all other solvents used were of analytical reagent grade and purchased from Loba chemicals (Mumbai, India).

Isolation of fenugreek mucilage

Fenugreek seed was weighed accurately and transferred into 500.0 ml beaker. Hexane was added to remove the lipid content from the seeds and it was soaked in hexane for 3-4 hrs. The supernatant was removed and seeds were dried in the sun light until it completely dry. The seeds are then soaked in the water and stirred for 8-9 hrs with mechanical stirrer (2100-2200 RPM) and the slurry was kept into the refrigerator for 3-4 hrs. Ethanol added to the slurry in 1:1 ratio and the solid material which is precipitated is separated. The material is then dried and powdered it into the grinder.

Viscosity determination of fenugreek mucilage

Viscosity of different concentration of fenugreek mucilage was determined that are shown in Table 2. Xanthan was then added to the fenugreek mucilage to increase the viscosity as both were found to be anionic in nature by zeta potential measurement.

Zeta potential measurement of fenugreek and xanthan gum

By zeta potential measurement, fenugreek mucilage and xanthan gum both were found to be anionic. So, xanthan gum was selected for increasing the viscosity of formulation because Fenugreek mucilage and xanthan gum shows the synergistic effect.

Formulation of Verapamil gel

Verapamil HCl, xanthan gum and fenugreek were weighed accurately. Verapamil HCl was dissolved in distilled water. Fenugreek was dispersed in drug solution at room temperature for 10 min by using magnetic stirrer then weighed quantity of xanthan gum to above solution was added. Temperature was maintained at 70ºC. The mixture was continuously stirred on magnetic stirrer until uniform gel was formed. Composition of all formulation batches were shown in Table 1.

The concentration of fenugreek mucilage was selected as 2.5% as it shows higher viscosity shown in Table 2. Since, in the concentration range of 0.5-2.5%, 2.5% solution of fenugreek mucilage showed the highest viscosity. And to study the synergistic effect, the concentration of xanthan gum was optimized in the 0.25-1.25% range.

Table 1 Composition of Verapamil HCl gel formulations

Ingredients	**Formulation composition (%W/V)**			
	FG1	FG2	FG3	FG4
Verapamil HCl	0.4	0.4	0.4	0.4
Fenugreek	2.5	2.5	2.5	2.5
Xanthan gum	0.25	0.75	1	1.25
Distilled water	q.s.	q.s.	q.s.	q.s.

Table 2 Viscosity of different concentration of fenugreek mucilage

Concentration (%)	Viscosity(cps)
1	2298
1.5	2442
2	2594
2.5	2878

EVALUATION OR CHARACTERIZATION OF VERAPAMIL HCL GEL

Viscosity measurements

Viscosities of all formulations were measured by Brookfield DV-E viscometer using spindle number 0 at 2.5 rpm because of maximum torque at this value.

Gel strength determination[8]

It is expressed in terms of time (in seconds) required by a 35.0g piston for penetration of 5.0 cm distance, through the 50.0g gel formulation. Test was performed using 'Gel strength apparatus modified at laboratory as mentioned by Yong, *et al*. Gel formulation (50.0g) was placed in a 100.0 mL measuring cylinder. The apparatus for measuring gel strength (weight: 35.0g) was then placed on to the gel. The gel strength was measured as the time (in seconds) required for moving the apparatus 5.0 cm down through the gel. In cases the formulation that took more than 5 min to drop the apparatus into the gel, suitable weights were placed on top of the apparatus and gel strength was described by the minimal weights that pushed the apparatus 5.0 cm down through the gel.

Drug content determination[9]

The selected formulation was weighed 100.00 mg accurately transferred to 100.00 mL volumetric flasks with a micropipette and the final volume was made up with phosphate buffer pH 6.8. Verapamil HCl concentration was determined at 279 nm (Shimadzu, UV-1700).

Mucoadhesive strength[10]

The mucoadhesive potential of each formulation was determined by measuring the force required to detach the formulation from buccal mucosal tissue using a modified method described by Murthy *et al*., In brief, buccal tissues were carefully removed from the buccal cavity of goat obtained from the local slaughter house. Tissues were immediately used after separation. At the time of testing, a section of buccal tissue was secured (keeping the mucosal side out) to the upper probe using a cyanoacrylate adhesive. The upper probe was attached to pre calibrated force displacement transducer SS12LA; (BIOPAC Systems Inc.) connected to the Student's physiograph apparatus (Medicaid systems, India). The surface area of each exposed mucosal membrane was 4.2 cm^2. At room temperature, fixed amount of samples of each formulation were

placed on the lower probe. Immediately, a force of 0.2 gm was applied for 2 minutes to ensure intimate contact between the tissues and the samples. The probe was then moved upwards at a constant speed of 0.15 mm/s. The mucoadhesive force, expressed as the detachment stress in dyne/cm^2, was determined from the minimal weights that detached the tissues from the surface of each formulation using the following equation.

$$\text{Detachment stress} = \frac{m \times g}{A}$$

Where,

m is the weight added to the balance in gram;

g is the acceleration due to gravity taken as 980 cm/s^2; and

A is surface area of mucosa tissue in cm^2.

***In vitro* diffusion**[8,14]

In-vitro diffusion study of formulated gels were carried out on Franz diffusion cell having 2.0 cm diameter and 16.0 mL capacity. Dialysis membrane having molecular weight 12000 – 14000 kDa (Himedia) was used as diffusion membrane. Dialysis membrane was soaked in phosphate buffer pH 6.8 for 24 hrs prior to experiment. Diffusion cell was filled with phosphate buffer pH 6.8; dialysis membrane was mounted on cell. The temperature was maintained at 34°C. After a pre-incubation time of 20 minutes, pure drug solution and formulation equivalent to 40.00 mg of verapamil HCl was placed in the donor chamber. At predetermined time intervals, 0.5 mL samples were withdrawn from the acceptor compartment, replacing the sampled volume with pH 6.8 after each sampling, for a period of 300 minutes. The samples withdrawn were filtered and used for analysis. Blank samples (without verapamil HCl) were run simultaneously throughout the experiment to check for any interference. The amount of permeated drug was determined using the UV spectrophotometer at 279 nm.

Differential scanning calorimetry

Drug-excipient compatibility study was carried out from 35ºC-350ºC at the rate of 10 ºC/min and four samples were evaluated for compatibility study. Thermogram of fenugreek mucilage, drug, xanthan gum and mixture of all ingredients were carried out.

Zeta potential measurement

Zeta potential of fenugreek mucilage and xanthan gum were measured by Malvern zeta sizer (Nano ZS-90). This was determined to study the charges present on the mucilage and xanthan gum.

RESULTS AND DISCUSSION

The final prepared gel formulation was evaluated for the different kind of its characteristics. The apparent viscosity values were measured for gel formulations by using Brookfield viscometer and it showed the marked increase in viscosity as concentration of xanthan gum is increased. Then, xanthan gum was mixed with the fenugreek as showed negative zeta potential and as a result it showed marked increase in the viscosity because of synergistic effect between xanthan gum and fenugreek. FG4 batch shown highest viscosity among the all batches shown in Table 3.

The gel strength was determined by using the gel strength measurement apparatus. The gel strength was found to be affected by concentrations of gelling polymers and viscosity enhancer. Optimal gel must have suitable gel strength so as to be administered easily and can be retained at buccal mucosa without leakage after administration. The gel strength values between 100 to 150 seconds were considered sufficient. The gel strength duration of less than 100 seconds may not retain its integrity and may erode rapidly while gels having strength greater than 150 seconds are too stiff and may cause discomfort to the mucosal surfaces. FG1 to FG4 formulation series showed the gel strength values in range 110 to135 seconds which are in acceptable for buccal delivery. FG4 batch shown the highest gel strength that shown in Table 3.

The further evaluation was done by measuring the mucoadhesion time. All the formulations were subjected to mucoadhesion studies by the method reported by Murthy *et al.* The mucoadhesion force is an important parameter for buccal formulations since it prolongs the buccal clearance of gels and increases its residence time in oral cavity. The stronger the bioadhesive force more is the buccal residence time. But if the mucoadhesion is too strong the gel can damage the mucosal membrane. Mucoadhesive property was increase with increase in the concentration of xanthan gum. Formulation FG4 showed optimum mucoadhesion required for buccal mucosa. FG4 batch shown highest mucoadhesion among the all batches shown in Table 3.

In vitro release studies of formulations were performed using the Franz diffusion cell with dialysis membrane, phosphate buffer of pH 6.8 used as diffusion media. Release profiles of FG formulation series are elaborated in Figure 1. The release profiles exhibited an inflection point, which indicated the gel formation in the donor compartment of diffusion cell. A portion of drug might be loaded in to the gel matrix, thus the cross linking of polymer reduces the drug release rate. The results showed that the formed gels had the ability to extend the release of verapamil HCl for the duration of about 300 to 360 min. Formulations of untreated xanthan gum showed the complete drug release up to duration of 180 min. Results of drug content are mentioned in Table 3. The drug content of the FG4 formulation was found to be 94.69 $\pm$ 2.01.

Table 3 Physical parameters of Verapamil HCl gel formulations.

Formulation	Viscosity(cps)	Mucoadhesion (dyne/cm^2)	Gel strength (sec)	% Drug content
FG1	3059	6723	115	92.45
FG2	4590	7150	121	97.23
FG3	5114	7580	130	95.76
FG4	5814	8013	134	94.69

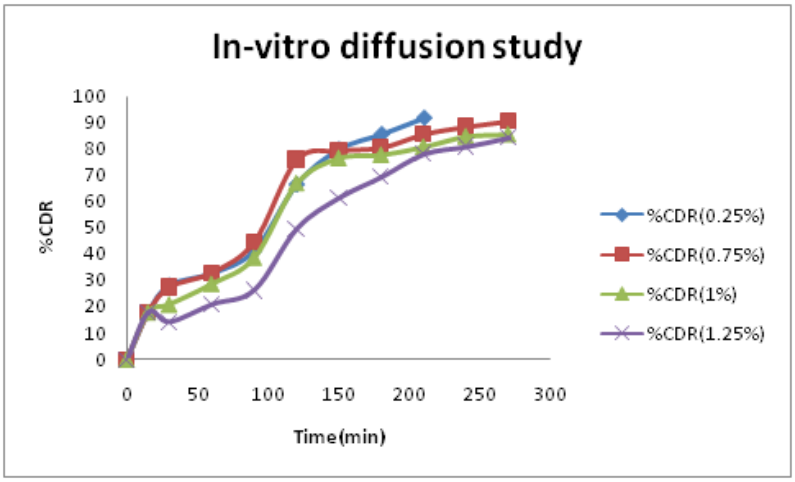

Figure 1 *In-vitro* diffusion study of verapamil HCl gel formulation from FG1-FG4

Formulation FG4 was further subjected to *ex-vivo* permeation study using the goat buccal mucosa and permeation profiles of FG4 formulation is shown in Figure 2. The percent drug permeated after 6 hours was found to be 82.86% ± 6.19, batch FG4 shows good permeation.

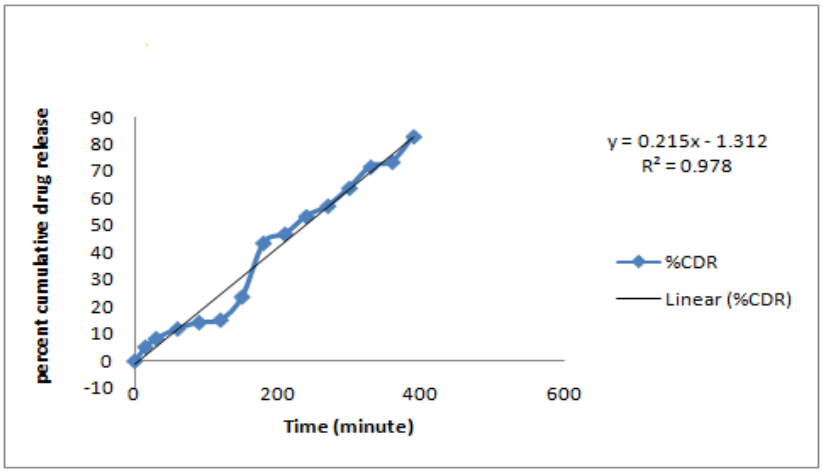

Figure 2 *Ex-vivo* permeation study of FG4 formulation through goat buccal mucosa

The DSC analysis (Figure 3) of pure verapamil HCl showed a characteristic, sharp intense peak at 146.90°C corresponding to its melting point. The DSC analysis of physical mixture of drug and excipients revealed negligible change in the melting point of verapamil HCl in the presence excipients, indicating no modification or interaction between the drug and excipients.

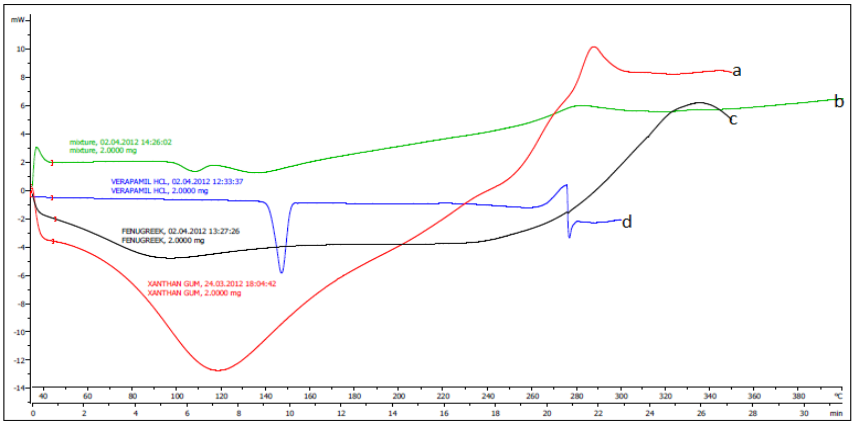

Figure 3 DSC Thermogram of verapamil HCl and physical mixture

(a- xanthan gum, b- mixture, c- fenugreek, d- verapamil HCl)

The zeta potential of fenugreek and xanthan gum were found to be -3.02 and -2.34 respectively that are shown in Figure 4.

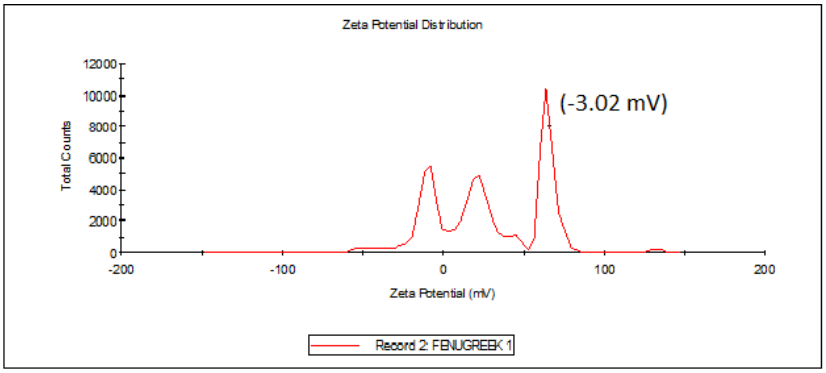

Figure 4(a) Zeta potential of Fenugreek

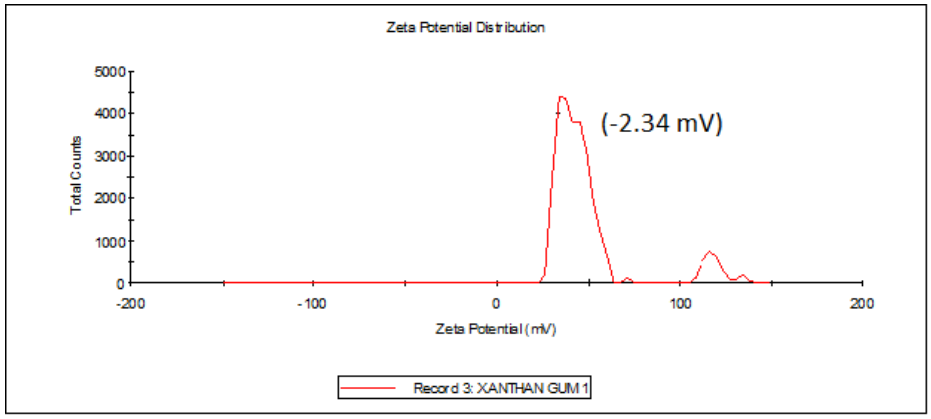

Figure 4(b) Zeta potential of xanthan gum

Release kinetic profiles of all formulations were carried out. The *in-vitro* diffusion study data was subjected to various kinetic models, viz. zero order, first order, Higuchi model, Korsmeyer & Peppas, Hixson-crowell. Results of kinetic models are shown in Table 4. The drug release kinetics follows the zero order which shown in Figure 5.

Table 4 Kinetic Models

Batch Code	Kinetic Models				
	Zero Order	First Order	Higuchi	Korsmeyer & Peppas	Hixson crowell
FG1	0.903	0.857	0.950	0.848	0.957
FG2	0.851	0.799	0.934	0.781	0.916
FG3	0.918	0.808	0.947	0.798	0.921
FG4	0.949	0.883	0.986	0.878	0.977

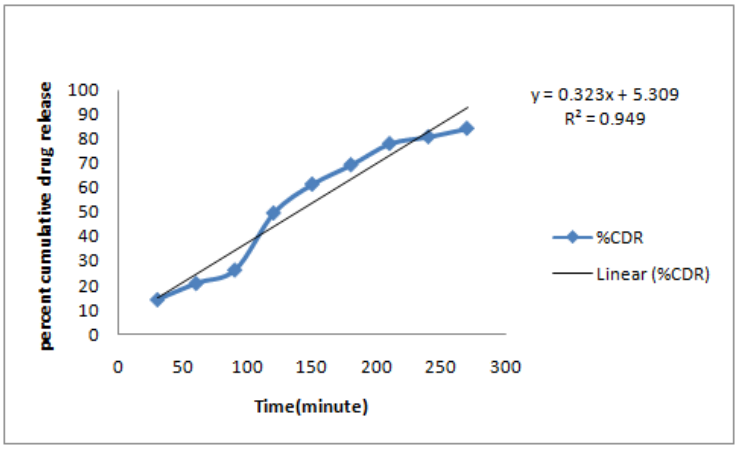

Figure 5 Drug release kinetic plot of FG4 formulation

CONCLUSION

The drug verapamil hydrochloride has biological half-life of 2 to 8 hrs and undergoes extensive first pass metabolism hence, it was selected as a model drug to formulate buccoadhesive drug delivery system. This is the easiest approach for technical and logical point of view among buccal retentive drug delivery system. Fenugreek in combination with xanthan gum successfully retards the release of verapamil HCl up to 6 hrs and it sustained the release of the drug at lower concentration.

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