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Synthesis, Optimisation and Evaluation of Okra Mucilage as Mucoadhesive Polymer

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ABSTRACT

In the present study okra mucilage is extracted and evaluated for mucoadhesive character. Buccal tablets containing metronidazole as model drug were prepared using okra mucilage as polymer, directly compressible lactose and PEG as excipient. The effect of concentrations of okra mucilage/lactose ratio and compression force were studied on ex-vivo bio adhesion time and on % release, employing two-factor, three-level central composite experimental design. The buccal tablets were evaluated for friability, weight variation, thickness variation and mucoadhesion time. The mucoadhesion was determined by observing the time taken by the tablets to detach from a chicken buccal pouch. It was found that the ratio (okra mucilage/lactose) has more pronounced effect on ex-vivo bioadhesion time than that of compression force. The optimized formulation hving okra mucilage/lactose ratio (0.5) and compression pressure (7 ton) provided adequate ex-vivo bioadhesion time (22 h) and percentage release of 98.23% after 24h.

Keywords: Metronidazole, Mucoadhesion, Okra mucilage.

INTRODUCTION

Mucilages and gums have contributed immensely to the development of technologies for delivering drugs over extended periods. Polymers have become an indispensable part of drug delivery system, be it conventional drug delivery or novel drug delivery. The development of novel drug delivery system is strictly dependent on the selection of appropriate carrier that is able to activate control of delivery. Polymers offer a wide range of properties such as diffusivity, permeability good biocompatibility and easy availability that are important in achieving controlled delivery. There are certain limitations like uncontrolled hydration, microbial contamination, pH dependent solubility and changes in physicochemical characters that restrict their use in the field [1]. These limitations of poysaccharides can be minimized by chemical modification like Ce (IV) induced copolymerisation of vinyl monomers [2], polyacrylomide and polyacrylonitrile grafted copolymers [3], carboxymethylation [4], thiolation [5], grafting by irradiation[6], polymeric chains grafting [7,8] modification by derivatization of functionl groups[9,10], and hydrolytic [11] or oxidative [12] degradation etc. Generally, mucilages have hydroxyl and carboxyl groups which favour their adhesion to mucosa. The buccal route of drug delivery has been employed for local delivery but the lack of adhesion of drug delivery system at the absorption site is the main drawback of buccal delivery route. To minimize or overcome this problem mucoadhesive dosages forms are preferred [13]. Mucilages are reported to possess mucoadhesive property, in the earlier studies of *Dillenia indica*[14], *Diospyros peregrine* [15] and *Mimosa* pudica[16] has been evaluated as buccal mucoadhesive dosage forms. The okra pods also yield mucilage that is composed of polysaccharide galactose, galacturonic acid and rhamnose [19]. Okra mucilage has already been explored as pharmaceutical excipients as binding, sustained release and emulsifying agent [20]. The present piece of research work is focussed on isolation and evaluation of okra mucilage mucoadhesive property and its potential in developing sustained release formulations**.**

MATERIALS AND METHODS

Materials

Metronidazole was obtained as a gift sample from Ranbaxy research lab. Pvt. Ltd (Gurgaon, India), Lactose was procured from Hi-Media lab. (Mumbai, India), PEG 4000 and sodium metabisulphite were purchased from SD Fine chemical Pvt. Ltd. (Mumbai, India). Okra fruit was obtained from local market (Hisar). All other chemical used were of reagent grade and were used as received.

Method of Extraction of okra mucilage

The fresh, washed okra fruits were cut into thin slices. The slices after removal of all seeds were soaked into the distilled water for 24h containing sodium-metabisulphite (1% w/v) as preservative. The swollen slices were then mashed and passed through muslin cloth to obtain aqueous extract. Aqueous extract of okra mucilage was precipitated with methanol and then dried in oven at 40^{o}C till constant weight. The okra mucilage was dried and powdered [21].

Experimental Design

A 2 factor 3 - level central composite experimental design was employed for optimisation as per the standard protocol. The okra mucilage/lactose ratio (X_1) and compression force (X_2) were taken as independent variables on the basis of previous trials and studied at 3 level (+1, 0 and -1) . Ex-vivo bioadhesion time (Y_1) and percent release after 24 h i.e. % Rel_{24h} (Y_2) were chosen as response variables. All other formulation and processing variables were kept invariant throughout the study. Table 1 displays the experimental design studied, their factor combination and the translation of coded levels to the experimental unit employed during study. The experimental design and statistical analysis of the data were done using the design expert software (Version 7.1.6, Stat-Ease Inc., Minneapolis MN)

Preparation of buccal tablets

The physical mixture of okra mucilage, metronidazole, PEG-4000 and directly compressible lactose was prepared homogeneously and the powder blend was compressed for 60s employing a 13 mm die on an IR hydraulic press (KP795, Kimaya Engineers, Thane, India) using different compression pressure. The preparation of metronidazole containing buccal tablets was optimized employing the central composite experimental design as shown in Table 1 [21].

EVALUATION

The buccal tablets of metronidazole were evaluated for friability, thickness, weight variation, *ex-vivo* bioadesion time and *in-vitro* release rate studies. The physico-chemical interaction of metroninazole with excipients was studied using FTIR.

Friability

The friability test was conducted on accurately weighed (n=20) buccal tablets using the Roche friabilator (Campbell Electronic, Mumbai, India) as per the method given in Indian Pharmacopoeia-2010 and percent friability was determined.

Thickness

The thickness of the buccal tablets was measured using digital vernier calliper (Aerospace, China). Buccal pellets (n=6) were randomly selected from each batch and thickness were measured.

Weight variation

For weight variation study, buccal tablets (n=20) of each batch was accurately weighed individually using an analytical balance (AND, Japan), and standard deviation was calculated (IP-2010) .

Ex-vivo bioadhesion time

The *ex-vivo* mucoadhesion time of buccal tablets containing metronidazole was determined by observing the time taken by the buccal tablets to detach from a chicken buccal pouch [21]. A freshly cut chicken buccal pouch was obtained from a local butcher house (Hisar, India) was cleaned and fixed on the internal side of a beaker (250ml) with cyanoacrylate glue. A side of buccal tablet was wetted with (6.8 pH) phosphate buffer, then tablets was adhered on chicken buccal tissue by applying a light force with the fingertip for 60s. The beaker was then filled with 200 ml phosphate buffer (6.8pH) and placed in the dissolution apparatus (Electrolab, Mumbai). The media was kept at 37^{0}C and stirred by means of paddle at 50 rpm .The time after which buccal tablets eroded or detach from the tissue was noted as *ex-vivo* bioadhesion time [14,15].

***In-vitro* release study**

In-vitro release of buccal tablets containing metronidazole was carried out by using type 2 dissolution apparatus. The dissolution medium phosphate buffer pH 6.8 (200 ml) was maintained at 37±0.5°C with constant stirring rate of 50 rpm and the release rate was determined for a period of 24 h. Aliquot of 5 ml sample were withdrawn from each dissolution vessel at regular time intervals and replaced with equal volume of fresh dissolution medium. The sample was filtered and analyzed spectrophotometrically at λ_{max} of 320 nm. All the dissolution studies were carried out in triplicate and standard deviation was calculated.

RESULTS AND DISCUSSION

In the present study tablets of okra mucilage were prepared containing metronidazole as a model drug and directly compressible lactose and PEG-4000 were used as excipients. Further, different formulations were optimised by using central composite experimental design and evaluated for mucoadhesive character.

It was observed from the trial runs that okra mucilage alone did not form pellet of adequate strength, so directly compressible lactose was used to prepare pellet of adequate strength. The results of physicochemical characterization of buccal tablets are depicted in Table 1. The buccal tablets were of uniform average weight and thickness. The thickness of buccal tablets was found to be in the range of 84 to 88 mm. The data of friability test represents that at lower value of ratio of okra mucilage to lactose the percent friability is more than 1% but as ratio of okra mucilage/ lactose decrease, percent friability was observed to be less than 1%. on the basis of preliminary trials the okra mucilage / lactose ratio and compression force were taken as independent variable, while % release and mucoadhesion time were selected as the responses for the optimization study.

Table 1 Central composite design used in formulations and independent variables influencing responses

Batch	Mucilage/lactose (X_1)	Compression Force (ton) (X_2)	Ex-vivo bioadh. Time (h) (Y_1)	% Rel_{24h} (Y_2)	Friability %	Thickness (mm)	Weight variation (mg)
1	0.25	7	3	100	1.02	0.844±0.031	147.8±1.69
2	0.375	8.5	18	89.13	0.98	0.85±0.0036	147.1±1.61
3	0.375	7	16	100	0.91	0.878±0.0432	146.0±3.65
4	0.5	7	18	98	0.85	0.882±0.0672	147.5±1.12
5	0.375	8.5	10	100	0.89	0.878±0.0303	148.3±1.43
6	0.375	8.5	12	96.14	0.94	0.862±0.0272	148.0±1.31
7	0.5	10	18	74.09	0.84	0.856±0.0391	146.6±2.07
8	0.375	10	10	81.61	0.92	0.84±0.0288	144.6±3.43
9	0.25	8.5	2	100	0.99	0.858±0.0130	144.9±5.12
10	0.375	8.5	10	97.10	0.90	0.878±0.0279	147.2±1.30
11	0.5	8.5	17	93.47	0.81	0.87±0.0374	146.0±3.53
12	0.25	10	4	100	1.12	0.838±0.0476	145.3±2.11
13	0.375	8.5	14	94.46	0.88	0.876±0.0151	145.5±3.41

The results of optimisation study using response surface methodology employing CCD for ex-vivo bioadhesion time (Y_1) and % release (Y_2) are being represented in Table 1. The data obtained were fitted into various polynomial models and ANOVA test was applied to models to estimate their significance. It was observed that the response Y_1 and Y_2 fitted best into the response surface quadratic model, The polynomial models showing relationship between the independent variables and the response Y_1 and Y_2 are expressed by the following equations:

$$Y_1 = +17.72 + 265.69\ X_1 - 15.54\ X_2 - 1.35\ X_1X_2 - 2.60.59\ X_1^2 + 0.95080\ X_2^2 \quad (2)$$

$$Y_2 = -66.79 + 155.20\ X_1 + 38.02\ X_2 - 33.12\ X_1\ X_2 + 108.19X_1^2 - 1.78X_2^2 \quad (3)$$

Table 2 represents the results of ANOVA for analysis of design models. It was observed that response surface models developed for the two response (Y_1 and Y_2) were significant and adequate with non-significant “lack of fit”. The value of “adequate precision” was greater than 4 which is desirable and indicates an adequate signal.

Table . 2. Statistical summary of the quadratic response surface model

Response Factor	Model					Lack of fit	
	F-value	Prob.>F	R^2	Adeq. Prec.	Std. dev.	F-value	Prob.>F
Y_1	11.33	0.0030	0.8900	9.673	2.57	0.59	0.9903
Y_2	10.62	0.0036	0.8835	11.586	3.64	0.33	0.8420

Table 3 displays the results of factor effects and P-values of responses Y_1 and Y_2. It can be observed from the data that the response Y_1 is significantly inferred by the linear contribution of X_1 and antagonistic quadratic contributions of X_1 .The response Y_2 was affected significantly by the synergistic linear contribution of X_1 and X_2, while the interaction effects X_1 and X_2 affected Y_2 antagonistically.

Table .3. Summary of factor effect and P-value of responses X_1 and X_2

Factor	Y_1		Y_2	
	Factor effects	P-value	Factor effects	P-value
X_1	+7.35	0.0002	-5.65	0.0067
X_2	+0.17	0.8760	-7.10	0.0020
X_1X_2	-0.25	0.8496	-6.21	0.0112
X_1^2	-4.07	0.0341	+1.69	0.4654
X_2^2	+2.14	0.2098	-4.02	0.1092

Figure 1 exhibits the combined effect of mucilage/lactose ratio and compression force on *ex-vivo* bioadhesion time of the buccal tablets. It can be observed from the 3-D plots of bioadhesion time that okra mucilage/lactose ratio had a greater influence on *ex-vivo* biodhesion time than the compression force. As the okra mucilage/lactose ratio is increased from 0.25 to 0.5, there occurs an increase in *ex-vivo* bioadhesion time and maximum ex-vivo bioadhesion retention occurs when okra mucilage/ lactose ratio is high i.e. higher proportion of mucilage.

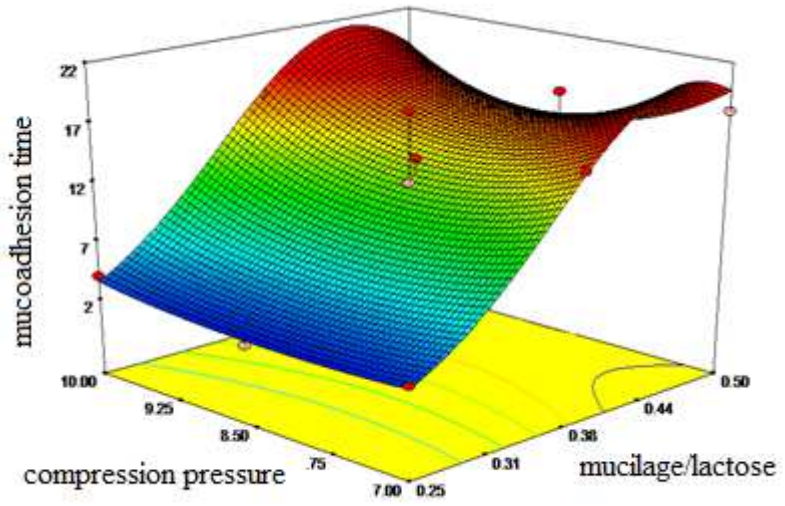

Fig. 1. Response surface graph showing the influence of okra mucilage / lactose ratio on mucoadhesion time

Figure 2 show the combined effect of okra mucilage/lactose and compression force on the % Rel_{24h}. It can be inferred from the plot that higher okra mucilage/lactose ratio (0.5) and the compression pressure (10 ton) causes a decrease in release rate. This decrease in release rate with the increase in okra mucilage/lactose ratio can be attributed to formation of more viscous gel layer with longer diffusion path length and also may be due to the formation of buccal tablets with greater strength at higher compression pressure.

A numerical optimization tool of design expert software using desirability approach was utilized for finding the value of independent variables for preparing optimized batch of okra mucilage buccal tablets containing metronidazole. The optimization of the okra mucilage/lactose ratio and compression pressure was done with the goal of preparing tablets with maximum % Rel_{24h} and *ex-vivo* bioadhesion time. The software provided three solution, one with highest desirability was selected for preparing the optimized batch of buccal tablets. The batch with okra mucilage/lactose ratio 0.5 and compression pressure 7 ton was observed to be optimised. The optimized batch provided *ex-vivo* bioadhesion (Y_1) of 22 h (predicted 19 h) and % Rel_{24h} (Y_2) of 98.23 % (predicted 100 %). Hence, a good correlation between the predicted and observed values was obtained, indicating the reliability of the model.

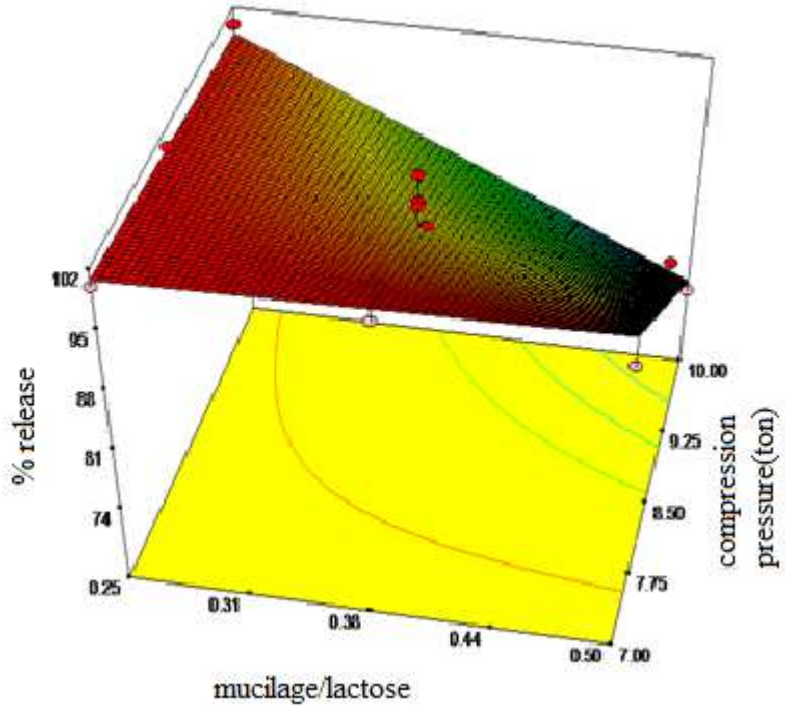

Fig. 2. Response surface graph showing the influence of okra mucilage / lactose ratio on relese rate of drug

CONCLUSION

Buccal tablets of metronidazole were prepared by direct compression method using okra mucilage as mucoadhesive polymer. Optimization of formulation of tablets was performed using central composite design. It was concluded from the results that polymer ratio (okra mucilage/lactose) has more pronounced effect on *ex-vivo* bioadhesion time than that of compression force. The optimized formulation of metronidazole had okra mucilage/lactose 0.5 and compression pressure 7 ton with adequate *ex-vivo* bioadhesion time and % Rel_{24h}. Thus, it can be concluded that okra mucilage possess the potential to be explored for its mucoadhesive property.

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