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RESEARCH ARTICLE

FORMULATION, EVALUATION AND OPTIMIZATION OF MUCOADHESIVE MICROSPHERES OF ACYCLOVIR

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Acyclovir-loaded mucoadhesive microspheres using gum tragacanth as a mucoadhesive polymer and barium chloride as cross-linker were prepared for the purpose of improving oral bioavailability of acyclovir. The prepared microspheres were characterized for parameters such as percent yield, percent mucoadhesion, entrapment efficiency, *in vitro* release and flow properties. The formulations were optimized using central composite design using two variables *viz.* gum tragacanth and sodium alginate at three levels. Pharmacokinetic based mathematical models applied to drug release data suggested that the release of drug from microspheres followed fickian diffusion.

Key words: Acyclovir, Mucoadhesion, Microspheres, Gum tragacanth.

INTRODUCTION

For controlled release systems, oral route of administration has received more attention and success because gastrointestinal physiology offers more flexibility in dosage form design than other route. A gastroretentive system means retention of the drug in the GIT for long period of time and sustaining the effect of drug. There are various approaches to increase the gastric retention time of dosage form and mucoadhesive drug delivery systems are one of the methods for drug delivery of drugs which are absorbed from stomach and upper small intestine (Shinde and More, 2008).

Acyclovir is a drug having high solubility at the stomach pH, short $t_{1/2}$, low bioavailability. It has narrow absorption window and absorbed from upper part of GIT. The drug is administered for long period of time and with high dosing frequency and drug amount is also high as 200-400 mg 5 times a day. Due to this, more drug is accumulated in the body, that increases the side effects. Many reports have been found in literature indicating potential role of multi-particulate systems for controlled drug delivery (Dahiya and Gupta, 2011; Kumar and Dureja, 2011; Tripathi *et al* 2011; Basarkar *et al* 2013) Mucoadhesive systems (tablets, capsules, microspheres) are prepared for minimizing the side effects of drug and to enhance the patient compliance (Wikipedia, acyclovir; Shinde *et al* 2010).

The aim of present study was to develop controlled release mucoadhesive gastro-retentive system using acyclovir as drug and Gum tragacanth as mucoadhesive polymer. This targeted delivery of the drug reduces the side effects and also provide an effective and safe therapy with reduced dose and dosing frequency.

MATERIALS AND METHODS

Acyclovir was a gift sample from Ranbaxy (Devas). Gum tragacanth and sodium alginate were obtained from S.D. Fine-Chem Limited, Mumbai and Loba Chemie Private Limited,

Mumbai respectively. Barium chloride was obtained from RFCL Limited, New Delhi.

Preparation of microspheres

Orifice ionic gelation method is one of the preparation technique widely used for microspheres for its ease of processing. In this method, microspheres were prepared from the gel blend of mucoadhesive polymer, Gum tragacanth (GT) and sodium alginate (SA) (**Table 1**) along with acyclovir (drug). Primarily homogeneous mixture of polymers was prepared in specified amount of water. Then, the drug was added in above homogeneous mixture and mixing was continued till homogeneous blend was obtained. This blend of polymers and drug was added dropwise into Barium chloride solution for cross-linking. After 10 min of cross-linking time, the prepared microspheres were filtered and washed with suitable solvent then, dried in vacuum oven at room temperature (Kotadiya *et al* 2009; Patil *et al* 2010).

Table 1. Factor Combination as per the chosen experimental design for GT microspheres

Formulation code	Coded factor levels	
	X_1	X_2
F1	+1	-1
F2	-1	-1
F3	0	-1
F4	+1	+1
F5	0	0
F6	-1	0
F7	0	0
F8	0	+1
F9	-1	+1
F10	0	0
F11	0	0
F12	+1	0
F13	0	0

Translation of coded levels in actual units			
Coded level	-1	0	+1
X_1: Sodium alginate (mg)	1000	1500	2000
X_2: Gum tragacanth (mg)	500	1000	1500

Evaluation of microspheres (Dhaliwal *et al* 2008; Tao *et al* 2009; Liu *et al* 2010)

Morphological characterization

Morphology of microspheres was examined by SEM. The sample was mounted onto an aluminium stub and sputter coated for 120s with platinum particles in an argon atmosphere.

Percentage yield

The yield was calculated as the weight of the microspheres recovered from each batch divided by total weight of drug and polymer used to prepare that batch by 100.

Flow properties of microspheres

Angle of repose

Weighed quantity of microspheres was passed through a funnel fixed on a stand at a specific height upon the graph paper. A static heap of powder with only gravity acting upon it was tending to form a conical mound. The height of the heap (h) and the radius (r) of the lower part of cone were measured. The angle of repose was calculated using the following formula:

$$\tan \theta = h/r \text{ and } \theta = \tan^{-1}(h/r) \qquad \text{Eq. 1}$$

where, θ = angle of repose, h = height of the cone and r = radius of the cone base

Carr's index

It is a simple test for evaluation of flow property of a powder by comparing the poured density and tapped density of a powder. It was determined by taking small quantity of microsphere samples in 10 ml measuring cylinder. The height of the sample was measured before and after tapping to get the poured and

tapped density. The Carr's index was calculated using the following formula:

$I = (V_b - V_t) \times 100 / V_b$ Eq. 2

where, V_b is the bulk volume and V_t is the tapped volume.

Hausner ratio

Hausner ratio was calculated using following formula:

Hausner's ratio = ρ_t / ρ_d Eq. 3

where, ρ_t is the tapped density and ρ_d is bulk density.

Drug content estimation

Drug loaded microspheres (100 mg) were powdered and suspended in 100 ml of solvent. The resultant dispersion was kept for 20 min for complete mixing with continuous agitation and filtered through a 0.45 μm membrane filter. The drug content was determined spectrophotometrically (UV-1700, Shimadzu Japan) at 254 nm using a regression equation derived from the standard graph (r^2=0.997).

Drug entrapment study

The drug entrapment efficiency (DEE) was calculated by the equation:

$DEE = (P_c / T_c) \times 100$ Eq. 4

P_c is practical content, T_c is the theoretical content. All the experimental units were analyzed in triplicate (n=3).

Particle size analysis

Particle size of the microspheres was determined by optical microscopy using stage micrometer and ocular micrometer. Microspheres were suspended in distilled water and mounted on a glass slide. A minimum of 100 microspheres per batch were counted for the determination of particle size.

Mucoadhesive studies by falling film technique

This method is suitable for testing mucoadhesion strength of mucoadhesive microspheres.

In weight percent method, a fixed wt. of microsphere sample was added over a fresh intestinal segment of sheep, mounted on a tilted slide with an angle of 45 degree and allowed to rest for 15 min. the effluent was run over the segment.

The effluent was collected in a whattman filter paper and wt. of detached particles was determined by using the equation:

% Mucoadhesion = (wt. of sample - wt. of detached particles / wt. of sample) × 100 Eq. 5

***In vitro* drug release**

In vitro drug release study was carried out in USP XXI paddle type dissolution test apparatus using 0.01 M HCl as dissolution medium. Volume of dissolution medium was 900 ml and bath temperature was maintained at 37±1° throughout study.

Paddle speed was adjusted to 50 rpm. At interval of 1 h, 5 ml of sample was withdrawn with replacement of five ml fresh medium and analyzed for drug content by UV-Visible spectrophotometer at 254 nm. All the experimental units were analyzed in triplicate (n=3).

***In vitro* drug release kinetics**

In order to study the exact mechanism of drug release from microspheres, drug release data was analyzed according to zero order, first order, higuchi model, korsmeyer's peppas model. The criterion for selecting the most appropriate model was chosen on the basis of goodness of fit test. All the experimental units were analyzed in triplicate (n=3).

RESULTS AND DISCUSSION

All the 13 formulations were evaluated for percentage yield, particle size, flow properties, entrapment efficiency (EE), mucoadhesion testing, *in vitro* drug release and kinetic modeling. Percentage yield of all the batches was found between 88.08 to 104.65%. The % yield more than 100 shows bound water content to the hydrophilic polymer which can't be removed easily. Particle size lied in between 174.93 and 316.94 μm.

Entrapment Efficiency ranged between 69.59 to 87.12% as depicted in **Table 2.** All the batches showed good flow properties according to values of carr's index, hausner ratio and angle of repose (**Table 3**), indicating that these microspheres can be compressed into tablets for easy administration. Response surface curves showed that GT (gum-tragacanth) had positive effect whereas SA (sodium alginate) had negative effect on entrapment efficiency (EE). The falling

Table 2. Characterization of MM (mucoadhesive microspheres) prepared using GT

Formumation code	Particle size (μm)	Yield (%)	Swelling index (%)	Entrapment efficiency (%)	Mucoadhesion (%)	% CDR in 10^{th} h (%)
F1	189.51	102.71	133.88	82.34	93.87	71.86
F2	191.59	90.4	117.94	69.59	90.57	87.03
F3	247.12	99.2	90.3	72.78	93.29	84.56
F4	262.39	101.73	126.72	68.00	99.07	63.61
F5	224.91	98.62	95.39	74.37	94.98	78.02
F6	174.93	95.86	148.83	76.765	94.07	76.81
F7	225.68	96.98	96.24	74.65	94.57	78.54
F8	316.54	95.5	163.33	77.56	98.21	65.04
F9	288.77	88.08	240.91	87.12	97.16	74.51
F10	220.89	98.57	96.98	73.21	93.99	78.13
F11	227.28	99.02	95.09	73.98	95.11	77.62
F12	249.9	104.65	129.38	71.58	95.71	67.67
F13	218.58	98.54	94.63	75.02	94.92	77.78

Table 3. Flow properties of mucoadhesive microspheres prepared using GT as mucoadhesive polymer

Formulation code	Bulk density (g/ml)	Tap density (g/ml)	Hausner's ratio	Carr's index (%)	Angle of repose (°)
F1	0.92	0.85	0.92	7.69	19.77
F2	1.59	1.30	0.82	18.18	20.04
F3	1.21	1.11	0.92	8.33	18.56
F4	1.51	1.31	0.87	13.16	20.12
F5	1.13	0.96	0.85	15.38	23.59
F6	1.31	1.07	0.82	18.18	25.24
F7	1.13	0.95	0.84	15.93	21.28
F8	1.07	1.00	0.94	6.08	22.66
F9	1.28	1.06	0.83	17.24	19.88
F10	1.14	0.96	0.84	15.79	25.76
F11	1.12	0.96	0.86	14.29	24.98
F12	0.95	0.87	0.91	9.09	24.18
F13	1.13	0.94	0.83	16.81	21.15

film test for % mucoadhesion varied from 90.57 to 99.07. Results of equation indicated that both the factors showed positive effect. At higher concentrations of both variables, mucoadhesion increased. Percent cumulative drug release showed that as the concentration of polymer increased, release of drug decreased from the microspheres indicating that polymer concentration had negative relation with that of drug release. Various evaluation parameters of prepared microspheres batches are compared in **Figure 1**.

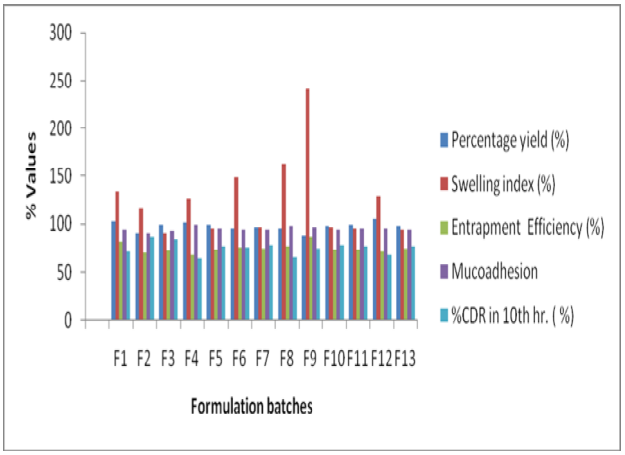


Fig. 1. % yield, SI, EE, % M and %CDR of batches having gum tragacanth (GT) as mucoadhesive polymer

In-vitro dissolution study

Dissolution profile of prepared mucoadhesive microspheres of formulation batches 1-4, 5-8 and 9-13 are depicted in **Table 4-6**, **Figure 2-4.**

Table 4. Dissolution profile of prepared mucoadhesive microspheres of formulation batches 1-4

Time (h) / Formulation code	F1	F2	F3	F4
0.5	26.87	40.28	36.57	21.9
1	33.35	53.85	41.27	26.02
2	41.28	59.65	49.89	33.35
4	47.25	65.18	57.85	40.28
6	53.43	71.09	63.29	47.52
8	62.62	77.79	71.78	52.08
10	71.86	87.03	84.56	63.61

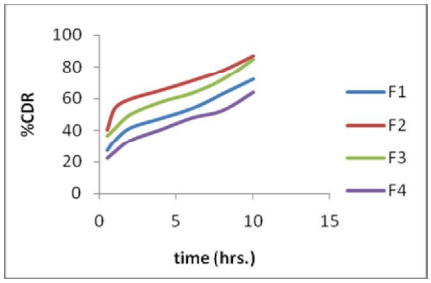


Fig. 2. Graphical representation of drug release profile of formulations batches 1-4 of GT microspheres

Table 5. Dissolution profile of prepared mucoadhesive microspheres of formulation batches 5-8

Time (h) / Formulation code	F5	F6	F7	F8
0.5	30.58	25.27	30.67	20.52
1	34.28	31.05	34.56	23.25
2	41.85	40.68	41.97	28.54
4	47.76	52.57	49.73	36.58
6	58.85	59.89	57.35	44.07
8	69.11	67.89	69.12	56.78
10	78.02	76.81	78.54	65.04

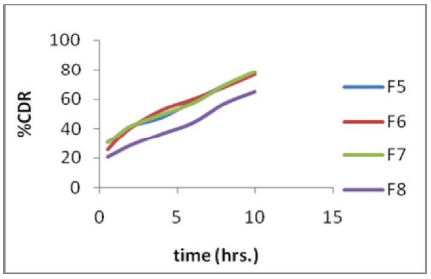


Fig. 3. Graphical representation of drug release profile of form. batches 5-8 of GT microspheres

Table 6. Dissolution profile of prepared mucoadhesive microspheres of formulation batches 9-13

Time (h)/Formulation code	F9	F10	F11	F12	F13
0.5	27.82	28.43	29.11	23.28	28.79
1	28.76	33.37	33.25	29.84	33.95
2	34.29	41.57	40.87	39.54	40.52
4	47.92	48.95	48.09	49.75	47.67
6	56.24	59.76	59.95	51.82	58.24
8	63.82	68.83	68.85	63.68	66.43
10	74.51	78.13	77.62	67.67	77.78

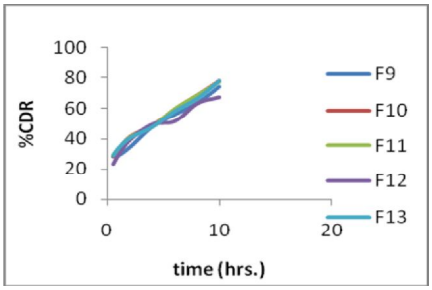


Fig. 4. Graphical representation of Drug release profile of form. batches 9-13 of GT microspheres

Optimization of formulations using face centered central composite design (FCCCD)

Mathematical modeling

Mathematical relationships generated using multiple linear regression analysis for the studied response variables are expressed as equations given below:

$$EE = +73.74 - 1.26\ X_1 - 1.33\ X_2 - 7.97\ X_1 X_2 - 0.30\ X_1^2 + 2.69\ X_2^2 \quad \text{Eq. 6}$$

$$\%M = +95.04 + 1.14\ X_1 + 2.79\ X_2 \quad \text{Eq. 7}$$

$$\%\ CDR = +75.48 - 5.87\ X_1 - 6.71\ X_2 \quad \text{Eq. 8}$$

The polynomial equations comprise the coefficients for intercepts, first-order main effects, interaction terms and higher order effects. The sign and magnitude of the main effects signify the relative influence of each factor on the response (**Figure 5-10**).

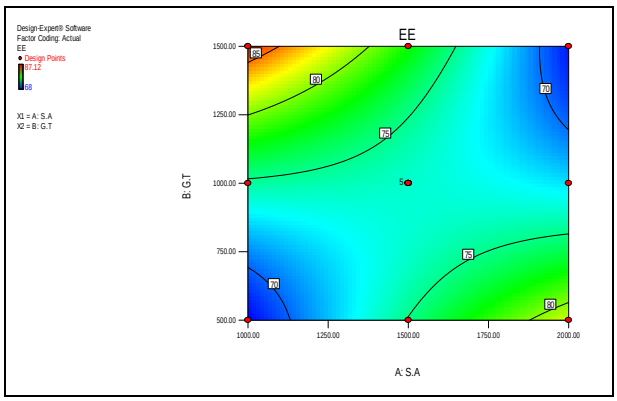


Fig. 5. Contour plot showing the influence of two factors on % EE

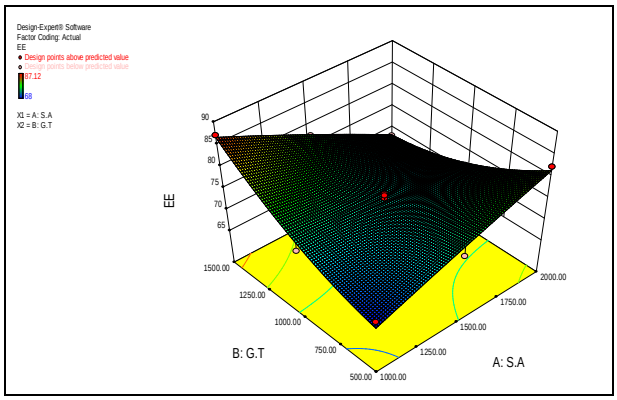


Fig. 6. Response surface plot showing the relationship between various levels of two factors on entrapment efficiency (EE)

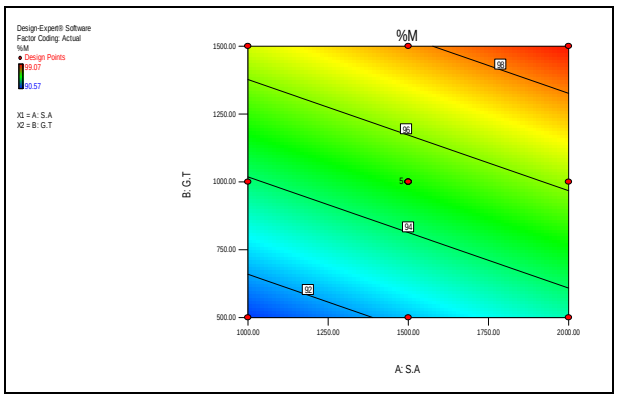


Fig. 7. Contour plot showing the influence of two factors on % mucoadhesion

The solution provided by the optimization software implied a new formulation which was prepared using 1000 mg of Sodium alginate and 1500 mg of Gum tragacanth. The optimized batch showed an entrapment efficiency of 87.02%, 96.64% mucoadhesion and in vitro release of 74.51%. On the basis of value of R^2 it was concluded that the optimized batch followed zero order release kinetics. Model providing the value nearest to 1 depicted order of drug

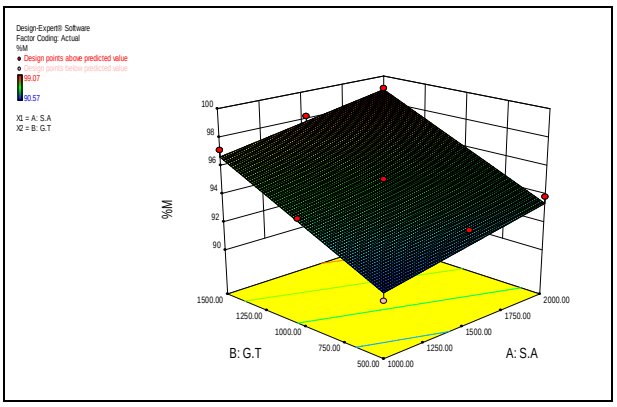


Fig. 8. Response surface plot showing the relationship between various levels of two factors on mucoadhesion (% M)

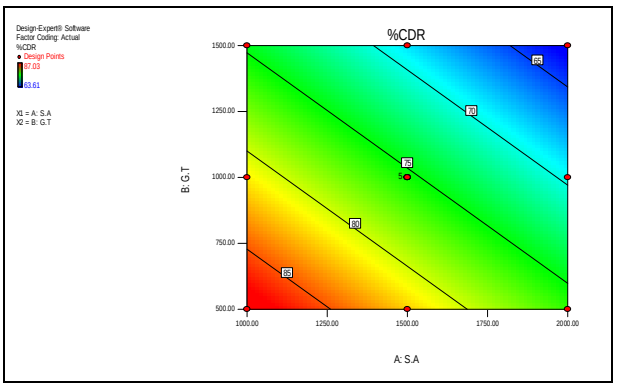


Fig. 9. Contour plot showing the influence of two factors on % CDR

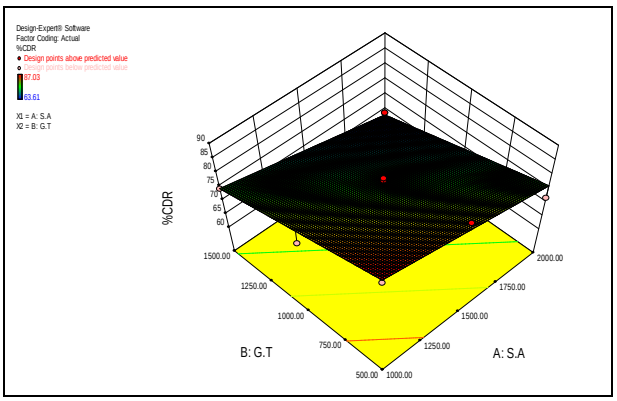


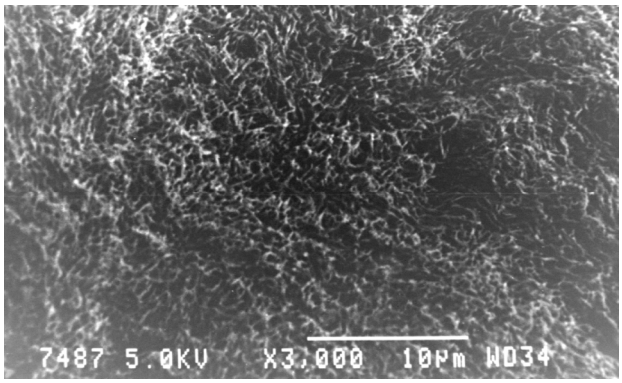
Fig. 10. Response surface plot showing the relationship between various levels of two factors on % cumulative drug release (% CDR)

release. The R^2 value for the zero order model was found out to be 0.993. Moreover, mathematical modeling of drug release data suggested that the release from microspheres followed fickian diffusion.

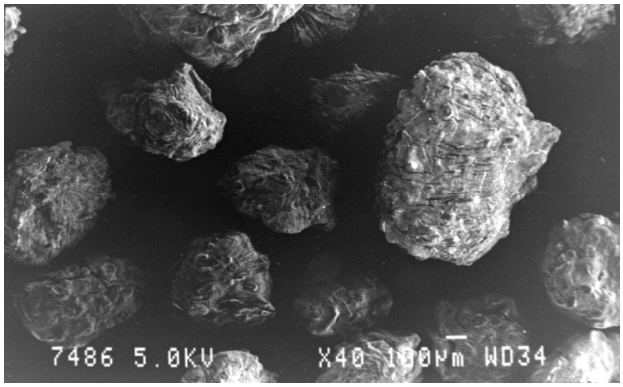
Particle characterization of optimized batch

According to SEM studies, the microspheres were found to be discrete, almost spherical and

free flowing. The surface was rough and porous which indicated fickian diffusion (**Fig. 11a, 11b**).



(a)



(b)

Fig. 11a, b. Surface morphology of the muco-adhesive microspheres prepared using GT as mucoadhesive polymer

CONCLUSION

The results of present study revealed that retention time of acyclovir at its absorption site *i.e.* the upper GIT, could be increased by formulating it into microspheres using gum tragacanth. Thus, mucoadhesive microspheres of acyclovir may represent a useful approach for targeting its release at its site of absorption, sustaining its release and improving its oral availability using sodium alginate and barium chloride along with gum tragacanth**.**

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