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FORMULATION AND EVALUATION OF NIZATIDINE MUCOADHESIVE MICROSPHERES BY IONIC-GELATION METHOD

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ABSTRACT

The present study involves preparation and characterization of mucoadhesive microspheres with Nizatidine as model drug for prolongation of gastric residence time. Mucoadhesive formulation has been accepted as a process to achieve controlled release and drug targeting. Mucoadhesion is a topic of current interest in the design of drug delivery systems. Mucoadhesive microspheres exhibited a prolonged residence time at the site of application or absorption and facilitate an intimate contact with the underlying absorption surface and thus contribute to improved and/or better therapeutic performance of drugs. In recent years such mucoadhesive microspheres have been developed for oral, buccal, nasal, ocular, rectal and vaginal routes for either systemic or local effects. The microspheres were prepared by Orifice Ionic-Gelation method using mucoadhesive polymers like HPMC K100 (hydroxy propyl methyl cellulose), CMC (carboxy methyl cellulose) , Carbopol 934 and a release controlling polymer Sodium alginate by using Calcium chloride(4%w/v) as curing agent. *In Vitro* drug release studies were performed and drug released was evaluated. The effect of polymer concentration on size of microspheres and drug release were observed. The prepared microspheres exhibited prolonged drug release the mean particle size increased as the concentration of copolymer increased, as the carbopol polymer concentration increases the mucoadhesion increased and the drug release rate decreased at higher concentration of HPMC K100.

INTRODUCTION

Nizatidine is a competitive inhibitor of histamineH2-receptors. The primary clinically important Pharmacologic activity of Nizatidine is inhibition of gastric secretion. Both the acid concentration and volume of gastric secretion are suppressed by Nizatidine, while changes in pepsin secretion are proportional to volume output [1, 2]. It is widely prescribed in gastric ulcers, duodenal ulcers, Zollinger- Ellison syndrome and gastro esophageal reflux disease in a dose of 20 mg b.i.d. The plasma half-life of drug was 2.5-3 hour as reported in literature, which may exhibits toxic effect in prolong use. Hence an attempt was made in this current study to evaluate the efficacy of different polymers in designing of sustained release mucoadhesive Nizatidine microcapsule for oral delivery.

In order to increase the gastric residence time of microspheres we developed mucoadhesive microspheres which consist of a drug (Nizatidine) and an adhesive polymer such as agents like Hydroxy Propyl Methyl Cellulose (HPMC), Carbopol 934, Carboxy Methyl Cellulose (CMC), and Sodium alginate dispersed in a curing agent [3-5]. It was confirmed that those mucoadhesive microspheres have the ability to adhere to the stomach wall in sheep and there by remain in the gastrointestinal tract for an extended period.

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MATERIALS AND METHODS

Nizatidine was obtained from Chandra Labs, Hyderabad (India), Sod. Alginate, Hydroxy Propyl Methyl Cellulose (HPMC K100), Carboxy Methyl Cellulose (CMC), and Carbopol 934, purchased from S.D.Fine chemicals, Mumbai. All the chemicals used were analytical grade.

METHOD OF PREPARATION

Ionotropic gelation method

Batches (Table 1) of microspheres were prepared by ionotropic gelation method which involved reaction between sodium alginate and poly cationic ions like calcium to produce a hydrogel network of calcium alginate.

Sodium alginate and the mucoadhesive polymer were dispersed in purified water (10 ml) to form a homogeneous polymer mixture. The API, Nizatidine (100mg) was added to the polymer premix and mixed thoroughly with a stirrer to form a viscous dispersion. The resulting dispersion was then added through a 18Gauge needle into calcium chloride (4% w/v) solution. The addition was done with continuous stirring at 200rpm. The added droplets were retained in the calcium chloride solution for 30 minutes to complete the curing reaction and to produce rigid spherical microspheres. The microspheres were collected by decantation, and the product thus separated was washed repeatedly with purified water to remove excess calcium impurity deposited on the surface of microspheres and then air-dried [6, 7].

MICROMERITIC PROPERTIES OF PREPARED MICROSPHERES:

Compressibility index

It was measured by tapped density apparatus for 100 taps for which the difference should be not more than 2 %. Based on the apparent bulk density and tapped density the percentage compressibility of the blend was determined using the following formula.

% Compressibility = [(Tapped density – Bulk density) / Tapped density] X 100

Hausner'sratio

It indicates the flow properties of the powder. The ratio of tapped density to the bulk density of microspheres is called Hausner ratio.

Hausner ratio= Tapped density / Bulk density

Angle of Repose

Angle of repose was determined using fixed funnel method. The blend was poured through funnel that can rise vertically until a maximum cone height (h) was obtained. Radius of the pile(r) was measured and angle of repose was calculated as follows [8].

$$Ø = \tan^{-1} h/r$$

Where, h= height of the pile, r= radius of the pile

EVALUATION OF PREPARED MICROSPHERES

The percentage of production yield was calculated from the weight of dried microspheres recovered from each batch and the sum of the initial weight of starting materials. The percentage yield was calculated using the following formula:

$$\% \text{ Yield} = \frac{\text{Practical mass (Microspheres)}}{\text{Theoretical mass (Polymer + Drug)}} \times 100$$

Drug entrapment efficiency

Microspheres equivalent to 15 mg of Nizatidine were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres. The powder was transferred to a 100 ml volumetric flask and dissolved in 10ml of methanol and the volume was made up using simulated gastric fluid pH 1.2. After 24 hours the solution was filtered through Whatmann filter paper and the absorbance was measured after suitable dilution spectrophotometrically at 269 nm. The amount of drug entrapped in the microspheres was calculated by the following formula,

% Drug Entrapment Efficiency

$$= \frac{\text{Experimental Drug Content}}{\text{Theoretical Drug Content}} \times 100$$

Particle size analysis

Samples of the micro particles were analyzed for particle size by optical microscope. The instrument was calibrated and found that 1unit of eyepiece micrometer was equal to 12.5µm. Nearly about 100 Micro particles sizes were calculated under 45 x magnifications.

The average particle size was determined by using the Edm ondson's equation:

$$D_{mean} = \frac{nd}{n}$$

Where,

n – Number of microspheres observed

d – Mean size range

Evaluation of mucoadhesive property

The mucoadhesive property of microspheres was evaluated by an in vitro adhesion testing method known as wash-off method. Freshly excised pieces of goat stomach mucous were mounted on to glass slides with cotton thread. About 20 microspheres were spread onto each prepared glass slide and immediately thereafter the slides were hung to USP tablet disintegration test apparatus, when the test apparatus was operated, the sample was subjected to slow up and down movement in simulated gastric fluid pH 1.2 at 37^0 C contained in a 1-litre vessel of the apparatus. At an interval of 1 hour up to 8 hours the machine is stopped and number of microspheres

still adhering to mucosal surface was counted.

$$\% \text{ Mucoadhesion} = \frac{\text{Number of microspheres adhered}}{\text{Number of microspheres applied}} \times 100$$

In vitro drug release study

The dissolution studies were performed in a fully calibrated eight station dissolution test apparatus (37 ± 0.5^0C, 50 rpm) using the USP type – I rotating basket method in simulated gastric fluid pH 1.2 (900ml). A quantity of accurately weighed microspheres equivalent to 15mg Model drug each formulation was employed in all dissolution studies. Aliquots of sample were withdrawn at predetermined intervals of time and analyzed for drug release by measuring the absorbance at 269nm. At the same time the volume withdrawn at each time intervals were replenished immediately with the same volume of fresh pre-warmed simulated gastric fluid pH 1.2 maintaining sink conditions throughout the experiment [9,10].

Release Kinetics

The analysis of drug release mechanism from a pharmaceutical dosage form is an important but complicated process and is practically evident in the case of matrix systems. As a model-dependent approach, the dissolution data was fitted to four popular release models such as zero-order, first-order, diffusion and Peppa's-Korseymeyers equations, which have been described in the literature. The order of drug release from matrix systems was described by using zero order kinetics or first orders kinetics. The mechanism of drug release from the matrix systems was studied by using Higuchi equation and Peppa's- Korsemeyer equation [11, 12].

Zero Order Release Kinetics

It defines a linear relationship between the fractions of drug released versus time.

$$Q = k_o t$$

Where, Q is the fraction of drug released at time t and k_o is the zero order release rate constant.

A plot of the fraction of drug released against time will be linear if the release obeys zero order release kinetics.

First Order Release Kinetics

Wagner assuming that the exposed surface area of a tablet decreased exponentially with time during dissolution process suggested that drug release from most of the slow release tablets could be described adequately by apparent first-order kinetics. The equation that describes first order kinetics is

$$\ln (1-Q) = - K_1 t$$

Where, Q is the fraction of drug released at time t and k_1 is the first order release rate constant. Thus, a plot of the logarithm of the fraction of drug remained against time will be linear if the release obeys first order release kinetics [13].

Higuchi equation

It defines a linear dependence of the active fraction released per unit of surface (Q) on the square root of time.

$$Q = K_2 t^{½}$$

Where, K2 is the release rate constant.

A plot of the fraction of drug released against square root of time will be linear if the release obeys Higuchi equation. This equation describes drug release as a diffusion process based on the Fick's law, square root time dependent [14].

Power Law

In order to define a model, which would represent a better fit for the formulation, dissolution data was further analyzed by Peppa's and Korsemeyer equation (Power Law).

$$M_t/M_\alpha = K.t^n$$

Where, M_t is the amount of drug released at time t and M_α is the amount released at time α, thus the M_t/M_α is the fraction of drug released at time t, k is the kinetic constant and n is the diffusion exponent. To characterize the mechanism for both solvent penetration and drug release n can be used as abstracted in Table. A plot between log of M_t/M_α against log of time will be linear if the release obeys Peppa's and Korsemeyer equation and the slope of this plot represents "n" value (diffusion coefficient) which describes mechanism of diffusion.

Stability Studies

To assess long-term stability, the optimized microsphere formulation was put in hard gelatin capsules and sealed in aluminum packaging coated inside with polyethylene. The studies were performed at 40°C/75% relative humidity (RH) in the stability chamber for two months. At the end of the storage period, the formulation was observed for physical appearance, size, shape, surface morphology, drug content and *in vitro* drug release .

RESULTS AND DISCUSSION

Compatibility studies

The FT-IR spectra of the formulations were compared with the FTIR spectra of the pure drug. The results indicated that the characteristic absorption peaks due to pure drug have appeared in the formulated microspheres, without any significant change in their position after successful encapsulation, indicating that there was no chemical interaction between pure drug and polymers.

Preparation of microspheres

The microspheres of various formulations were prepared by ionic gelation method.

Micrometrics of prepared microspheres

The micrometric studies (Table 2) of prepared microspheres (F1 to F11) revealed that all the formulations possessed good flow properties with angle of repose values ranging from 14-22^{o},compressibility index values from 3.01-16.57 and hausner's ratio values from 1.03 to 1.19.

Evaluation and characterization of microspheres:

Percentage yield

The percentage yield (Table 3 & Fig 6) of optimized formulation F7 (Nizatidine: Sod. Alginate: Carbopol: HPMC K100= 1:1:0.75:0.25) was found to be 95%.

Drug entrapment efficiency

Formulation F7 showed best entrapment efficiency (Table 3).The higher viscosity of the polymer solution at the highest polymer concentration would be expected to decrease the diffusion of the drug into the external phase which would result in higher entrapment efficiency.

***In-vitro* mucoadhesion test**

The rank of order of mucoadhesion (Table 4 &Fig 10) was carbopol 934 > HPMC K 100 > CMC.

Particle size analysis

The particle size (Fig7) of the microspheres increased with increased polymer concentration.

***In-vitro* drug release studies**

These release studies (Fig 8**)** show the effect of environment of the body on the drug release pattern from the prepared microspheres. The *In-vitro* release was observed in HCl (pH 1.2) for 12 hrs. It was found that the release rate from the all formulation was found to be different for the different polymer proportion used in the formulation 86.4%, 84%, 70.4%, 76.8% 79.4%,86.9%,85.7%, 83.9%, 80.6%,84.5% and 86.4% for formulation F1, F2, F3, F4, F5,F6,F7,F8,F9,F10 and F11 respectively. The F7 has highest proportion of polymer Sod. Alginate: Carbopol: HPMC (K100M) in the ratio of (1:0.75:0.25) showed maximum release.

***In-vitro* drug release kinetics**

The kinetic data analysis of all the formulations reached higher coefficient of determination with the Korsmeyer - Peppas model (R^2 = 0.911 to 0.989) whereas release exponent value (n) ranged from 0.498 to 0.743. From the coefficient of determination and release exponent values, it can be suggested that the mechanism of drug release follows Korsmeyer -Peppas model along with non-Fickian diffusion mechanism which leading to the conclusion that a release mechanism of drug followed combination of diffusion and spheres erosion.

Stability studies

Stability studies (Table 7) revealed that there was no significant change in the drug release after two months study.

Table 1. Composition of different formulations

Batch Code	Coat Composition	Ratio
F1	Nizatidine : Sod.Alginate	1:1
F2	Nizatidine : Sod.Alginate	1:2
F3	Nizatidine : Sod.Alginate	1:3
F4	Nizatidine : Sod.Alginate : carbopol	1:1:1
F5	Nizatidine : Sod.Alginate : HPMC(K100M)	1:1:1
F6	Nizatidine : Sod.Alginate : CMC	1:1:1
F7	Nizatidine: Sod. Alginate: Carbopol : HPMC(K100M)	1:1:0.75:0.25
F8	Nizatidine : Sod.Alginate: Carbopol : HPMC(K100M)	1:1:0.5:0.5
F9	Nizatidine : Sod.Alginate: Carbopol : HPMC(K100M)	1:1:0.25:0.75
F10	Nizatidine : Sod.Alginate : Carbopol : CMC	1:1:0.75:0.25
F11	Nizatidine : Sod.Alginate : Carbopol : CMC	1:1:0.5:0.5

Table 2. Flow properties of different formulations

Formulation	Angle of Repose	Bulk density (g/ml)	Tapped Density(g/ml)	Hausner ratio	Compressibility index
F1	16	0.428	0.456	1.065	6.14
F2	14	0.772	0.796	1.03	3.01
F3	17	0.656	0.772	1.17	15.02
F4	19	0.536	0.596	1.1	9.06
F5	22	0.817	0.871	1.06	6.19
F6	17	0.297	0.356	1.19	16.57
F7	14	0.604	0.679	1.12	11.04
F8	20	0.672	0.717	1.06	6.27
F9	16	0.690	0.718	1.04	3.89
F10	18	0.659	0.716	1.086	7.96
F11	17	0.452	0.496	1.097	8.87

Table 3. Percentage yield, percentage drug entrapment efficiency , average particle size and Drug Content of the prepared microspheres

S.No.	Formulation code	% yield	Drug Content (mg)		% Drug entrapment efficiency	Average Particle size(µm)	% muco-adhesion
			Theoretical	Practical			
1	F1	60	50	24.78	49.56	512	62
2	F2	65	50	28.51	57.02	617	74
3	F3	69	33.33	27.02	81.06	711	69
4	F4	82	25	17.76	71.04	826	69
5	F5	80	20	14.9	74.5	517	73
6	F6	53.2	50	29.5	59.0	642	86
7	F7	95	20	18.92	89.1	792	88
8	F8	89	25	16.61	66.46	834	78
9	F9	85	25	15.73	62.92	664	75
10	F10	75.3	50	25.5	51.0	774	79
11	F11	84.9	33.33	18.8	56.4	812	84

Table 4. RELEASE KINETICS: Coefficient Of Correlation (R^2) values of different batches of Nizatidine Mucoadhesive Alginate Microspheres

Formulation code	Release model			
	Zero order	First order	Higuchi matrix	Koresmeyer-peppas
F1	0.985	0.953	0.960	0.989
F2	0.911	0.945	0964	0.969
F3	0.984	0.953	0.964	0.992
F4	0.94	0.994	0.996	0.975
F5	0.982	0.986	0.996	0.911
F6	0.986	0.997	0.998	0.952
F7	0.973	0.772	0.993	0.991
F8	0.960	0.997	0.993	0.990
F9	0.963	0.983	0.982	0.963
F10	0.962	0.978	0.974	0.965
F11	0.993	0.970	0.964	0.973

Table 5. Stability study of optimized formulation (F7)

Time (hr)	Cumulative % drug dissolved					
	At 25°C / 60% RH			At 40°C / 75% RH		
	0 month	1 month	2 months	0 month	1 month	2 months
0	0	0	0	0	0	0
1	17.1	16.9	16.5	16.02	16	15.9
2	25.9	25.8	25.8	25.2	25.1	24.8
3	38.08	38.01	38	37.5	37.2	37.09
4	45.48	45.35	45.23	44.8	44.5	44.4
6	54.97	54.88	54.6	53.9	53.6	53.6
8	67.8	67.5	67.3	66	65.9	65.9
10	74.3	74.2	74.1	74.0	73.9	73.6
12	84.9	84.5	84.5	83.2	83.0	83.0

Fig 1. Photograph of prepared microspheres

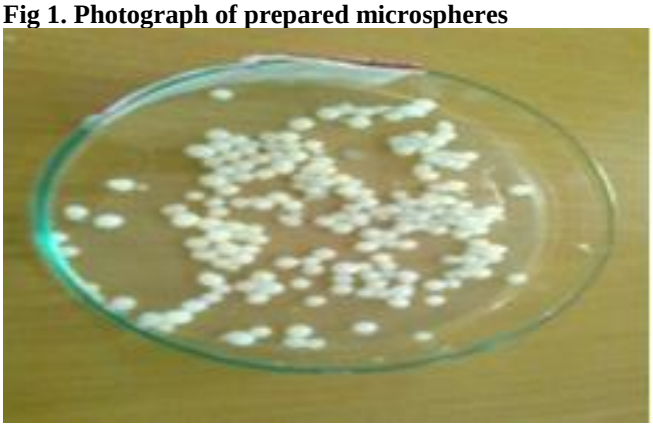


Fig 2. FT-IR spectra of Pure drug

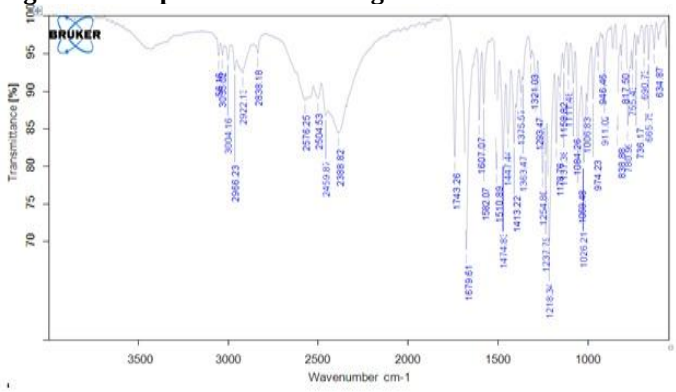


Fig 3. FT-IR spectra of Sodium alginate

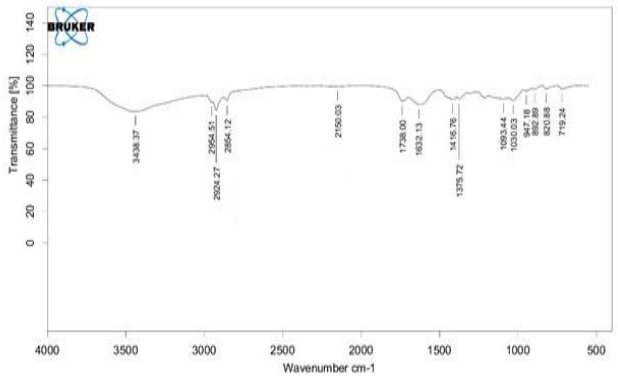


Fig 4. FT-IR spectra of Carbopol 934

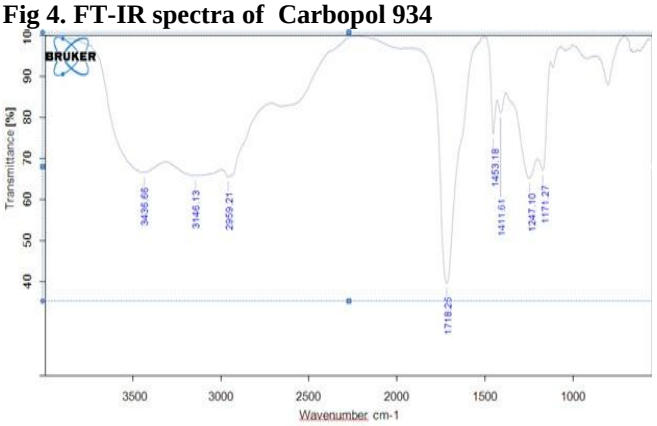


Fig 5. FT-IR spectra of optimized formulation(F7)

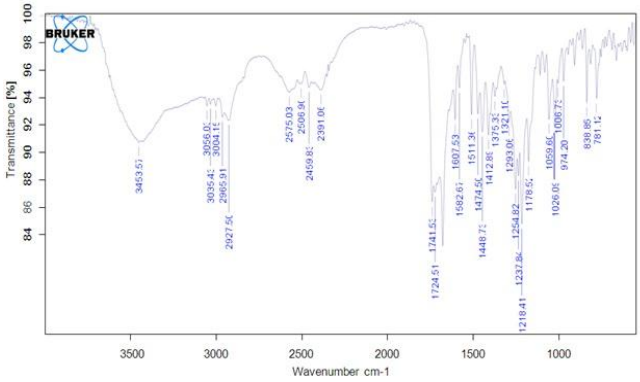


Fig 6. Showing % yield,% drug entrapment efficiency and % mucoadhesion

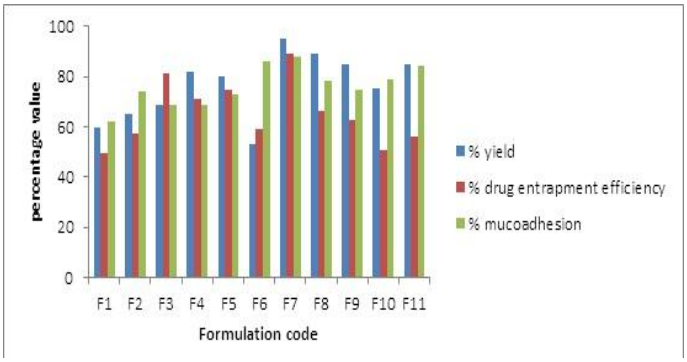


Fig 7. Average particle size of prepared microspheres for all formulations

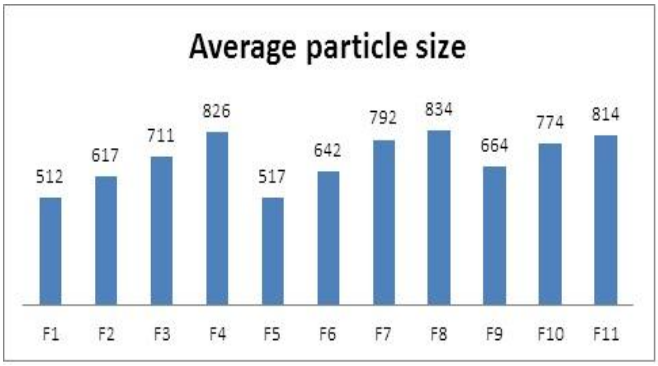
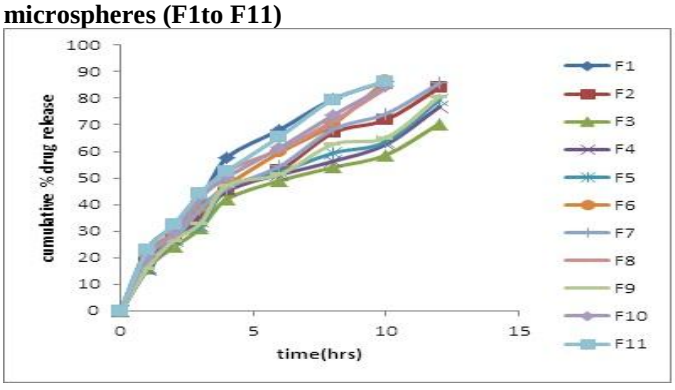


Fig 8. *In-vitro* drug release profile of Nizatidine microspheres (F1to F11)



CONCLUSION

The present study shows that the microspheres prepared polymer sod. Alginate, carbopol and HPMC K100 have a significant effect on the mucoadhesion, drug entrapment efficiency and drug release. carbopol is hydrophilic polymer has good entrapment efficiency and good mucoadhesion but it releases the drug immediately therefore HPMC K100 was used to control the release rate as well as the other factors to match the acceptance criteria. Sod.alginate offers rigidity to microspheres. After evaluating all the formulation, the formulation F7 which is containing the higher percentage of sod. CMC showed the good entrapment efficiency about 89.1%, mucoadhesion about 88% and good drug release profile in 12hrs.Therefore it was selected as the best formulation.

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