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Formulation and Evaluation of Oral Mucoadhesive Microspheres of Ofloxacin for Peptic Ulcer Use

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Abstract

A peptic ulcer is a disease that affects the stomach lining caused by *Helicobacter pylori.* The present research aimed to formulate and evaluate the mucoadhesive microsphere of ofloxacin for the effective and safe treatment of peptic ulcers. The mucoadhesive microspheres were developed using natural polymers like Guar gum, Sodium alginate, and Chitosan using the emulsification cross-linking method. A total of 8 batch formulations were prepared using 2^3 full factorial designs. The prepared microspheres were evaluated various parameters such as % yield, entrapment efficiency, drug release study, *in vitro* wash-off test, optimization was done by the design of expert which was further subjected to statistical analysis that was kinetic release mechanism, compatibility studies were performed such as FTIR and DSC. Using factorial design 2^3 for the maximum possible formulation, the optimized formulation was found to be F5 in terms of better release for a longer time and with better mucoadhesive properties on the use of guar gum, sodium alginate, and chitosan in ratio 1:1:2. Second-order polynomial equations were derived for absorbing responses after the application of ANOVA, after omitting the non-significant ($p \geq 0.05$) coefficients. %CDR 56.10 % for an extended period of 8 h, % EE exhibited 86.25±1.16. The sustained release activity of F5 might be due to the adhesion of the polymer to the gastric mucosa for more extended periods of 71 % to 8 h. it is also favoured by the suitable particle size of the optimized formulation. The result indicated that the prepared mucoadhesive microspheres can release the ofloxacin in sustained manner and helps in the management of peptic ulcer.

Keywords: Microsphere, Swelling index, Peptic ulcer, Factorial design, Gastro-retentive system, Entrapment efficiency

Introduction

Peptic ulcers are wounds in the mucous lining of the stomach or proximal duodenum. Usually, a peptic ulcer is a problem characterized by duodenal mucosa that extends into the submucosa [1]. This disease is one of the bacterial diseases which are mainly caused by *Helicobacter pylori. (H. pylori)* infections. Various antibacterial drugs are used to treat bacterial infections like- ciprofloxacin, nalidixic acid, norfloxacin acid, rosoxacin, etc., but these drugs are effective or work on some particular bacteria [2,3]. However, ofloxacin is a drug of 1st-generation fluoroquinolones which is works on both grams (-) & gram (+) bacteria [4]. The bioavailability of the ofloxacin is highly dependent on the specific physiology in the digestive system and is ideally consumed in the higher intestinal region. In the acidic medium, ofloxacin is easily soluble. While in the gut, in which pH-neutral to mildly alkaline conditions are strong; however, accumulation of the drug molecule occurs, which negatively affects absorption in lower bowel parts. A system is required that remains in the stomach for a reasonably long period and constantly releases the active compound [5].

Gastro-retentive drug delivery systems are the most potent approach designed to retain the dosage form in the stomach for a more extended period and release their active ingredients for targeting their specific site. This technology has gained massive attention in recent years in the field of oral drug delivery. Gastro-retentive dosage forms can hold drugs in the gastrointestinal region for an extended period, significantly sustaining drug retention [6,7].

There are various ways to distribute the drugs within the body, namely-oral, parenteral, transdermal, inhalational, transmucosal, and subcutaneous. The oral route is the easy, uncomplicated, secure, and most suitable route for administering the drug to patients. This oral route is suitable especially for chronic

therapies where repeated administration is essential. To establish a drug delivery system for the oral route, it is necessary to improve the release rate of an active compound from the system and the gastric transit time of the GIT [8]. Owing to this failure to suppress and identify the device at the gastrointestinal tract, oral administration of full medications in the traditional dosage type has short-range limitations. Microspheres have been an essential trial of these delivery systems due to their lesser size and effective carrier capacity [9,10]. Due to its smaller size and valuable transporter characteristics, the microsphere constitutes an important part of the particulate drug delivery system. Because of their less residence period at the absorption site, the effectiveness of this novel drug delivery system is limited [11]. The coupling of mucoadhesion properties to microspheres will accomplish this. Oral sustained release dosage types are the most widely developed but provide the maximum knowledge in the matter of novel delivery systems. The Bio-adhesive delivery system is one of the innovative approaches in this field. The formulations of the mucoadhesive-controlled release gained significant interest because of their capacity to bind to the mucosal layer and continuously release the loaded product. Using these dosage formulations would increase the intimate duration of contact with the mucus membrane, resulting in increased medication retention at the source, resulting in enhanced therapeutic effect for the management of digestive tract infections [12]. Natural polymers are the polymers that are obtained from plants, microorganisms, and animals. Natural polymers have several benefits, including as biocompatible, biodegradable, non-toxic, economical, and abundantly present in nature compared to synthetic polymers. Some examples of natural polymers like pectin, chitosan, gaur gum, dextran, hyaluronic acid, sodium alginate, etc.,

Sodium alginate an anionic polysacchhraides, which is obtained from seaweads (Ascophyllum nodosum, Macrocystis pyrifera and, Laminaria hyperborea) and bacteria (Azotobacter sp. and Pseudomonas sp.). Sodium alginate have significant gelling properties in aqoueos medium due to it consists α-1,4-L-guluronic acid and β-1,4-D-manurunic acid unit. Sodium alginate have some unique properties such as biodegradable in nature, mucoadhesiveness, cross linking capacity, pH sensivity, low toxicity and more suitable for colon drug-targeting.

Guar guar is derived from the Cyamopsis tetragonolobus plant seeds. GG is a polysaccharide of the reverse type made up of mannose [(1→4)-β-D-mannopyranosyl] and galactose [α-D-galactopyranosyl] units joined by (1→6) linkage. Gaur gum have some unique features such as excellent gelling efficiency, biodegradability, Mucoadhesiveness, suitable for colon drug-targeting, pH responsive polymer and sustained release properties.

Chitosan is produced commercially by deacetylation of chitin composed N-acetylglucosamine , and it is positively charge polycationic polymers, has become an important biomaterial and pharmaceutical excipient for drug delivery due to low cost, more biocompatibility, and poor immunogenisity. There are many uses for chitosan in the pharmaceutical and medical industries. Chitosan has a lot of functions as a film coating material, a tablet excipient, a drug disintegrant that improves drug dissolution, and a drug release controller. Additionally, it has been used for gel, beads, films, capsules, microspheres, and nanoparticles. Chitosan is successfully used in the targeted delivery of various organs, including the colon, liver, kidney, and lung. Additionally, it is mucoadhesive and can facilitate macromolecule permeation through epithelia with good organisation. The interaction of this polycationic polymer with negatively charged materials can result in the formation of a core shell microparticles, which has been shown to be a promising drug delivery system.

In this work, mucoadhesive microspheres were prepared using natural polymers such as Sodium alginate Gaur gum and Chitosan as a polymer with different concentrations of polymers by using the emulsification cross-linking technique. DSC checked the compatibility study. The mucoadhesive microspheres were characterized for particle size, morphology through SEM, % mucoadhesive, % Entrapment efficiency, and *in vitro* drug release.

Materials and methods

Materials

Ofloxacin (assay 99 %) was procured by Finecure Pvt. Ltd. (Uttarakhand, India). Chitosan (assay 95 %) was obtained by the Central Institute of Fisheries Technology (Cochin, India) as a gift sample. Sodium alginate (assay 91 - 106 %) and Guar gum were purchased from CDH, New Delhi, India. Glacial acetic acid was procured from Merck, Mumbai, India. Distilled water was used in all the preparations. All other reagents were used for analytical grade and purchased from Merck, Mumbai, India.

Preparation of ofloxacin mucoadhesive microspheres

The emulsification cross-linking technique was used to prepare the mucoadhesive microspheres of ofloxacin by Wong *et al.* [13] with minor modification. The guar gum and sodium alginate were dissolved in 200 mL of distilled water and allowed to swell for 24 h at room temperature. The weighed amount of chitosan was mixed and dissolved in 2 % of glacial acetic acid and kept aside for 24 h to swell or dissolve properly. After 24 h, the swelled mixture was mixed in a magnetic stirrer at a constant speed for 1 h to get a homogenous mass of both the gums. Similarly, the suspension of chitosan was homogenized for half an hour. The weighed amount of ofloxacin was added to the chitosan solution and mixed properly. This dispersion is sonicated for 30 min to remove the entrapped air bubbles. Then the gas-forming agent sodium bicarbonate with alginate was appropriately mixed. The slurry of sodium alginate and guar gum was put into chitosan solution and mixed adequately at an appropriate stirring rate using a magnetic stirrer, as shown in (**Table 1**) [12,14,15]. Cross-linking solution containing span 80 was stirred at 2,000 rpm using a megnatic stirrer (Remi, India), and 0.1 mL of concentrated H_2So_4 and 0.75 mL of glutaraldehyde were poured into a separate beaker. The above solution was added into the span 80. This cross-linking solution was added to the dispersion through a disposable syringe needle (24G size), followed by stirring at a constant speed under 2,000 rpm for 4 h at 50 °C. Using the sedimentation process, the microspheres were produced and collected, followed by oil decantation, then rinsed with multiple fractions of isopropyl alcohol. In the reaction of glutaraldehyde with sodium bisulphate, the remaining quantity was removed. The microspheres were filtered and dried. The dried microspheres were kept for 24 h using Vacuum desiccators at room temperature [16-19].

Table 1 2^3 factorial designs for forming ofloxacin-loaded mucoadhesive microspheres by the emulsification-cross linking method. (A) Gaur gum (B) Sodium alginate (C) Chitosan.

Formulation batches	Composition of factors with their level according to factorial design				Stirring speed(rpm)
	Drug (mg)	A (mg, w/w)	B (mg, w/w)	C (mg, w/w)	
F1	100	250	1,500	2,500	2,000
F2	100	500	3,000	2,500	2,000
F3	100	250	1,500	2,500	2,000
F4	100	500	3,000	2,500	2,000
F5	100	250	1,500	5,000	2,000
F6	100	500	3,000	5,000	2,000
F7	100	250	1,500	5,000	2,000
F8	100	500	3,000	5,000	2,000

Design of experiment (DOE)

Experiment design is a systematic approach used for determining the relationship between the dependent and independent variables, affecting the formulation process and the output of that process. DOE is a tool for developing a plan for exploration that maximizes learning using a minimum of resources. Experimental design techniques in such cases are becoming increasingly important in developing new products and processes cost-effectively and confidently.

Microspheres were made utilizing 2^3 full factorial designs. Eight experimental batches were made by altering the amount of guar gum, sodium alginate, and chitosan concentration at 2 distinct levels. In this formulation, the amount of the drug was kept constant. The polymers concentration and stirring speed play a significant role in particle size, % EE, and % buoyancy. The independent variables were the guar gum, sodium alginate, chitosan, and stirring speed. Particle size, % entrapment efficiency, % buoyancy, and % cumulative drug release were selected as dependent variables [20].

Particle size determination

Digital microscopy (XSZ-107 METZER, Range 100× to 1,000× magnitude) was used to measure the particle size of each formulation. Firstly, in dry microspheres, a small amount was suspended in glycerine. After that, a small drop of the prepared suspension was deposited on a glass slide, covered with slip, then mounted to the microscope stage and observed. Around 100 microspheres were calculated for particle size using a standardized ocular microscope [20-22].

Swelling index

For the determination of the swelling index, the pre-weighted quantity of microspheres was added in pH 1.2 buffer solution for 24 h. The final weight was recorded and calculated for the selling index using the following formula [23].

$$\text{DEGREE OF SWELLING } (\alpha) = \frac{Ws - W0}{Ws}$$

where, Ws = weight of microspheres after swelling
W_0 = initial weight of microspheres.

Percentage yield

The prepared mucoadhesive microspheres were precisely weighed after drying. The percentage yield of all the prepared formulations was calculated using the following equation [24].

$$\text{Percentage (\%)yield} = \frac{\text{Actual weight of microspheres obtained}}{\text{Total weight of drug and polymer}} \times 100$$

Estimation of drug entrapment efficiency

Entrapment efficiency and drug loading capacity were determined to check the amount of drug present in the prepared mucoadhesive microspheres. The microspheres were put into the mortar and pestle and crushed into fine powder. An accurately weighed quantity of crushed microspheres was suspended in 0.1 N HCl (pH 1.2) to extract the drug from the microsphere. It was then shaken in a mechanical shaker. After 24 h, the filtrate was investigated spectrophotometrically at 293 nm using UV spectrophotometer (Shimadzu, UV-1800, Japan) for drug content against 0.1 N HCl as blank [25].

$$\text{\% Drug entrapment efficiency} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

***In-vitro* wash-off test**

The *in-vitro* wash-off technique was used to evaluate the properties of the mucoadhesive microspheres by Malik *et al*. Intestinal mucosa from goats was initially obtained from a nearby slaughter house and a 2×2-cm piece of intestinal mucosa/mucosal membrane was tied onto a glass slide (3×1 inch) with thread and cleaned with 0.1N HCL. The prepared slide was put onto the arm of a USP tablet disintegration test apparatus containing 900 mL of 0.1 N HCl at 37±0.5 °C. Microspheres were dispersed (100) over a wet-washed tissue specimen. The apparatus is operated slowly, up and down. The apparatus was stopped at 30, 60 and 8 h intervals. The number of microspheres still in the following tissue was calculated [26,27].

$$\text{\% Mucoadhesiveness} = \frac{\text{Quantity of microspheres adhered}}{\text{Quantity of microspheres spread}} \times 100$$

Buoyancy study

USP dissolution type II apparatus (Hicon, ET-1916, India) was used to calculate the buoyancy of the floating microsphere. About 200 mg of microspheres were placed on the surface of the apparatus and taken 900 mL of 0.1 N hydrochloric acid containing 0.02 % of tween 80. The paddle was moving at 100 rpm on his axis, and the floating and settled microspheres quantity were collected separately, dried, and weighed [28-30].

$$\text{\% Buoyancy} = \frac{Wf}{Wf + Ws} \times 100$$

where, W_f and W_s are the weight of floating and settled microspheres, respectively.

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

U.S.P. dissolution (paddle type II) apparatus (Hicon, ET-1916, India) was used for *in-vitro* drug release study investigations. In this study, mucoadhesive microspheres were spread on the surface of the dissolution apparatus. The apparatus was filled with 900 mL of 0.1 N HCL as dissolution medium at 37±0.5 °C and agitated at 100 rpm for 8 h. A 5 mL sample was withdrawn at predetermined time intervals up to 8

h, filtered with the help of a 0.45µm membrane filter (Millipore), and diluted to a proper concentration with an equivalent dissolution medium. The sample was analyzed using a UV spectrophotometer (Shimadzu, UV-1800, Japan) at 293 nm. An equal volume of fresh dissolution medium was replaced immediately after the withdrawal of the test sample to maintain sink condition [31].

Statistical analysis

By using Design expert software (version 8.0.5.2, Stat-Ease, Inc., Minneapolis, USA) to identify the effect of the independent variable on the response. For the dependent variable, % EE, % mucoadhesive, % CDR_{8h}, and polynomial equations were established. The obtained equations were minimized by discarding non-significant coefficients using one-way ANOVA at a 95 percent confidence level ($p > 0.05$) to check for significant coefficients. High entrapment efficiency, mucoadhesion, CDR8h, and maximum desirability were used to identify the optimal formulation.

Analysis of drug release kinetics and mechanism

The general mechanism and drug release of the kinetic profile of ofloxacin microspheres were determined using different kinetic release models like 0 order, 1st order, Higuchi, Korsmeyer- Peppas. The given data were fitted with these models. The correlation coefficient (R2) was estimated for the drug release kinetics for each model [30,32].

Characterization of optimized formulation

Drug-excipient compatibility studies

Excipients are essential ingredients for all pharmaceutical formulations. This is necessary to identify any physical and chemical interaction between the drug and excipients, which may influence the bioavailability and consistency of the drug. Generally, drugs and excipients are combined to produce a pharmaceutical product that is stable, effective, attractive, safe, and simple to use.

FTIR (Shimadzu, Japan) and DSC (Mettler Toledo) methods are employed to identify the compatibility study among the drug and different kinds of excipients that are useful for pharmaceutical formulation. So, this technique was preliminary to the selection of the drug and polymer [33-35].

Results and discussion

Microsphere preparation and optimization

By using a suitable ionic-gelation approach as described by Wong *et al.* with minor modifications, ofloxacin-loaded different mucoadhesive microspheres were successfully developed. Microspheres were prepared by using drug: polymer ratios (w/w), which is given in **Table 1**. The quantity of cross-linking agent, polymers proportion, and stirring speed had a crucial effect on the formulation of microsphere characteristics. The optimized moving rate was 2,000 rpm for fabricating ofloxacin-loaded appropriate mucoadhesive microspheres.

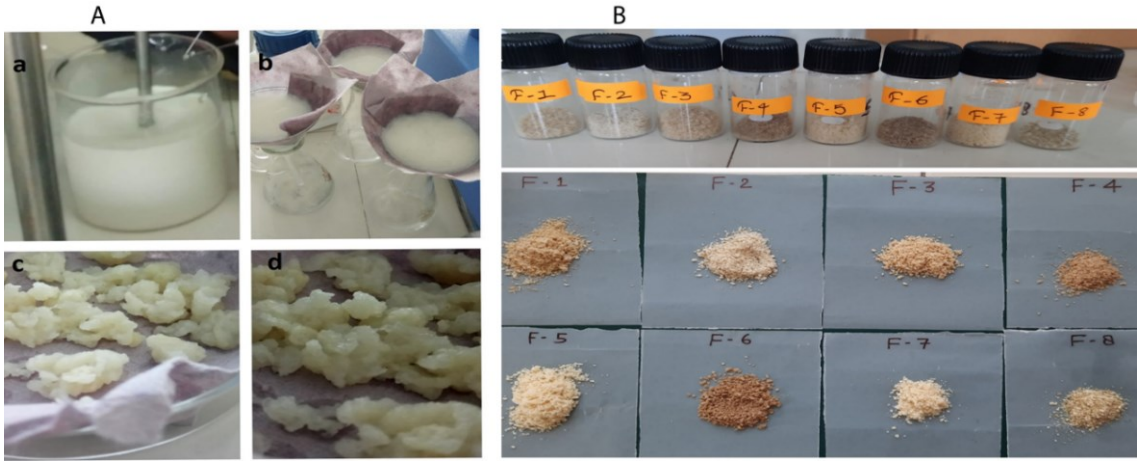

Figure 1 A) Preparation of ofloxacin-loaded microspheres, and B) Prepared Mucoadhesive Microsphere.

Fourier transform infrared spectrophotometer (FTIR)

The FTIR spectra are shown in **Figure 2**. In the IR spectrum of pure drug and drug with various polymers, no significant difference was observed in the mixture. Ofloxacin Includes one OH, -NH, CH_3, C-F, and one C=O which have characteristics peak values range around 3,050 - 3,000 cm^{-1}, 1,650 - 1,600 cm^{-1}, 2,750 cm^{-1}, 1,050 - 1,000 cm^{-1}, 1,750 - 1,700 cm^{-1} respectively. The identification of ofloxacin was made via infrared spectroscopy. The sample (1 mg) was triturated with the KBr and crushed into the pellet; the spectra were recorded on an FTIR spectrophotometer (Shimadzu, Japan) and scanned under the wavelength region of 4,000 - 400 (cm^{-1}). In IR Spectra, characteristic peak was observed at 3,478.79 cm^{-1}(OH) of chitosan, 3,447.61cm^{-1}(OH) of Guar gum, and 3,250 cm^{-1}(OH) of Sodium alginate, -NH, CH_3, C-F, C=O all of them are also in their range in all spectra. In contrast, the new band or shift in the characteristic band was not seen in the mixtures. The result indicated that there is no chemical incompatibility found between the selected drug and polymers.

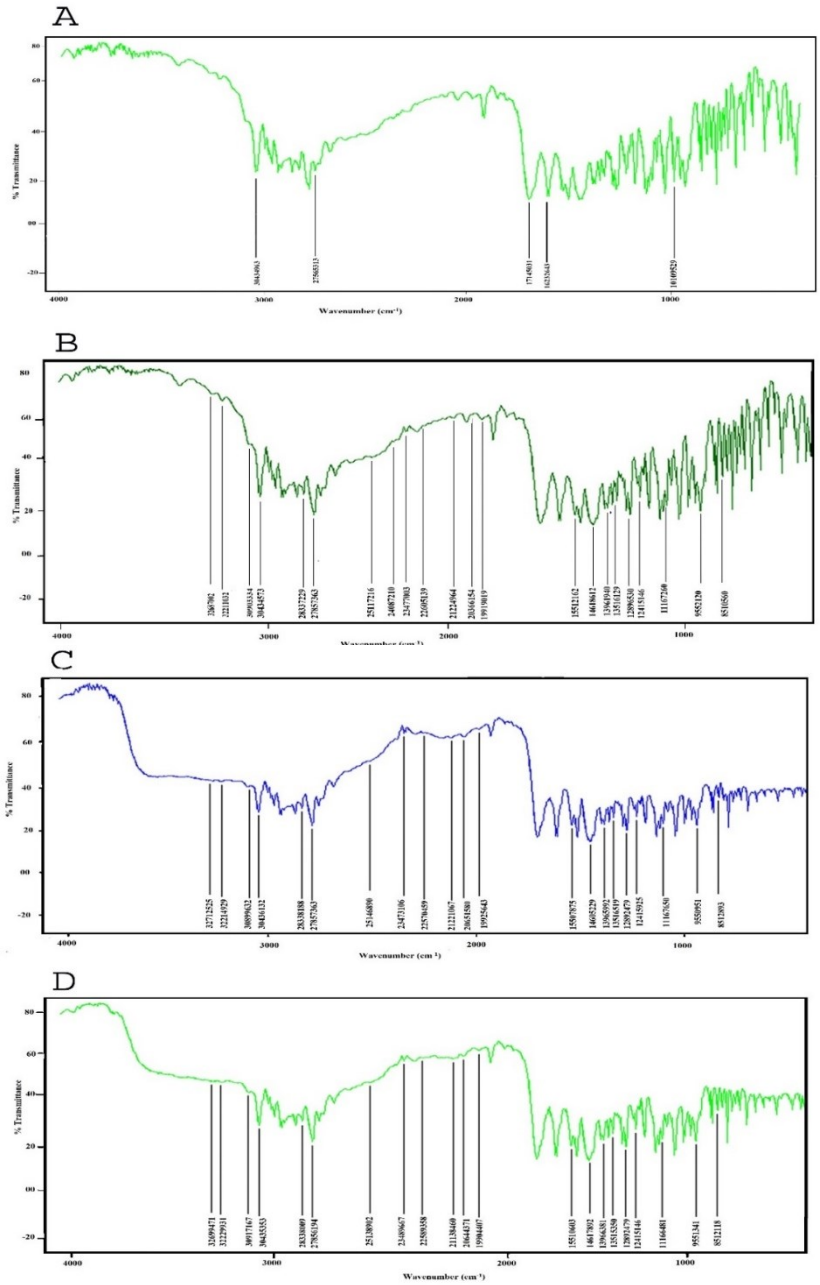

Figure 2 FTIR spectra of (A) Ofloxacin (B) Ofloxacin and Chitosan (C) Ofloxacin and Guar gum (D) Ofloxacin and Sodium alginate.

Differential scanning calorimetry (DSC)

To obtain the DSC thermograms of the drug, the METTLER TOLEDO instrument was employed. The sample (5 mg) was accurately weighed and sealed hermetically into an aluminum pan. The sample's heating runs were kept from 50 - 300 °C at a heating rate of 10 °C / min. under a nitrogen atmosphere. The DSC value of ofloxacin is 254, as shown in **Figure 3A**.

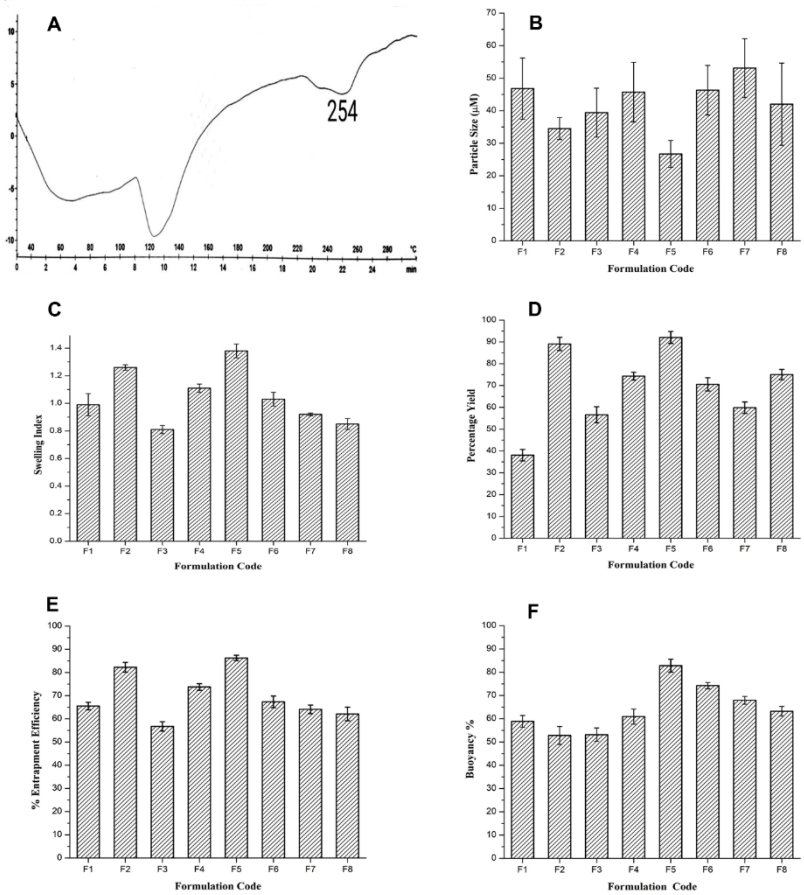

Figure 3 A) DSC of Ofloxacin B) Particle size of all the formulations from F1 to F8. C) Swelling index of all microsphere formulations from F1 to F8. D) Percentage yield of all the prepared formulations from F1 to F8. E) % Entrapment efficiency of all the formulations F1 – F8. F) % Buoyancy of all the formulations F1 to F8.

Particle size determination

The particle size of the different formulations of ofloxacin microspheres were obtained between the range of 26.27±4.14 to 53.10±9.04 µm. It was found that the microspheres particle size significantly increased when the polymer concentration increased. The increase in polymer concentration was attributed to an increase in viscosity, which resulted in the development of big droplets, thus increasing the size of microspheres. All the prepared formulations' particle size is shown in **Figure 3B**.

Swelling index

The swelling properties of the various formulations were tested in a Simulated gastric fluid medium (pH 1.2), and the results are shown in **Figure 3C**. Swelling indexes were achieved between the range of 0.85±0.04 to 1.38±0.05 %. The best degree of swelling (1.38±0.05 %) value was achieved with formulation F5 for an extended period (24 h), as compared with other formulations, which is suitable for retaining in the colon.

Percentage yield (% yield)

The percentage yield of all the microspheres were found to be between 38.07±2.63 and 92.02±2.76 %. It was observed that the polymers ratio in the formulation was increased, and the % yield was also increased. Some formulations were shown low percentage yield because of blocking of needle and wastage

of the drug-polymer solution, and microspheres were mislaid during the filtration and washing process, which ultimately reduced the production yield of some formulations. The production yield of all the prepared formulations is displayed in **Figure 3D**. The production of mucoadhesive microparticles at a constant speed at 2,000 rpm may be facilitated by an appropriate polymers ratio (**Table 1**). At this ratio, % yield of optimized formulation was found to be good for formulation F5.

Estimation of drug entrapment efficiency

The entrapment efficiency of all the formulations of ofloxacin microspheres were obtained between 56.74±2.01 and 86.25±1.16 %. The formulation F5 showed maximum entrapment efficiency due to the appropriate ratio of polymers (**Table 1**) in the formulation. The entrapment efficiency of ofloxacin in various formulations is represented in **Figure 3E**.

Percentage buoyancy

The % buoyancy strength of the prepared microsphere was found to be good. The % buoyancy of all formulations of microspheres was found to be between 52.80±3.86 to 82±2.78 %. The percentage buoyancy of microspheres was float and reached their targeted site. The F5 batch showed the highest % buoyancy. The % buoyancy of all the prepared formulation was shown in **Figure 3F**.

***In-vitro* Muco-adhesion Study**

The mucoadhesive strength of the prepared microsphere was found to be good. The mucoadhesive properties of all the formulations were found to be 42±1.23 to 71±1.79 %. It was spotted that the mucoadhesive strength of the prepared formulations increased because of increasing the polymer concentration. An increase in concentration was attributed to increasing in viscosity; producing a more robust mucus gel network, which helps to increase mucoadhesion. The highest mucoadhesive strength was achieved in formulation F5, which is shown in **Figure 4B**.

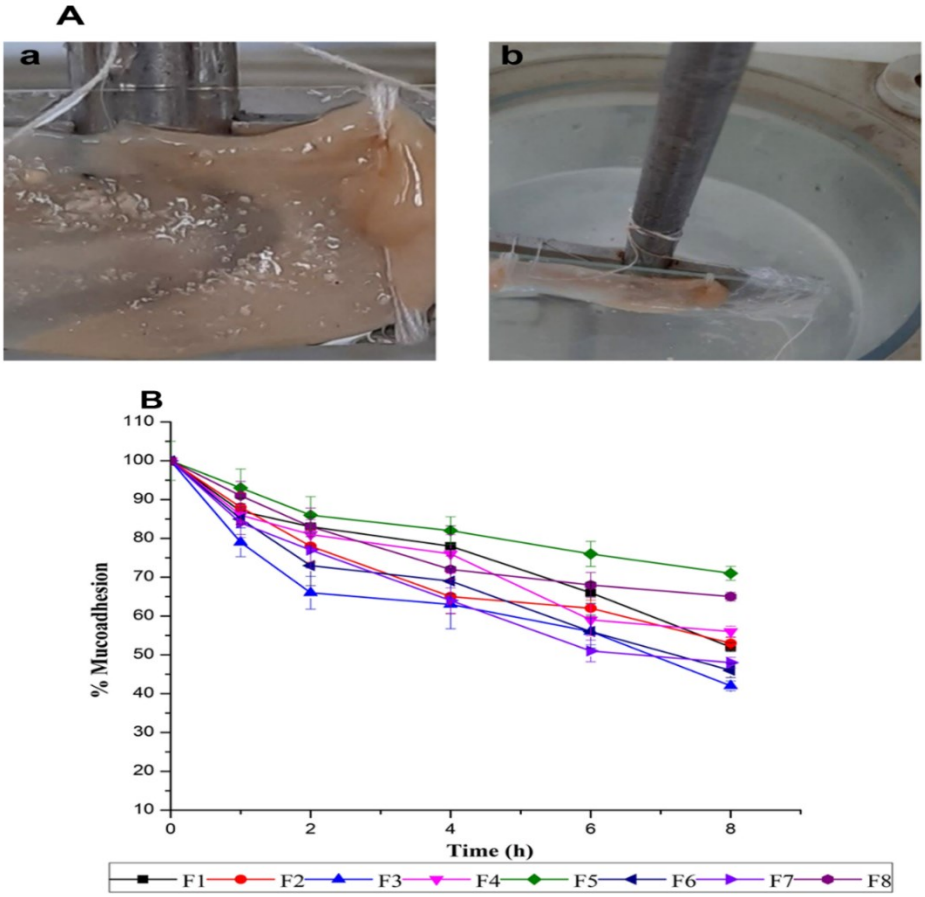

Figure 4 A) Images of different stages of the Mucoadhesive test, and B) *In-vitro* Mucoadhesive strength of all the formulations from F1 to F8.

***In-vitro* drug release study**

In-vitro drug release study was performed for different formulations for 8 h to ensure the release characterstics of the developed mucoadhesive micrpsheres. Release was gradual and evenly distributed out over time. This could be explained by the formulation's matrix structure. Poorly water-soluble drugs initially appear to flow into the interstices of the polymer, where the aqueous phase forces the drug into the gel portion of the polymer. The hydrophilic membrane then expands and serves as a flux-controlling barrier. As a result, the drug could be delivered over time using alginate microspheres combined with chitosan that were produced using a simple ionic gelation procedure. Diffusion and erosion both served to restrict the drug release [31]. The release studies of all the formulation is shown in **Figure 5**.

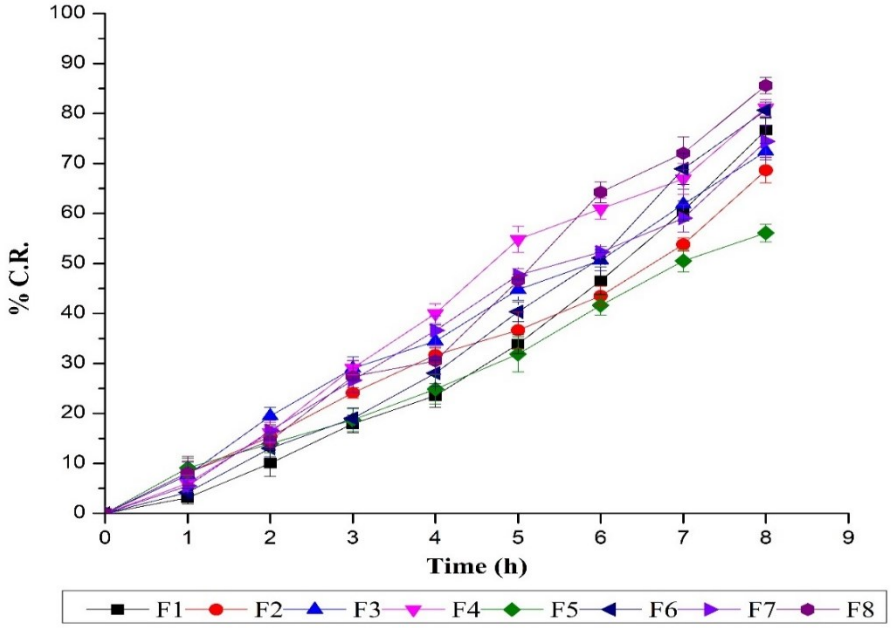

Figure 5 *In-vitro* drug release profile from F1 to F8.

Statistical analysis

A general statistical modal can be shown based on the data obtained from the formulation. Design expert software (version 8.0.5.2, Stat-Ease, Inc., Minneapolis, USA) was used for statistical analysis. After using one-way ANOVA and eliminating non-significant ($p > 0.05$) variables, the 2nd-order polynomial equations were derived from observed responses. The modified equations are,

The final equation in terms of fundamental factor:

$$\% \text{ Entrapment efficiency} = +27.62 + 31.27A - 16.49B + 48.97C + 8.62AB - 27.33AC - 5.07BC \quad (1)$$

$$\% \text{ CDR} = +109.82 - 19.68A - 5.88B - 38.05C + 1.68AB + 17.52AC + 7.50BC \quad (2)$$

$$\% \text{ Mucoadhesion} = -12.63 + 36.75A - 4.25B + 54.75C + 1.50AB - 28.50AC - 1.50BC \quad (3)$$

where A = Guar gum, B = sodium alginate, C = chitosan

After considering the coefficient magnitude and its mathematical symbol (i.e., positive and negative), polynomial equations may be utilized to find the conclusion. A positive sign indicates the synergistic effect of the variable on the response, whereas a negative sign represents an antagonistic effect. The mathematical relationship is expressed as the factor coefficient, corresponding p-value for measured responses, and the correlation coefficient.

Multiple linear regression analysis indicated that coefficients A and C have a positive sign ($R2 = 0.9524$) regarding % EE. It can be concluded from equation 1 that A showed a less positive effect compared to B. The coefficients A, B, AB, AC, and BC were non-significant at $p > 0.05$.

Concerning percent CDR, multiple linear regression analysis outcomes indicate that the coefficients combination of AB and AC show positive signs ($R^2 = 0.996$). It can be concluded from equation 2 that the combination of AB so fewer positive effects compared to BC than AC. The coefficients A, B, C, AB, and BC were non-significant at $p > 0.05$.

Concerning % Mucoadhesion, multiple linear regression analysis results showed that coefficients A and C bear a positive sign ($R^2 = 0.998$). It can be concluded from equation 3 that A showed a less positive effect compared to B than C. The coefficients A, B, AB, AC, and BC were non-significant at $p > 0.05$. The data of statical analysis is shown in (**Table 2**) and the coefficient table is shown in (**Table 3**).

Table 2 Summary of results of regression analysis for responses % EE, % CDR and % Mucoadhesion.

Model	R^2	Adjusted R^2	Predicted R^2	SD	% CV
% EE response	0.9524	0.6670	–2.0446	5.88	8.44
% CDR response	0.9961	0.9726	0.9694	2.39	4.78
% Mucoadhesion	0.9998	0.9987	0.9879	0.35	0.65

Table 3 Coefficient table.

Response	Intercept	A	B	C	AB	AC	BC
% EE	27.6172	31.2659	–16.486	48.976	8.61927	–27.3323	–5.06669
p =		0.3346	0.5303	0.2258	0.4888	0.1881	0.6518
% CDR	109.824	–19.6825	–5.8825	–38.0525	1.675	17.515	7.505
p =		0.6564	0.8872	0.4534	0.9296	0.4524	0.7059
% Mucoadhesion	–12.625	36.75	–4.25	54.75	1.5	–28.5	–1.5
p =		0.0189	0.1598	0.0127	0.2048	0.0112	0.2048
Legend		$p < 0.01$	$0.01 \leq p < 0.05$	$0.05 \leq p < 0.10$	$p \geq 0.10$		

Optimized ofloxacin mucoadhesive microsphere

Numerical optimization employing the desirability method was used to find the best values for the formulation variables to get the desired outcome. Based on dependent and independent variables, the optimized formulation F5 was developed. Formulation F5 showed the highest desirability factor, % EE, % CDR, % Mucoadhesion till 8 h.

Kinetic release studies

In vitro, drug release data of optimized formulation was evaluated using different mathematical models like 0 order, 1st order, Higuchi's and Korsmeyer to predict their release mechanism. The best fit kinetic model is 0 order achieved with the optimized formulation. The results of the kinetic release study of the optimized formulation are given in (**Table 4**). The fiction diffusion was followed by applying the model value of n less than 0.45.

Table 4 Kinetic release study of the optimized formulation.

Batch	0-order		1st-order		Higuchi model		Korsmeyer- Peppers	
	r^2	k_0	r^2	k_1	r^2	K_H	K_{HP}	n
F 5	0.9596	6.911	0.9179	0.026	0.8599	33.28	0.8336	0.43

Scanning electron microscopy

The surface morphology of the optimized formulation of the microsphere was analyzed by using SEM. The unloaded drug mucoadhesive microsphere was rough in shape, while the drug-loaded microsphere was smooth and spherical.

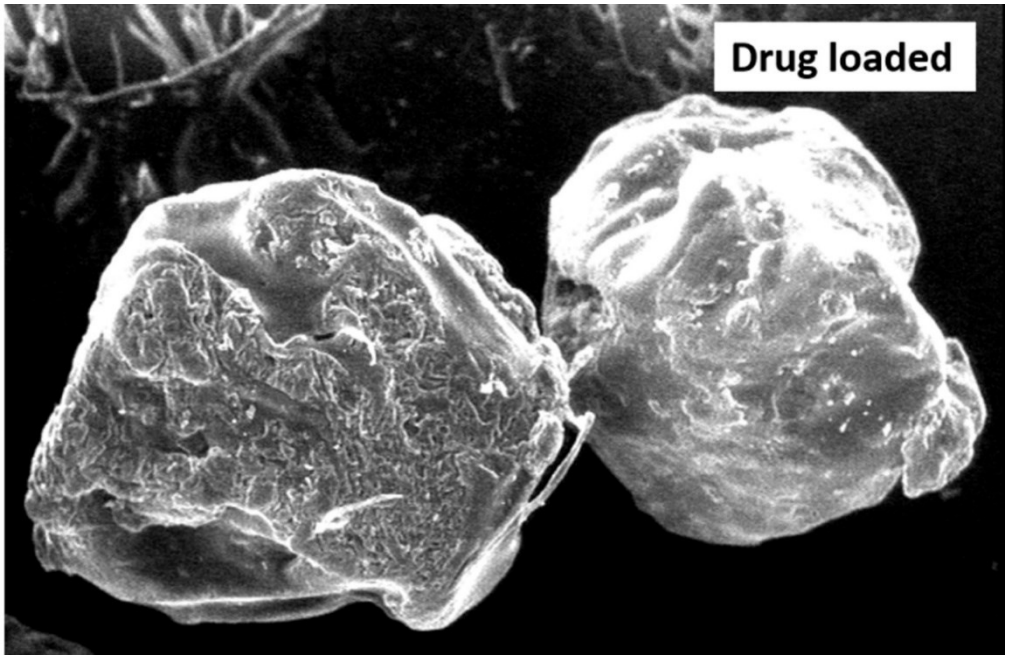

Figure 6 SEM images of the optimized formulation.

Conclusions

It was concluded that the ofloxacin mucoadhesive microspheres were prepared successfully using the emulsification cross-linking method and optimized through factorial design. The concentration of the different polymers significantly impacted the % mucoadhesive and drug release. The results of the 2^3 full factorial design revealed that the stirring speed, and concentration of other polymers significantly affects the dependent variable such as Particle size, % Entrapment efficacy, drug release, and % mucoadhesive strength. After evaluating all formulations, the F5 formulation showed optimum particle size, better entrapment efficiency, good mucoadhesive power, and a better drug release profile.

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