ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

Formulation, Statistical Optimization, and *In Vivo* Pharmacodynamics of *Cydonia oblonga* Mucilage/Alginate Mucoadhesive Microspheres for the Delivery of Metformin HCl

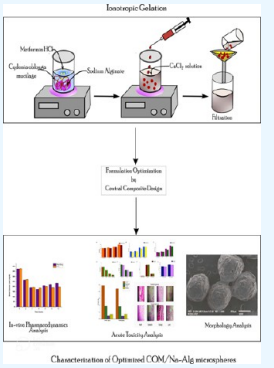
Sobia Noreen,* Sara Hasan, Shazia Akram Ghumman, Shoaib Anwar, Humaira Yasmeen Gondal,* Fozia Batool, and Shazia Noureen

Cite This: *ACS Omega* 2023, 8, 5925−5938

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: In recent years, attention has shifted toward the utilization of natural polymers for encapsulation and sustained release of health-hazardous drugs. The purpose of this work is to define and assess the sustained delivery potential and mucoadhesive potential of a *Cydonia oblonga* mucilage (COM) and sodium alginate (Na-Alg)-constituting polymeric delivery carrier of antidiabetic drugs with a specific end goal to retain metformin HCl in the stomach while expanding the drug's bioavailability. Metformin HCl was encapsulated in mucoadhesive microspheres by an ionic gelation method. Polymers with different combinations were tried, and the resulting mucoadhesive COM/Na-Alg microspheres were assessed for particle size (mm) PS/Y_1, drug encapsulation efficiency DEE (%)/Y_2, and *in vitro* percentage cumulative drug release R_{12h}/Y_3 using Drug Design Expert software version 10. The response surface methodology by a 3^2-central composite design predicted optimal synthesis parameters for the microspheres to be 295 mg for COM and 219 mg for Na-Alg. An optimized formulation was prepared under these conditions and used to evaluate the micrometric properties, morphology and structural characteristics, swelling behavior, *in vitro* drug release, and kinetics. Acute toxicity studies were carried out on blank COM/Na-Alg microspheres to deem them safe for *in vivo* studies. The DEE (%) was calculated to be 85.8 ± 1.67, whereas scanning electron microscopy (SEM) showed a coarse surface with characteristic wrinkles and cracks with an optical microscopic particle size of 0.96 ± 2.45. The *ex vivo* tests showed great mucoadhesive properties and good swelling behavior with pH-responsive drug release and a significant reduction in *in vivo* blood glucose levels. The results advocated the use of optimized microspheres to enhance the bioactivity with a possible dose reduction, making it less symptomatic, reducing the expense of the treatment, and subsequently facilitating better patient compliance.



1. INTRODUCTION

The absorption barriers, particularly mucosal membrane and P-gp efflux (P-glycroprotein), an efflux transporter involved in drug transportation, need to be pondered for the perioral administration of hydrophilic agents.[1] Several predicaments associated with such drugs eminently arise because of their increased concentration in the intestinal lumen, which in turn constrains the bioavailability and the drug's half-life.[2] In the gastrointestinal tract (GIT), hydrophilic antidiabetic drugs are known to pose gastrointestinal tract (GIT) intolerance with several toxic effects, with hyperlactatemia being the primary toxicity in response to chronic administration that widely influences the dosage frequency and therapeutic efficacy of the drugs.[3] In this regard, various GIT extended-release delivery systems, for instance, pH-sensitive swelling systems, low/high-density systems, high-porosity hydrogel systems, bioadhesive systems, and magnetic delivery vehicles, have become desirable, as these allow improved glycemic level control, in addition to minimalizing side effects.[4] Among the aforementioned carriers, bioadhesive polymer-based microspherical tools have gained profound acclaim owing to their ability to enable drug localization by accelerating absorption proficiency via supplementing the drug−mucosa contact. Furthermore, the small size of the microspheres enables them to be trapped inside the gastric folds, prolonging the residence time.[5]

Bioadhesive (natural/synthetic)polymer selection is quite often based on their biological membrane adherence potential.[5] Recently, plant-derived polymers have shown escalating utilization in drug delivery excipients due to their abundance, renewability, biodegradability, inexpensiveness, and biocompatibility.[6] Several ionic polysaccharides, for instance, sodium alginate, chitosan, xanthan gum, and dextran, have drawn a substantial deal of attention as a mucoadhesive carrier matrix for drug transport.[7] Additionally, the approach to

Received: December 6, 2022
Accepted: January 16, 2023
Published: February 5, 2023

blend such ionic biopolymers with other natural polysaccharides is still ongoing to acquire a coveted concentration of polysaccharide microspheres to increase the drug's encapsulation efficiency, release, etc.[8]

Sodium alginate (Na-Alg), a highly effective biodegradable polyanionic polysaccharide, is extracted from the Phaeophyceae species and comprises 1 → 4 linked α-L glucuronic acid and β-D-mannuronic acid monomers.[9] The potential application properties of Na-Alg, namely, gelation ability, low toxicity, ease of handling, versatility, and chemical modification, make it a suitable polymer in drug delivery tools. Its ability to gel by sodium ion substitution with a divalent cation such as calcium, characterized by "egg-box" geometry formation, makes it more desirable.[10] Also, the use of $CaCl_2$ as a cross-linker for Na-Alg has been reported to possess high biocompatibility with no effect on cell viability.[11] Despite their promising applications, Na-Alg microspheres as drug delivery tools still face several drawbacks in their usage, including low stability, rapid release in response to high pH stimulus, drug leakage, etc.[12] A popular current practice prevailing is the blending of alginate microspheres with suitable mucoadhesive biopolymers by ionotropic gelation.[13,14] To increase the drug delivery intelligence of these delivery tools and make them more stable, the use of plant mucilage seems to be a way. The diversity in functionalities such as NH_2, COOH, and OH groups, allowance of chemical alteration in regards to the target site, and potential for enzymatic modification makes coupling of alginate with mucilage efficacious in terms of desired biochemical and mechanical characteristics, in addition to regulating apt drug release and swelling.[15]

Cydonia oblonga mucilage (COM) is extruded from quince seed coats, traditionally known as baihi-dana. It is a hydrogel in nature with an increased manifold swelling index in comparison with that of various other medicinally revered polysaccharides such as guar gum. This glucuronoxylan has been established as a smart material, as it qualifies all required criteria of advanced delivery tools in terms of mucoadhesive nature, bioavailability, biodegradability, and stimuli responsiveness. Moreover, a high percentage of glucuronic and galacturonic acid offers additional reaction sites to monomers and cross-linkers.[16] COM not only is economical but also possesses excellent water absorption potential, which potentiates its use as a stabilizer, drug-encapsulating agent, and sustained-delivery module. Previously, COM/Na-Alg-based beads have been used for sustained transport of cefixime-HCl.[17]

Metformin HCl (MFH) is a chemically derived biguanide anti-hyperglycemic drug, frequently used in the management of diabetes mellitus type II. A frequent high (1.5−3 g/day) dose is usually administered due to its poor pharmacokinetics such as a low bioavailability of 50−60%, a short half-life of 3−4 h, and ≤5 protein binding, which lead to side effects (lactic acidosis and GIT irritation).[2,18,19] It has been reported that MFH mainly gets absorbed in the small intestine.[20] Achieving controlled and prolonged gastric-retentive release of MFH will surely enable the acquisition of better bioavailability and constant plasma concentration, increasing the therapeutic effect and in return improving patient compliance.[21]

In the present research work, we developed optimized MFH-loaded COM/Na-Alg microspheres with calcium chloride as a cross-linker by a 3^2 central composite design. The particle size, encapsulation efficiency, and drug release were selected for the prediction of polymer concentrations for optimized microsphere formulation. Furthermore, the optimized microspheres were characterized for size, chemical characterization, morphology, and encapsulation efficiency by optical microscopy, FTIR, SEM, and UV−Vis, respectively, followed by the *in vivo* assessment of the acute toxicity of blank COM/Na-Alg and MFH-loaded COM/Na-Alg pharmacodynamics in alloxan-induced mice.

2. MATERIALS AND METHODS

2.1. Materials. Active salt of metformin HCl (MFH) was received as a gift from Werrick Company, Islamabad. Sodium alginate (Sigma-Aldrich, France) was used. Calcium carbonate, potassium dihydrogen phosphate, and dipotassium hydrogen phosphate were purchased from Merck, Germany. Different analytical-grade solvents such as methanol, ethanol, and acetone were purchased from local suppliers. Distilled water was used for the extraction of mucilage and for solution preparation.

2.2. Mucilage Extraction. Quince (*C. oblonga*) seeds were purchased from the local herbal store "Chinnoti Dawa Khana", Sargodha, Pakistan. COM was extracted from ripened quince seeds by following the previously reported literature.[22] Initially washed with water, quince seeds were soaked in double-distilled water for 3 h at room temperature and then heated for 2 h on a low flame to allow mucilage discharge. Once the slurry was formed, it was cooled at room temperature.

The mixture was passed through a multilayer muslin cloth and stored in a beaker. Ethanol was added to this solution in 3:1 ratio to precipitate pure mucilage. Once washed with ethanol and demineralized with water, the precipitate was oven-dried at 45 °C overnight. The dried COM was ground using a pastel mortar, passed via a sieve number-80, and sealed in a vial for future use.

2.3. Physicochemical Analysis of COM. COM was characterized physicochemically (swelling index, pH, total ash percentage) and rheologically (bulk and tapped density, Carr's index, angle of repose and Hauser's ratio) by following the BP guidelines.[23]

2.4. Preparation of MFH-Loaded COM/Na-Alg Microspheres. MFH-loaded COM/Na-Alg microspheres were formulated via the ionotropic gelation method with the cross-linker being calcium chloride ($CaCl_2$). The aqueous dispersion of COM was prepared with Na-Alg in various proportions (Table 1), in distilled water with constant stirring at 1000 rpm for 30 min.[11] Later, MFH was added to this polymeric mixture keeping the drug concentration constant, i.e., 100 mg in each formulation. The homogenization of the suspension was carried out for 20 min at 1000 rpm speed and debubbled by ultrasonication. This mixture was then taken in a dispensable syringe with a No. 22 needle and added dropwise to the solution of cross-linker, i.e., $CaCl_2$ (10%), for curing reaction completion. The rigid microspheres formed were kept in the $CaCl_2$ solution for 30 min, filtered, and washed with distilled water twice. The prepared COM-Na-Alg microspheres were oven-dried at 40 °C for 24 h and stored in a desiccator for further use.[24]

2.5. Experimental Design. An essential criterion in drug delivery is adequately improving and optimizing the experimental design while enhancing the practical drug delivery carrier fabrication efficiency. For formulation optimization of COM/Na-Alg microspheres, a 3^2 central composite design (CCD) was employed with COM (X_1, 100−300 mg) and Na-Alg (X_2, 100−300 mg) polymer concentrations chosen as

https://doi.org/10.1021/acsomega.2c07789
ACS Omega 2023, 8, 5925−5938

Table 1. Independent Variables and Responses Observed in CCD[a]

		independent variables		dependent responses		
run	formulation codes	X_1Na-Alg (mg)	X_2 COM (mg)	Y_1 PS (mm)	Y_2 DEE (%)	Y_3 R_{12h} (%)
1	F1	100 (−1)	200 (0)	0.79	74.7	57
2	F2	300 (+1)	100 (−1)	0.85	78	51
3	F3	200 (0)	200 (0)	0.91	80.7	62
4	F4	300 (+1)	200 (0)	0.95	86.9	47
5	F5	100 (−1)	300 (+1)	0.81	79.93	55.7
6	F6	300 (+1)	300 (+1)	0.96	84.7	53
7	F7	200 (0)	100 (−1)	0.87	69	65.7
8	F8	100 (−1)	100 (−1)	0.73	60.7	64.8
9	F9	200 (0)	300 (+1)	0.96	81	61

[a]Na-Alg: sodium alginate; COM: *Cydonia oblonga* mucilage; PS (mm): mean particle size (mm); DEE (%): drug encapsulation efficiency; and R_{12h} (%): cumulative drug release for 12 h. Data expressed as mean ± S.D.; n = 3.

independent factors by utilizing the published literature and preliminary trials.[14,25] These factors were varied at three levels, i.e., −1, 0, and 1, for low, medium, and high levels, respectively, whereas the particle size (mm) (PS/Y_1, mm), drug encapsulation efficiency (DEE%/Y_2), and cumulative drug release (%) at 12 h (R_{12h}/Y_3) were used as dependent variables (Table 1). The statistical design model and numerical optimization were generated using Design Expert Software (DES) (10.0.1.0), while ANOVA was applied for drug model significance estimation ($p < 0.05$).[26] A generalized quadratic mathematical model was utilized for evaluating the relation between dependent and independent variables.[27]

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2$$

2.6. Mean Particle Diameter as Y_1. An optical microscopy technique was adopted to calculate the mean particle diameter (PS) in mm. For it, 50 random microspheres were selected from each formulation, and PS in mm was examined via an optical microscope (Olympus, Tokyo, Japan) accompanied with an ocular micrometer with calibration via a stage micrometer.[28]

2.7. Drug Encapsulation Efficiency (DEE%) as Y_2. It is essential that the technique utilized for microencapsulation can incorporate an increased amount of the drug. The technique often used is the dissolution of microspheres in buffer solution followed by measurement of the drug with spectroscopic methods or the nonencapsulated drug with the measurement from the supernatant by the degradation of the microspheres via centrifugation. For assurance of drug encapsulation effectiveness of the antidiabetic load, 50 mg of microspheres was taken from all formulations and ground with the assistance of a mortar and pestle. The ground powder of MFH-loaded COM/Na-Alg microspheres was taken in a 250 mL volumetric cup containing 7.4 pH phosphate buffer (PBS), kept for 24 h, and shaken once in a while at 37 ± 0.5 °C. After the specified time, the COM/Na-Alg blend was mixed (speed = 500 rpm; time = 20 min) using a magnetic stirrer and sifted via Whatman filter paper No. 40. The drug's amount in the filtrate was determined using a UV−Vis spectrophotometer (λ_{max} = 233 nm) by the following equation.[29]

$$\text{DEE (\%)} = \frac{\text{actual drug content in microspheres}}{\text{theoretical drug content in the microspheres}} \times 100$$

2.8. *In Vitro* Drug Release as Y_3. The *in vitro* release of MFH from each formulation was analyzed using a dialysis apparatus USP-II (Pharm max. TEST). For this, an accurately weighed quantity, i.e., 100 mg of microspheres, was placed inside the dialysis apparatus cell, which contained 900 mL of acidic buffer solution of 0.1 N HCl (hydrochloric acid) with pH 1.2 for a time duration of 2 h. Afterward, the release was continued in basic medium (0.1 M phosphate buffer with pH = 7.4) till 12 h with an aliquot (5 mL) taken out of the apparatus for analysis and replacing it with an equal amount of buffer. The aliquot was filtered via a filter membrane of 45 μm. Using a UV-spectrophotometer, the absorbance of the sample was measured at a wavelength of 233 nm.[14]

2.9. Optimization of the Formulation. The desirability approach was used to optimize the microsphere formulations mathematically and graphically. The model was established using DES to attain an appropriate set of independent factors for optimizing all responses. The value constraints, i.e, maximum, minimum, and in the range, were selected for the calculation of desirability/D, and a desirability graph was obtained as a result, where D is the dependents' variable mean, suggested by software. The parameters that provided minimum PS (mm), highest DEE (%), and maximum R_{12h} were selected. Additionally, the comparison of the actual and predicted values was carried out to acquire the best PS (mm), DEE (%), and R_{12h} (%) for the successful optimization of COM/Na-Alg microspheres.[30]

2.10. Evaluation of the Optimized Formulation.

2.10.1. Micrometric Property Analysis. For characterizing the micrometric properties of the optimized formulation, bulk density/ρ_b, tapped density/ρ_t, Hausner's ratio, % compressibility, and the angle of response were analyzed. For this, ρ_b of the optimized formulation was resolved, i.e., quotient of the weight to the bulk volume of every cluster. The bulk density was calculated as the quotient of weights to the microspheres' volumes followed by tapping 100 times the measuring barrel containing the samples for determining the tapped volume, which in turn led to ρ_t calculation. Hauser's ratio was determined as the proportion of bulk density to the mass density, while % compressibility was determined as the proportion between the ρ_b and mass density to the ρ_t. The microspheres were passed from the funnel fixed in a stand. Microspheres fell from 6 cm height, i.e., the distance between the funnel top and surface, forming a heap at the bottom. The radius and height of the heap were determined for measuring the angle of response/θ with all values taken in triplicate and then compared with the standards to assess the micrometric properties.[31]

$$\tan\theta = \frac{h}{r}$$

where θ = angle of response; h = height, and r = radius of the heap.

2.10.2. Morphology Analysis. The surface morphology of the optimized MFH-loaded COM/Na-Alg microspheres (F-O) was examined by scanning electron microscopy/SEM (JEOL/JSM-6100, Japan). For this, the microspheres were mounted on a brass stub using double-sided adhesion tape.

https://doi.org/10.1021/acsomega.2c07789
ACS Omega 2023, 8, 5925−5938

Sputtering of the sample with a thin gold layer for a duration of 75 s for electrical conduction activation at 20 kV was done, and the microspheres' morphology was analyzed.[32]

2.10.3. Fourier Transform Infrared Spectroscopy (FTIR). The MFH-loaded COM/Na-Alg microsphere samples were ground to a fine powder form and mixed with potassium bromide/KBr i.e., 2 mg of powdered formulation to 200 mg of KBr to prepare pellets. The pellets were placed in a sample holder and analyzed by FTIR in the wavelength range of ∼4000−400 cm^{-1}. Similarly, the individual spectra of Na-Alg, COM, and MFH were taken to determine the interaction between the drug and excipient polymers.[17]

2.10.4. Investigation of the In Vitro Drug Release and Kinetic Mechanism. The *in vitro* drug release from the F-O COM/Na-Alg microspheres was studied using a USP-II dissolution apparatus (Pharma max. TEST). An accurately weighed amount (100 mg) of F-O microspheres was added to vials of a dissolution apparatus enclosing 900 mL of acid buffer (0.1 N HCl; pH = 1.2). The analysis was initially performed in an acidic buffer for 2 h, after which the test was continued in PBS (pH = 7.4) for 12 h. Next, 5 mL of the sample was taken out from the dissolution apparatus at fixed intervals with subsequent replacement with the same volume (5 mL) of freshly prepared PBS. Sample filtration with a 0.45 μm membrane filter was done followed by the measurement of the absorbance of the filtered samples at max 233 nm using a UV−Vis spectrophotometer.[33]

Furthermore, to evaluate the *in vitro* discharge behavior of MFH from the optimized COM-alginate microspheres, it is essential to fit the release data into reasonable kinetic models, i.e., zero, first, Higuchi, Korsmeyer−Peppas, and Hixson−Crowell models. For this, the *in vitro* drug release information was assessed dynamically in all kinetic models. The precision of these models was compared by calculation of the squared correlation coefficient/R^2 in addition to zero with first Higuchi and Hixson−Crowell constant, i.e., k_0, k_1, k_H, and k_{HC}, calculation using DD-Solver software.[34] Additionally, the Korsmeyer−Peppas model was used in the *in vitro* drug release performance study of F-O microspheres to determine the release mechanisms, i.e., Fickian release with $n \leq 0.43$ (diffusion-controlled release), non-Fickian discharge with n between 0.43 and 0.85 (anomalous transport), and case-II transport with $n \geq 0.85$ (relaxation-controlled release).[35]

2.10.5. Swelling Behavior Estimation. Swelling behavior assessment of MFH-loaded COM/Na-Alg microspheres was accomplished in basic medium (PBS; pH = 7.4). The COM/Na-Alg-based optimized formulation (50 mg) was added in vessels of a disintegration mechanical assembly (Pharma max, Germany) containing 500 mL of separate medium. The analysis was made at 37 ± 1 °C with a paddle speed of 50 rpm. The swollen microspheres were taken out of the medium after preset intervals, dried, and weighed.[13]

2.10.6. Ex Vivo Mucoadhesive Analysis. The *ex vivo* mucoadhesive property of the F-O microspheres was assessed by an *in vitro* wash-off test.[36] A 1 cm-by-1 cm bit of each mouse gastric mucosa was knotted on a glass slide (2 in./2 in.) with a string. The microspheres were spread onto a wet-washed tissue specimen, and the slide was clung to one of the cells of a USP-tablet disintegrating apparatus. The disintegrating test mechanical apparatus was worked with the end goal of recurrently moving the tissue specimens up and down in measuring cells containing the simulated gastric liquid USP with pH = 1.2 and 7.4, respectively. After 30 and 60 min and at hourly intervals up to 12 h, the microspheres that were still adhered to the tissue were tallied, to determine the mucoadhesiveness of the F-O microspheres.

2.10.7. Toxicology Analysis. The acute oral toxicity of the optimized microsphere (F-O) was characterized via the "acute toxic class method" according to the OECD (Guidelines for Economic Cooperation and Development).[37] The approval of the complete experimental procedure was obtained from the Biosafety and Ethical Review Committee of the University of Sargodha under the reference number ref no. 143-2021/PREC/UOS. For toxicology studies, healthy Wistar mice weighing 20−30 g were housed in an animal house in clean cages under a 12 h cycle (light/dark) for acclimation with a standard water and diet supply for 2 weeks. A random division of animals was done into control and treated groups (n = 3). The treated group was orally administered 2 g/kg of body weight COM/Na-Alg microspheres, while the control group received only standard rat feed and water. Two times-a-day observation of the mice for 14 days for signs of ailment and mortality was made. Parameters such as body weight and food and water intake were examined on the 1st, 7th, and 14th days. At the end of the trial, blood samples were taken for hematological and biochemical studies. Finally, the mice were sacrificed and the vital organs were removed for histological observations, weighed, and stored in a 10% V/V solution. Tissues were sectioned to prepare slides for microscopic examination.[38]

2.10.8. Pharmacodynamic Analysis. For pharmacodynamic analysis, alloxan-induced diabetic male Wistar mice of either sex with weight ranging between 31 and 36 g were used. The mice were made to fast for 1 day with H_2O-added libitum. All trials were done between 8 A.M. and 12 P.M. to limit quotidian impacts. For clearance, the trial convention was put through the examination of the Institutional Animal Ethical Council. The mice were taken care of according to the rules of the PREC (Pharmacy Research Ethics Committee). The mice were made diabetic by interperitoneal administration of alloxan at 150mg/kg dose in citrate buffer (2 mM; pH = 3.0). After administrating alloxan for 7 days, the alloxanized mice with a fasting blood glucose level of 300 mg/dL or more were deemed as diabetic and used in a 12 h examinational period. After the initial collection of the blood samples from the trial mice, the mice were randomly divided into two groups, i.e., control and treated groups, five in each group, with the former one being administered 100 mg of pure MFH per kg of body weight and the latter one being given a similar amount of MFH-loaded COM/Na-Alg microspheres (F-O) via oral administration. Next, 0.1 mL samples were taken out from the tail of every mouse at specific intervals under gentle anesthesia and studied via the oxidase/peroxidase technique for assessing the glucose range fluctuation using a marketed kit. After oral administration of pure MFH and F-O microspheres, the relative *in vivo* glucose concentration in alloxan-instigated diabetic mice was evaluated.[29]

3. RESULTS AND DISCUSSION

3.1. Extraction of COM. In the present study, the seeds of *C. oblonga* were soaked in distilled water for 4−5 h, and mucilage was extracted by some mechanical process. The mucilage was precipitated from the resin in the solvent (water and ethanol). The percentage of the yield of gel was found to be 38.4%.

https://doi.org/10.1021/acsomega.2c07789
ACS Omega 2023, 8, 5925−5938

3.2. Physicochemical and Rheological Characterization. The physicochemical, micrometric, and mechanical evaluations, shown in Table 2, support the binding capability, appreciable compressibility, and flow properties of the oven-dried COM.[14,39]

Table 2. Physicochemical and Rheological Characteristics of COM

serial no.	parameters	results
1	solubility in water	soluble
2	solubility in non-polar solvents	insoluble
3	swelling index (%)	21 ± 1.6
4	pH	6.1 ± 0.2
5	total ash percentage (%)	3.1 ± 0.14
6	bulk density (g/cm^3)	0.8 ± 0.01
7	Carr's index (g/cm^3)	2.4 ± 0.04
8	angle of repose	22 ± 1.01
9	Hauser's ratio	1.1 ± 0.03

3.3. Preparation of MFH-Loaded COM/Na-Alg Microspheres. The MFH-loaded COM/Na-Alg microspheres were formulated using $CaCl_2$ as a cross-linking agent by ionic gelation. The presence of calcium ions for accommodating the gelation of the polymeric blend is due to the multivalent cation-induced ionic gelation potential of Na-Alg. The method used to ensure the COM/Na-Alg gelation has been applied previously for several polysaccharides, for instance, chitosan, pectin, etc. The ionic gelation characteristics of Na-Alg are due to the formation of double-helical junction areas, multivalent cationic metal exchange, and its hydrogen bonding of water (H_2O). In the present work, we prepared mucoadhesive COM/Na-Alg ionically gelled microspheres for sustained release of MFH. COM was used as a mucoadhesive polymer and cross-linked with Na-Alg to overcome the latter's defects in varying ratios, and $CaCl_2$ aided in the formation of compact microspheres by penetrating inside the droplets, which resulted in squeezing out of H_2O from the polymeric matrix.

3.4. Experimental Design and Optimization. The most critical parameter in pharmaceutical design is the minimalistic trials. To overcome the time consumption and inadequate optimization in conventional processes, the central composite design seems to be an effective statistical tool. It encompasses individual factors while correlating them with each other and dependent responses too. In the present study, nine runs of COM/Na-Alg microspheres were suggested by Design Expert Software/DEE software (version 8.0.6.1). The effects of variation in polymer concentration on PS (mm), DEE (%), and R_{12h} (%) were investigated, as shown in Table 1. One-way ANOVA was applied for the evaluation of statistical models (Tables 3 and 4). The further elucidation of factors with varying levels of variables/response was made using 3D response surface graphs and 2D contour graphs using response surface methodology (RSM), as shown in Figure 1.[40]

Table 4. Summary of ANOVA Response Parameters

dependent variables	actual R^2	predicted R^2	adjusted R^2	PRESS
PS (mm)	0.984	0.865	0.958	0.007
DEE (%)	0.998	0.979	0.995	11.07
R_{12h} (%)	0.985	0.868	0.96	43.28

3.5. Mean Particle Size as Y_1. The mean particle size (PS) in mm of MFH-loaded COM/Na-Alg microspheres (F1−F9) was in the range of 0.81 ± 0.05−1.29 ± 0.15 mm (Table 1). The equation and Figure 1 were acquired from DDS with coded coefficients.

$$\mathrm{PS\ (mm)} = 0.92 + 0.07X_1 + 0.046X_2 + 0.007X_1X_2 - 0.06X_1^2 - 0.02X_2^2$$

The equation showed that an increase in particle size is greatly influenced by the polymer blend solution. This can be attributed to the relative viscosity accruing, leading to large microparticle formation upon adding a polymeric dispersion to the gelating agent solution.[41] In addition, an increase in the polymer ratio can lessen the free sites available for the cross-linker; hence, the COM/Na-Alg concentration was incited.[42]

3.6. Drug Encapsulation Efficiency as Y_2. The drug encapsulation efficiency (DEE) of the MFH-loaded COM/Na-Alg formulations varied between 60 ± 1.2 and 86.9 ± 5.2% w/w (Table 1). The equation and 2D and 3D graphs (Figure 2) obtained from DDS were used to establish the relation between the concentration of polymers and DEE (%).

$$\mathrm{DEE\ (\%)} = 80.37 + 5.71X_1 + 6.32X_2 - 3.13X_1X_2 + 0.58X_1^2 - 5.21X_2^2$$

The mathematical relation showed that an increase in the concentration of polymers, i.e., Na-Alg and COM, greatly influenced drug encapsulation within microspheres, as their high content caused the formation of a highly viscous cross-linked polymeric matrix. Moreover, a lesser increase in the encapsulation degree was observed by increasing the Na-Alg amount in comparison to that of COM due to more hydration and weaker electrostatic bonding.[43]

3.7. *In Vitro* MFH Release from COM/Na-Alg Microspheres as Y_3. The *in vitro* cumulative drug release (%CDR) of all formulated COM-Na-Alg microspheres showed a prolonged release of MFH for a time frame of 12 h. The

Table 3. ANOVA Results for Responses

	terms			responses					
	PS (mm)			DEE (%)		R_{12h} (%)			
source	sum of squares	F value	p value	sum of squares	F value	p value	sum of squares	F value	p value
quadratic model	0.0534	38.04	0.0065	529.79	317.46	0.0003	324.38	39.85	0.0061
X_1	0.0308	109.84	0.0019	195.74	586.46	0.0002	117.04	71.89	0.0034
X_2	0.0131	46.57	0.0064	239.78	718.42	0.0001	23.21	14.25	0.0325
$X_{1\times2}$	0.0002	0.802	0.4365	39.25	117.6	0.0017	30.8	18.92	0.0224
X_1^2	0.0084	30.12	0.0119	0.6923	2.07	0.2454	132.85	81.6	0.0029
X_2^2	0.0008	2.85	0.1899	54.32	162.76	0.001	20.48	12.58	0.0382

https://doi.org/10.1021/acsomega.2c07789
ACS Omega 2023, 8, 5925−5938

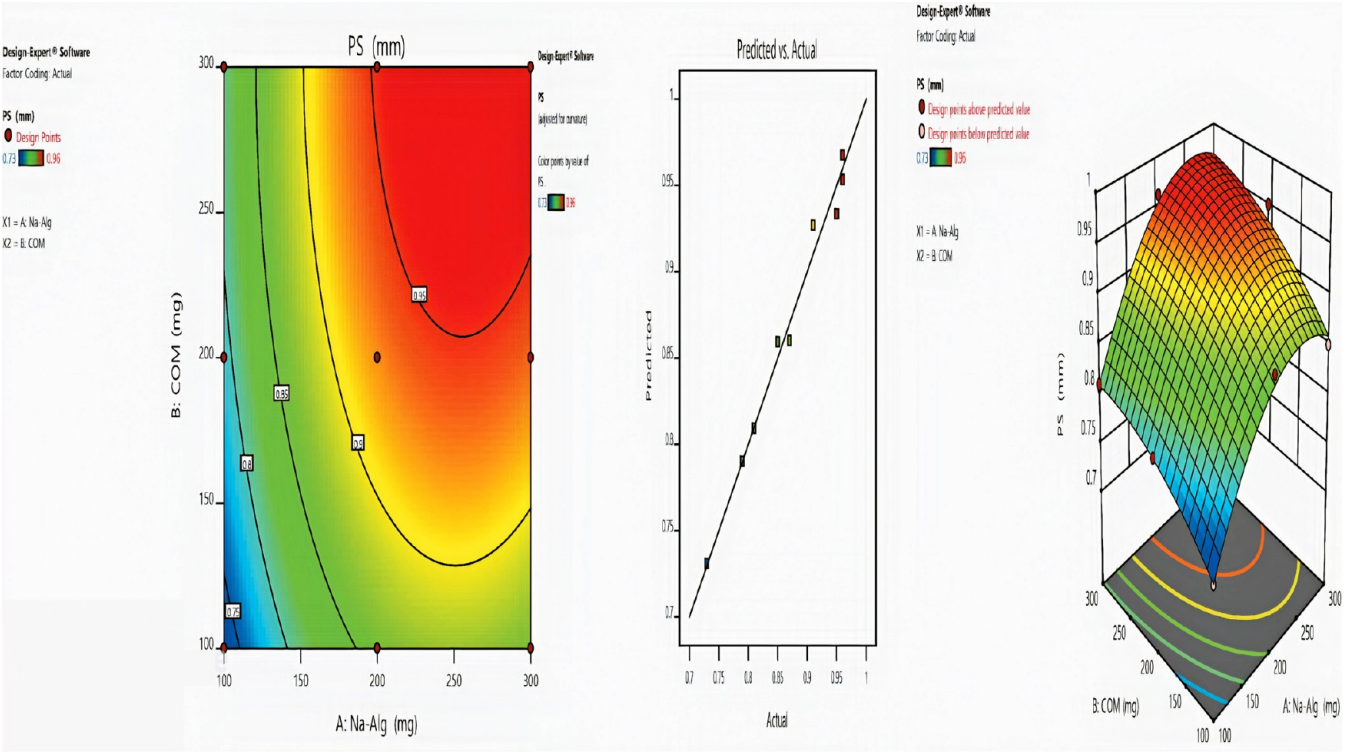


Figure 1. 3D response surface, predicted vs actual, and 2D contour graphs illustrating the influence of COM and Na-Alg content on PS (mm) of COM/NA-Alg microspheres.

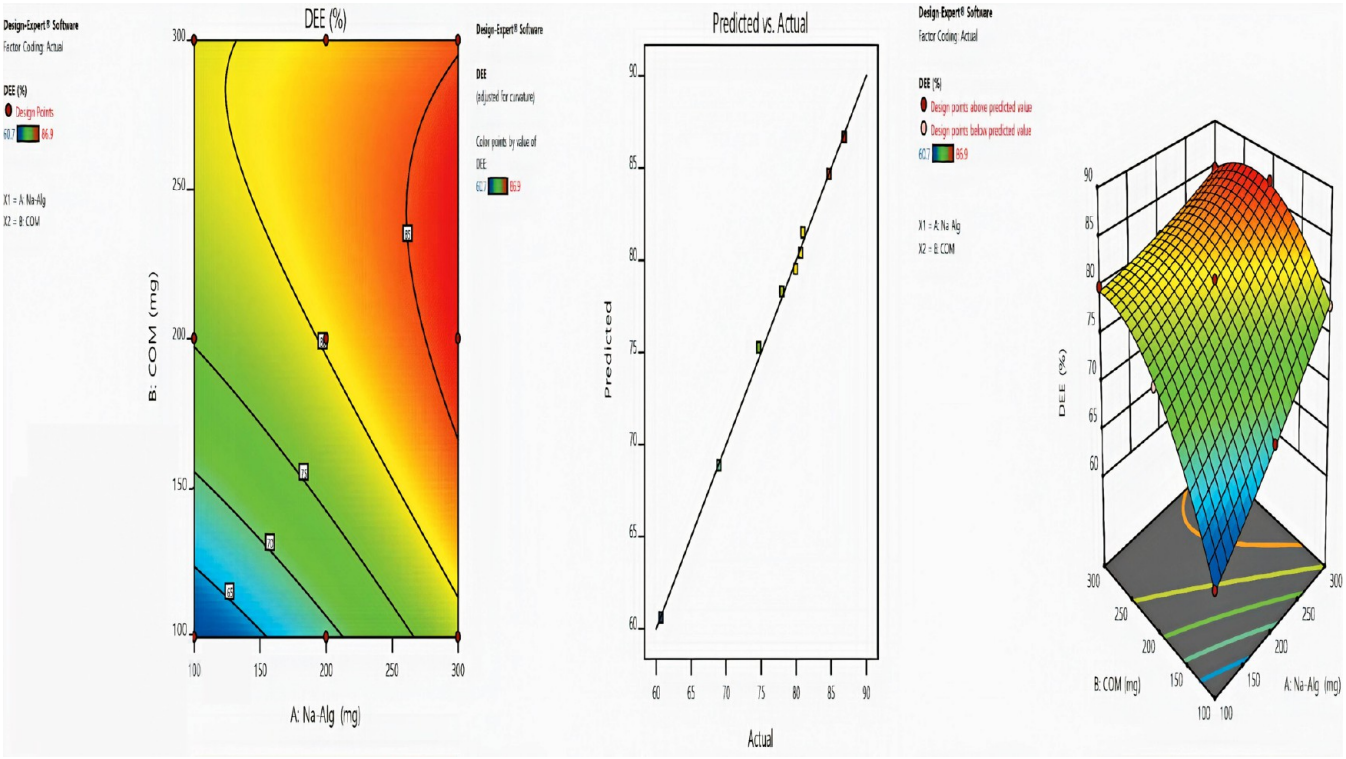


Figure 2. 3D response surface, predicted vs actual, and 2D contour graphs illustrating the influence of COM and Na-Alg content on the DEE (%) of COM/NA-Alg microspheres.

release was carried out in both acidic dissolution medium (0.1 N HCl at pH = 1.2) and basic medium (PBS at pH 7.4). The %CDR after 12 h (R_{12h}) varied in a range of 47 ± 1.12−65.7 ± 2.32. The equation and 2D and 3D graphs (Figure 3) constructed using DDS revealed that an increase in the polymer ratio caused a decrease in R_{12h}; however, this decrease is more pronounced with the Na-Alg increase.

https://doi.org/10.1021/acsomega.2c07789
ACS Omega 2023, 8, 5925−5938

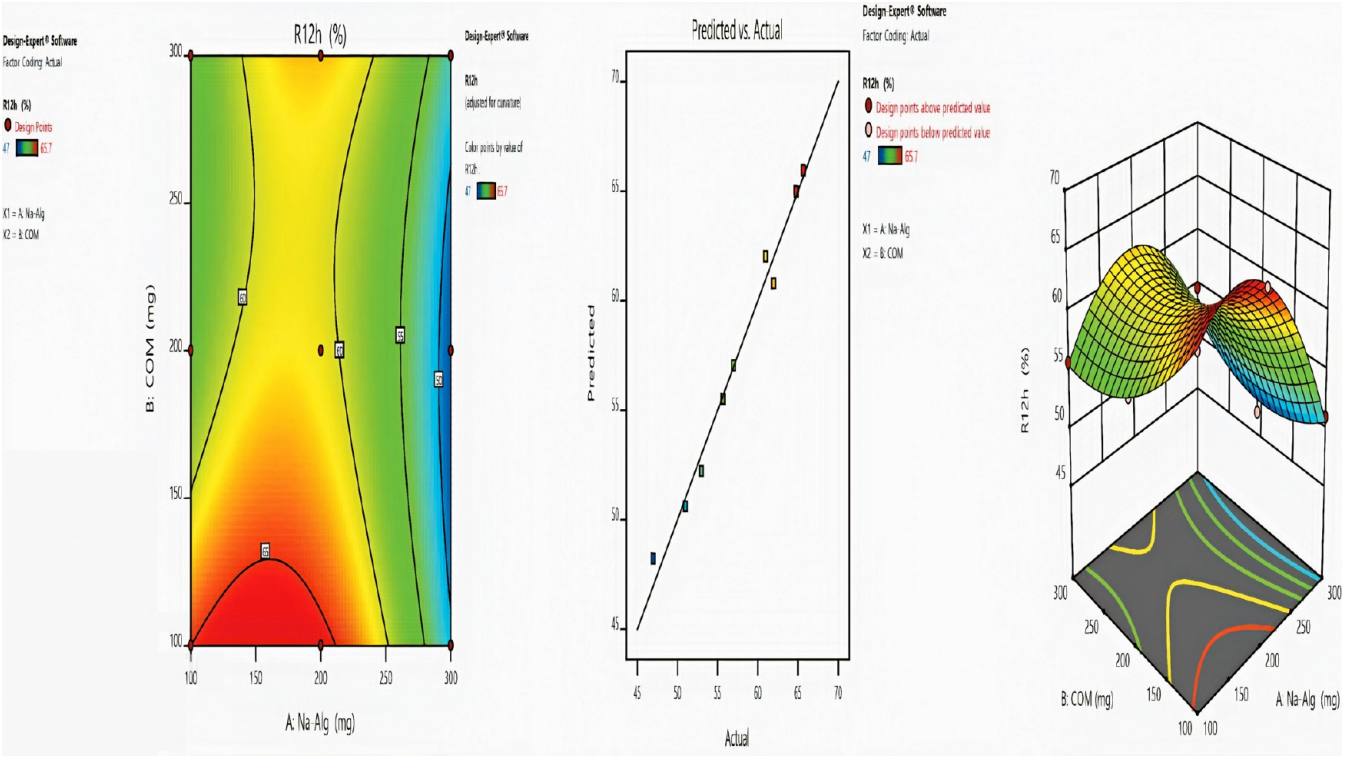


Figure 3. 3D response surface, predicted vs actual, and 2D contour graphs illustrating the influence of the COM and Na-Alg content on on R_{12h} (%) of COM/NA-Alg microspheres.

Table 5. Constraints of Independent and Dependent Factors for Optimizing the COM and Na-Alg Concentrations

factors	goal	lower limit	upper limit
	Constraints		
X_1: Na-Alg	in range	100	300
X_2: COM	in range	100	300
	Responses		
PS (mm)	in range	0.8	0.96
DEE (%)	in range	60.7	86.9
R_{12h} (%)	in range	47	65.7

Optimized Formulation with Response Values											
			PS (mm)			DEE (%)			R_{12h} (%)		
number	Na-Alg (mg)	COM (mg)	predicted value	actual value	% error	predicted value	actual value	% error	predicted value	actual value	% error
F-O	295	218	0.954	0.96 ± 2.45	0.62	86.7	85.8 ± 1.67	1.04	49.36	48.23 ± 3.23	2.34

$$R_{12h}\,(\%) = 60.77 - 4.42X_1 - 1.97X_2 + 2.77X_1X_2 - 8.15X_1^{\,2} + 3.20X_2^{\,2}$$

A comparatively sustained release was attained by augmenting the COM concentration; this is due to COM's hydrophilic potential that allows the polymer to interact with water effectively, forming a viscous structure that consequently blocks microspheric surface pores.[14,44]

3.8. Formulation Optimization. For attaining an optimized formulation with the desired responses, the desirability approach was utilized. The independent factors (X_1 and X_2) were constraints between 100 and 300 mg, where response values were restricted to the range of $0.8 \le$ PS (mm) ≤ 0.96, $60.7 \le$ DEE (%) ≤ 86.9, and $47 \le R_{12h}$ (%) ≤ 65.7 (Table 5). The optimized formulations were devised with the quadratic model using DDE-version 11 software, and the selection of one formulation among them, i.e., F-0, was based on the desirability/*d* value. The optimized formulation, i.e., F-O was formulated using the ionic gelation technique, and dependent responses were evaluated (Tables 3 and 4). The actual and predicted values of PS (mm), DEE (%), and R_{12h} of F-O were analyzed to be 0.96 ± 2.45, 85.8 ± 1.67, and 48.23 ± 3.23, respectively, and compared to values predicted using DDE software. The percentage error was determined for describing the applicability domain of optimizing the mathematical model. The % error values, i.e., 0.62, 1.04, and 2.34, revealed the suitability of the quadratic model obtained by 32-factorial design.[30,45]

3.9. Evaluation of Microspheres. *3.9.1. Micrometer Properties of the Microspheres.* The micrometric characterization, i.e., bulk density/p_b, tapped density/p_t, Hausner's ratio, Carr's compressibility index, and response angle, was carried out. The p_b and p_t were analyzed to be 1.134 ± 0.054 and 1.146 ± 0.037 g/cm^3, respectively, whereas Carr's compressi-

https://doi.org/10.1021/acsomega.2c07789
ACS Omega 2023, 8, 5925−5938

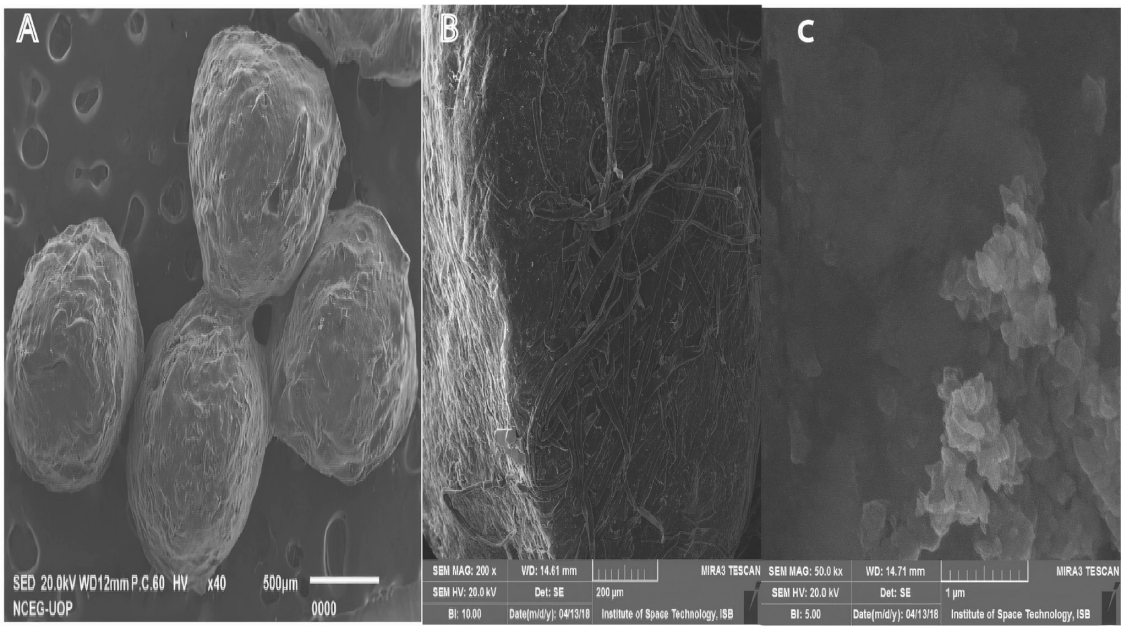


Figure 4. Scanning electron micrograph; (A) microspheres with a 500 μm scale and 12 mm working distance; (B) microspheres with a 200 μm scale and 14. 01 mm working distance; and (C) microspheres with a 1 μm scale and 14.71 mm working distance.

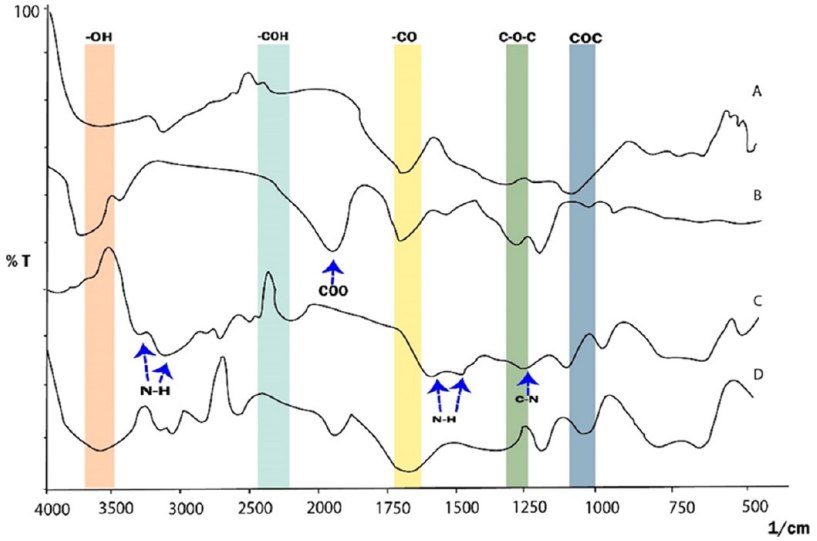


Figure 5. FTIR spectra of (A) COM; (B) Na-Alg; (C) MFH; and (D) MFH-loaded COM/Na-Alg.

bility index was found to be 10.47%, indicating satisfactory flow characteristics. Hausner's ratio and angle of response were calculated to be 1.03 ± 0.012 and 28.34 ± 2.56°, respectively. All the parameters analyzed indicated excellent compressibility and flow properties.[14]

3.9.2. Scanning Electron Microscopy. Scanning electron microscopy (SEM) of the surface of MFH-loaded COM/Na-Alg microspheres showed a rough surface morphology with characteristic large wrinkles and cracks (Figure 4). These splits and wrinkles may be caused by the partial collapse of the polymer network amid drying.[46] The COM-Nail debris, in a very small amount, can be seen on the microsphere surface due to simultaneously preparing and forming the polymeric dispersion, whereas the presence of MFH crystals on the surface of the microspheres can be attributed to perhaps the migration of the drug with water during drying.[47]

3.9.3. FTIR Spectroscopy. The FTIR spectra of COM, Na-Alg, MFH, and drug-loaded COM-alginate microspheres were used to characterize the chemical structure of the formulated microspheres. The Na-Alg FTIR spectra showed peaks at 3540, 2350, 1640, 1440, and 1110 cm^{-1}, which were due to the −OH stretch, COO− asymmetric, COO− symmetrical stretch, and COC group presence, respectively.[14] The isolated COM FTIR spectra presented characteristic peaks at 3530, 2470, and 1710 cm^{-1} for −OH, −COH, and CO, respectively. The peaks in the region of 1200−1000 indicate glycosides linked to COM.[17] The FTIR of MFH displayed the peaks at 3936.12, 3784.23, 3278.65, and 3172.73 cm^{-1} representing 1° N−H stretching vibrations, while 1565.54 and 1067.67 cm^{-1} indicated the presence of 1° amine bending and nitrile functional group vibrations, respectively.[14,48] In the FTIR range of COM-alginate microspheres containing MFH, different representative peaks of Na-Alg, COM, and MFH were visible without any

https://doi.org/10.1021/acsomega.2c07789
ACS Omega 2023, 8, 5925−5938

significant shift of these peaks, proposing that there was no association between the MFH and the polymers utilized (Na-Alg and COM) as shown in Figure 5.

3.9.4. Evaluation of the Swelling Behavior. Swelling is considered the most important parameter of microspheres, as it helps determine the rate of drug release from drug excipients. The swelling behavior of the optimized COM/Na-Alg microspheres was investigated at pH 1.2 and 7.4 for 12 h at 37 °C. In acidic medium, the swelling index was low, mainly due to the shrinkage of Na-Alg, whereas the swelling index augmented in PBS (pH = 7.4) because of the dissolution of the polymeric matrix after 5−6 h (Figure 6). In accordance with

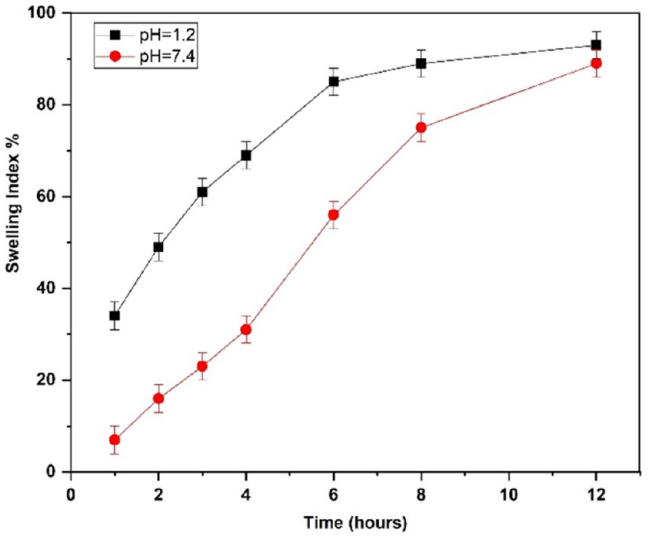


Figure 6. Swelling index of the optimized batch at pH = 1.2 in 0.1 N HCl and pH = 7.4 in PBS with values presented as $n = 3 \pm$ S.D.

the previously reported literature, this behavior is in agreement with the fact that a high polymer concentration aids in a tight and dense network formation, allowing dipole interactions with the drug-loaded microsphere's polymeric constituents. Interactions like these halt the acidic solution from penetrating the microsphere.

However, the microspheres swelled comparatively more when exposed to an intestinal PBS environment at pH 7.4, owing to the Ca^{2+} and Na^{+} ion exchange, as PO_4^{3-} acts as a calcium sequestrate, resulting in erosion and disintegration of the swollen COM/Na-Alg ionotropically gelled microspheres. These findings suggest that COM/Na-Alg microspheres slightly swell in the gastric environment and the swelling index increases as these microspheres travel subsequently to an upper intestinal region where MFH absorption occurs.[49]

3.9.5. In Vitro Release and Kinetic Evaluation. The *in vitro* release and kinetics of the optimized formulation were studied as shown in Figure 7. The drug discharge was found to be comparatively low in acidic buffer, i.e., less than 10% at the initial 2 h. The quantity release might be due to the drug molecules adhering to the microsphere surface. Contrary to this, the microspheres swelled rapidly in alkaline medium, leading to an effective drug release of 47.48 ± 1.25. This phenomenon could be due to the high swelling index and high solubility of polymer blends, permitting drug release. Also, the hydrophilic characteristics of COM might have resulted in the formation of a viscous gelating network on contacting water, which would have acted as a partial barricade to the

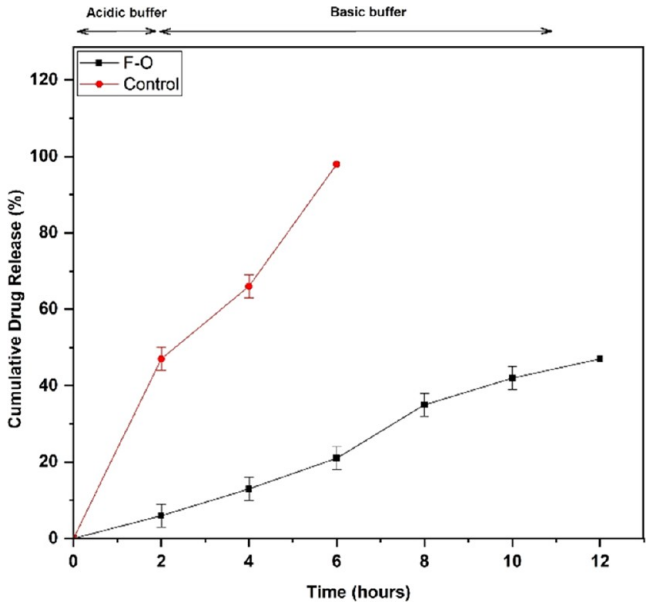


Figure 7. Cumulative drug release (%) of control/marketed drug and MFH-loaded COM/Na-Alg optimized formulation for 12 h; mean ± SD and $n = 3$.

microsphere's surface pores, permitting a sustained release of the drug.[50] Moreover, the %CDR of the control was calculated to be 98.45 ± 1.84 at 6 h. The release of the control shows that the drug has an immediate gastric release, which necessitates repeated dose administration,[51] whereas the release of COM/Na-Alg-encapsulated MFH was effectively controlled in acidic pH due to the formation of a dense matrix, which prevents water from penetrating within the carrier, which in turn protects the COM/Na-Alg matrix.[52] This advocates the use of COM/Na-Alg microspheres in comparison to the control (marketed as MFH) for prolonged drug release, which will be helpful in reducing the commercial MFH high concentration demand, reducing its gastric side effects.

Several release mechanisms have been suggested by researchers in previous years. It has been suggested that the drug is released from the polymer matrix via penetration of water followed by carrier swelling and drug diffusion from it or via erosion of the polymeric layer. The drug release kinetics of the optimized COM/Na-Alg microspheres (F-O) were studied by application of multiple well-known mathematical models, for instance, zero-order, first-order, Higuchi, Korsmeyer−Peppas, and Hixson−Crowell models; equations are presented in Table 6. With R^2 as the correlation coefficient, the release data were fitted in models depicted in Table 6. The zero-order model is utilized when the drug delivery module allows a slow release of the drug. The first-order model's kinetics are applied usually for porous matrix-based delivery vehicles, whereas the Higuchi model is used when the drug release solely follows a diffusion mechanism. The Hixson−Crowell model application to release data explain the change in the diameter of the drug release systems. The results showed that the above-mentioned models fit comparatively less to the experimental drug release.[53] The MFH experimental release from the COM/Na-Alg microspheres could be best described by the Korsmeyer−Peppas model, as R^2 values are closest to unity followed by the zero-order kinetic model.[14,54] All the data suggest that MFH controlled release from COM/Na-Alg, which is advantageous for provision of prolonged drug effects

https://doi.org/10.1021/acsomega.2c07789
ACS Omega 2023, 8, 5925−5938

Table 6. Kinetic Model Data of Cumulative Drug Release of the Optimized MFH-Loaded COM/Na-Alg Microspheres[a]

	zeroth-order model[b]		first-order model[c]		Higuchi model[d]		Krosmeyer−Peppasmodel[e]		Hixson−Crowellmodel[f]	
code	k_0	R^2	k_1	R^2	k_H	R^2	n	R^2	k_{HC}	R^2
F-O	4.83	0.92	0.059	0.851	12.58	0.646	1.47	0.978	0.015	0.8736

[a]Definitions: Q_t = amount of drug dissolved at time t; Q_0 = initial amount of drug in solution; k_0 = zeroth-order rate constant; k_1 = first-order rate constant; k_H = Higuchi model constant; M_t/M_∞ = drug fraction release at time t; k_r = release constant; n = release-mechanism-dependent exponent; k_{HC} = Hixson−Crowell model constant. [b]Zeroth-order model: $Q_t = Q_0 + k_0t$. [c]First-order model: $\log Q_t = \log Q_0 - k_1t/2.303$. [d]Higuchi model: $Q_t = k_Ht^{1/2}$. [e]Krosmeyer−Peppas model: $M_t/M_\infty = k_rt^n$. [f]Hixson−Crowell model: $Q_t^{1/3} = k_{HC}t^{1/2}$.

in addition to being useful in the improvement of patient compliance that is difficult to acquire in clinical practice with chronic ailments (e.g., diabetes).[2] Similar release kinetic results have been observed by various authors from time to time. Moreover, the value of "*n*" indicated that the release of MFH followed a super case-II transportation mechanism, mainly controlled by the COM/Na-Alg microsphere swelling and relaxation.[14]

3.9.6. Ex Vivo Mucoadhesive Studies. An *ex vivo* wash-off technique was used to examine the mucoadhesion capability of F-O microspheres with goat intestinal mucosa in both types of buffers, i.e., acidic medium (0.1 N HCl/pH = 1.2) and basic medium (PBS/pH = 7.4). The mucoadhesion results are presented in Figure 8, indicating that microsphere (F-O)

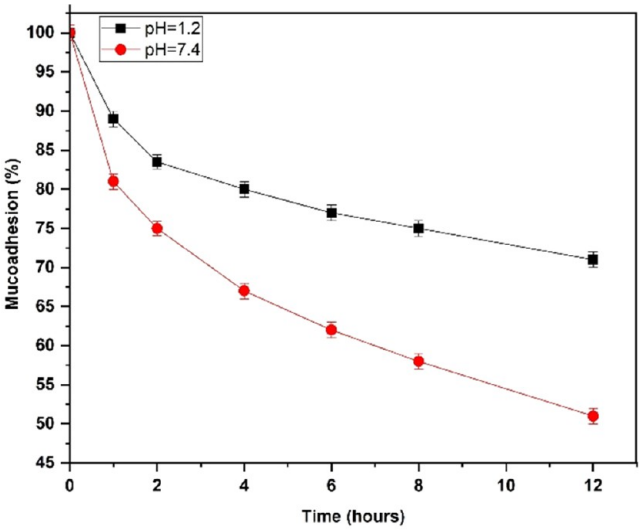


Figure 8. Mucoadhesive evaluation of the optimized (F-O) microspheres at pH = 1.2 in 0.1 N HCl and pH = 7.4 in phosphate buffer saline.

adherence to the intestinal mucosa showed a significant difference between gastric and intestinal pH varying from 71.01 ± 4.26 to 51 ± 3.57 over a time duration of 12 h. The presence of hydroxyl functionalities in both of the hydrophilic polymers could be credited for noncovalent interactions such as hydrogen bonding with mucin molecules, affirming an increase in the mucoadhesion of the optimized microspheres. Additionally, the rapid release of microspheres from mucus molecules in intestinal pH buffer could be due to the calcium ion erosion and carboxylic and other functionality ionization in the polymeric matrix.[18,55]

3.9.7. Acute Toxicity Analysis. Acute toxicity analysis was carried out to evaluate any toxic effect caused by COM/Na-Alg. The results showed the absence of toxicity in mice. No signs of mortality or any ailments were observed in either the F-O-treated or control groups throughout the time of the trial. Nonsignificant weight fluctuations were noted in either of the groups, as depicted in Figure 9. The vital organs were unaltered in all mice, and no significant variations were observed in either treated or control groups. Subsequently, after oral ingestion of MFH-loaded COM/Na-Alg, parameter changes related to food/H_2O consumption and variations in the total body weight were also noted in the F-O treated group in comparison to the control group (Figure 9). Values related to food/H_2O consumption in the blank COM/Na-Alg microsphere-administered group were slightly lower than those in the control one, which is related to gastric fullness from blank COM/Na-Alg microspheres (Figure 9).

3.9.8. Biochemical Studies. Biochemical analysis was performed in blank COM/Na-Alg microsphere-treated and control groups to examine the difference among the physiological conditions. Numerous biochemical factors were examined in both treated and control groups for corroborating normal hepatic, cardiac, renal, and spleen function ao as to affirm the safe use of COM/Na-Alg excipients (Figure 9).

3.9.9. Histological Studies. The organs were examined for the absolute weight calculation in F-O microspheres in treated and control groups (Figure 10A). No pathological changes were observed in any of the organs (Figure 10B), signifying the COM/Na-Alg formulation to be safe for oral administration as a MFH carrier.

3.9.10. Pharmacodynamic Evaluation. The pharmacodynamic study of the F-O-optimized microspheres containing MFH was carried out in alloxan-induced diabetic Wistar mice by estimating the lessening of the plasma glucose concentration. The *in vivo* plasma glucose content and mean glucose rate decrease after orally administrating pure MFH and MFH-loaded COM/Na-Alg microspheres, as presented in Figure 11. A fast diminishment in the plasma glucose level was seen for up to 3 h in the group administered with the pure drug, and from that point forward, the glucose level recuperated quickly toward the standard value. Contrary to it, the lessening in the plasma glucose level came to over 25−30% in the initial 1−2 h and stayed curtailed over a 30−35% decrease in the blood glucose level till 12 h with MFH-loaded COM/Na-Alg microspheres. A 25% decrease in the glucose level has been reported to have a substantial hypoglycemic impact.[56] Notably, there were critical contrasts between the glucose levels in the blood after the pure MFH and COM/Na-Alg F-O microspheres were administered correspondingly.

4. CONCLUSIONS

The ionic gelation technique was used to successfully prepare COM/Na-Alg microspheres for MFH delivery. The RSM based on 3^2 CCD was used for the optimization of the polymer concentrations used in MFH-loaded COM/Na-Alg microspheres. The optimized conditions were obtained with 215 mg of COM and 295 mg of Na-Alg. Furthermore, the predicted

https://doi.org/10.1021/acsomega.2c07789
ACS Omega 2023, 8, 5925−5938

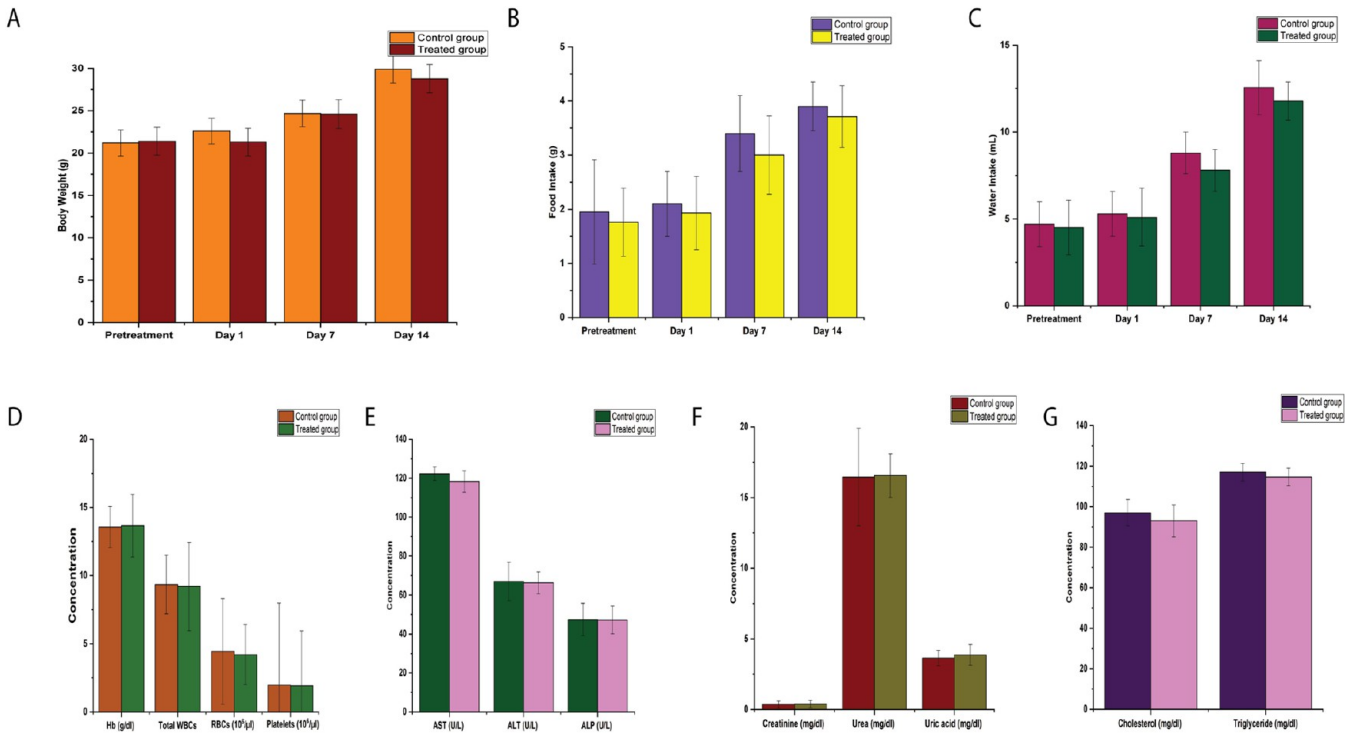


Figure 9. Effect of COM/Na-Alg microsphere in a Wistar mice model; (A) body weight (g); (B) food intake (g); (C) water intake (mL); (D) blood profile; (E) hepatic profile; (F) renal profile; and (G) cholesterol and triglycerides levels.

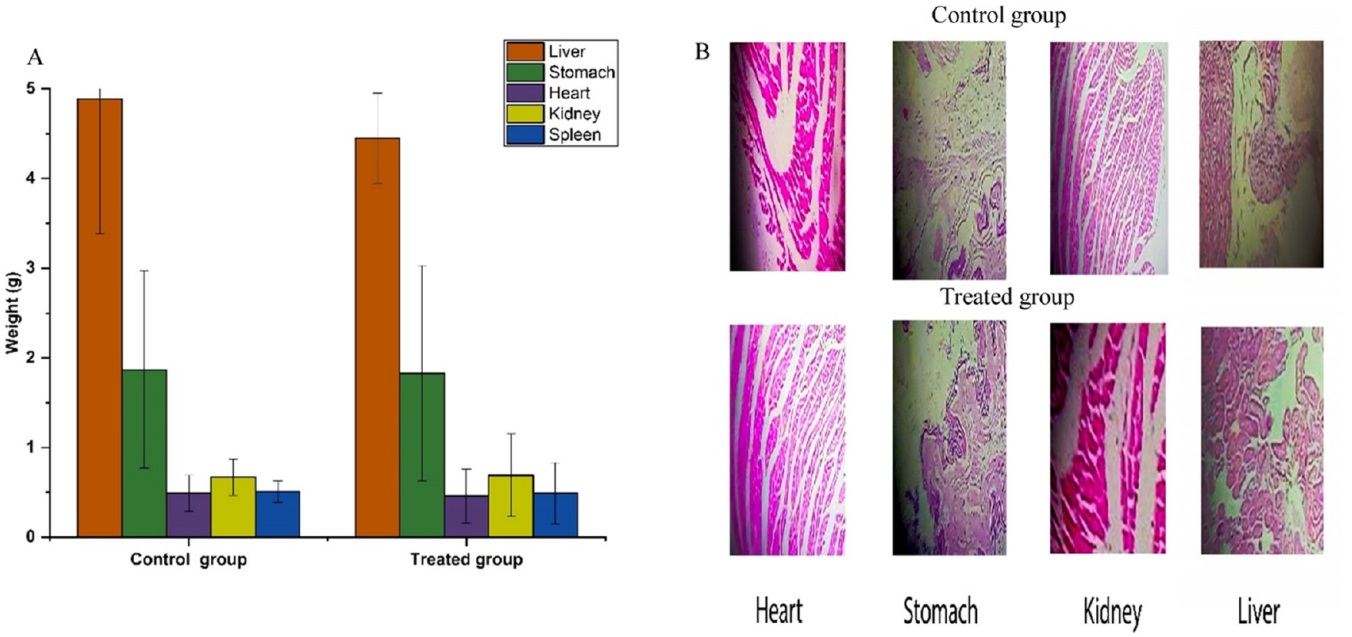


Figure 10. (A) Absolute organ weight and (B) microscopic images of the rat's liver, stomach, heart, kidney, and spleen from the control and treated group after COM-Na-Alg microsphere administration after 14 days.

and actual values of responses (PS (mm), DEE (%), and R_{12h} (%)) of the optimized formulation (F-O) were close to one another. The F-O microspheres were characterized by FTIR, and SEM for characterizing the morphology and structure of the microspheres. The results suggested a gradual gastric swelling index and upraised swelling intestinal behavior of the microspheres, affirming the potential of COM as a sustained and gastro-retentive drug delivery tool. The optimized F-O microspheres showed a significant mucoadhesive behavior with better encapsulation efficiency and yield. Our work suggested that COM/Na-Alg (F-O) can offer a sustained drug release for 12 h accompanied by glucose level maintenance by systematic absorption control of our model antidiabetic drug. We strongly believe that the natural polymer, i.e., COM, utilization for MFH will make its delivery more targeted, also enabling the prevention of stomach-based side effects. This natural polymer-based formulation will certainly be an efficacious

https://doi.org/10.1021/acsomega.2c07789
ACS Omega 2023, 8, 5925−5938

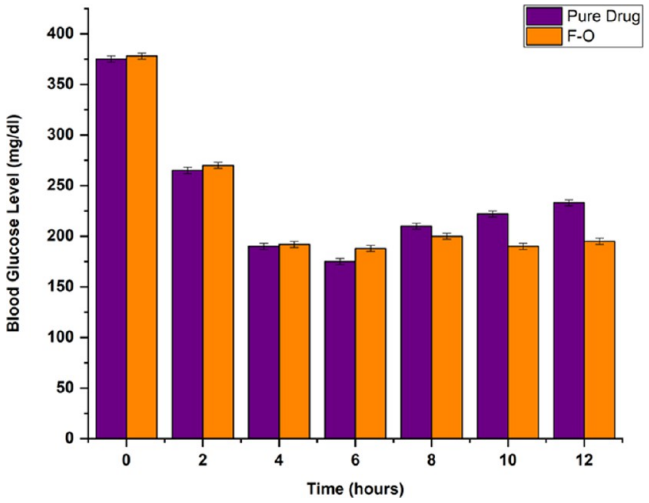


Figure 11. Glucose level *in vivo* analysis in alloxan-induced mice by orally administrating pure MFH and optimized COM-Na/Alg-encapsulated MFH. Values are calculated as $n = 6$ and presented as mean ± S.D. with bars denoting the significant difference ($p < 0.05$) between batches.

replacement for traditional diabetes medicines with a decreased dosage and limited dosing frequency as well.

AUTHOR INFORMATION

Corresponding Authors

Sobia Noreen − *Institute of Chemistry, University of Sargodha, Sargodha 40100, Pakistan;* orcid.org/0000-0002-0815-2223; Phone: +92301843400; Email: sobia.noreen@uos.edu.pk

Humaira Yasmeen Gondal − *Institute of Chemistry, University of Sargodha, Sargodha 40100, Pakistan;* orcid.org/0000-0001-9532-681X; Email: humaira.yasmeen@uos.edu.pk

Authors

Sara Hasan − *Institute of Chemistry, University of Sargodha, Sargodha 40100, Pakistan; Department of Chemistry, The University of Lahore, Sargodha 40100, Pakistan*

Shazia Akram Ghumman − *College of Pharmacy, University of Sargodha, Sargodha 40100, Pakistan*

Shoaib Anwar − *Institute of Chemistry, University of Sargodha, Sargodha 40100, Pakistan*

Fozia Batool − *Institute of Chemistry, University of Sargodha, Sargodha 40100, Pakistan*

Shazia Noureen − *Institute of Chemistry, University of Sargodha, Sargodha 40100, Pakistan*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c07789

Author Contributions

Conceptualization: S. Noreen and S.A.G. Methodology and formal analysis: S.H., S. Noreen, and S.A. Software: S.H. and F.B. Supervision: S. Noreen. Validation: S.A.G. Histopathological data visualization: S. Noureen and H.Y.G. All authors participated in writing, reviewing, and editing of the article.

Notes

Experiments were performed according to the guidelines and were approved by the Institutional Research Ethics Committee of the University of Sargodha (Approval Number: SU/ORIC-394).

The authors declare no competing financial interest.

REFERENCES

(1) Finch, A.; Pillans, P. P-glycoprotein and its role in drug-drug interactions. *Aust. Prescr.* **2014**, *37*, 137−139.

(2) Khalid, S.; Abbas, G.; Hanif, M.; Shah, S.; Shah, S. N. H.; Jalil, A.; Yaqoob, M.; Ameer, N.; Anum, A. Thiolated sodium alginate conjugates for mucoadhesive and controlled release behavior of metformin microspheres. *Int. J. Biol. Macromol.* **2020**, *164*, 2691−2700.

(3) Lopes, C. M.; Bettencourt, C.; Rossi, A.; Buttini, F.; Barata, P. Overview on gastroretentive drug delivery systems for improving drug bioavailability. *Int. J. Pharm.* **2016**, *510*, 144−158.

(4) Layek, B.; Mandal, S. Natural polysaccharides for controlled delivery of oral therapeutics: a recent update. *Carbohydr. Polym.* **2020**, *230*, No. 115617.

(5) Chakraborty, S.; Khandai, M.; Sharma, A.; Khanam, N.; Patra, C. N.; Patro, V. J.; Sen, K. Preparation, in vitro and in vivo evaluation of algino-pectinate bioadhesive microspheres: An investigation of the effects of polymers using multiple comparison analysis. *Acta Pharm.* **2010**, *60*, 255−266.

(6) Barclay, T. G.; Day, C. M.; Petrovsky, N.; Garg, S. Review of polysaccharide particle-based functional drug delivery. *Carbohydr. Polym.* **2019**, *221*, 94−112.

(7) Uyen, N. T. T.; Hamid, Z. A. A.; Ahmad, N. B. Synthesis and characterization of curcumin loaded alginate microspheres for drug delivery. *J. Drug Delivery Sci. Technol.* **2020**, *58*, No. 101796.

(8) Jouki, M.; Khazaei, N.; Rashidi-Alavijeh, S.; Ahmadi, S. Encapsulation of Lactobacillus casei in quince seed gum-alginate beads to produce a functional synbiotic drink powder by agro-industrial by-products and freeze-drying. *Food Hydrocolloids* **2021**, *120*, No. 106895.

(9) Lakkakula, J. R.; Gujarathi, P.; Pansare, P.; Tripathi, S. A comprehensive review on alginate-based delivery systems for the delivery of chemotherapeutic agent: Doxorubicin. *Carbohydr. Polym.* **2021**, *259*, No. 117696.

(10) Nayak, A. K.; Pal, D.; Santra, K. Swelling and drug release behavior of metformin HCl-loaded tamarind seed polysaccharide-alginate beads. *Int. J. Biol. Macromol.* **2016**, *82*, 1023−1027.

(11) Mondal, A.; Gebeyehu, A.; Miranda, M.; Bahadur, D.; Patel, N.; Ramakrishnan, S.; Rishi, A. K.; Singh, M. Characterization and printability of sodium alginate-gelatin hydrogel for bioprinting NSCLC co-culture. *Sci. Rep.* **2019**, *9*, No. 19914.

(12) Wu, Y.; Zhang, W.; Huang, J.; Luo, Z.; Li, J.; Wang, L.; Di, L. Mucoadhesive improvement of alginate microspheres as potential gastroretentive delivery carrier by blending with Bletilla striata polysaccharide. *Int. J. Biol. Macromol.* **2020**, *156*, 1191−1201.

(13) Ghumman, S. A.; Bashir, S.; Noreen, S.; Khan, A. M.; Riffat, S.; Abbas, M. Polymeric microspheres of okra mucilage and alginate for the controlled release of oxcarbazepine: In vitro & in vivo evaluation. *Int. J. Biol. Macromol.* **2018**, *111*, 1156−1165.

(14) Ghumman, S. A.; Noreen, S.; tul Muntaha, S. Linum usitatissimum seed mucilage-alginate mucoadhesive microspheres of metformin HCl: Fabrication, characterization and evaluation. *Int. J. Biol. Macromol.* **2020**, *155*, 358−368.

(15) Yari, K.; Akbari, I.; Yazdi, S. A. V. Development and evaluation of sodium alginate-basil seeds mucilage beads as a suitable carrier for controlled release of metformin. *Int. J. Biol. Macromol.* **2020**, *159*, 1−10.

(16) Maroufi, L. Y.; Ghorbani, M. Injectable chitosan-quince seed gum hydrogels encapsulated with curcumin loaded-halloysite nanotubes designed for tissue engineering application. *Int. J. Biol. Macromol.* **2021**, *177*, 485−494.

(17) Ghumman, S. A.; Mahmood, A.; Noreen, S.; Rana, M.; Hameed, H.; Ijaz, B.; Hasan, S.; Aslam, A.; ur Rehman, M. F. Formulation and evaluation of quince seeds mucilage−sodium

https://doi.org/10.1021/acsomega.2c07789
ACS Omega 2023, 8, 5925−5938

alginate microspheres for sustained delivery of cefixime and its toxicological studies. *Arab. J. Chem.* **2022**, *15*, No. 103811.
(18) Nayak, A. K.; Pal, D. Formulation optimization and evaluation of jackfruit seed starch−alginate mucoadhesive beads of metformin HCl. *Int. J. Biol. Macromol.* **2013**, *59*, 264−272.
(19) Chinnaiyan, S. K.; Deivasigamani, K.; Gadela, V. R. Combined synergetic potential of metformin loaded pectin-chitosan biohybrids nanoparticle for NIDDM. *Int. J. Biol. Macromol.* **2019**, *125*, 278−289.
(20) Ubaid, M.; Murtaza, G. Fabrication and characterization of genipin cross-linked chitosan/gelatin hydrogel for pH-sensitive, oral delivery of metformin with an application of response surface methodology. *Int. J. Biol. Macromol.* **2018**, *114*, 1174−1185.
(21) Huh, H. W.; Na, Y.-G.; Kang, H.; Kim, M.; Han, M.; Pham, T. M. A.; Lee, H.; Baek, J.-S.; Lee, H.-K.; Cho, C.-W. Novel self-floating tablet for enhanced oral bioavailability of metformin based on cellulose. *Int. J. Pharm.* **2021**, *592*, No. 120113.
(22) Jouki, M.; Mortazavi, S. A.; Yazdi, F. T.; Koocheki, A. Optimization of extraction, antioxidant activity and functional properties of quince seed mucilage by RSM. *Int. J. Biol. Macromol.* **2014**, *66*, 113−124.
(23) Haile, T. G.; Sibhat, G. G.; Molla, F. Physicochemical Characterization of grewia ferruginea hochst. ex A. Rich Mucilage for potential use as a pharmaceutical excipient. *BioMed Res. Int.* **2020**, *2020*, No. 4094350.
(24) Chowdary, K.; Rao, Y. S. Preparation and evaluation of mucoadhesive microcapsules of indomethacin. *Indian J. Pharm. Sci.* **2003**, *65*, 49.
(25) Okunlola, A.; Odeniyi, M. A.; Arhewoh, M. I. Microsphere formulations of ambroxol hydrochloride: influence of Okra (Abelmoschus esculentus) mucilage as a sustained release polymer. *Prog. Biomater.* **2020**, *9*, 65−80.
(26) Mehta, D.; Parejiya, P.; Barot, B.; Suthar, D.; Shelat, P. Polyethylene oxide based pH-independent drug delivery system: Formulation and optimization by central composite design. *Drug Delivery Lett.* **2013**, *3*, 220−232.
(27) Pande, V.; Patel, S.; Patil, V.; Sonawane, R. Design expert assisted formulation of topical bioadhesive gel of sertaconazole nitrate. *Adv. Pharm. Bull.* **2014**, *4*, 121.
(28) Pal, D.; Nayak, A. K. Novel tamarind seed polysaccharide-alginate mucoadhesive microspheres for oral gliclazide delivery: in vitro−in vivo evaluation. *Drug Delivery* **2012**, *19*, 123−131.
(29) Nayak, A. K.; Pal, D.; Pradhan, J.; Hasnain, M. S. Fenugreek seed mucilage-alginate mucoadhesive beads of metformin HCl: Design, optimization and evaluation. *Int. J. Biol. Macromol.* **2013**, *54*, 144−154.
(30) Noureen, S.; Noreen, S.; Ghumman, S. A.; Batool, F.; Hameed, H.; Hasan, S.; Noreen, F.; Elsherif, M. A.; Bukhari, S. N. A. Prunus armeniaca Gum-Alginate Polymeric Microspheres to Enhance the Bioavailability of Tramadol Hydrochloride: Formulation and Evaluation. *Pharmaceutics.* **2022**, *14*, 916.
(31) Lachman, L.; Lieberman, H. A.; Kanig, J. L.*The Theory and Practice of Industrial Pharmacy*, 2nd ed.; Lea & Febiger: Philadelphia, 1976; pp 412−428.
(32) Jana, S.; Das, A.; Nayak, A. K.; Sen, K. K.; Basu, S. K. Aceclofenac-loaded unsaturated esterified alginate/gellan gum microspheres: in vitro and in vivo assessment. *Int. J. Biol. Macromol.* **2013**, *57*, 129−137.
(33) Martínez-Gómez, F.; Guerrero, J.; Matsuhiro, B.; Pavez, J. In vitro release of metformin hydrochloride from sodium alginate/polyvinyl alcohol hydrogels. *Carbohydr. Polym.* **2017**, *155*, 182−191.
(34) Mendyk, A.; Jachowicz, R.; Fijorek, K.; Dorozynski, P.; Kulinowski, P.; Polak, S. KinetDS: an open source software for dissolution test data analysis. *Dissolution Technol.* **2012**, *19*, 6−11.
(35) Pal, D.; Nayak, A. K. Development, optimization, and anti-diabetic activity of gliclazide-loaded alginate−methyl cellulose mucoadhesive microcapsules. *AAPS PharmSciTech* **2011**, *12*, 1431−1441.
(36) Nayak, A. K.; Pal, D.; Das, S. Calcium pectinate-fenugreek seed mucilage mucoadhesive beads for controlled delivery of metformin HCl. *Carbohydr. Polym.* **2013**, *96*, 349−357.
(37) Barbosa, J. A. P.; Franco, E. S.; Silva, C. V.; Bezerra, T. O.; Santana, M. A.; Júnior, C. H.; Silva, T. G.; Santos, N. P.; Maia, M. Poly-ε-Caprolactone microsphere polymers containing usnic acid: acute toxicity and anti-inflammatory activity. *J. Evid.-Based Complement. Altern. Med.* **2017**, *2017*, No. 7392891.
(38) Bera, K.; Sarwa, K. K.; Mazumder, B. Metformin HCl loaded mucoadhesive agar microspheres for sustained release. *Asian J. Pharm.* **2013**, *7*, No. e29.
(39) Moghbel, A.; Tayebi, M. Quince seeds biopolymer: extraction, drying methods and evaluation. *Jundishapur J. Nat. Pharm. Prod.* **2015**, *10*, No. e25392.
(40) Li, G.-y.; Zhong, M.; Zhou, Z. d.; Zhong, Y.-d.; Ding, P.; Huang, Y. Formulation optimization of chelerythrine loaded O-carboxymethylchitosan microspheres using response surface methodology. *Int. J. Biol. Macromol.* **2011**, *49*, 970−978.
(41) Nayak, A. K.; Das, B.; Maji, R. Calcium alginate/gum Arabic beads containing glibenclamide: development and in vitro characterization. *Int. J. Biol. Macromol.* **2012**, *51*, 1070−1078.
(42) Sinha, P.; Ubaidulla, U.; Hasnain, M. S.; Nayak, A. K.; Rama, B. Alginate-okra gum blend beads of diclofenac sodium from aqueous template using ZnSO4 as a cross-linker. *Int. J. Biol. Macromol.* **2015**, *79*, 555−563.
(43) Pardeshi, S.; Patil, P.; Rajput, R.; Mujumdar, A.; Naik, J. Preparation and characterization of sustained release pirfenidone loaded microparticles for pulmonary drug delivery: Spray drying approach. *Drying Technol.* **2021**, *39*, 337−347.
(44) Dey, S.; Pramanik, S.; Malgope, A. Formulation and optimization of sustained release stavudine microspheres using response surface methodology. *Int. Scholarly Res. Not.* **2011**, *2011*, No. 627623.
(45) Wu, X.-G.; Li, G.; Gao, Y.-L. Optimization of the preparation of nalmefene-loaded sustained-release microspheres using central composite design. *Chem. Pharm. Bull.* **2006**, *54*, 977−981.
(46) Hasnain, M. S.; Nayak, A. K.; Singh, M.; Tabish, M.; Ansari, M. T.; Ara, T. J. Alginate-based bipolymeric-nanobioceramic composite matrices for sustained drug release. *Int. J. Biol. Macromol.* **2016**, *83*, 71−77.
(47) Yadollahi, M.; Farhoudian, S.; Namazi, H. One-pot synthesis of antibacterial chitosan/silver bio-nanocomposite hydrogel beads as drug delivery systems. *Int. J. Biol. Macromol.* **2015**, *79*, 37−43.
(48) Kalpna, M.; Dev, D.; Shahnaz, M.; Parkash, J.; Prasad, D. Preparation of controlled release metformin hydrochloride loaded chitosan microspheres and evalaution of formulation parameters. *J. Drug Delivery Ther.* **2018**, *8*, 378−387.
(49) Sinha, P.; Udhumansha, U.; Rathnam, G.; Ganesh, M.; Jang, H. T. Capecitabine encapsulated chitosan succinate-sodium alginate macromolecular complex beads for colon cancer targeted delivery: In vitro evaluation. *Int. J. Biol. Macromol.* **2018**, *117*, 840−850.
(50) Mady, F. M.; Ibrahim, S.; Abourehab, M. Development and evaluation of alginate-gum blend mucoadhesive microspheres for controlled release of Metformin Hydrochloride. *J. Adv. Biomed. Pharm. Sci.* **2021**, *4*, 111−118.
(51) Rebitski, E. P.; Darder, M.; Carraro, R.; Aranda, P.; Ruiz-Hitzky, E. Chitosan and pectin core−shell beads encapsulating metformin−clay intercalation compounds for controlled delivery. *New J. Chem.* **2020**, *44*, 10102−10110.
(52) Chen, S.; Yang, X.; Zhang, Y.; Liu, Y.; Lu, H.; Qiu, Y.; Cheng, L.; Li, C.; Wang, C. Inhalable Porous Microspheres Loaded with Metformin and Docosahexaenoic Acid Suppress Tumor Metastasis by Modulating Premetastatic Niche. *Mol. Pharmaceutics* **2021**, *18*, 2622−2633.
(53) Dash, S.; Murthy, P. N.; Nath, L.; Chowdhury, P. Kinetic modeling on drug release from controlled drug delivery systems. *Acta Pol. Pharm.* **2010**, *67*, 217−223.

https://doi.org/10.1021/acsomega.2c07789
ACS Omega 2023, 8, 5925−5938

(54) Nayak, A.; Jain, S. K.; Pandey, R. S. Controlling release of metformin HCl through incorporation into stomach specific floating alginate beads. *Mol. Pharmaceutics* **2011**, *8*, 2273−2281.
(55) Nayak, A. K.; Pal, D.; Santra, K. Tamarind seed polysaccharide−gellan mucoadhesive beads for controlled release of metformin HCl. *Carbohydr. Polym.* **2014**, *103*, 154−163.
(56) Nayak, A. K.; Pal, D. Development of pH-sensitive tamarind seed polysaccharide−alginate composite beads for controlled diclofenac sodium delivery using response surface methodology. *Int. J. Biol. Macromol.* **2011**, *49*, 784−793.

https://doi.org/10.1021/acsomega.2c07789
ACS Omega 2023, 8, 5925−5938