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Development of Spray-Dried Enteric Omeprazole Microparticles Using O-Carboxymethyl Chitosan and Eudragit® S100: Central composite Design Optimization and In Vitro-In Vivo evaluation

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ABSTRACT

Purpose: Gastroesophageal reflux is a common disease in infants and children. However, effective pediatric pharmacotherapy with omeprazole is limited by the lack of child-friendly dosage forms and physicochemical drawbacks of omeprazole. To provide a cost-effective, one step alternative to conventional enteric coated pellets we developed spray-dried enteric omeprazole microparticles (OME-MP) using O-carboxymethyl chitosan (CMC) and Eudragit® S100 (ES100).
Methods: A central composite design was applied to optimize the spray dried OME-MP. The independent variables were the w/w ratios of CMC/OME (0.5, 5) and ES100/OME (0.5, 10). The dependent variables were considered as EE %, and drug release percentage after 10 min and 60 min. The optimized microparticles were characterized for their size, morphology, thermal behavior and in vitro release. Further, an ethanol induce gastric ulcer model in rat was applied to investigate the in-vivo anti-gastric ulcer efficacy of the optimized OME-MP.
Results: The optimised formulation with CMC/OME = 1.92 and ES100/OME = 5.30 produced smooth free flowing microparticles with a size range of 5.42 ± 0.9 µm. In vitro testing showed minimal drug release under simulated gastric conditions (<10 % over 2 h), followed by rapid and extensive release in simulated intestinal medium (> 90 %) confirming pH-triggered protection and delivery. In-vivo study revealed OME-MP reduced ulcer severity compared with the commercially available generic omeprazole pellet formulation, and histological assessment supported improved mucosal preservation.
Conclusion: This one-step, spray-dried enteric OME-MP platform offers a scalable enteric system for omeprazole and other acid labile drugs, with potential relevance to pediatric oral administration.

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Introduction

Gastroesophageal reflux is common among children, particularly because infants often have an immature lower esophageal sphincter (LES). Omeprazole, a proton pump inhibitor with a biological half-life of about one hour, is frequently used to treat reflux-related symptoms in infants and young children.[1,2] Developing omeprazole for pediatric use presents significant challenges due to its low solubility in water and its heightened sensitivity to factors like acid, light, and moisture. Additionally, there is a limited selection of dosage forms that are suitable and appealing for children, making it crucial to address these issues for effective treatment.[3,4]

Eudragit® S100 (ES100) is commonly used to protect acid-labile drugs from the harsh conditions found in the stomach. In addition, biodegradable and mucoadhesive polymers, such as O-carboxymethyl chitosan (CMC), can enhance mucosal residence time and potentially improve therapeutic performance. However, it is essential to optimize the selection of polymers and their blend ratios to avoid negative outcomes, such as burst drug release or inadequate bioavailability.[5-7]

Spray drying is a straightforward, reproducible, and scalable technique used to produce microparticles with well-controlled particle size distributions and morphologies.[8,9] Unlike multi-step pellet-coating processes, spray drying provides a continuous workflow that is promising for scaling up production. Additionally, it allows for precise control over particle characteristics, which can enhance flowability and ensure uniformity in dosing.

The primary objective of this study was to develop spray-dried enteric omeprazole microparticles using CMC and ES100. We aimed to optimize the polymer-to-drug ratios through response surface methodology based on a central composite design. Encapsulation efficiency and drug release percentage at 10 and 60 minutes were used as criteria for optimization. The optimized formulation was then characterized for its physicochemical properties, pH-dependent in vitro release, and in vivo gastroprotective efficacy in an ethanol-induced ulcer model.

Materials and Methods

Omeprazole (OME) was provided by Temad, Iran. The o-carboxymethyl chitosan (CMC), Eudragit S100 (ES100), ethanol, sodium phosphate dibasic, phosphate monobasic, hydrochloric acid (HCl), and sodium hydroxide (NaOH) were sourced from Merck, Germany.

Preparation of omeprazole loaded microparticles by spray drying

The omeprazole-loaded microparticles (OME-MPs) were prepared using a spray drying technique. First, 400 mg omeprazole was dissolved in a mixture of 20 mL ethanol (96 %) and water mixture (1: 3 v/v). Subsequently, CMC and ES100 were added sequentially under continuous stirring to obtain the desired polymer-to-drug ratios. The dispersion was stirred continuously and protected from light before proceeding to spray drying. A bench-top spray dryer (Dorsa Tech®, Iran) was utilized for this process. The spray drying conditions included an inlet temperature of 110 °C, an aspiration rate of 70 %, a feed flow rate of 2.5 mL/min, and a nozzle time gap of 9 seconds. The outlet temperature ranged from 53 to 56 °C. The dried powders were collected and stored in a light-protected, tightly sealed container for further analysis. The powder recovery percentage was calculated as the ratio of the collected powder to the initial solid content of the feed solution.

Experimental design and optimization

OME-MPs were prepared using CMC and ES100 polymers through a spray drying process. To achieve optimal encapsulation efficiency (EE %) and a desirable drug release profile, the formulations were optimized using a central composite design (CCD). The independent variables were the weight ratios of CMC/OME (A) and ES100/OME (B), each evaluated at three different levels. The dependent variables in this study are the EE % and the drug release percentages at 10 minutes (DR-10) and 60 minutes (DR-60), as outlined in Table 1. A total of 13 experimental runs were designed using CCD. The composition of the independent variables and their corresponding responses are detailed in Table 2.

The midpoint levels of CMC/OME (2.75) and ES100/OME (5.25) corresponded to the center points of the selected factor ranges. According to the basics of central composite design, these center points were included and replicated to estimate experimental error, evaluate the presence of curvature in the response surfaces, and enhance the robustness of the fitted models.

An analysis of variance (ANOVA) was performed on the dependent variables (EE, DR-10, and DR-60), followed by numerical optimization using Design Expert® 13 (Stat-Ease Inc.). The data were fitted to both linear and quadratic models, as outlined in equations 1 and 2.

Equation 1 : $Y_i = b_0 + b_1A + b_2B$

Equation 2 : $Y_i = b_0 + b_1A + b_2B + b_{12}AB + b_{11}A^2 + b_{22}B^2$

In these equations, Yi represents the dependent variable, while b0 is the intercept. The coefficients b1 through b22 are the regression coefficients. A and B symbolize the independent variables, and AB indicates the interaction term between these variables. Furthermore, A^2 and B^2 refer to the quadratic terms associated with A and B, respectively.

The models were statistically validated using ANOVA. After this, the optimal values for the independent variables were determined through numerical optimization based on established criteria. An optimized formulation was then developed and characterized accordingly. Finally, we compared the experimental values with the predicted values to assess the prediction error and verify the validity of the model.

Table 1. Parameters and constraints in central composite design (CCD).

Factors (independent variables)	**levels**		
	-1	0	+1
A: CMC/OME (w/w ratio)	0.5	2.75	5
B: ES100/OME (w/w ratio)	0.5	5.25	10
Responses (dependent variables)	**Constrains**		
Y_1: Encapsulation efficiency (EE) (%)	100 %		
Y_2: Drug release after 10 minutes (DR-10) (%)	Minimize		
Y_3: Drug release after 60 minutes (DR-60) (%)	Maximize		

CMC: O-carboxymethyl chitosan; ES100: (Eudragit S100); OME: Omeprazole

Table 2. Composition of independent variables and the corresponding measured dependent variables.

Batch	Independent variables		Dependent variables		
	A (CMC/OME)	B (ES100/OME)	Y_1 (EE %)	Y_2 (DR-10 %)	Y_3 (DR-60 %)
F1	2.75	11.9675	107.5 *	38.5	76.2
F2	0.5	10	102.6 *	42.4	80.1
F3	0	5.25	85.3	57.6	83.4
F4	2.75	5.25	101.7 *	55.3	85.5
F5	5	10	100.5 *	11.4	91.7
F6	2.75	5.25	101.5 *	45.5	88.7
F7	5	0.5	102.6 *	47.4	60.4
F8	5.9319	5.25	94.7	39.2	75.1
F9	2.75	5.25	105.6 *	59.5	95.2
F10	2.75	5.25	100.6 *	64.3	87.3
F11	2.75	5.25	102.5	57.4	88.1
F12	0.5	0.5	89.8	86.5	91.2
F13	2.75	0	94.5	68.6	72.3

EE= encapsulation efficiency; DR-10 = drug release in 10 min; DR-60 = drug release in 60 min
CMC: O-carboxymethyl chitosan; ES100: (Eudragit S100); OME: Omeprazole
* Values greater than 100% were regarded as indicative of complete encapsulation within the limits of experimental uncertainty.

Characterization of omeprazole loaded microparticles

Encapsulation efficiency and drug loading

A precisely weighed 50 mg sample of OME-MPs was dissolved in 50 mL of ethanol and stirred continuously for 24 hours. After this period, 1 mL of the solution was withdrawn and diluted to 25 mL using phosphate buffer at pH 6.8. The amount of omeprazole was quantified through UV spectrophotometry at a wavelength of 304 nm. The encapsulation efficiency and drug loading were calculated using Equations 3 and 4, respectively.

Equation 3 :

$$\text{EE}\ (\%) = \frac{\text{Encapsulated drug}}{\text{Total added drug}} \times 100$$

Equation 4 :

$$\text{DL}\ (\%) = \frac{\text{Encapsulated drug}}{\text{Total mass of microparticles}} \times 100$$

Drug release profile

The release profile of OME-MPs was evaluated using a USP Type II dissolution apparatus (NOAVARAN IND, Iran). In the 13 runs of the Central Composite Design (CCD), 100 mg of microparticles were dispersed in 900 mL of phosphate buffer at pH 7.4. The test was conducted at a stirring speed of 100 rpm and a temperature of 37 ± 0.5 °C for 60 minutes. At predetermined time intervals, 2 mL of the medium was collected and replaced with an equal volume of fresh medium. The concentration of omeprazole was determined by UV spectrophotometry at a wavelength of 304 nm.

The release behavior of optimized OME-MPs was further evaluated under sequential pH conditions. Initially, the dissolution medium was set to pH 1.2 using 0.1 N HCl. After 2 hours, the medium was replaced with a phosphate buffer at pH 7.4, and the test continued for an additional 2 hours. The concentration of omeprazole was analyzed using the previously described method.

Characterization of the optimized microparticles

Particle size analysis of the optimised microparticles

Particle size distribution of the optimised OME-MPs was measured using Mastersizer 3000E (Malvern, UK). Powders were resuspended in water and sonicated for 5 minutes prior to analysis.

Morphology of optimised microparticles

The surface morphology of the optimized OME-MPs was evaluated using scanning electron microscopy (SEM). The powder was carefully dispersed onto double-sided adhesive tape that was mounted on an aluminum stub and was then sputter-coated with gold for 30 seconds. Images were captured using an SEM (XL30, Netherlands) operating at an accelerating voltage of 20 kV.

Thermal analysis of the optimised microparticles

The thermal behavior of the optimised OME-MPs, OME, CMC, and ES100 was characterized by differential scanning calorimetry (DSC, Mettler Toledo, Switzerland). Accurately weighed samples were sealed in aluminum pans and heated from 0 to 250°C at a rate of 10°C per min. Thermograms were recorded to identify thermal events.

***In-vivo* studies**

The anti-ulcer activity of the optimized OME-MPs was evaluated using an ethanol-induced gastric ulcer model in rats. We used Wistar male rats, aged 6 weeks and weighing between 150-200 g (n=25), sourced from the Pasteur Institute in Tehran, Iran. All in-vivo experiments were conducted in accordance with the approved ethical guidelines of the National Research Council's Guide and received approval from the ethics committee of Tehran University of Medical Sciences (Code of Ethics: IR.TUMS.AEC.1402.036).

Rats were randomly assigned to three experimental groups, with each group consisting of eight rats (n=8 per group). Before the experiment, the animals were fasted for 24 hours to ensure gastric emptying, while they had free access to water. Treatments were administered orally by gavage, as detailed in Table 3. The gastric ulcer

control group (GU-control) received water, while the OME-generic group was given 20 mg/kg of omeprazole from a commercial generic pellet formulation. The OME-MP group received 20 mg/kg of omeprazole from optimized microparticles. Additionally, one rat served as a normal control and remained untreated without any induced ulcer.[10] One hour after the treatment, gastric ulcers were induced in the three experimental groups by administering absolute ethanol orally at a dose of 5 ml/kg.

Table 3. Different treatment groups for anti-ulcer activity study in rats with ethanol induced gastric ulcer.

Group	Treatment
Control	Normal rat without treatment
GU-control	Oral water + ethanol induced gastric ulcer
OME-generic	Oral generic omeprazole pellet (20 mg/kg) + ethanol induced gastric ulcer
OME-MP	Oral omeprazole microparticle (20 mg/ kg) + ethanol induced gastric ulcer

GU-control: gastric ulcer control; OME: omeprazole; MP: microparticle

Afterward, the animals were euthanized by decapitation. The stomachs were excised, opened along the greater curvature, and gently washed with normal saline. Ulcerative lesions were examined under 3× magnification, and lesion size was measured. The ulcer index (UI) and the percentage of ulcer protection were calculated according to Equations 5 and 6, respectively. The scoring criteria applied in Equation 5 are defined in Table 4.[11]

Equation 5 : UI= Score 1+ [(Score 2) × 2] + [(Score 3) × 3] + [(Score 4) × 4] + [(Score 5) × 5]

Equation 6 : Ulcer protection (%)= $\frac{UI_{control} - UI_{tratment}}{UI_{control}} \times 100$

Table 4. Scoring system for gastric lesions.

Score number	Definition
Score 1	$\frac{\text{The number of petechial lesions with less than 1 mm in length}}{5}$
Score 2	The number of lesions with 1 to 2 mm in length
Score 3	The number of lesions with 2 to 4 mm in length
Score 4	The number of lesions with 4 to 6 mm in length
Score 5	The number of lesions with more than 6 mm in length

For histological evaluation, gastric tissues were fixed in 10% formalin for 48 hours, embedded in paraffin, and sectioned at 5 µm thickness using an automated microtome. The resulting sections were then stained with hematoxylin and eosin (H&E)

Results

Characterization of omeprazole microparticles

The OME-MPs formulations were successfully prepared by spray drying according to formulations generated using a central composite design (CCD). The resulting microparticles were subsequently characterized and analyzed as described previously.

The powder yield of the microparticles is presented in Table 5. The highest yield was observed in F2, at 77.15%, while the lowest yield was recorded in F12, at 38.25%. According to Table 5, the particle sizes of the microparticles ranged from 1.73 µm (F4) to 25.92 µm (F7). Drug loading varied significantly, ranging from 6.88% to 44.61%, with the highest loading observed in F12, which is consistent with its relatively low polymer content.

Table 5. Characterization of various microparticle formulations based on runs generated by the Central Composite Design (CCD).

Formulation	Yield (%)	Particle Size (µm)	Drug loading (%)
F1	75.63	11.23	6.88
F2	77.15	21.93	8.90
F3	60.28	10.65	15.04
F4	60.36	1.73	11.20
F5	75.18	4.02	7.71
F6	67.89	7.68	11.10
F7	52.69	25.92	15.67
F8	63.93	18.37	7.00
F9	69.28	8.13	11.71
F10	67.22	7.51	8.88
F11	65.33	6.57	8.88
F12	38.25	22.84	44.61
F13	56.33	8.88	24.20

Optimization of omeprazole microparticle

Response Surface Methodology (RSM) provides a more efficient optimization strategy compared to the traditional one-factor-at-a-time approach. It minimizes the number of experimental runs while effectively capturing interaction effects. In this study, thirteen formulations were created using a central composite design to assess how the ratios of CMC/OME (A) and ES100/OME (B) influence three outcomes: encapsulation efficiency (Y1), drug release at 10 minutes (Y2), and drug release at 60 minutes (Y3). The responses were analyzed using Design-Expert software and are summarized in Table 2.

Effect of independent variables on Y_1

As presented in Table 6, the ANOVA regression analysis for Y_1 (%EE) indicated that the quadratic model was significant ($p< 0.005$). Adjusted R^2 of 0.85 and Predicted R^2 of 0.56 were found, which signifies that while the model explained the variability in the experimental data very well, the predictive ability of the same was just moderate. A significant difference between the Adjusted R^2 and Predicted R^2 indicates that making predictions outside the explored design space should be performed carefully. But still, the model proved useful enough for optimizing formulations in the given range of experimental region, since the prediction error observed was less than 5%.

Table 6. Statistical analysis of responses containing proposed models and equations.

Predicted model	Y_1		Y_2		Y_3	
	Quadratic (p-value<0.005)		**Linear (p-value<0.005)**		**Quadratic (p-value<0.005)**	
Parameter	CE	p-value	CE	p-value	CE	p-value
Intercept	101.80	0.0011	51.38	0.0006	88.60	0.0032
A	2.97	0.0140	-11.93	0.0046	-3.91	0.0341
B	4.38	0.0020	-15.30	0.0009	3.21	0.0684
AB	-3.75	0.0227	-	-	10.50	0.0016
A^2	-5.59	0.0007	-	-	-3.80	0.0490
B^2	0.9125	0.3818	-	-	-6.30	0.0056
Lack of fit		0.1680		0.2222		0.3294

As shown in Table 2, the EE values varied from 85.3 % to 107.5. Higher values of EE over 100 could be considered approximately 100 %.

To determine the level of the independent variables that maximise EE, the following reduced model equation was obtained after applying ANOVA:

Equation 7: $Y_1 = 101.80+2.97A+4.38B-3.75AB-5.59A^2$

In this analysis, A represents the CMC/OME ratio, while B denotes the ES100/OME ratio. According to Equation 7, both variables significantly influence the emulsification efficiency percentage (EE%). Notably, the ES100/OME ratio has a greater impact, with a coefficient of 4.38, compared to the CMC/OME ratio, which has a coefficient of 2.97. Overall, increasing the amounts of both CMC and ES100 tends to enhance EE%. However, the negative interaction term (AB) and the negative quadratic term for A suggest diminishing returns, indicating there is an optimal region rather than a strictly linear increase. The response surface curve (Figure 1A) illustrates the combined effects of A and B. The curvature of the surface indicates that optimal emulsification efficiency (EE of 100%) is achieved at approximately intermediate levels of both independent variables.

Effect of independent variables on Y_2

Drug release within 10 minutes in a basic buffer was classified as Y2. The fitted model, as indicated in Table 6, was linear and statistically significant ($p < 0.005$). The predicted R^2 and adjusted R^2 values were 0.59 and 0.73, respectively. The mathematical relationship between the independent variables and Y2, derived from ANOVA, is represented by equation 8.

Equation 8 : $Y_2 = 51.38-11.93A-15.30B$

Where, A represents the CMC/OME ratio, while B represents the ES100/OME ratio. Since the model is linear, no interaction terms were identified. It was observed that increasing the levels of both excipients decreased drug release within the first 10 minutes. Furthermore, the inhibitory effect of ES100/OME was greater than that of CMC/OME, as indicated by the larger coefficient magnitude associated with ES100/OME.

Effect of independent variables on Y_3

Y3 was defined as the percentage of drug released (%) within 60 minutes in a basic buffer. As presented in Table 6, the proposed model for Y3 was found to be quadratic ($p < 0.005$) as shown below:

Equation 9 :

$$Y_3 = 88.60 - 3.91A + 3.21B + 10.50AB - 3.80A^2 - 6.30B^2$$

The predicted R^2 value was 0.58, while the adjusted R^2 value was 0.80, indicating a satisfactory agreement between the experimental and predicted values. An increase in the CMC/OME ratio resulted in a decrease in drug release within 60 minutes, whereas an increase in the ES100/OME ratio enhanced drug release under basic conditions. The combination of these two excipients had a significant impact on the release profile of omeprazole from the microparticles (Figure 1B). The curvature observed in the 3D response surface plot confirmed the existence of an optimal level for each variable, necessary to achieve a desirable release profile in a basic medium.

Prediction of optimised formulation by CCD

According to the previously established constraints for the dependent variables (Y1 = 100%, Y2: minimize, Y3: maximize), the central composite design (CCD) predicted an optimized formulation with a ratio of CMC/OME equal to 1.92 and ES100/OME equal to 5.30 (w/w). The experimental data collected from three replicates of this optimized formulation were then compared with the model predictions, as shown in Table 7. The predicted error was less than 5% for all responses, which confirms the reliability of the model. Additionally, the optimized sample was further characterized in terms of surface morphology, thermal behavior, and in vivo anti-ulcer efficacy.

Table 7. Characterization of optimised formulations and the measure of predicted error.

Dependent variables	Y_1 (% EE)	Y_2 (% DR-10)	Y_3 (% DR-60)
Experimental response (n=3)	102.5	27.9	88.9
	97.8	26.3	91.2
	96.5	24.5	85.6
Predicted response	100	25.6	90.5
Predicted Error (% PE)	1.06	2.47	2.14

EE= encapsulation efficiency; DR-10 = drug release in 10 min; DR-60 = drug release in 60 min

Physical and chemical properties of the optimum sample

The powder recovery yield of the optimal microparticle over three runs was 64.3 ± 1.1%, and the average particle size (d50) measured 5.42 ± 0.9 μm. The sample's morphology was analyzed using SEM (Figure 2A). The particles were predominantly spherical, featuring nearly smooth surfaces and well-dispersed, discrete entities. Minimal aggregation was observed, and the size range was consistent with the Master size analysis.

Thermal behavior of the optimum sample

The thermal behavior of omeprazole, ES100, CMC, and the optimal OME-MP formulation is illustrated in Figure 2B. The thermogram of omeprazole, which is an anhydrous crystalline drug, shows a sharp endothermic peak at 158.3°C, indicating its melting point, followed by an exothermic peak at 173.2°C that is associated with thermal decomposition. Similarly, ES100 displays a melting point at 72.3°C and a degradation temperature at 222.3°C. The thermogram of CMC reveals an endothermic melting peak at 134.3°C. In contrast, the optimal OME-MP formulation has a thermal profile similar to those of CMC and ES100, but its peaks are broader and less intense. Notably, the endothermic and exothermic peaks of omeprazole are absent in the OME-MP thermogram. This disappearance indicates that omeprazole is molecularly dispersed (and likely amorphous) within the microparticles, which prevents its thermal degradation.

Drug release study

The release behavior of the microparticles was evaluated. Figure 2C presents the release profiles of 13 different microparticle formulations in simulated intestinal medium (pH ~ 7.4), revealing significant variability among the formulations. Microparticles from formulation F12, which contained the lowest polymer content, demonstrated the highest drug release at 10 minutes (DR-10 %) at 86.5%. After this point, the release plateaued for the remainder of the 60 minutes. In contrast, microparticles from formulation F5, which had the highest polymer content, showed the lowest DR-10 % at 11.4%. However, these microparticles displayed a continuous increase in drug release over the full 60-minute period, ultimately reaching 91.7%.

Figure 2D illustrates the release profile of OME from the optimized OME-MP in simulated gastric medium (pH 1.5) and simulated intestinal fluid (pH 7.4). After 2 hours in the simulated gastric medium, the drug release was

ideally below 10% of the loaded amount, at 6.22 ± 0.83 %. Upon transferring the microparticles to the simulated intestinal fluid for an additional 2 hours, a rapid burst release was observed. The total percentage of drug released in the simulated intestinal medium was approximately 97%, with minimal variability.

anti-ulcer activity of the microparticles

The anti-ulcer effectiveness of the optimized OME-MPs was tested in a rat model of ethanol-induced gastric ulceration. The macroscopic appearance of the freshly excised gastric tissues is displayed in Figure 3A. In the normal control group, which did not receive ethanol or any treatment, no detectable hemorrhagic spots or lesions were found. In contrast, the GU-control group showed multiple ulcerative lesions characterized by linear hemorrhage, erythema, and edema, confirming the successful induction of gastric ulcers. The severity of ulcers was reduced in the OME-generic group, with a more significant reduction observed in the OME-MP group compared to the GU-control group.

The distribution of lesion scores (ranging from 1 to 5) is shown in Figure 3B. In all categories of ulcer scores, treatment with OME-MPs led to a greater reduction in the number of lesions compared to treatment with OME-generic when measured against the GU-control group. Notably, the OME-MP group experienced a significant decrease in the number of high-severity lesions (score 5; length greater than 6 mm) compared to the GU-control group ($p < 0.01$).

UI values for each group are shown in Figure 3C. The GU-control group had the highest UI (47.4 ± 12.2), while the OME-generic and OME-MP groups recorded scores of 37.4 ± 22.3 and 25.1 ± 12.09, respectively. The reduction in the OME-MP group was statistically significant compared to the control ($p < 0.01$, Tukey's post-hoc test). Moreover, OME-MP demonstrated superior gastroprotective efficacy, with an ulcer protection rate of 46.97% versus 21.14% for OME-generic."

Histopathological images of gastric tissue sections from different groups are presented in Figure 3D, which support the macroscopic findings. In the normal control group, no pathological lesions were observed in the mucosal, submucosal, or muscular layers (indicated by the orange arrow in Fig. 7). In the GU-control group, however, severe erosive lesions were evident on the epithelial surface, characterized by necrosis, edema, and hemorrhage. The infiltration of inflammatory cells in the mucosa was relatively low, and the damage did not extend beyond the lamina propria, where only a few inflammatory cells were noted. The muscle layer remained intact.

In the OME-generic group, mild erosive lesions were observed on the epithelial surface. Pathological changes in the mucosa manifested as mild necrotic edema, with minimal bleeding and limited inflammatory infiltration. However, infiltration of mononuclear inflammatory cells was noted in the mucosal layer, indicated by red arrows. No pathological alterations were found in the submucosal or muscular layers.

The OME-MP group showed gastric tissue that was nearly normal. Only very mild, non-erosive changes were noted in a few areas of the epithelium, which included limited edema without any signs of necrosis or hemorrhage. Additionally, there were no pathological lesions found in the submucosal or muscular layers.

Discussion

Omeprazole, a proton pump inhibitor widely prescribed for the treatment of gastro-oesophageal reflux disease, peptic ulcer disease, and Zollinger–Ellison syndrome, is a highly acid-labile and lipophilic compound.[5] Its therapeutic performance critically depends on maintaining physicochemical stability in the upper gastrointestinal tract and achieving an appropriate site-specific release profile. Multiparticulate delivery systems, such as polymeric microparticles, can confer several advantages for omeprazole, including pH-dependent release, improved stability and bioavailability, prolonged duration of action, and reduced dosing frequency.[12-14] Given previous investigations into spray-dried omeprazole microparticles and the drug's well-documented sensitivity to acid, moisture, and heat, CMC and ES100 were selected as complementary polymers to formulate enteric microparticles with enhanced in vivo efficacy. To simultaneously optimize critical formulation variables and assess potential interaction effects, a central composite design–based response surface methodology (RSM) was employed.

It was observed that increasing the CMC ratio resulted in larger microparticle sizes. At higher CMC levels, a greater amount of polymer is present within a similar volume of atomized droplets, leading to the formation of larger particles. These findings align with a previous study that involved the preparation of spray-dried albumin microparticles using PLA as the matrix polymer.[15] In this study, the particles displayed a smooth, spherical morphology. In contrast, another study on spray-dried chitosan microparticles reported spherical particles with imperfect surfaces. In that study, surface roughness increased at higher drug concentrations due to the crystallinity of the drug, an effect that was not observed in our formulations.[16]

Encapsulation efficiency (EE) increased with higher ES100-to-OME ratios. As shown in Equation 7, the coefficient for ES100/OME was 4.38, indicating a strong positive influence of ES100 concentration on EE. In the optimal formulation, an ES100/OME ratio of 5.3 yielded an EE of 100 %.

It should be mentioned that EE values above 100 % are not physically meaningful. The observed values slightly exceeding 100 % are most likely attributable to experimental and analytical variability associated with UV quantification, including potential matrix effects from formulation excipients. Therefore, these values could be interpreted as indicating essentially complete encapsulation (approximately 100 %) not a true physical phenomenon.

Encapsulation efficiency is governed by multiple factors, notably polymer concentration and the nature of drug–polymer interactions. In a representative study, omeprazole-loaded microparticles were formulated using Eudragit RS 100, hydroxypropyl methylcellulose (HPMC), and ethyl cellulose (EC) at various concentration levels. The effect of polymer-to-drug ratio was evaluated using ES100-to-omeprazole ratios of 2.5:1 and 5:1 (w/w). Results demonstrated that both drug loading and encapsulation efficiency decreased significantly with increasing ES100 concentration, suggesting a concentration-dependent inhibitory effect on encapsulation performance.[17] In contrast, another study found that higher ratios of RS100 and ethyl cellulose enhanced the encapsulation efficiency (EE) of spray-dried microparticles.[18] These discrepancies may be attributed to differences in the microsphere preparation methods, specifically solvent diffusion versus solvent evaporation. In another study, the combination of ES100 with sodium alginate or glyceryl behenate, used to prepare lansoprazole microparticles via spray drying, resulted in relatively lower encapsulation efficiencies (EE) of 50–60%.[10] In the present work, however, the combination of ES100 and CMC afforded an EE of 100 % following CCD optimization.

The in vitro release profile of omeprazole from the enteric-coated microparticles was assessed using a sequential two-stage dissolution method, in accordance with United States Pharmacopeia (USP) guidelines. Initially, the microparticles were incubated in simulated gastric fluid (pH 1.5) for 2 hours, followed by transfer to simulated intestinal fluid (pH 7.4) for an additional 2 hours. Given the marked acid-lability of omeprazole, an enteric formulation strategy is essential to preclude drug degradation in the gastric environment and to enable targeted release within the upper small intestine, the primary site of drug absorption.[5] Previous studies reported that desirable formulations limit drug release in gastric medium to <10% over the first 2 h, while achieving >80% release within 45 min after transfer to intestinal medium.[19]

In the present study, the cumulative release of omeprazole from the developed microparticles (MPs) in phosphate-buffered saline (PBS, pH 7.4) over 60 minutes ranged from 60.4% to 95.2%, demonstrating a strong dependence on the formulation composition. The optimized formulation exhibited an appropriate biphasic release profile in both simulated gastric and intestinal media. Notably, no yellow discoloration, indicative of omeprazole degradation, was observed in the simulated gastric fluid.

In the intestinal medium, the optimized MPs displayed an initial burst release within the first 10 minutes, followed by a sustained release phase lasting over 2 hours. This behavior is attributed to the pH-dependent solubility of the enteric polymer Eudragit® S100 (ES100). The solubility threshold of ES100 at pH 7.0 minimizes drug release in the gastric environment; however, upon transition to the intestinal pH, the polymer rapidly dissolves, thereby triggering the initial burst and subsequent release phases. In the context of our formulation, the rapid dissolution of ES100 at pH values exceeding 7.0 in the distal intestine facilitates a significant increase in omeprazole release.

Furthermore, the inclusion of CMC in the formulation modulated the release kinetics, resulting in a more sustained drug release profile. This is likely due to the enhanced swelling of CMC under the alkaline conditions of the intestinal tract. The swollen polymer network restricts drug diffusion and promotes the in-situ formation of a hydrogel or microgel matrix, which serves as a diffusion barrier. This gel network enables a controlled and prolonged release of the encapsulated omeprazole over several hours, thereby supporting extended drug delivery in the intestinal environment.[20,21]

This release profile is particularly advantageous, given that omeprazole is primarily absorbed in the duodenum[15]. In the present study, increasing the CMC ratio beyond the optimal level resulted in diminished omeprazole release at both 10 and 60 min. At the optimal CMC concentration, the non-crosslinked polymer matrix appeared insufficient to suppress the initial burst release, which may be attributed to the rapid dissolution of surface-adsorbed omeprazole, despite its poor water solubility.[8] Further elevation of the CMC ratio led to a marked reduction in release rates at both time points, likely due to enhanced drug–polymer interactions, such as adsorption of omeprazole onto CMC chains via van der Waals forces. Additionally, higher CMC content may increase the diffusional barrier for the drug, thereby extending the duration of omeprazole action.[22] Additionally, CMC water absorption and swelling behavior may close the micropore channels and slow the release rate via polymer degradation.[23] Following burst release, the sustained release was observed as a result of both ES100 and CMC. Microparticles with a size of less than 23-38 μm would have mucoadhesive properties.[24] Additionally, Chitosan and its derivatives could bind to the mucosal membranes and provide the matrix for sustained drug delivery systems with elongated duration of drug release [25]. The release rate decreased by increasing the ratio of ES100. In the current study, the 10-minute release was reduced by ES100 (coefficient value of -15.3) but the 60-minute release was enhanced at higher ES100 ratios (coefficient value of 3.21). It could be concluded that a thin wall of

polymer is created around the microparticles, and the optimum pattern of release is highly dependent on the ratio of the enteric polymer. A similar sustained release profile was also observed using ES100 due to its mucoadhesiveness.[14,26] It is, however, difficult to simulate the biological environment of humans and we just did the release test *in vitro* as an indicator for screening the ratio of ES100 and CMC.

DSC was done to provide detailed data about the physical and thermal features of the omeprazole optimum sample. The thermogram indicated the disappearance of characteristic endotherm and exotherm of omeprazole in the final microparticles. This result confirmed that omeprazole was no longer crystalline. Similar omeprazole amorphization was reported following the spray drying of omeprazole microparticles.[14] It could be assumed that omeprazole was molecularly dispersed in CMC matrix but did not interact with ES100 where the location of the ES100 peaks is not changed, whereas, their corresponding intensity or broadness has changed due to the dilution effect by formulation composition.

The *in vivo* anti-ulcer effects of omeprazole microparticles were observed upon ulcer induction by oral ethanol. Gastric lesions induced by ethanol are mainly due to stasis in the mucosa leading to hemorrhage and necrosis. These lesions are attributed to the formation of free radicals and lipid peroxidation products.[27] Treatment with OME-generic and OME-MP reduced the ulcer index, while OME-MPs showed a significant reduction in the ulcer index and recovered histopathological features compared to the GU-control. The improved therapeutic outcome of OME-MPs may be due to their mucoadhesive properties, delayed and prolonged drug release, and potentially higher drug bioavailability.

O-carboxymethyl chitosan (CMC), employed in the present formulation, exhibits pH-dependent charge characteristics. At acidic pH values below approximately 6.5, the amino groups of the polymer become protonated ($-NH_3^+$), conferring a net positive charge. This cationic property facilitates gastric mucoadhesion of the microparticles, thereby forming a protective physical barrier that mitigates the mucosal damage induced by ethanol. Previous investigations have consistently demonstrated that mucoadhesive polymers can prolong gastric retention beyond one hour, significantly enhancing in vivo protection against ethanol-induced gastric ulceration[28] In this context, Cao et al. reported that O-CMC coating on calcium phosphate nanoparticles promoted in vitro mucus penetration and improved in vivo intestinal mucosal delivery.[29] Similarly, Joshi et al. observed that conjugation with N,O-CMC significantly enhanced mucin adsorption in nanovesicles compared to unmodified counterparts ($p < 0.05$).[30] Furthermore, Huang et al. demonstrated that O-CMC coating of omeprazole-loaded gum Arabic substantially increased the oral bioavailability of omeprazole.[6]

In a case study, the omeprazole loaded enteric coated nanoparticles revealed significantly higher ulcer protection compared to omeprazole suspension (86.10 % vs 25.61%). They suggested such more gastric mucosal protection can be caused by increasing bicarbonate production or reducing the volume of gastric acid secretion.[19] In another attempt, microparticles of pantoprazole sodium sesquihydrate were prepared in the presence of ES100 by the solvent evaporation method; The UI by microparticles was statistically lower than the control group and the group received a pantoprazole solution.[31] These results confirmed that the microparticles were stable enough not only facilitated a release of omeprazole, but also enhanced bioavailability, which are crucial factors to protect the stomach against ulcer formation by ethanol. The UI reduction is due to the formation of a physical barrier protecting the gastric tissue against ethanol. Similarly, omeprazole nanoparticles composed of CMC provided ulcer protection up to 74%.[32] A limitation of the current research is the absence of a dedicated positive control sample, such as a commercial omeprazole suspension, as well as the lack of a vehicle-treated control group.

Although comparison with a marketed generic omeprazole pellet formulation provides clinically relevant information considering the performance of the prepared microparticles, inclusion of additional control groups would allow a more comprehensive interpretation of the in vivo protective effects. Future studies should incorporate such controls to further validate the efficacy of the proposed formulation.

Microparticle-based formulations utilizing CMC and ES100, along with the spray drying technique, can create an omeprazole formulation that offers a delayed and sustained-release profile, enhancing its therapeutic efficacy. The formulation parameters, including the concentration and type of polymer used, have a direct impact on particle size, release duration, and encapsulation efficiency. Encapsulating omeprazole within both hydrophilic and hydrophobic polymers helps prevent its premature release in the stomach. This is particularly important for omeprazole, as it is acid-labile and degrades quickly in gastric acid.

Conclusion

In this study, a single-step spray-drying technique was employed to fabricate enteric-coated omeprazole-loaded microparticles (OME-MPs) utilizing carboxymethyl chitosan (CMC) and Eudragit® S100 (ES100) as polymeric carriers. Formulation optimization was systematically performed via response surface methodology (RSM) to identify the ideal polymer ratio. The optimized formulation achieved a balanced trade-off between encapsulation efficiency and pH-responsive drug release, effectively minimizing omeprazole liberation in simulated gastric fluid while facilitating rapid and complete drug release under intestinal pH conditions. In vivo evaluation in an ethanol-induced gastric ulcer model demonstrated that the optimized OME-MPs significantly attenuated gastric mucosal injury compared to the reference omeprazole formulation, a finding corroborated by histopathological examination. Collectively, these results indicate that the CMC-ES100 spray-dried microparticulate system constitutes a promising and scalable platform for pediatric oral drug delivery, particularly as a reconstitutable suspension with enhanced stability and patient acceptability.

Ethics approval and consent to participate

All experiments adhered to the ethical guidelines of the National Research Council's Guide and were approved by the ethical committee of the Tehran University of Medical Sciences (Code of Ethics: IR.TUMS.AEC.1402.036).

Authors' Contribution

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Competing interests

The authors declare that they have no competing interests.

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