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Original Article

Preparation and characterization of Eudragit L 100-55/chitosan enteric nanoparticles containing omeprazole using general factorial design: *in vitro/in vivo* study

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Abstract

Background and purpose: Omeprazole (OMP) is broadly used for the treatment of gastroesophageal reflux and other acid-related diseases. The current study aimed to prepare enteric-coated nanoparticles containing OMP to achieve a stable powder formulation easily prescribed in children.
Experimental approach: The nanoparticles were formed by complex coacervation method using chitosan (CTS) and Eudragit L100/55 (EU) and the impact of various formulation variables (the concentrations of EU solution and its volume ratio to CTS solution) were assessed using 3^2 fractional design. The mean particle size (PS), zeta potential (ZP), encapsulation efficiency (EE), and drug loading (DL) were determined. Finally, the pharmacological effects of the optimized OMP enteric nanoparticles were evaluated by an *in vivo* antiulcer study using Sprague-Dawley rats.
Findings/Results: The highest desirability value was for formulation F5 (containing EU concentration 4 mg/mL and EU/CTS volume ratio 2:1). PS, ZP, EE, and DL of the optimized OMP-loaded nanoparticles were confirmed 810 ± 14 nm, -38.2 ± 1.8 mV, 83.1± 4.2%, and 13.1± 1.5%, respectively. *in vitro* release studies showed the pH sensitivity of nanoparticles and OMP release was pH-dependent. *in vivo* pharmacological assessment revealed that the optimized formulation was able to protect rat stomach against ulcer formation induced by indomethacin compared to the group that received normal saline which demonstrated severe peptic ulcer and hemorrhagic spots.
Conclusion and implication: Our results indicated that the enteric EU/CTS nanoparticles were successfully prepared *via* a complex coacervation method and their efficacy could be comparable with commercial OMP pellets.

Keywords: Animal study; Chitosan; Eudragit L 100-55; Omeprazole.

INTRODUCTION

Omeprazole (OMP), a proton pump inhibitor, is broadly used for the treatment of gastroesophageal reflux eradication of helicobacter pylori and other acid-related diseases such as peptic ulcer disease, and Zollinger-Ellison syndrome (1). OMP effectively suppresses the secretion of gastric acid by specific inhibition of the H/K ATPase enzyme system found at the secretory surface of the gastric parietal cell. It is sparingly water-soluble and easily destroyed in an acidic environment. Therefore, it is necessary to protect OMP from acidic harsh stomach medium when orally administered. OMP is commercially available as solid dosage forms included coated granules and tablets in doses of 20 and 40 mg for adults and there is no other friendly dosage form to prescribe in children (2).

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All of the different novel drug delivery formulations, polymeric nanoparticles, have attracted more attention. Enteric nanoparticles not only protect the drug content from degradation in the gastric acid environment but also possess the typical advantages of nanoparticles including higher intracellular penetration and retention time in the site of action (3). The enteric nanoparticles have been previously used for the delivery of peptides, proteins, and some acid-labile drugs such as OMP and lansoprazole. For instance, in the study conducted by Jelvehgari *et al.* Eudragit L100-55 nanoparticles loaded with insulin was successfully decreased the release rate of the incorporated drug in an acidic environment (4). In other studies, Eudragit polymers were used for the delivery of OMP and lansoprazole (5,6). Although the results of those studies revealed the ability of Eudragit polymer in controlling the release rate of the drugs and protecting the incorporated drugs from the harsh gastric environment, there is no *in vivo* study to demonstrate the therapeutic efficacy of Eudragit nanoparticles. Eudragits are known to be extremely used in sustained- and controlled-release formulations. Among the group of Eudragits, Eudragit L 100-55 (EL 100-55; methacrylic acid-ethyl acrylate copolymer type A, 1:1) is an enteric pH-dependent copolymer with freely water solubility above pH 5.5 medium, so EL 100-55 has been commonly used for the preparation of enteric solid-dosage forms (7). Different techniques have been employed for the preparation of enteric nanoparticles such as the emulsification-diffusion method (5), emulsification-evaporation method (8), electrospray deposition method (9), and aerosol flow reactor method. In the emulsification method, an organic solution of polymer is emulsified in an aqueous solution with or without surfactant. Subsequently, the organic solvent is removed by different methods such as evaporation or diffusion to allow particle formation. Based on the literature review, OMP is very sensitive to heat, humidity, light, and organic solvent (10). Thus, emulsification methods for the fabrication of OMP nanoparticles would result in the degradation and inactivation of the drug. Electrospray and aerosol flow methods avoid using organic solvents, however, they are complicated processes and are not easily commercialized. The current study aimed to fabricate enteric-coated nanoparticles of OMP to be easily administrated as freeze-dried powders in children and also geriatric patients who have swallowing difficulties. Here, to avoid organic solvent the nanoparticles were prepared by complex coacervation method which is more applicable and accessible than previously mentioned methods. In this approach, two water-soluble and oppositely charged polymers are employed to fabricate nanoparticles. In this study, Eudragit and chitosan (CTS) were used as anionic and cationic polymers, respectively. Besides Eudragit, CTS is a biodegradable, biocompatible, nontoxic, and mucoadhesive polymer that is extensively used for drug delivery (11). Mucoadhesive nanoparticles can prolong the residence time of the carriers at the absorption site and improved drug absorption properties. High drug-loading capacity and complete coating against an acidic environment are also crucial for effective clinical response. The current work focused on the preparation and characterization of nanoparticles and the effect of two variables including CTS concentration and CTS/Eudragit weight ratio on the properties of nanoparticles such as particle size (PS), zeta potential (ZP), loading capacity, and release pattern of the nanoparticles were determined using fractional design. In addition, their physicochemical properties of EL 100-55 nanoparticles were investigated using various analytical equipment such as scanning electron microscope (SEM) and Fourier transform infrared spectroscopy (FT-IR). Finally, the pharmacological effects of the optimized OMP enteric nanoparticles were evaluated by an *in vivo* antiulcer study using Sprague-Dawley (SD) rats.

MATERIALS AND METHODS

Materials

EL-100-55 (EU) was obtained from Rohm Pharma GMBH (Weiterstadt, Germany). OMP, CTS (deacetylation degree: 85%, viscosity: 20 cps (5 g/L)), and tween 80 were purchased from Sigma Chemical Co. (St. Louis, MO,

USA). Glacial acetic acid, hydrochloric acid (HCL), potassium dihydrogen phosphate, and sodium hydroxide were all from Merck (Germany). All solvents and reagents were of analytical grade. Sprague-Dawley rats (5-6 weeks old, 200-250 g body weight) were obtained from the laboratory animal center of the Faculty of Pharmacy and Pharmaceutical Science, Isfahan University of Medical Science, Isfahan, Iran. All animal experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals approved by the Research Ethics Committee of Isfahan University of Medical Science (Ethic No. 395498).

Experimental design and analysis

In this study, a 3-level factorial design using Design Expert 8 (Stat-Ease Inc., Minneapolis, MN) was employed to evaluate the effect of variables on the characteristics of the OMP-loaded nanoparticles and obtain the optimized formulation. Two factors including the EU solution concentration (2, 4, and 6 mg/mL) and EU/CTS ratio (1:3, 2:1 to 1:1 v/v) were defined in three levels to fulfill the characterization, optimization, and prediction propose. In this study, the concentration of CS solution was 2 mg/mL. All formulations were prepared and subsequently evaluated for responses, such as PS, ZP, entrapment efficiency percent (EE%), drug loading percent (DL%), and dissolution efficiency (DE%).

Preparation of OMP-loaded EU/CS nanoparticles

The colloidal suspension of EU/CTS nanoparticles was obtained through the electrostatic interaction between a solution of CTS at pH 4 (solution concentration of CTS was 2 mg/mL in acetic acid 0.2 M) and EU (solution concentrations: 2, 4, and 6 mg/mL in NaOH solution pH 11) as reported by Jelvehgari *et al.* (4) with minor modifications. Five mL of CTS solution was slowly added to the EU solution (at different volume ratios: 1/1 to 3/1 v/v) while the mixture was homogenizing by magnetic stirrer at 1200 rpm. The resulting suspension was ultrasonicated using a probe sonicator (Baldelin, Berlin Germany) by probe TT13 in amplitude 40% to form EU/CTS nanoparticles. OMP-loaded nanoparticles were prepared by adding the constant amount of OMP (8 mg) to the EU solution prior to the interaction with the CTS solution. The resulting nanoparticle suspension was transferred to Falcon tubes and centrifuged for 10 min at 3000 rpm (Hettich Zentrifugen Model Routine 420 g) at 25 °C and then lyophilized to obtain a white powder of OMP-loaded EU/CTS nanoparticles using a freeze dryer (Model ALPHA 2-4 LD plus, Christ Company, Stuttgart, Germany).

Characterization of nanoparticles

Determination of PS and ZPs of nanoparticles

Five hundred µL of each nanoparticle suspension was diluted in 1 mL water, then PS and ZPs were determined by the dynamic light scattering instrument (Zeta Sizer 3000HS, Malvern Instruments Ltd., Malvern, UK). All measurements were carried out at 25 °C and performed in triplicate.

Determination of DL% and EE%

The drug-loaded nanoparticle suspension (0.4 mL) was placed into the centrifugal ultrafiltration unit (Amicon Ultra-15, Ireland, molecular weight cut-off: 3 kDa) and centrifuged at 25 °C for 5 min at 3000 rpm using microcentrifuge (Microcentrifuge Sigma 30k, UK). The filtrate was collected and the drug concentration was determined by a UV-visible spectrophotometer (UV-mini 1240, Shimadzu, Kyoto, Japan) at wavelength 300 nm. Unloaded nanoparticles were used as control (12). A calibration curve was constructed over the range of 2-20 µg/mL of OMP in distilled water and a linear correlation ($r^2 > 0.998$) with high precision and accuracy was obtained (CV%: 0.74-15.23%, error%: 0.89-9.47%).

The drug EE% and DL% of the nanoparticles were calculated by the following equations:

$$EE\% = \frac{Wi - Wf}{Wi} \times 100 \quad (1)$$

$$DL\% = \frac{Wi - Wf}{Wi - Wf + Wp} \times 100 \quad (2)$$

where, W_i was the weight of the drug initially added in the system, W_f was the drug content in the filtrate after centrifugation, W_p was the weight of polymers added in the system.

in vitro drug release studies

In release experiments, 5 mL of an aqueous dispersion of each formulation was added to the dialysis bags with molecular cut-off 12000 Da and sealed. In the first 2 h, the sealed bags were placed into 50 mL HCl solution (pH 1.2) and shaken at 100 rpm at 37 °C. In the remaining time, the release medium was changed to pH 6.8 by adding phosphate buffer solution 0.2 M. At predetermined time intervals, samples were withdrawn and replaced with fresh phosphate-buffered saline maintained at the same temperature. The content of OMP in the samples was determined spectrophotometrically at 300 nm. Based on the release profiles, DE% was calculated from the area under the curve at time t (measured using the trapezoidal rule) and expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time following equation:

$$DE\% = \frac{\int \mathrm{yt.dt}}{\int 100.\mathrm{T}} \quad (3)$$

Optimization

The optimized formulation was selected by Design Expert 8 and corresponding dependent variables including PS, ZP, EE%, DL%, and DE% predicted based on the previous modeling achieved by the software. The optimized formulation was then prepared and all the responses were practically evaluated. Based on the predicted and actual responses, the error percent was calculated.

SEM

The morphology of the freeze-dried optimized nanoparticles was analyzed by SEM. An LEO 1450 VP SEM (Leo Electron microscopy Ltd., Cambridge, UK) was used with an acceleration voltage of 1.00 kV and a secondary detector.

FT-IR

The FT-IR analysis of all samples was performed by KBr pellets. Approximately 150 mg of pure polymers, OMP, and the drug-loaded nanoparticles were macerated with a sufficient amount of KBr to form a tablet. IR spectra for all samples were obtained in the region of 4000-400 cm^{-1} by a Varian spectrophotometer FT-IR WAS-510/520 (Rayleigh, China) with a resolution of 4 cm^{-1} by an average of 32 scans.

Induction of gastric ulcer

Twenty-two Sprague-Dawley rats were divided randomly into four groups. They were caged individually and already fasted for 24 h before gastric ulcer induction using indomethacin. Four rats in the normal control group (group 0) received no indomethacin, whereas the remaining 18 rats randomly divided into three groups (6 each) and received a single intraperitoneal injection (i.p.) of indomethacin (25 mg/kg) with needle insulin. Group 1 served as negative control and received normal saline before ulcer induction. Groups 2 and 3 received optimized OMP-loaded nanoparticles and commercial enteric-coated pellet (manufactured by Abidi, Tehran, Iran) 1 h before ulcer induction, respectively. Six h later, the animals were sacrificed by inhalation of an overdose of diethyl ether. The abdomens were opened and the stomachs were excised while both sides (cardiac and pyloric) were ligated appropriately (13). Photographs were taken from the stomachs and the areas were evaluated for ulcer severity as described below. The gastric ulcer tissues were then fixed in 10% (v/v) formalin and stained with hematoxylin and eosin (H&E) in the 5-µm thickness sections for histopathological studies.

Assessment of macroscopic parameters

A pathologist unaware of treatments recorded macroscopic scoring parameters. The macroscopic score ranged from 0-4 was performed based on a validated scoring system by Minaiyan *et al*. (13). The scores were: 0 = no ulcer, 1 = mucosal erythema only, 2 = mild mucosal edema, slight bleeding or slight erosion, 3 = moderate edema, bleeding ulcers or erosions, and 4 = severe ulceration, erosions, edema, and tissue necrosis. The ulcer area was measured using 3M® (USA) scaled surgical transpose tape, which was fixed on a light and transparent sheet. Each cell on the tape was 1 mm^2 in the area and the number of cells was counted for determining the ulcer area for the stomach section (14). Ulcer index was the later parameter, measured by summing the ulcer score and the ulcer area for each tissue specimen.

Assessment of pathologic parameters

The specimens, fixed in 10% (v/v) formalin and stained with H&E, were examined using light microscopy (×400) and received scores of 0-3, described by Rezazadeh *et al*. (15) as follow:

Score 0: normal epithelium and connective tissue without vasodilation; absence of bleeding, and inflammatory infiltrate. Score 1: mild cellular infiltration; no hemorrhagic areas, abscesses, or ulceration. Score 2: area of epithelial degeneration; the prevalence of neutrophils infiltration; distinct hemorrhagic areas, edema, and ulceration. Score 3: extensive ulceration and abscesses; severe vascular vasodilation and hemorrhage.

Measurement of gastric pH

In each group, the gastric contents were collected and centrifuged at 2000 rpm for 10 min. Next, the supernatant was examined for pH by a digital pH meter.

Data analysis

The experimental results were analyzed by Design Expert 8. One-way analysis of variance (ANOVA) was also used to determine which factors were statistically significant. A *P*-value <0.05 was considered statistically significant in all cases.

RESULTS

Particle size and zeta potential of nanoparticles

The PS and ZP of the different formulations are listed in Table 1. The PS changed from 618 to 996 nm for various factor-level combinations. As shown in Fig. 1A and B, the PS was significantly increased by increasing the concentration of the EU solution and its volume ratio. Fig. 1C and D also indicated that increasing the concentration of EU solution and its volume ratio has led to the increase in the absolute value of ZP of the nanoparticles.

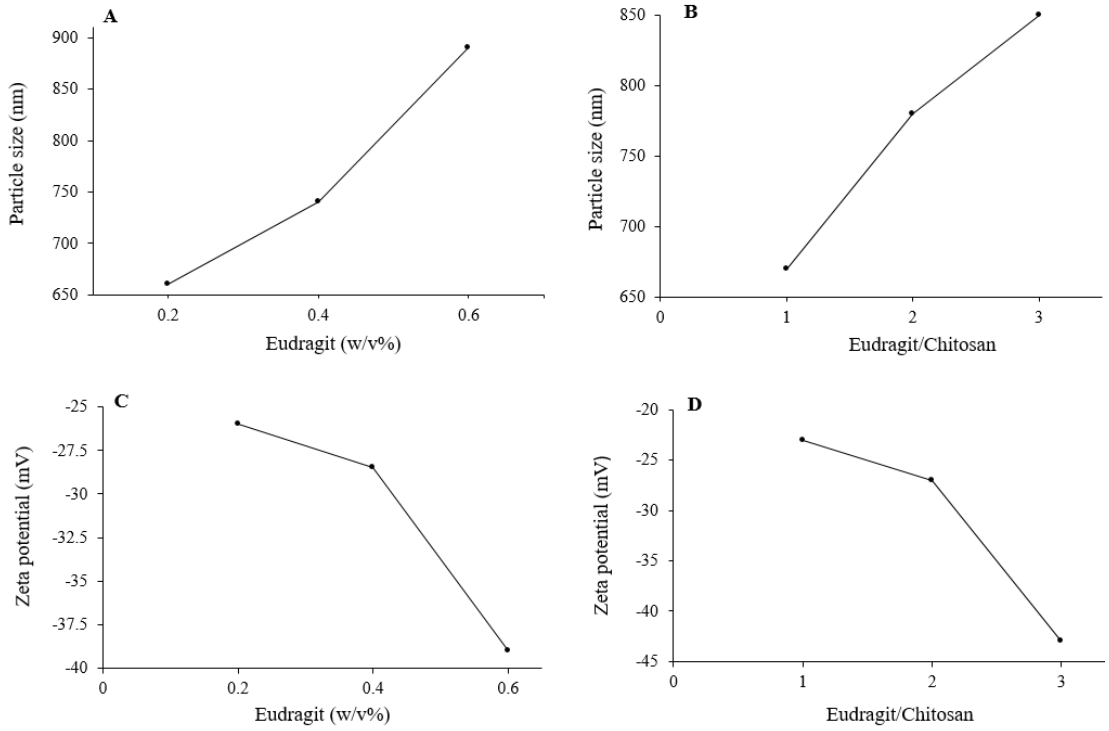

Fig. 1. The effect of (A) EU solution concentration and (B) EU/CTS ratio on particle size, (C) EU solution concentration and (D) EU/CTS ratio on zeta potential. CTS, Chitosan; EU, Eudragit L100/55.

Table 1. Formulations generated by the general factorial design along with their respective responses.

Formulations	Particle size (nm)	Zeta potential (mV)	Encapsulation efficiency (%)	Drug loading (%)	Dissolution efficiency $_{2\text{-}12}$%	%Drug released at 2h
F1 (2*, 1:1**)	618.1 ± 22.8	-18.3 ± 2.5	56.6 ± 6.2	18.9 ± 1.3	55.4 ± 2.9	8.4 ± 0.7
F2 (4, 1:1)	670.2 ± 17.7	-19.6 ± 2.5	61.2 ± 2.6	12.8 ± 0.5	50.9 ± 0.71	19.4 ± 1.1
F3 (6, 1:1)	738.6 ± 16.4	-30.6 ± 2.8	65.4 ± 2.6	10.9 ± 0.4	51.2 ± 3	7.3 ± 0.4
F4 (2, 2:1)	619.8 ± 19.8	-18.6 ± 1.5	77.9 ± 4.5	16.4 ± 0.9	64.7 ± 1.8	6.4 ± 0.7
F5 (4, 2:1)	411.4 ± 27.1	-34.5 ± 3.1	82.2 ± 1.3	11.3 ± 0.1	55.3 ± 0.5	14.5 ± 0.4
F6 (6, 2:1)	911.2 ± 37.1	-43.3 ± 2.5	87.2 ± 3.4	8.9 ± 0.3	58.7 ± 2.1	16.3 ± 1.7
F7 (2, 3:1)	794.3 ± 39.1	-27.6 ± 3.2	90.4 ± 2.6	15.1 ± 0.4	47.5 ± 1	ND
F8 (4, 3:1)	757.6 ± 42.3	-40.3 ± 4.5	71.4 ± 0.9	7.3 ± 0.09	63.2 ± 0.7	ND
F9 (6, 3:1)	996.2 ± 61.5	-66.3 ± 3.6	73.7 ± 3.7	5.5 ± 0.2	58.8 ± 3.9	7.9 ± 0.4

*, Eudragit L100/55 concentration (mg/mL); **, Eudragit L100/55/chitosan volume ratio (v/v); ND, not detectable.

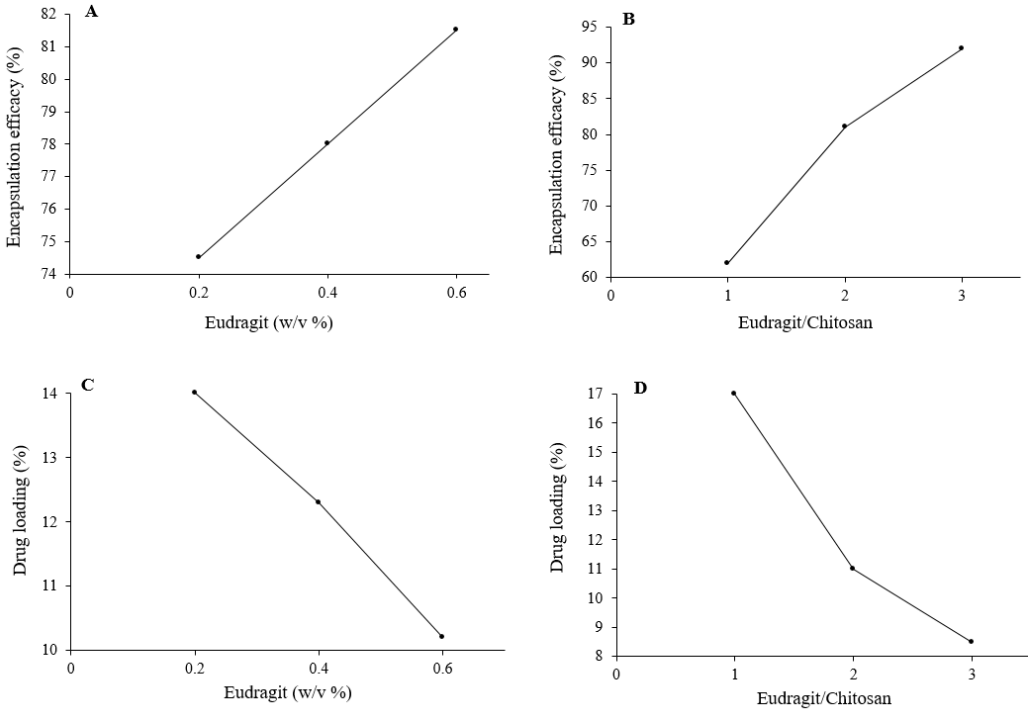

Fig. 2. The effect of (A) EU solution concentration and (B) EU/CTS ratio on encapsulation efficacy%, (C) EU solution concentration and (D) EU/CTS ratio on drug loading%. CTS, Chitosan; EU, Eudragit L100/55.

Entrapment efficiency and drug loading

As listed in Table 1, the EE of different formulations was obtained between 55-93%. As revealed from Fig. 2A and B, increasing the concentration of EU and its volume ratio increased the EE%. DL, on the other hand, changed markedly based on the amount of polymers used in each formulation ranging from 5.5 to 18.9%. (Table 1, Fig. 2C and D).

in vitro drug release studies

The drug release profiles from the formulations are shown in Fig. 3A and B. In the formulations F7 and F8 no drug was detectable in the acidic medium after 2 h, for the rest of the formulations less than 15% OME was released in the first 2 h. However, the release rate of OME was significantly increased in the buffer with pH 6.8 and approximately 70% OME was released from the nanoparticles at 24 h. $DE_{2\text{-}12}$% were calculated for different formulations and listed in Table 1. Figure 4 A and B also depicts the effects of two variables on $DE_{2\text{-}12}$% of OMP in pH 6.8.

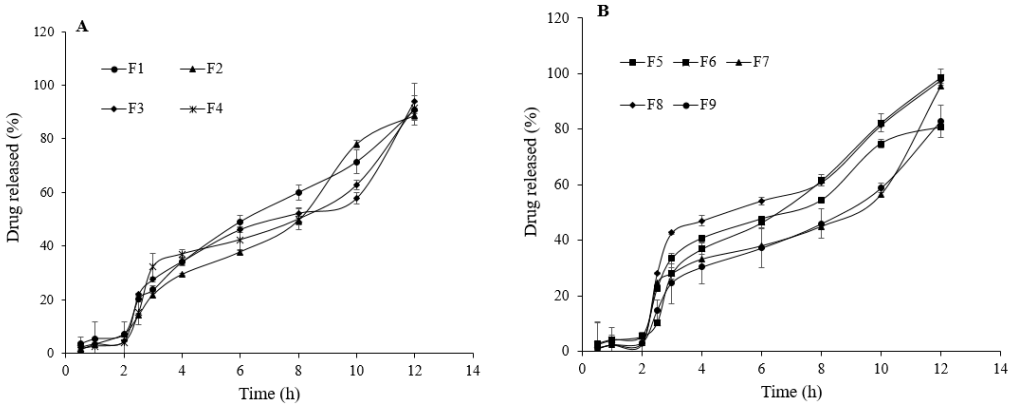

Fig. 3. *in vitro* release profiles of omeprazole from nanoparticle formulations F1-F4 (A) and F5-F9 (B) (n = 3).

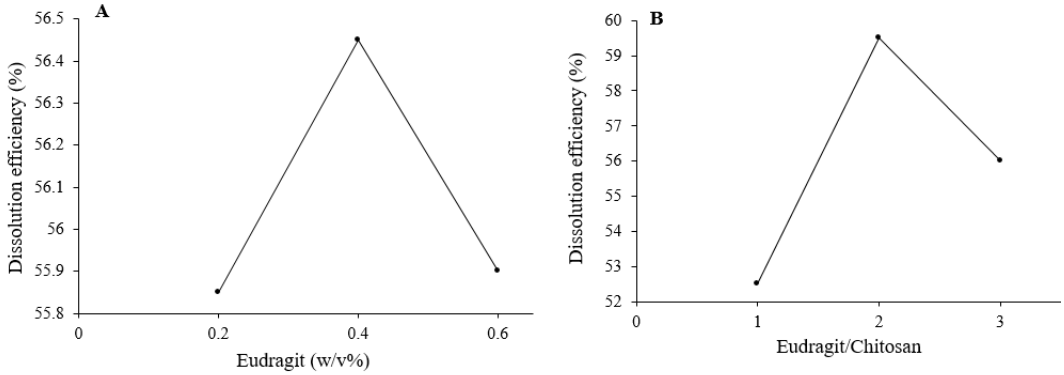

Fig. 4. The effect of Eudragit solution concentration (A) and Eudragit L100/55/chitosan ratio (B) on the percentage of dissolution efficiency.

Optimization and validation

The desirability function was explored using Design-Expert software to achieve the optimized formulation. PS and DL were fitted in the quadratic model while ZP and DE% were fitted in the linear model and EE% corresponded to a 2F1 model. Based on the modeling by Design-Expert software and a desirability factor of 0.85, the F5 formulation was suggested by the software as an optimized formulation. The optimal formulation was then prepared in our laboratory and all responses were evaluated to confirm the validity of the optimization procedure. The error percent for PS, ZP, EE, DL, and DE% were calculated 2.65, 15.7, 12.3, and 15.4%, respectively which confirm the adequate precision of our method for the prediction of optimized conditions. The SEM micrographs (Fig. 5) revealed the morphology of the optimized nanoparticles. The particles were found to be spherical having a size of less than 300 nm.

FT-IR

Figure 6 shows the FT-IR spectra of pure CTS, EU, OMP, and the drug-loaded CTS/EU nanoparticles. The characterization peaks in the CTS spectrum (Fig. 6a) are 1640 and 3445 cm^{-1} which are related to NH and NH_2 groups, respectively. The characterization peaks in EU spectrum (Fig. 6b) are 1740 cm^{-1} C=O stretching) and 1700 cm^{-1} (COO stretching). The characterization peaks of the OMP spectrum (Fig. 6c) are 1626 cm^{-1} (C=C-N and S-C=N stretching), 1079 cm^{-1} and 1025 cm^{-1} related to benzimidazole OCH_3. In the spectrum of OMP-loaded CTS/EU nanoparticles (Fig. 6d), the signal related to NH group of CTS was shifted to 1700 cm^{-1}, evidence of the interaction between both polymers. Characteristic peaks of OMP with a negligible shift were also observed in the OMP-loaded CTS/EU nanoparticles spectrum.

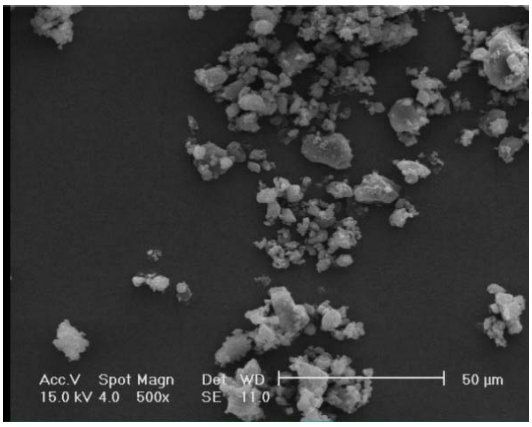

Fig. 5. Scanning electron microscope image of freeze-dried optimized omeprazole-loaded chitosan/Eudragit L100/55nanoparticles (F5).

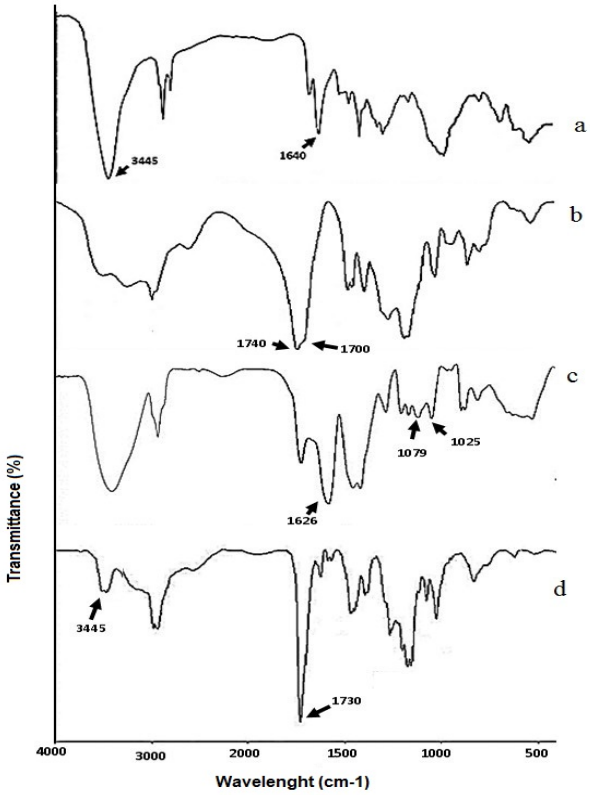

Fig. 6. Fourier transform infrared spectra of (a) chitosan, (b) Eudragit L100/55, (c) omeprazole, and (d) optimized omeprazole-loaded nanoparticles (F5).

in vivo anti-ulcer activity

Macroscopic features of gastric tissue

In the normal control group, no ulcer and hemorrhagic spots were found (Fig. 7a). In the negative control group (peptic ulcer induced by indomethacin and received normal saline), several ulcer and hemorrhagic spots together with erythema, inflammation, and edema especially in the antrum region were evident (Fig. 7b). In OMP-treated groups in the form of nanoparticles and pellet (groups 2 and 3, respectively), the ulcer area (cm^2), as well as its severity (scores), were reduced compared to the negative control group ($P < 0.05$), and there

were no significant differences between group 2 and group 3 based on ulcer area, its severity, and ulcer index (Table 2). Macroscopic observation of different groups has been shown in Fig. 7A-D

Histopathological features of gastric tissue

Histopathological examination of stomach sections obtained from the normal control group showed normal mucosa and sub-mucosal layers as shown in Fig. 8A. In the negative control group, severe histopathological changes such as erythema, edema, inflammation, and congestion were observed (Fig. 8B). In OMP groups, necrosis and edema of the mucosal layer meaningfully decreased and normal mucosal appearance and considerable reduction in ulcerative injuries were seen (Fig. 8C and D). The histopathological scores for the negative control, OMP loaded nanoparticles and OMP pellet was obtained 2.3 ± 0.24, 0.66 ± 0.34, and 0, respectively. Over the whole treatment period, histopathological scores were significantly lower in the group that received OMP compared with the negative control group. The pH value of stomach in normal control, negative control, OMP nanoparticles, and pellets were obtained 3.4 ± 0.5, 2.1 ± 0.1, 7.2 ± 1.3, and 6.8 ± 0.9, respectively. In the groups that received OMP, the acid secretion in the stomach was decreased and elevated the pH value of its content was observed.

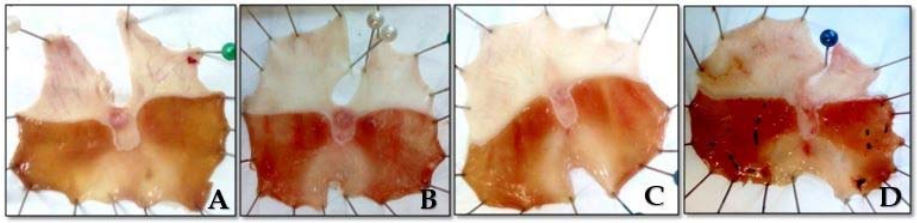

Fig. 7. Macroscopic presentation of gastric tissue injuries induced by indomethacin, in rats. (A) Normal; (B) gastric ulcer induced and treated by omeprazole pellet; (C) gastric ulcer induced and treated by omeprazole-loaded nanoparticles; and (D) negative control group gastric ulcer induced and received normal saline.

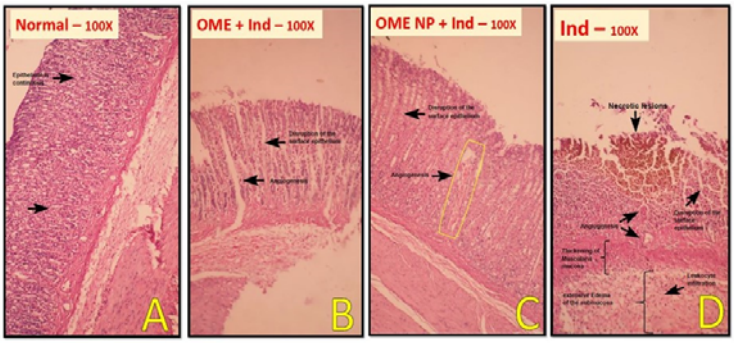

Fig. 8. Microscopic evaluation of gastric tissue injuries induced by indomethacin in rats. (A) Normal tissue; (B) gastric ulcer induced and treated by omeprazole pellet; (C) gastric ulcer induced and treated by omeprazole-loaded nanoparticles; and (D) gastric ulcer induced and received normal saline. Stomach ulcer formation induced by indomethacin.

Table 2. Effects of omeprazole on ulcer are, ulcer score, and ulcer index of gastric ulcer induced by indomethacin, in rats. The normal control group received normal saline without ulcer induction, whereas the negative control received normal saline before ulcer induction. Stomach ulcer formation induced by indomethacin.

Groups	Ulcer area	Ulcer score	Ulcer index
Normal control group	0	0	0
Negative control group	2.56 ± 0.42	3 (3)	5.56
Group received omeprazole nanoparticles	1.21 0.19	1 (0-1)	2.21
Group received omeprazole pellets	1.05 0.18	0.5 (0-1)	1.55

DISCUSSION

The commercial formulation of OMP is available in enteric-coated granule tablets or capsules to protect the drug from the acidic environment of the stomach. Enteric nanoparticles have more advantages compared to granules and tablets such as higher intracellular entrance because of their smaller size, more residence time in the gastrointestinal tract, and higher stability (16). Eudragit is a well-known enteric-coated polymer that is widely used in the preparation of enteric-coated formulations. Enteric nanoparticles of OMP and other acid-labile agents have been previously reported (5,6,8). However, in most earlier published reports emulsion-diffusion techniques using organic solvent have been used for the preparation of nanoparticles. For instance, Bendas et al. (8), prepared OMP-enteric nanoparticles by dissolving enteric polymers (hydroxypropyl methylcellulose phthalate or polyvinyl acetate phthalate) into a mixture of ethanol and acetone. In another similar study, OMP was dissolved in dichloromethane and injected into the aqueous solution containing Eudragit to form an O/W emulsion. Alai *et al*. dissolved lansoprazole and Eudragit RS100 in the mixture of dichloromethane/methanol which was subsequently added into an aqueous solution containing poly(vinyl alcohol) to form O/W emulsion (6). As previously mentioned, OMP is very sensitive to heat, humidity, light, and organic solvent. Moreover, the residual organic solvent in the final product is dangerous. In the current study to avoid organic solvent, we employed a complex coacervation method to prepare enteric-coated nanoparticles. Here, the nanoparticles were formed through the electrostatic interaction between positively charged CTS and negatively charged EU under continuous shearing stress. A general factorial design with two factors and three levels was applied to optimize the nanoparticles. As shown in Table 1 and Fig. 1a and b, the PS was significantly increased by increasing the concentration of EU and its volume ratio. Higher concentrations of EU led to a higher density of negatively carbocyclic groups (-COO), resulting in greater repulsion among them, and, consequently, an increase in PS. Figure 1b and c indicate that increasing the concentration of the EU solution and its volume ratio led to an increase in the absolute value of ZP. As stated above, increasing the amount of EU attributed to an increase in the COO on the EU surface causes a significant increase in the absolute value of ZP. In the work conducted by Jelvehgari *et al*. (4), CTS/Eudragit nanoparticles were prepared for oral delivery of Insulin. The PS and ZP of the nanoparticles were significantly increased in higher concentrations of the EU solution, which is in accordance with our results. Rezazadeh *et al.* (17) evaluated the effect of different CTS concentrations on PS and ZP of the CTS/chondroitin sulfate nanoparticles. The results revealed that increasing the concentration of CTS solution and its volume ratio significantly increased the PS and ZP of the nanoparticles which were attributed to the higher density of positively charged NH_3 groups on the surface of nanoparticles. As listed in Table 1, in most of the formulations, the EE and DL changed markedly based on the amount of polymers used in each formulation. Increasing the concentration of the EU solution and its volume in the formulation increased the EE% and decreased the DL. It can be clarified that since the amount of the drug is constant and equal in all formulations, increasing the amount of EU polymer reduces the drug/polymer weight ratio and causes DL to decrease. At the same time, higher polymer concentration promotes better drug entrapment between polymer chains. The *in vivo* anti-ulcer evaluation demonstrated that the enteric-coated nanoparticles were able to reduce ulcer formation induced by indomethacin. The gastric mucosal protection against indomethacin can be mediated through a number of mechanisms that include enhancement of the gastric mucosal defense through the increase in mucus and/or bicarbonate production, reducing the volume of gastric acid secretion or by simply neutralizing the gastric acidity and increasing the pH value of gastric content (18). Pretreatment of rats with OMP effectively increased the value of stomach and reduced the severity of injury compared to the negative control group. Based on macroscopic and microscopic evaluation of samples, the developed formulation could be a

successful alternative for commercial OMP pellets. However, in a previous study regarding the preparation of enteric-coated OMP, ulcer protection was only evaluated based on macroscopic examination (8).

CONCLUSION

In the current study OMP enteric nanoparticles were successfully prepared by complex coacervation method and optimized using general factorial design. The least PS, highest ZP and EE values were obtained for the formulation (F5) containing EU concentration 4 mg/mL and EU/CTS volume ratio 2:1. The *in vivo* evaluation corroborated with the *in vitro* results demonstrating that OMP-loaded enteric nanoparticles were efficient in protecting the stomach against ulcer formation. Further accelerated stability study and comparative bioequivalence study in human volunteers are needed to be conducted in the future to confirm the formulation's physical and chemical stability and its therapeutic efficacy.

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Conflict of interest statement

The authors declared no conflict of interest in this study.

Authors' contribution

M. Rezazadeh and A. Mostafavi proposed the idea and developed the initial proposal, M. Minayian developed animal study, R. Safaran collected the data in the laboratory and analyzed the data, M. Rezazadeh searched the literature and developed the initial draft of the manuscript, all the authors have read and contributed in the final version of the manuscript.

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