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Instantaneous enteric nano-encapsulation of omeprazole: Pharmaceutical and pharmacological evaluation

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ABSTRACT

Recently, great attention has been paid to nanocapsules. The interest of these structures is due to their promising applications as drug delivery systems. The objective of this study was to develop novel enteric coating technique based on instantaneous encapsulation of the acid-labile drug, omeprazole in innovative enteric nanocapsules. Omeprazole enteric nanocapsules were formulated by varying the type and amount of the enteric polymer. The particle size (PS), polydispersity index (PDI), zeta potential (ZP) and encapsulation efficiency (EE) values of the prepared enteric nanocapsules were determined. A full $2^1 \times 3^1$ factorial design was used for planning and analysis of the experimental trials to select the optimized formulation. The highest desirability value was 0.7463 for formula E3 (containing 200 mg hydroxypropyl methylcellulose phthalate (HPMCP)). The stability of omeprazole was reflected by the absence of the exothermal peak when the drug was encapsulated as detected by differential scanning calorimetry (DSC) thermograms. In vitro drug release study confirmed the USP specifications required to meet the key formulation characteristics of gastro-resistance. In vivo pharmacological assessment showed that the optimized nanocapsules were able to protect rat stomach against ulcer formation compared to the aqueous suspension of the drug which showed less significant protection.

1. Introduction

Research in nanotechnology has powerfully increased in the last decades. Many colloidal carriers, such as liposomes (Gregoriadis, 1995), niosomes (Rajera et al., 2011), nanoemulsions (Tiwari and Amiji, 2006) and nanoparticles (Agnihotri et al., 2004) have been extensively investigated for drug delivery.

The main advantages of nanoparticles are biocompatibility and biodegradability, control of the drug release, increase of drug selectivity and effectiveness, improvement of drug bioavailability and decrease of drug toxicity and the ability to target specific tissues (Malam et al., 2009). Polymeric nanoparticles are named nanocapsules, when they contain a polymeric wall and an oil core (Jager et al., 2007), where the core acts as a liquid reservoir for several molecules or drugs, and the wall as a protective membrane. They have special use for encapsulating and delivering hydrophobic drugs (Sanchez-Moreno et al., 2012). The versatility of these nanocapsules for an efficient encapsulation in their oily core of several anti-cancer drugs has been previously demonstrated (Beduneau et al., 2007).

Omeprazole, a substituted benzimidazole, is a powerful inhibitor of gastric acid secretion in man probably without a direct effect on pepsin secretion (Festen et al., 1986). It acts by non-competitive inhibition of parietal cell H^+/K^+-ATPase (Fellenius et al., 1981). Omeprazole has a half-life of less than 1 h and is almost entirely cleared from plasma within 3–4 h. Omeprazole is metabolized in the liver. The two major plasma metabolites are sulphone and hydroxyomeprazole, neither of which contributes to the antisecretory activity (Cederberg et al., 1989). Omeprazole seems to be well absorbed from the gastrointestinal tract (GIT). However, its oral bioavailability in humans is about 40–50% due to a marked first-pass metabolism before entering the systemic circulation (Watanabe et al., 1994). Omeprazole is widely used in doses of 20–80 mg in treatment of duodenal and gastric ulcers, reflux oesophagitis and in the Zollinger–Ellison Syndrome (Clissold and Campoli-Richards, 1986; Watanabe et al., 1994).

Omeprazole is sensitive to heat, humidity, light, and organic solvents (Turkoglu et al., 2004). It degrades rapidly in acid solutions (Quercia et al., 1997). To prevent acid degradation in stomach, the drug is formulated as enteric-coated formulations such as enteric coated granules (Andersson et al., 1991) and enteric-coated tablets and capsules (Thomson et al., 1997). Differences in the quality of the granules coating are a potential limiting factor of the in vivo performance of the tested

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formulations. It was found that omeprazole degradation is more pronounced in aqueous polymer dispersions than in organic polymer solutions (Riedel and Leopold, 2005). The influence of organic polymer solutions on the stability of omeprazole depends on the amount of acidic groups in the polymeric structure, whereas the influence of aqueous polymer dispersions depends on the pH value of the dispersion. However, there are several concerns over the use of organic solvents, which usually are toxic, flammable, and explosive in addition to, the ecological and the economic problems (Rafati et al., 2006). Also there are still some potential problems associated with the use of the aqueous dispersions in pharmaceutical coating, comprising microbial contamination, the instability of the dispersions or the active ingredient, long processing time and adjusting optimal processing conditions. These critical parameters can cause a non-uniformity of the applied coating layer and up-scaling problems (Mehuys et al., 2005). To overcome the limitations of aqueous coating and to reduce process time, a dry coating method was developed (Obara et al., 1999).

The aim of this study was to develop novel enteric coating technique based on instantaneous encapsulation of the chemically labile drug, omeprazole in innovative enteric nanocapsules and find optimal production parameters for a stable, highly concentrated omeprazole formulation. Moreover, the pharmacological effects of the omeprazole enteric nanocapsules were evaluated by an in vivo antiulcer study using Wistar male rats.

2. Materials

Omeprazole was kindly provided by Aventis Pharma, Cairo, Egypt. Lecithin and Pluronic F68 were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Hydroxypropyl methylcellulose phthalate (HPMCP) was purchased from Samsung Fine Chemicals Co. (Seoul, Korea). Polyvinyl acetate phthalate (PVAP) was obtained from Colorcon (West Point, PA, USA). Miglyol® 812 was supplied by Sasol Germany GmbH (Germany). Sodium bicarbonate was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Absolute ethanol, acetone and acetonitrile (HPLC grade) were purchased from Fisher Scientific Co. (Pittsburgh, PA, USA). All other chemicals were of analytical grade.

3. Methods

3.1. Preparation of omeprazole enteric nanocapsules

Omeprazole enteric nanocapsules were prepared according to the following procedures: 20 mg of omeprazole, 50 mg of lecithin and different amounts of enteric polymers (HPMCP or PVAP) were dissolved into a mixture of 2.5 mL of ethanol and 12.0 mL of acetone and then 315 μL of Miglyol® 812 was added. Immediately after that, this organic phase was poured into 25 mL of aqueous solution containing 62.5 mg of Pluronic F68 and 50.0 mg of sodium bicarbonate. Instantly upon addition of the organic phase into the aqueous solution, it turned milky. The milky mixture was evaporated under reduced pressure, in a rotary evaporator (Rotavapor, Type R 110, Büchi, Switzerland) at 45 °C for 15 min to a final volume of 20 mL. The nanocapsules formulae containing HPMCP were coded (E1–E3) while those containing PVAP were coded (E4–E6).

3.2. Characterization of enteric nanocapsules

3.2.1. Particle size and zeta potential

The particle size of omeprazole enteric nanocapsules was measured by photon correlation spectroscopy (PCS) using a Zetasizer Nano ZS-90 instrument (Malvern Instruments, Worcestershire, UK) after suitable dilution with distilled water. The measurements were conducted in triplicate. The average size of the nanoparticles was expressed as a median diameter (Dv50), which is particle diameter at 50% cumulative volume. The polydispersity index was also determined. The particles surface charge was quantified as zeta potential (ζ) using a Zetasizer Nano ZS-90 instrument (Malvern Instruments, Worcestershire, UK). Measurements were also performed after suitable dilution with distilled water and were conducted in triplicate.

3.2.2. Drug encapsulation efficiency (EE)

The encapsulation efficiency (EE) of omeprazole was determined indirectly by calculating the difference between the total amount of omeprazole added in the formulation and that remaining in the aqueous medium after separating the enteric nanocapsules by ultracentrifugation at 14,000 rpm for 1 h at 4 °C using cooling ultracentrifuge (Model 8880, Centurion Scientific Ltd., W. Sussex, UK). Omeprazole contents were determined by an accurate validated HPLC method. Encapsulation efficiency was calculated using the equation:

$$\mathrm{EE} = \frac{\text{Drug content in nanocapsules}}{\text{Drug amount used}} \times 100 \quad (1)$$

3.3. HPLC determination of omeprazole

An isocratic HPLC method was employed for the quantification of omeprazole (Nataraj et al., 2012). A Thermo Separation HPLC system (Fremont, California) equipped with a P4000 pump unit, an AS3000 autosampler including an injection valve with a sample loop of 50 μL volume, and a UV2000 detector was used. A Zorbax Extend-C18 column (4.6 mm × 250 mm) containing 3.5 μm size adsorbent as stationary phase (Agilent Technologies, Santa Clara, California) was used. The column was maintained at room temperature (25.0 ± 2.0 °C). The mobile phase consisted of a mixture of phosphate buffer (pH 7.4) and acetonitrile (60:40 v/v). The flow rate and the UV detector were set at 1.0 mL/min and 302 nm, respectively. Omeprazole was eluted at 6.3 min under these conditions. An external calibration curve was established in the range of 10–70 μg/mL. The assay procedures were validated in terms of linearity, precision, and accuracy.

3.4. Optimization using statistical design

For systematic optimization of enteric nanocapsules formulae, full factorial experimental design methodology was employed with Design-Expert® software (Version 7, Stat-Ease Inc., MN) to investigate the effect of formulation variables on enteric nanocapsules properties (Table 1). Two independent variables were evaluated which were: type (X_1) and amount (X_2) of the polymer. The particle size (Y_1: PS), polydispersity index (Y_2: PDI), zeta potential (Y_3: ZP) and encapsulation efficiency (Y_4: EE) were

Table 1
Full factorial design for optimization of the enteric nanocapsules formulae.

Factors (independent variables)	Levels		
X_1: type of polymer	HPMCP		PVAP
X_2: amount of polymer (mg)	50	100	200
Responses (dependent variables)	**Constraints**		
Y_1: particle size (nm)	Minimize		
Y_2: polydispersity index	Minimize		
Y_3: zeta potential (mV)	Maximize (least negativity)		
Y_4: encapsulation efficiency (%)	Maximize		

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