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Research Article

Development and *In Vitro* Evaluation of Eudragit RLPO based Polymeric Nanoparticles of Lansoprazole

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ABSTRACT

The objective of present research work was to formulate nanoparticles of lansoprazole using Eudragit RLPO® (ERLPO) as carrier to protect it from acidic pH and to improve its solubility. The nanoparticles were prepared by the solvent displacement method (nanoprecipitation). Lansoprazole is a benzimidazole derivative, which is used as a representative proton pump inhibitor. The compatibility of lansoprazole and Eudragit RLPO was evaluated by the Fourier transform infrared spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC). The formulated nanoparticles were evaluated by Powder X Ray Diffractometry (PXRD) and Scanning Electron Microscopy (SEM). DSC results showed that there is not any drug- excipient interaction. Saturation solubility and dissolution studies indicated that dissolution rate was remarkably increased in lyophilized Formulation as compared to drug alone. The in vitro drug release study showed that very less drug was released in the pH 1.2 dissolution medium within 2h whereas 90 % of the drug was released in the pH 6.8. In conclusion Eudragit RLPO can be suitably utilized to increase the solubility and acid resistance of lansoprazole.

INTRODUCTION

Lansoprazole, 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl] methyl] sulfinyl]benzimidazole, is a benzimidazole derivative. It shows its proton pump inhibiting action by selectively inhibiting the H^+/K^+-ATPase of the parietal cell of the stomach. Lansoprazole has been clinically used in the therapy of gastric and duodenal ulcerative diseases [1-5]. Limited solubility of lansoprazole and the variation in the genotype of CYP2C19 are attributed to wide inter-individual variation in bioavailability of lansoprazole.

Adsorbents prepared by depositing lansoprazole and surfactants on porous adsorbents have been employed to improve the dissolution and oral bioavailability of lansoprazole. Nanoparticles have been well used to improve the solubility of poorly water-soluble drugs [6, 7].

Lansoprazole is the least stable among the other inhibiters of the H^+/K^+-ATPase such as rabiprazole, pentaprazole and omeprazole. It is also the most potent drug used in the therapy of gastric and duodenal ulcerative diseases. The drug is having more stability in basic pH as compared to acidic pH [8].

The pH of the colonic region is toward neutral to basic and therefore targeting to this region of gastro- intestinal tract (GIT) may improve the bioavailability of the lansoprazole, but the dosage form should be capable of protecting the drug to reach to colon. There should not be any drug release in stomach.

The simplest method to prepare drug loaded nanoparticles is the solvent displacement method also known as nanoprecipitation method, developed by Fessi et al 1989. This method is based on the interfacial deposition of a polymer following displacement of a semi-polar solvent miscible with water [9]. The technique is easy, less complex, less energy consuming as well as widely applicable without any additives. However, entrapment of hydrophilic drug substances is very difficult in this method [6]. Carriers of nanoparticles with high drug entrapment efficiency would reduce the quantity of carrier required for the administration of a

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sufficient amount of drug at the target site, as well as drug wastage during manufacture [10]. There are several methods already reported in the literature to improve drug entrapment efficiency of the nanoprecipitaion method [11]. Eudragit RLPO(ERLPO) was selected based on its capability to form nanodispersions with submicron particle size, positive surface charge, and good stability [12]. ERLPO is an acrylic and methacrylic acid-based polymer having hydrophilic properties which may be due to the presence of quaternary ammonium groups (QAGs). It is less soluble in water but swells in the digestive fluids, independent of the pH and become permeable [13]. It is used mainly in film coating of tablets, granules, and other small particles and can be used in matrix formulation as well. Eudragit nanoparticles appear as inert carriers suitable for oral drug delivery [14]. In the present investigation an attempt is made to prepare and characterize lansoprazole loaded ERLPO (LSP/ERLPO) nanoparticles formulations intended for the treatment of gastric and duodenal ulcerative diseases. Physicochemical characterization of the nanoparticles formulations was performed by measuring particle size, zeta potential, drug entrapment efficiency and in vitro drug release. Solid state characterization of the freeze dried nanoparticles formulations was performed by Fourier Transform Infrared spectroscopy (FTIR), Differential Scanning Calorimeter (DSC) and Powder X-Ray Diffraction (PXRD) analysis. These techniques allow in understanding the thermal behavior, drug crystallinity and possible occurrence of drug polymer interaction for the freeze dried nanoparticles formulations. Freeze drying and redispersibility of the lyophilized samples were performed for the selected formulation. Short term stability for 1 month for the selected formulation was also carried at room temperature 20 ^{0}C and at 40 ^{0}C [8].

MATERIALS AND METHODS

Materials

Lansoprazole was obtained as gift samples from Alkem Laboratories Pvt. Ltd. (Mumbai, India). ERLPO was purchased from Evonik Industries Pvt. Ltd. (Mumbai, India). Poloxamer 188 (P-188) was purchased from Sigma-Aldrich Corporation (Bangalore, India). All other solvents and chemicals used were of analytical grade and obtained from S.D Fine Chemicals (Mumbai, India).

Preparation of Nanoparticles

The LSP/ERLPO nanoparticles Formulations were prepared by the solvent displacement method similar to that employed by Fessi et al 1989. Four different weight ratios of drug to polymers were used as shown in Table 1, ERLPO (100 mg) and various proportions of drug (10-40% by weight of the polymer) were dissolved in 10 ml of acetone. This organic phase was poured drop wise into 20 ml of a 1% w/v of Poloxamer 188 solution with moderate magnetic stirring at room temperature. Nanoparticles were spontaneously formed and turned the solution slightly turbid. Then, acetone was removed by continuous stirring for 20 h. The resulting particle suspension was filtered through a 1.2 µm cellulose nitrate membrane filter in order to remove larger particle aggregates. The prepared suspension was centrifuged at 19,000 RPM at 15 °C for 60 min (Beckman coulter Optima Max –Xp Ultracentrfuge). For all Formulations, the supernatant was removed and the sediment was freeze dried for 48 h.

The freeze drying process was carried out in the Virtis Freeze mobile model bench top K (SP, Industries INC, NY, USA). Temperature was kept about - 75°C and the vacuum were kept at 21.33 Pa. After 48 hours, the lyophilized samples were collected and stored in a desiccator for further analysis [15]. All of the four Formulations (L1, L2, L3 and L4) were freeze dried to obtain a dry powder.

Characterization of the Nanoparticles

Particle size analysis and zeta potential measurement

The lyophilized samples were dispersed in distilled water and mean particle size for the Formulations was determined by Photon Correlation Spectroscopy (PCS) with a Zeta sizer Nano ZS-90 (Malvern Instruments Ltd., UK) equipped with the DTS software. The reading was carried out at a 90° angle with respect to the incident beam. The zeta potential was measured by a laser Doppler anemometer coupled with the same instrument. A potential of ±150 mV was set in the instrument. Disposable cuvettes of 0.75 ml capacity were used for all measurements. All measurements were carried out in triplicate [1].

Scanning Electron Microscopy (SEM)

In order to examine the particle surface morphology and shape, SEM was used. A concentrated aqueous suspension of lyophilized formulation was spread over a slab and dried

under vacuum. The sample was shadowed in a cathodic evaporator (also known as "Sputter coater") with a gold layer of 20 nm thick in an argon gas environment at 45 mA current for 5 seconds. Photographs were taken using a JSM-5200 Scanning Electron Microscope (Tokyo, Japan) operated at 10 kV [16].

Differential Scanning Calorimetry (DSC)

DSC (model 822e, Mettler Toledo, OH, USA) was used in order to analyze the thermal behavior of different samples. Indium (3-5 mg, 99.99% pure, onset 156.6 °C, heat of fusion of 107.5 J/g) was used to calibrate the instrument. Selected lyophilized formulation, Eudragit RLPO, lansoprazole drug substance, Poloxamer 188 and physical mixture of all the excipients, (2 -5 mg) were accurately weighed into separate 100 µl aluminum pans and then crimped. The thermograms were recorded over a temperature range of 40-300 °C at a rate of 10 °C /min under nitrogen purge gas at 30 ml/min. Mettler Toledo STARe software (version 8.10) was used to analyze data [17].

Powder X-Ray Diffractometry (PXRD)

The crystalline state of the drug in the lyophilized formulation was evaluated by PXRD analysis. The X ray spectra were recorded with an X'Pert-PRO multipurpose powder X-Ray diffractometer (PANalytical, Tokyo, Japan) using Ni-filtered, CuKα radiation with a voltage of 45 kV, and a current of 40 mA with a scintillation counter. The instrument was operated in the continuous scanning speed of 4°/min over a 2θ range of 5° to 40°. Formulation and lansoprazole drug samples were ground using a mortar and pestle, placed into the cavity of an aluminum sample holder and packed smoothly using a glass slide. The results were evaluated using the X-Pert Data collector version 2.1 software.

Fourier Transform Infrared spectroscopy (FTIR)

The Fourier transform infrared analysis was conducted to verify the possibility of interaction of chemical bonds between drug and polymer. The FTIR spectrum was performed using a FTIR-8400 S spectrophotometer by using IR Solution software with a resolution of 1 cm^{-1}.The samples of Eudragit RLPO, lansoprazole drug substance, physical mixture and selected lyophilized formulation were scanned in the spectral region between 4000 and 400 cm^{-1} by taking an average of 8 scans per sample. Solid powder samples were oven dried, finely crushed, mixed with potassium bromide (1:10 ratio by weight) and pressed at 15000 psig (using a 15 tons motorized pellet press, Kimaya Engineers Pvt. Ltd, Mumbai, India)) to form pellet.

Drug Entrapment Efficiency (DEE)

Separately prepared nano-suspension in distilled water (20 ml) of lyophilized formulations were centrifuged at 19,000 rpm for 60 min at 15 °C temperature using a Beckman coulter Optima Max –Xp Ultracentrifuge. The amount of unincorporated drug was measured by taking the absorbance of the appropriately diluted supernatant solution at a 260 nm using single beam UV spectrophotometer (Shimadzu 1700, Japan) against blank/control nanosuspension. DEE was calculated by subtracting the amount of free drug in the supernatant from the initial amount of drug taken. The experiment was performed in triplicate for each formulation and the average was calculated.

Drug Content

The content of lansoprazole drug substance in all formulations was estimated by UV spectrophotometric method using Shimadzu 1700 spectrophotometer. An accurately weighed quantity equivalent to 10 mg of lansoprazole lyophilized nanoparticles (L1-L4) were taken and dissolved separately in 100 ml ethanol. From the resulting solution 1 ml was diluted to 10 ml and assayed for drug content at 260 nm.

Saturation Solubility Study

The solubility of lansoprazole in the lyophilized formulation using distilled water and pH 6.8 buffer was determined. An excess amount of lansoprazole lyophilized formulation was placed in different glass bottles containing 20 ml of distilled water and pH 6.8 buffer solvent. The bottles were thoroughly shaken for 24 h and at the end of this period the solutions were filtered and the filtrate was collected into dry containers. The solutions were suitably diluted and assayed for drug content [18].

***In vitro* Drug Release Study**

The release of lansoprazole from lyophilized formulations was evaluated for 5 h (2h acidic buffer solution of pH1.2 and for next 3h in 6.8 buffer solution) by using dialysis bag (Himedia labs, cut off weight 12,000–14,000 Da) diffusion technique. In this technique, each bag was loaded with the freeze dried formulation (equivalent to 10 mg of lansoprazole), hermetically sealed and dialyzed against 900 ml acidic buffer solution

(pH 1.2) contained kept on a thermostatically controlled magnetic stirrer maintaining temperature of 37±0.5°C for 2 h and then acidic buffer was completely replaced by the 900 ml phosphate buffer solution (pH 6.8) contained; kept on a thermostatically controlled magnetic stirrer maintaining temperature of 37±1 °C for 3 h stirring at 500 rpm. Samples (5 ml) were collected at predetermined time intervals till 5 h, and immediately replaced with 5 ml of fresh buffer to maintain sink condition. The cumulative percent release of lansoprazole was calculated by analyzing the samples on UV spectrophotometer at 260 nm.

Kinetics of Drug Release

Data obtained from in vitro drug release studies in phosphate buffer solution (pH 6.8) i.e. after 2h drug release study were plotted in various kinetic models as zero order, first order and Higuchi's model [19, 20].

Mechanism of Drug Release

Mechanism of drug release from drug-loaded nanoparticles was evaluated by subjecting the data obtained from in vitro drug diffusion studies to Korsmeyer–Peppa's model as log cumulative percentage drug released versus log time. Release exponent (n) and kinetic constant (k) were calculated from slope of straight line:

$$\frac{Mt}{M\infty} = Kt^{n} \qquad (1)$$

Where M_t represents the amount of released drug at time t, M∞ is the total amount of drug released after an infinite time, K is the diffusional (kinetic) constant of drug–polymer system and n is the release exponent that determines the mechanism of drug release from drug delivery system [20, 21].

Effect of cryoprotectant on redispersibility of Formulations

Selected Formulation was studied for the effect of cryoprotectant on freeze drying as well as the redispersibility of the drug loaded nanosuspension. Two cryoprotectants were used; sucrose and mannitol both at 5% w/v concentration level. The freeze drying process was carried out as described in nanoparticles preparation section.

Redispersibility of lyophilized products was carried out by manually shaking the powder in small glass vial with distilled water. Visual observation was done to investigate formation of any aggregates or precipitates after shaking. Particle size and size distribution after redispersion of the sample was performed using Zeta potential/Particle sizer (model NicompTM 380 ZLS, USA).

Short Term Stability Study

The selected lyophilized formulation was used to perform short term stability study of the nanoparticles. Samples were stored in glass vials for 1 month at room temperature (20 °C) and at 40 °C [22]. After 1 month, particle size determination was performed using Zetasizer (Nano ZS90, Malvern ltd., UK).

RESULTS

Particle size and Zeta potential analysis

The effect of the drug-to-polymer ratio on the size of the nanoparticles was studied using four different weight ratios of drug and polymer, namely10:100 (L1), 20:100 (L2), 30:100 (L3) and 40:100(L4), as shown in Table 1. Blank formulation in which no drug was added showed a mean particle size of 429 nm and mean polydispersity index (PDI) of 0.36. The mean particle size for drug loaded Formulations (L1 to L4) varied in the narrow range from107 to 146 nm. The mean PDI values for the drug loaded formulations varied in the range of 0.24 to 0.36. Formulations showed slightly more PDI than 0.2. The PDI value demonstrates particle size distribution of nanoparticles population, the lower the PDI, higher the uniformity of the nanoparticles. The PDI value less than 0.2 indicates that the formulation is approaching toward the monodisperse system. The prepared formulations showed slightly more PDI than 0.2 which indicate that Formulation L1-L4 are approaching towards the monodisperse stable system.

It could be inferred from the results that, there was no significant impact of the drug-to-polymer ratio on the mean particle size of the drug loaded nanoparticles formulations ($p < 0.05$). One way ANOVA followed by the Turkey test showed that Blank formulation showed significant difference in particle size as compared to drug loaded Formulations ($p< 0.05$). A trend of increasing drug to polymer ratio, in the formulations with decreasing mean size was observed [1].

In the measurement of zeta potential there was a presence of bluish opalescence, which indicated the formation of colloidal nanosuspension [12]. Zeta potential remained in the range of positive

values for all Formulations and varied between +11.1mV to 21.7 mV (Table 1), the positive surface charge for the nanoparticles was observed may be due to the presence of the quaternary ammonium groups of ERLPO.

Scanning Electron Microscopy (SEM)

Nanoparticles surface morphology and shape were visualized using SEM, which revealed the distinct, rod shaped, drug loaded nanoparticles with a smooth surface, **Fig. 1**.

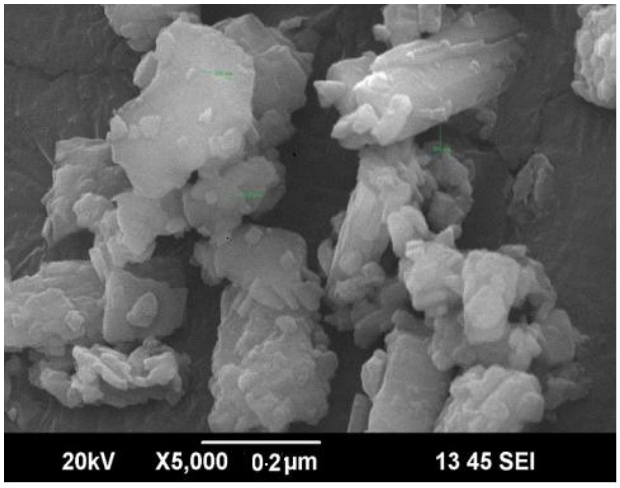

Figure 1: SEM image of lyophilized Formulation L3

Differential Scanning Colorimetry

The DSC thermogram of lansoprazole drug substance exhibited a sharp endothermic peak at 179.6 °C **(Fig.2. a)** indicating the melting point of lansoprazole, followed by a sharp exothermic peak at 181.7 °C **(Fig.2. b)**, which may be ascribed to decomposition of lansoprazole. The physical mixture of LSP/ERLPO showed the endothermic and decomposition peak of lansoprazole **(Fig.2. f, g)**. The lyophilized formulation (L3) showed similar type of peaks **(Fig.2. k, l)** as compared to that of physical mixture. Shifting of endothermic peak 'a' of lansoprazole drug substance in both the physical mixture sample as well as in the lyophilized Formulation (L3) may be due to the mannitol. Eudragit RLPO showed broad endothermic peaks at 45.3°C, 65°C and at 165 °C **(Fig.2. m, n** and **o** respectively**)**[11, 12]. Out of these peaks of Eudragit RLPO, the endothermic peak **(Fig.2. n and o)** are also found to be shifted in the physical mixture and Formulation sample thermogram. These observations of DSC study indicated absence of significant interactions between drug and polymer in LSP/ERLPO Formulation, **Fig 2**.

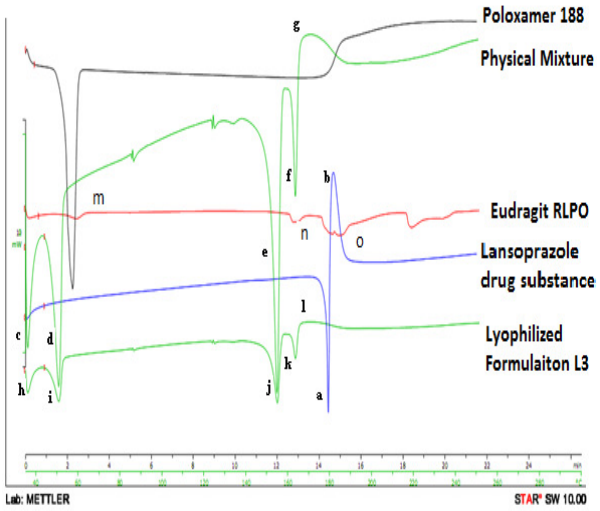

Figure 2: DSC thermograms of Poloxamer 188 (A), Physical mixture of LSP/ERLPO (B), Eudragit RLPO (C), Lansoprazole drug substance (D), Lyophilized Formulation L3 (E)

Powder X-ray Diffractometry (PXRD)

Lansoprazole showed characteristic peaks at 6.1°, 14.6°, 17.3°, 18.9°, 22.7°, 25.3°, 25.9°, 26 °, 26.1° and 28.1°. The diffractogram of LSP/ERLPO lyophilized formulation is slightly like lansoprazole indicating slight presence of lansoprazole crystalline [1]. The prominent peaks from lansoprazole drug substance at 2θ of 6.1°, 17.3°, 19.0° and 26 ° etc. were clearly seen at the same position in the nanoparticles but the peak intensities were decreased nearly half extent of lansoprazole drug substance.

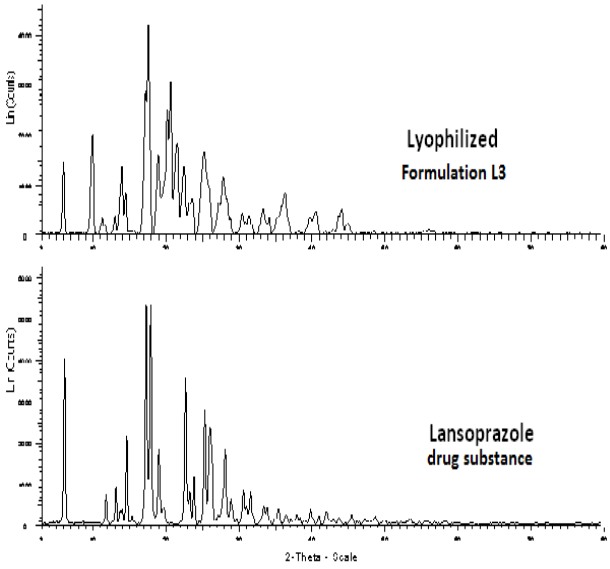

Figure 3: PXRD spectra of lansoprazole (A), Lyophilized Formulation L3, (B)

From these observations, it can be concluded that the crystalline nature of the drug was partly present, but the relative reduction of diffraction

intensity LSP/ERLPO nanoparticles suggested reduction in quality of the crystals and/or change in crystal size, **Fig. 3**.

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of lyophilized formulation of LSP/ERLPO (L3) and physical mixture of lansoprazole drug substance with ERLPO were compared with the standard spectrum of lansoprazole drug substance, **Fig.4**. FTIR spectrum of lansoprazole is characterized at the characteristic absorption peaks appeared at 3222, 2983,1580,1282 and 1117 cm−1, respectively [23], denoting stretching vibration of –NH, –CH2–, the aromatic ring, C–N on the pyridyl ring and the ether bond. In spectra of lyophilized formulation of LSP/ERLPO and physical mixture of lansoprazole with ERLPO, these bands are clearly seen at the same position in the nanoparticles and in the physical mixture. From the stated observations, it can be conclude that there is no any interaction between the drug and polymer observed.

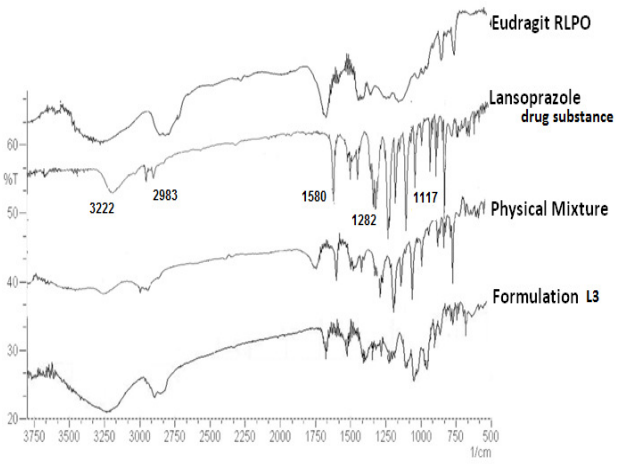

Figure 4: FTIR spectrograms of Eudragit RLPO (A), Lansoprazole drug substance (B), Physical mixture of ERLPO-LSP(C), lyophilized Formulation L3 (D).

Drug Entrapment Efficiency (DEE)

DEE for the lansoprazole loaded nanoparticles was found to be in the range of 47.68 – 72.62 % for all formulations. The low DEE values indicated that as the polymer concentration increased there was decrease in entrapment efficiency (EE), due to the less availability of surfactant, which was unable to reduce interfacial tension between the polymer and aqueous phase at high polymer concentration. Hence the EE decreased with increase in polymer amount, **Table 1** [1].

Drug Content

The drug content in all the lansoprazole loaded nanoparticles were found to be in the range of 94.53 -98%. **Table 1** shows the percent drug content of lansoprazole loaded nanoparticles.

Saturation Solubility Study

The results for saturation solubility of lansoprazole and all its lyophilized nanoparticles formulations are shown in **Table 2**. Based on the saturation solubility data, the Formulations showed an increase in solubility of lansoprazole as compared to lansoprazole drug substance. It was observed that there was about 16 folds increase in solubility as compared to the drug alone in distilled water and pH 6.8. Saturation solubility study clearly indicated that formulation by solvent displacement method (nanoprecipitation) enhanced the solubility of lansoprazole greatly because of synergistic effect of reduced particle size and solubilization due to Poloxamer 188 in slightly pH 6.8 solvent leading to improvement in solubility.

Table 2: Saturation solubility study of lansoprazole and of drug loaded ERLPO nanopaticles formulations in distilled water & 6.8 phosphate buffer system

Formulations	Distilled Water(µg/ml) (mean ± sd)	6.8 phosphate buffer(µg/ml) (mean ± sd)
Drug	56.71±0.57	59.12±0.44
L1	96.59±0.76	102.22±0.79
L2	320.22±0.43	412.72±0.38
L3	1068±0.33	1401±0.45
L4	957.10±0.47	1039±0.74

***In vitro* Drug Release Study**

In the first 2h all formulations showed 8.04 to 11.84% drug releases, hence protection of lansoprazole from acidic pH can be claimed. The amount of drug incorporation in the formulation and drug entrapment efficiency has a direct effect on the drug release profile from the four formulations. As the content of the drug in the formulation increased, the release rate also increased. Formulation L4 had the drug entrapment efficiency (DEE) of 68% with a smaller average particle size (107 nm) and gave 100% drug release within 4 h.

Table 1: Mean particle size, Polydispersity index , zeta potential, Degree of entrapment efficiency, Drug content of blank and lansoprazole-loaded ERLPO nanoparticles (n=3)

Formulation	Drug to Polymer ratio (by wt)	Mean Particle size ± sd (nm)	Polydispersity Index (mean ± sd)	Zeta potential (mean ± sd) (mV)	DEE (%) (mean ± sd)	% Drug content (mean ± sd)
Blank Formulation	0:100	429.2 ± 18.83	0.355±0.087	20.9±0.12	-	-
L1	10:100	146.2 ± 16.77	0.260±0.066	21.7±0.42	47.68±0.31	94.53±0.55
L2	20:100	135.7 ± 13.32	0.244±0.121	23.0±0.31	58.0±0.44	95.82±0.72
L3	30:100	124.4 ± 9.27	0.262±0.047	11.1±0.26	72.62±0.23	98.03±0.49
L4	40:100	107.8 ± 19.79	0.267±0.164	15.2±0.39	68.0±0.29	96.02±0.67

Table 3: Kinetic release rate constants, correlation coefficient and diffusion exponent of various models (n=3)

Formulation	Zero Order	First Order	Higuchi model	Korsemeyer- Peppas		
	R^2	R^2	R^2	K	n	R^2
L1	0.971	0.998	0.950	7.5	4.18	0.972
L2	0.973	0.994	0.987	2.83	1.87	0.981
L3	0.960	0.996	0.936	9.53	5.03	0.954
L4	0.984	0.999	0.972	8.03	4.54	0.973

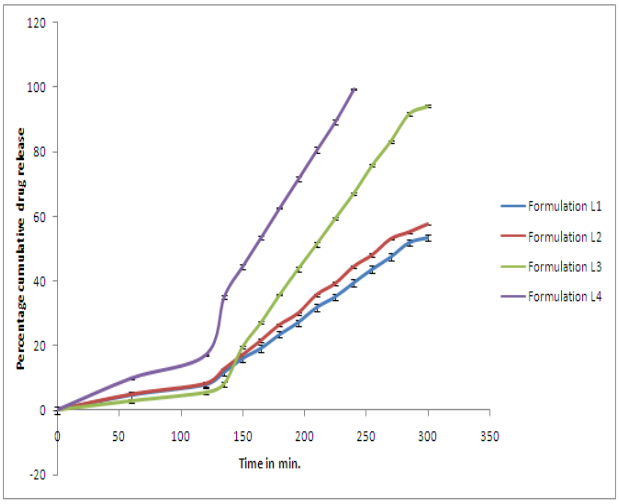

Figure 5: In vitro release of lyophilized Formulations in acidic buffer pH1.2 and phosphate buffer pH 6.8 at 37^{0}C (n=3).

Formulation L1 had a DEE of 47.68 % with a larger average particle size (146 nm) and exhibited a prolonged drug release profile with only about 53.54% drug release after 5 h. A similar tendency was observed for Formulation L2 (DEE 58% and particle size 135 nm) which released about 56.71% of the drug after 5 h [9, 24, 25]. Formulation L3 with a particle size of 124 nm and DEE of 72.62% showed 93.138% drug release after 5 h, **Fig.5.**

Kinetics of Drug Release

The release data were fitted to various kinetic models in order to calculate the release constant and coefficient of determination (R^2) as seen in Table 3. Among the models tested, the drug release profiles for all Formulations were best explained with the first order model with the coefficients of determination (R^2) in the range of 0.998 - 0.999.

Drug Release Mechanism

Release data were fitted to Peppa's exponential model to investigate the mechanism of drug release from formulation L3. The corresponding plot of Korsmeyer–Peppa's equation indicated a good linearity of coefficients of determination (R^2=0.954). Release exponent (n) was found to be 5.3 while kinetic constant (k value) was found to be 9.5. The n value indicated that nanoparticles formulation followed the non-Fickian super case II diffusion mechanism of drug release [12] which represents a drug release governed by a combined mechanism of diffusion and erosion.

Effect of Cryoprotectant on Redispersibility of Formulations

The Formulation L3 was selected to study the effect of cryoprotectant during freeze drying process as well as the redispersibility of the drug loaded nanosuspension. Formulation L3 was selected because it had the highest drug

entrapment efficiency with a smaller particle size and sustained release behavior. The effect of cryoprotectants on redispersibility in distilled water was investigated visually to observe the formation of any aggregates upon manual shaking. Freeze dried nanoparticles without cryoprotectants appeared as off-white fluffy and sheet-like materials. Using sucrose as cryoprotectant resulted in the formation of a white, brittle, crystalline material with perforated structure. Mannitol formed white spongy, cotton like material upon lyophilization [7]. The freeze dried sample without cryoprotectants did not redisperse in water after manual shaking. Large aggregates were observed. Sample containing 5% mannitol showed good redispersibility upon manual shaking. Sucrose containing samples showed redispersibility after a few minutes of shaking with slight turbidity and foaming upon shaking for samples was observed. The average particle size of the 5% sucrose containing formulation was 314 ± 26 nm, whereas the 5% mannitol containing formulation has 161 ± 14 nm particle size. Therefore, the 5% mannitol appeared to be the most suitable cryoprotectant.

Short term stability study of nanosuspension

The physical appearance of the lyophilized formulation did not change when samples were stored at 20 °C and at 40 °C for 1 month. The average particle size of formulation L3 was 132 ± 22 nm and 129 ±11nm respectively after 1 month. The particle size for the formulation L3 was 124 ±8 nm before performing the stability study. It can be inferred from the observed data that the prepared Formulation L3 was stable after 1 month of storage at room temperature [5].

DISCUSSION

Eudragit RLPO nanosuspensions were prepared by the solvent displacement technique. In this process, nanoparticles were spontaneously formed when the organic phase (acetone) containing ERLPO with/without lansoprazole was added drop wise into stirred aqueous surfactant solution (1% Poloxamer 188), resulting in a transparent solution with a bluish opalescence. Instantaneous formation of a colloidal suspension occurred as a result of the polymer deposition on the interface between the organic phase and water when partially water miscible organic solvent (acetone) diffused out quickly into the aqueous phase from each transient particle intermediate. According to the "Marangoni effect", the transient particle intermediate causes a size reduction to the nano range. Formation of a colloidal nanodispersion can be visualized by the bluish opalescence. This phenomenon is known as the Tyndall effect which results from scattering of light caused by the dispersed colloidal particles [24].

Trends of increasing drug content in the formulation with decreasing mean size of nanoparticles were observed. The probable spatial interaction (due to electrostatic charges) between drug and polymer forming more compacted structure at higher drug concentrations have resulted in decrease in particle size. The phenomenon may be related to viscosity. Scanning Electron microscopy (SEM) revealed that particles are rod shaped, smooth, and regular in nature.

The positive surface charge on the nanoparticles can allow a longer residence time due to the ionic interaction between lansoprazole nanoparticles and the negatively charged sialic acid residues present in mucous of the intestinal lumen [1, 12]. The relative constancy of zeta potential with slight variation indicates that lansoprazole was encapsulated within the nanoparticles and a major part of the drug is not present on the nanoparticle's surface.

Solid state characterization of freeze dried nanosuspension was performed by DSC, PXRD and FTIR techniques. These techniques allow us to confirm the possibility of any chemical or physical interaction among drug, polymer and surfactants or other additives of the formulation. No significant chemical interaction was observed among lansoprazole, ERLPO and Poloxamer 188. Therefore, the formulation ingredients are compatible with the drug.

After preparing the fresh nanosuspension, it was centrifuged and the free drug present in the supernatant was analyzed by UV-Visible spectrophotometer. Subtracting this value from the initial amount of drug, DEE was calculated. The method is suitable for determining entrapment efficiency of nanosuspension when fairly high concentrations of free drug are present in the supernatant after centrifugation [26]. Lansoprazole is slightly soluble in water and has an ionization constant of 4.7. The aqueous 1% poloxamer 188 (surfactant) solution has a pH of about 8. Therefore, when the organic phase is added drop wise into the aqueous surfactant solution, part of the drug is ionized and escapes from the nanoparticles during diffusion of the acetone into the aqueous phase. Increasing the

drug content in the formulation increased the DEE inside the nanoparticles, saturation of the polymer particles occurs with such a high drug loads. The excess drug escapes from the acetone phase into the water. Therefore, DEE dropped in formulation L4. Another possibility for the decreased DEE at high drug content in the formulation can be explained by possible saturation of the cationic sites on the Eudragit by anionic drug molecules. Therefore, excess drug is being lost from the particles during its formation process.

Lansoprazole to Eudrgait RLPO ratio and average particle size has impact on drug release profile of nanoparticles Formulations. As the content of the drug in the formulation increased, the release rate also increased. Formulation with the lowest drug entrapment efficiency (DEE) and smaller average particle size showed faster drug release. The progressive saturation of the quaternary groups in the polymer by drug molecules (occurred at high drug content) increased drug release from the formulation [21, 22]. On the other hand, formulation with a larger average particle size exhibited a prolonged drug release profile. A correlation between drug release from the nanoparticles and mean particle size is observed [10]. Thus, it can be inferred that larger particles have a small initial burst release and a longer sustained release than smaller particles.

Among the models tested, the drug release profiles were best explained by first order release for all four Formulations (L1-L4), as these models were having the highest correlation coefficient values. The diffusion exponent (n) values calculated from Korsemeyer-Peppas equation for all formulations were above 1 which indicated that the drug release mechanism followed non-Fickian super case II diffusion mechanism of drug release [18,27] showing the drug release from Eudragit RLPO nanoparticles was complex in nature which involved the occurrence of dissolute and diffusive phenomena. Overall the drug release rate was faster which were due to the high water permeability and low swellability characteristics of ERLPO. The presence of a high content of quaternary ammonium groups may have made the polymer more permeable to water.

CONCLUSION

From the above study, it is evident that ERLPO polymer successfully retarded the release of lansoprazole for 5h. The ERLPO polymer proved to be capable of entrapping high quantities of drug (up to 72.62%) depending on drug to polymer ratio. DSC and XRD studies revealed the enhanced solubility of drug which can be attributed to partial loss of crystallinity of drug. High stability was observed when nanoparticles were stored at room temperature. Acidic pH resistance to the drug lansoprazole due to conversion in polymeric nanoparticles is evident. Therefore, lansoprazole nanoparticles can be expected to gain considerable attention for improved therapeutic activity for the treatment of gastric and duodenal ulcer.

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