ORIGINAL ARTICLE

Development of pH sensitive polymeric nanoparticle of erythromycin

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Abstract

Introduction: Erythromycin is a macrolide antibiotic used for the treatment of various infections caused by bacteria. It is also used for Respiratory tract infection, skin infection, syphilis, etc. Erythromycin base was a poorly water-soluble and acid-labile drug. The bioavailability of Erythromycin was very less about (15–35%). The absorption site of Erythromycin is the small intestine. pH-responsive polymeric drug delivery system is efficient delivering the acid instable drugs. **Aim:** The main objective of the study is to formulate Erythromycin loaded pH-responsive polymeric nanoparticles was prepared using pHresponsive polymer to prevent the degradation of acid-labile drug and to evaluate the Polymeric nanoparticle. **Materials and Methods:** The Erythromycin Polymeric nanoparticles were prepared by Nano precipitation method using Eudragit L100 as a Polymer and polyvinyl alcohol (PVA) as stabilizer. The particle size, polydispersity index (PDI), zeta potential, entrapment efficiency and *In-vitro* drug release studies were performed for nanoparticle. The compatibility between drug and polymer was studied by Fourier transform infrared. The nanoparticle was lyophilized and surface morphology was determined by scanning electron microscope. Excipients were added and formulated into tablet. The Precompression evaluation of lyophilized powder was carried out. The post-compression evaluation for tablets and *In-vitro* release study was performed and compared with marketed tablet. **Results:** Erythromycin nanoparticle was formulated and evaluated. It showed the particle size of 270.2 nm with PDI of 0.166 and the zeta potential was found to be −32.5. The Entrapment efficiency of the ENP 9 Nanoparticle was 96%. Erythromycin loaded tablet was formulated and evaluated for hardness, weight variation, disintegration, friability, thickness, and diameter. It was present within the limit. *In vitro* drug release of the formulated tablet showed 91% drug release in phosphate buffer pH6.8 at the end of 60 min. **Conclusion:** Erythromycin nanoparticles were in the nanometer range and it was stable. Polymeric nanoparticle tablet had the ability to protect the acid-labile drug and it gets dissolved in the intestine pH 6.8. The nano particulate tablet showed the better release when compared with marketed tablet.

Key words: Erythromycin, pH responsive polymer, polymer nanoparticle

INTRODUCTION

Macrolide antibiotics are made of macro cyclic lactone ring with attached with amino or neutral sugar. Erythromycin is a macrolide antibiotic, obtained from *Streptomyces erythreus*.[1] It has been used clinically for 50 years. In most cases, macrolide antibiotics have been used predominantly for treating bacterial infections including respiratory tract infections, skin infections, genital infections and gastro intestinal infection.[2,3] The aim of any drug delivery is to achieve the therapeutic amount of drug to the targeted site or action.[4] Oral route is the common route of drug administration due to the convenience of drug administration, patient compliance, and non-invasiveness.[5] It destroys the bacteria at higher concentration. It may be used as alternative to patients with serious penicillin allergy.[6] Erythromycin is available as free base, Erythromycin estolate, Erythromycin ethyl succinate, Erythromycin lactobionate, Erythromycin stearate, and Erythromycin gluceptate.[7] Erythromycin destroys the bacteria by inhibiting the protein synthesis. Erythromycin base is acid labile and it is degraded by gastric juice. The oral bioavailability of Erythromycin base is about 15–35% and most of the drug being degraded and it is absorbed as microbiologically inactive form as anhydro erythromycin.

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Erythromycin base is acid labile and it is destroyed by gastric acid in the stomach. Acidic media degrade the erythromycin to derivatives with poor antimicrobial activity. Erythromycin gets absorbed in the small intestine.[4] pH responsive drug delivery system act as an excellent therapeutic system due to the stability of drug in the stomach and offers drug release in the intestine.[8] Polymer Nanoparticles have the ability to protect the instable drugs and improve the delivery of therapeutically active agent to the target site.[9] Polymeric Nanoparticles are colloidal solid particles made from the biodegradable and non-biodegradable polymers in the size range of 10–1000 nm. Nanoparticles can be in the form of spherical, branched, and shell structure. The drug molecule can be entrapped, adsorbed, and attached or encapsulated inside the Nanoparticle.[10] The polymeric nanoparticle can be formulated by various methods. The potential benefits of improving bioavailability of poorly water-soluble drug through nanoparticle. It increases the saturation solubility and dissolution of poorly water-soluble drug. It improves the mucoadhesion characteristics of drug to the mucosa in the gastrointestinal tract.[11] The main objective of the study is to fabricate pH-responsive Polymeric Nanoparticle of Erythromycin using Eudragit L 100 as polymer and polyvinyl alcohol (PVA) as Stabilizer by Nano precipitation Technique. The formulated nanoparticles should protect the drug degradation in acidic environment is determined.

MATERIALS AND METHODS

Excipients for Polymeric Nanoparticle

Erythromycin was obtained as gift sample. Eudragit L 100 was procured from YarrowPharma (Mumbai). PVA was procured from Lobacheime Pvt. Ltd (Mumbai). Solvent like Ethanol was purchased from Lobacheime Pvt. Ltd.

Excipients for Erythromycin Loaded Tablet

Magnesium Stearate (Himedia), Lactose (Kemphasol), Talc (Lobacheime Pvt. Ltd), Microcrystalline cellulose (Loba cheime Pvt. Ltd), Mannitol (Loba Cheime Pvt. Ltd) were used.

Preformulation Studies

Melting point determination

Erythromycin melting point was determined by the capillary tube method with the aid of the Digital melting apparatus.

Solubility Studies

The solubility of Erythromycin was determined by the saturation solubility method. The drug was dissolved in various solvents like water and methanol.

Determination of λmax

The erythromycin drug solution was prepared and scanned by double beam ultraviolet (UV) visible spectrometer between the wavelengths ranges of 200 nm and 400 nm.

Standard Curve of Erythromycin

Erythromycin stock solution was prepared using methanol and phosphate buffer and it was serially diluted and analyzed at 284 nm by double beam UV visible spectrometer.

Fourier Transform Infrared (FTIR) Spectroscopy

Infrared spectra of the drug and polymer were recorded by KBr pellet method using FTIR Spectrophotometer.

Method of Preparation

Polymeric nanoparticles were prepared by nanoprecipitation Technique. Different concentration pHresponsive polymer Eudragit L 100 and fixed concentration of Erythromycin base was dissolved in Ethanol to form the organic phase which is kept in ultrasonic bath for 10 min. PVA at different concentration was dissolved in distilled water to form aqueous phase, which is kept in magnetic stirring at 400 rpm [Table 1]. The organic phase is added dropwise to the aqueous phase and the nanoparticle were formed spontaneously and turned into milky colloidal dispersion. The milky dispersion is kept in magnetic stirring for 3–4 h to evaporate the organic solvent.[11-13]

Characterization of Polymer Nanoparticle

Characterization of particle size, polydispersity index (PDI) and zeta potential

The average particle size was determined using dynamic light scattering principal of Malvern Zeta seizer (Malvern Instrument, Malvern, UK). Malvern zeta seizer allows the sample measurement in the range of 0.1–10,000 nm. All the measurement was performed on the diluted suspension in low volume disposable sizing cuvette at 25°C.[12]

The Zeta potential was determined by dipping an additional electrode used for particle size analysis (Malvern Instrument, Malvern, UK). The samples was diluted with water and placed in the electrophoretic cell.[12]

Percentage Entrapment Efficiency

The amount of erythromycin entrapped within the nanoparticles was determined by measuring the amount of non-entrapped drug in supernatant recovered after centrifugation. The supernatant was analyzed spectrometrically at 284 nm.

Percentage drug entrapment was determined using the following formula.[14,15]

%EE = [1- (Drug in supernatant liquid/Total drug added)] × 100

Morphology Study

Scanning electron microscopy (SEM)

The surface morphology of polymer nanoparticles was carried out using SEM. The lyophilized powder sample was sprinkled on one side of the double adhesive stub. The nanoparticles were viewed at an accelerating voltage of 15–20 kv.

Lyophilization of polymer nanoparticle

The polymer nanoparticles were lyophilized using lyophilizer (Lyodel Freeze drier). The nanoparticulate dispersion was kept at −80°C and it was freeze dried. 1% mannitol was used as cyroprotectant.

Drug Content

The lyophilized nanoparticle powder 50 mg was transferred to 10 ml volumetric flask. The nanoparticulate powder was dissolved in 1 ml of methanol and volume was made up to mark in volumetric flask with phosphate buffer pH6.8 and analyzed by spectrophotometrically at 284 nm. The amount of drug present was determined with the help of standard graph.

Preparation of Tablet[16]

Pre-compression evaluation for the nanoparticle powder was characterized by different parameters such as bulk density, tapped density, angle of repose, carrs' index, and hausner's ratio. Tablet was prepared by direct compression method. Different excipients were used like magnesium stearate as lubricant, microcrystalline cellulose as disintegrant, lactose as binder, talc as glidant [Table 2]. Formulated tablets were characterized by different parameters such as weight variation, thickness, hardness, disintegration, *in vitro* drug release.

Evaluation of Pre Compression characteristics of Tablets[17]

Bulk density

The nanoparticle powder was accurately weighed and transferred into the measuring cylinder. The initial volume (i.e. bulk volume) was noted.

$$\text{Bulk density} = \frac{\text{Weight of the powder}}{\text{Bulk volume}}$$

Tapped Density

The nanoparticle powder in the measuring cylinder was tapped for 100 times and the volume was noted. This was repeated for 3 times and the average values were taken.

$$\text{Tapped density} = \frac{\text{Weight of the powder}}{\text{Tapped volume}}$$

Carr's Index

Carr's index is used to determine the flow property of powder. The unit of Carr's index is percentage.

$$\text{Carr's index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}}$$

Angle of Repose

The angle of repose was determined by fixed funnel method. The funnel was placed at a constant height of 2 cm horizontally with the help of burette stand. The powder was passed slowly through the funnel till the tip of the pile touches the edges of the funnel.

Angle of repose = $\tan^{-1}(h/r)$

h -height of the pile

r- radius of the pile

Hausner's Ratio

It is also used to characterize the powder flow property.

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Evaluation of Post Compression Characteristics of Tablets[16,17]

Hardness

The hardness determination of the formulated tablet was evaluated using Erweka hardness tester. The hardness was expressed in kg/cm^2.

Weight Variation

The formulated tablets were weighed individually. Weight was measured by analytical digital balance and the average

weight, mean and standard deviation were calculated. The weight of the pill being made is measured to make sure that it carries predetermined quantity of drug.[17]

Friability

Roche Friabilator was used for testing the friability of the Tablets. Tablets were weighed accurately and placed in friabilator and rotated at 25 rpm for 5 min. Tablets were again reweighed and the percentage weight loss was determined by using formula.

$$\%F = \frac{W\,(\text{Initial}) - W\,(\text{Final})}{W\,(\text{Initial})} \times 100$$

Thickness and Diameter

The prepared tablets were evaluated for their thickness and diameter using Vernier caliper. Average values are taken.

Disintegration Test

The Disintegration time for the formulated tablet was determined using the Lab India disintegration apparatus. The tablets were placed in the tube and disc was added. The beaker was filled with phosphate bufferH 6.8 and the temperature was maintained at 37 ± 0.5°C. The time taken for the tablet to disintegrate was noted and reported.

In vitro Drug Release

In vitro dissolution studies were performed with the aid of USP Type II (Paddle) apparatus. The tablet was kept in the jar containing 900 ml of dissolution medium and the medium was stirred at 75 rpm and the temperature of the medium was maintained at 37 ± 0.5°C. For first 1 h, the dissolution medium was 0.1N HCl, and then it was replaced with Phosphate buffer pH 6.8.

RESULTS AND DISCUSSION

Preformulation Studies

The melting point of Erythromycin was 136°C. Solubility was determined using saturation solubility method. It indicates that the drug was slightly soluble in water (0.179 mg of drug dissolved in 1 ml of water) and soluble in methanol (57.6 mg of drug dissolved in 1 ml of methanol). Erythromycin showed the absorption maximum at 284 nm. The calibration curve of Erythromycin showed the regression value of 0.9899. FTIR spectra of drug, polymer, physical mixture, and other excipients used in the formulation are compatible. There was no interaction between the excipients.

Formulation and Evaluation of Nanoparticles[18,19]

The nanoparticle was formulated by nanoprecipitation method. The formulation appeared as a milky dispersion indicates the formation of nanoparticle. The nano sized particle was produced by continuous magnetic stirring for 3–4 h. At low concentration of polymer, the size of nanoparticle was above 1000 nm. The size of nanoparticle was reduced when the polymer concentration get increased. The ENP 9 of polymer and drug ratio (0.75:1) was selected as the best formulation and the results were shown in Table 3. PDI determines the average uniformity of particle solution. Larger PDI value indicates the larger size distribution in the particle solution. For polymer-based drug delivery, the acceptable range of PDI is below 0.2. The ENP 9 showed the PDI of 0.166. Zeta potential determines the surface charge of the particle which helps in understanding the stability of nano suspension. PVA was used as a stabilizer. The value of Zeta potential was shown in the Table 3. The images of particle size and zeta potential were shown in the [Figure 1]. The percentage drug entrapped in the nanoparticle was shown in Table 3. The entrapment efficiency of the nanoparticle was in the range of 90–96%.

In vitro drug release was studied for polymeric nanoparticle dispersion by dialysis bag method. The formulation that

Table 1: Composition of polymer nanoparticle

Formulation code	Erythromycin (mg)	Polymer (mg)	
		Eudragit L100 (mg)	PVA (mg)
ENP 1	250	62.5	125
ENP 2	250	62.5	187.5
ENP 3	250	62.5	250
ENP 4	250	125	125
ENP 5	250	125	187.5
ENP 6	250	125	250
ENP 7	250	187.5	125
ENP 8	250	187.5	187.5
ENP 9	250	187.5	250
ENP 10	250	250	125
ENP 11	250	250	187.5
ENP 12	250	250	250

PVA: Poly (vinyl alcohol)

Table 2: Composition of tablets

Excipients	Weight (mg)
Lyophilized Nanoparticle of ENP 9	380 mg
Microcrystalline cellulose	25 mg
Talc	5 mg
Lactose	80 mg
Magnesium Stearate	10 mg

Table 3: Evaluation of polymeric nanoparticle

Formulation code	Particle size (nm)	Zeta potential	PDI	% Entrapment
ENP 9	270.2 nm	–32.5	0.166	96%

PDI: Polydispersity index

Table 4: Pre compression parameters of nanoparticle powder

Formulation code	Bulk density (g/cm³)	Tapped density (g/cm³)	Carr's index (%)	Hausner's ratio	Angle of repose (Θ)
ENP 9 Nanoparticle powder	0.043±0	0.052±0.002	0. 173±0.017	1.11±0.02	26º56×±1.14

Table 5: Evaluation test of erythromycin loaded polymeric tablet

Formulation code	Hardness (kg/cm²)	Friability (%)	Thickness (mm)	Diameter (mm)	Weight variation (%)	Disintegration (mins)
ENP 9	5.75±0.5	0.4±0	0.4±0.03	1.11±0	0.2±0. 1	12±0. 5

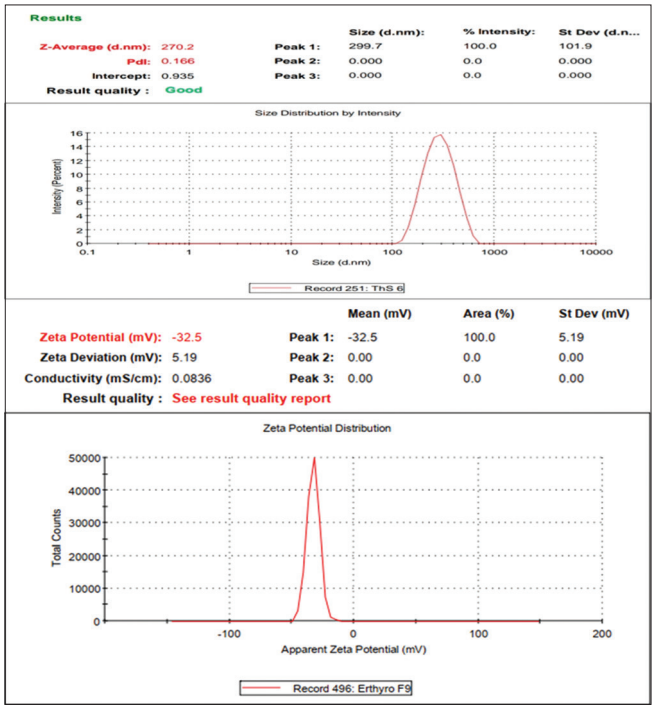

Figure 1: (a) Particle size of ENP nanoparticle (b) Zeta Potential ENP nanoparticle

had the high entrapment of drug molecules showed the better release. It has the ability to protect the drug in acidic medium and it releases about 70% in phosphate buffer within 1 h.[20,21] For improving the stability and convert to the dosage form the polymeric nanoparticle dispersion needs to be lyophilized. Based on the entrapment efficiency and *in vitro* release of nanoparticle dispersion, ENP 9 was lyophilized. Mannitol was widely used agent in lyophilization process and it promotes efficient freeze-drying and improves the appearance of freeze-dried product. ENP 9 was kept in deep freezer at –80°C and kept in lyophilizer with and without mannitol for 74 h. Addition of mannitol inhibits the aggregation of nanoparticles.[19]

Morphological Evaluation by SEM

The morphological evaluation was determined for lyophilized nanoparticle powder with SEM and it was shown in Figures 2 and 3. The formulations without cryoprotectant lead to the formation of aggregates. It is difficult to redisperse and it greatly affects the size of nanoparticles lyophilized powder without mannitol is irregular in shape and some spherical-shaped clusters have been identified. The presence of mannitol showed the spherical particles in the nanometer range.

Drug Content[22,23]

The drug content of the lyophilized powder was evaluated. 380 mg of lyophilized powder contains 250 mg of Erythromycin. Excipients were added accurately and the tablet was formulated by direct compression method.

Evaluation of Tablet

Pre-compression evaluation parameter for the nanoparticle powder was carried out. The flow characteristic of powder is important in converting a dosage form. Bulk density, Tapped density, Carr's index, Angle of repose, Hausner's ratio for the powder was calculated and shown in Table 4. The nanoparticle powder showed the excellent flow property.

Post compression evaluations of formulated tablets were carried out. The formulated tablet was evaluated for Hardness, Weight variation, Friability, thickness, diameter, disintegration, and the results are shown in Table 5.

In vitro dissolution study was carried out in 0.1N Hcl and phosphate buffer pH6.8 for the formulated and marketed

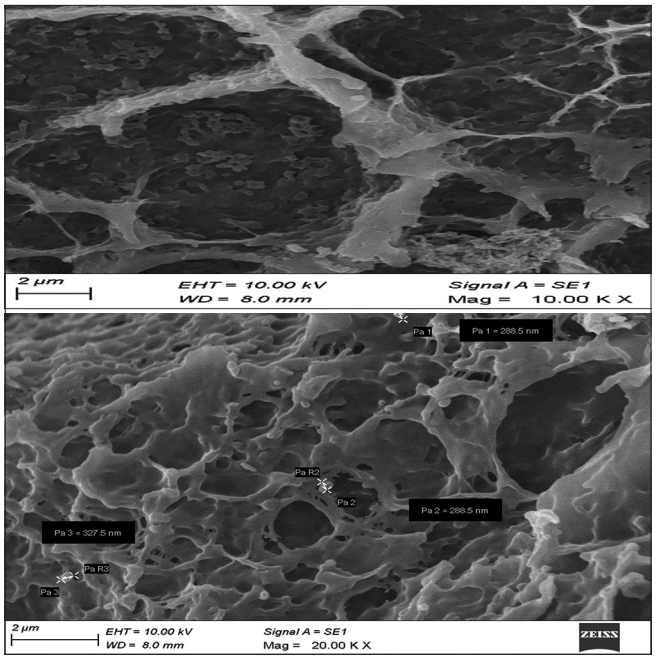

Figure 2: Scanning electron microscopy images (a) without mannitol b) with mannitol

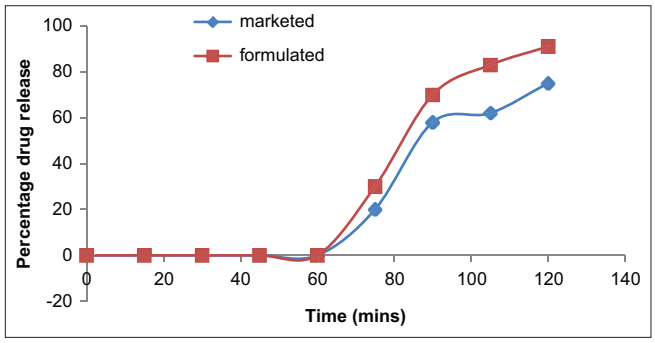

Figure 3: Comparison of *in vitro* drug release of ENP 9 formulated and marketed tablet

tablet. The tablet offers protection in acidic media and drug release in phosphate buffer was found to be 91% and 75% at the end of 60 min in phosphate buffer pH 6.8, respectively for formulated and marketed tablet. The formulated tablet showed the better release. The graph was shown in Figure 3.

CONCLUSION

Erythromycin nanoparticle was prepared with pH-responsive polymer Eudragit L100.[24] It was lyophilized and converted into tablet dosage form.[25] Eudragit L100 act as a carrier molecule and it protects the drug in acidic medium and it get dissolved in the intestinal pH6.8. The *in vitro* release of formulated tablet showed better results when compared with the marketed tablet. 0.21 fold increases in bioavailability of nanoparticle loaded tablet was observed.

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