DEVELOPMENT AND EVALUATION OF HOLLOW MICROSPHERES OF CLARITHROMYCIN AS A GASTRORETENTIVE DRUG DELIVERY SYSTEM USING EUDRAGIT POLYMERS

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ABSTRACT

The present study deals with an attempt to develop hollow floating microspheres of Clarithromycin for gastro retention using Eudragit polymers prepared by emulsion solvent diffusion method. Eudragit S 100, RS 100, RL 100, L 100 and L 100 55 were used to prepare hollow microspheres. A drug – excipients compatibility study was performed using FTIR. The microspheres were characterized for shape and surface morphology by scanning electron microscopy. They were evaluated for particle size, flow properties, bulk density, % drug entrapment efficiency, floating properties and in-vitro drug release. The residual solvent content was determined after varying the stirring time. Drug to polymer ratio was varied from 1: 1 to 1: 3 and its effect on particle size and drug release was studied. The microspheres exhibited round shape, porous surface, prolonged drug release and buoyancy for more than eight hours. As the drug to polymer ratio increased the particle size increased and the drug release rate decreased.
KEYWORDS
Clarithromycin, Hollow Microspheres, Eudragits, Gastro retention, emulsion solvent diffusion

INTRODUCTION
Gastro retentive dosage forms are drug delivery formulations that are designed to be retained in the stomach for a prolonged time slowly releasing active materials and thereby enable sustained and prolonged input of the drug to the upper part of the gastrointestinal tract. This technology has generated enormous attention over the last few decades owing to its potential application to improve the oral delivery of some important drugs for which prolonged retention in the upper gastrointestinal tract can greatly improve their oral bioavailability and their therapeutic outcome. Prolonged gastric retention improves bioavailability, reduces drug waste, and improves solubility for drugs that are less soluble in a high pH environment. It has applications also for local drug delivery to the stomach and proximal small intestines. Gastro retention helps to provide better availability of new products with new therapeutic possibilities and substantial benefits for patients. Among the various approaches used the multiple unit formulations show advantages over single unit formulations such as more predictable drug release kinetics, less chance of localized mucosal damage, insignificant impairing of performance due to failure of few units, co-administration of units with different release profiles or containing incompatible substances and larger margin of safety against dosage form failure.

Hollow microspheres, a multiunit gastro retentive drug delivery system have been developed for different drugs using various materials like polycarbonate, calcium alginate, PEGylated Rosin derivatives, Methocel K 100, porous calcium silicate, HPMC phthalate,Eudragit S100, Eudragit L 100 and Eudragit L 100 55. Clarithromycin (CLR) is a macrolide antibiotic with broad spectrum of activity. It is given in the treatment of respiratory tract infections and in the skin and soft tissue infections. CLR may be given to eradicate H. pylori in treatment regimens for peptic ulcer diseases. CLR is rapidly absorbed from the gastrointestinal tract and undergoes first pass metabolism. The bioavailability of the drug is about 55%. It is given in the doses of 250 mg and 500 mg as tablets and suspension. The terminal half life of CLR is reportedly about 3-4 hours. Thus CLR has all the requisites of gastro retentive drug delivery system. Helicobacter pylori (H. pylori) are a Gram negative organism that grows in the gastric mucosa of humans. It has been shown that a stomach specific drug delivery that can prolong the residence time of antibacterial agents such as tetracycline, amoxicillin and CLR and continuously release the contained antibiotic provides better antimicrobial effects. Various gastro retentive drug delivery systems have been developed for CLR. Floating- bioadhesive microparticles of CLR have been developed using ethyl cellulose and chitosan. A hydro dynamically balanced system (i.e. floating tablets) of CLR was formulated using HPMC K4M. Floating capsules of CLR were developed using HPMC K4M and Carbopol 934. Floating- bioadhesive microspheres were prepared by emulsification – solvent evaporation method using ethyl cellulose as a matrix polymer and Carbopol 934 P as a mucoadhesive polymer. Floating microspheres of CLR using HPMC K4M, HPMC 100 LV were prepared and formulations were statistically optimized. Concanavalin- A conjugated gastroretentive multiparticulate drug delivery system was prepared using chitosan and ethyl cellulose.
Eudragit polymers have an ability to resist the acid environment of stomach and retain there for prolonged period. Hence an attempt was made to develop hollow microspheres of CLR using Eudragit polymers with an aim to retain the microspheres in the stomach for prolonged period.

MATERIALS AND METHODS

Clarithromycin was obtained as a gift sample from Cipla Ltd. (Kurkumbh, India). Eudragit S 100, Eudragit L 100, Eudragit RS 100, Eudragit RL 100 and Eudragit L 100 55 were provided by Evonik Degussa Ltd., Mumbai as gift samples. Dichloromethane was purchased from SD fine chemicals. All other chemicals used were of analytical grade.

i. Characterization of Drug (Clarithromycin):

The IR spectrum of clarithromycin was taken using Shimatzu FTIR spectroscope. The spectrum was compared with the reference spectrum given in I.P. The characteristic peaks are shown in the table 1.

Table 1

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>IR Frequency V (cm⁻¹)</th>
<th>Functional Group Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3487-3468</td>
<td>Tertiary –N stretching.</td>
</tr>
<tr>
<td>2.</td>
<td>1730.21</td>
<td>Carbonyl stretching</td>
</tr>
<tr>
<td>3.</td>
<td>1170</td>
<td>Aliphatic –CH stretching.</td>
</tr>
<tr>
<td>3.</td>
<td>2937</td>
<td>Hydroxyl (OH) stretching.</td>
</tr>
</tbody>
</table>

ii. Drug-Excipients Compatibility Studies:

Clarithromycin and five grades of Eudragits were subjected to drug-excipients compatibility studies. The drug and polymer were mixed physically in 1:1 ratio and the mixtures were placed in sealed vials for 3 months at room temperature. FTIR measurements of drug, individual polymer and drug-polymer mixtures were obtained on Shimatzu FTIR. Samples were prepared by mixing with KBr and placing in the sample holder. The spectra were scanned over the wave number range of 4000-400 cm⁻¹ at the ambient temperature. The IR of Clarithromycin and compatible Drug- Eudragit mixtures are given in Fig.2 to 5.

iii. Preparation of Hollow Microspheres¹¹:

Hollow microspheres containing Clarithromycin drug in their outer polymeric shell were prepared by emulsion solvent diffusion method used by Kawashima et al.

Weighed amount of Clarithromycin was mixed with polymers (Eudragit-S 100 and RS 100) (in ratios of 1:1, 1:2 and 1:3) in a mixture of Dichloromethane and Ethanol (1:1) at room temperature. Glycerol Monostearate was added as the emulsifying agent. The resulting drug-polymer solution was poured gradually into 200 ml of water containing polyvinyl alcohol (0.2%, 0.5%, 0.75% w/v), maintained at constant temperature of 40°C and the preparation was stirred at 300 rpm with a three- blade propeller (Remi, Mumbai) for one hour to obtain o/w emulsion. The obtained microspheres were filtered, washed with water and dried in air for 24 hrs. The method is pictorially represented in fig.1. The representative formulations are given in Table 2. The concentration of polyvinyl alcohol was changed from 0.2- 0.75 % w/v and its effect on the particle size was studied. The formulae are given in Table 3.
Emulsion Solvent Diffusion Method

**Fig. 1**
*Emulsion Solvent Diffusion Method for Preparation of Hollow Microspheres*

**Table 2**
*Microsphere Formulations of Eudragit S 100, Eudragit RS 100 and Eudragit RL 100*

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>RS1</th>
<th>RS2</th>
<th>RS3</th>
<th>RL01</th>
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</thead>
<tbody>
<tr>
<td>Clarithromycin</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Eudragit S 100</td>
<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Eudragit RS 100</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
<td>-----</td>
</tr>
<tr>
<td>Eudragit RL 100</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>1.0</td>
</tr>
<tr>
<td>Glycerol Monostearate</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Polyvinyl alcohol 0.2% w/v (200 ml)
DCM + Ethanol (1:1 15-20 ml)
Temperature 40°C
Stirring Speed 300 rpm
Stirring Time 1 Hour
Drug: Polymer Ratio 1:1 1:2 1:3 1:1

**Table 3**
*Microsphere Formulations prepared with different concentrations of PVA*

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clarithromycin</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Eudragit S 100</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Glycerol Monostearate</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Polyvinyl Alcohol</td>
<td>0.2%</td>
<td>0.5%</td>
<td>0.75%</td>
<td>5%</td>
</tr>
</tbody>
</table>

% Yield 86.67 98.33 81.67 Viscous & foaming solution
Particle Size (micron) 100-150 40-60 20-35 ------
EVALUATION OF MICROSPHERES:

Micromeritic Properties:
The microspheres were characterized for their micromeritic properties, such as particle size, tapped density, bulk density, compressibility index and flow properties. The size was measured using an optical microscope, and the mean particle size was calculated by measuring 100 particles with the help of a calibrated ocular micrometer. The bulk density apparatus (Meta Lab, Mumbai) was used to determine the tapped density, bulk density and percent compressibility index as follows:

Weight of microspheres
Bulk Density = -------------------------------
Volume of microspheres before tapping

Weight of microspheres
Tapped density = -------------------------------
Volume of microspheres before tapping

Tapped density - Untapped density
% Compressibility Index = ----------------------------------- X 100
Tapped density

Shape and Surface Morphology:
The shape and surface morphology of Eudragit microspheres were investigated using scanning electron microscopy (SEM). The samples for SEM study were prepared by lightly sprinkling the formulation on a double adhesive tape stuck to an aluminum stub. The stubs were coated with gold to a thickness of about 300 Å under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with a scanning electron microscope.

Drug Entrapment Efficiency and Percent Yield:
The dried microspheres were weighed and yield was calculated as follows:

Total weight of microspheres obtained
% yield = ----------------------------------- X 100
Total wt of drug, polymer and other nonvolatile solids

The % Clarithromycin entrapment of Eudragit microspheres was determined by dispersing accurately 50 mg of microspheres in 10 ml of 0.1 N hydrochloric acid followed by sonication for 30 minutes to dissolve the polymer and to extract the drug. After filtration, the drug concentration was analyzed spectrophotometrically at 760 nm using UV-visible spectrophotometer (Jasco Double Beam UV-VIS Spectrophotometer, Japan). 1 ml of filtrate was mixed with 1 ml of Folin-Ciocateau Phenol reagent (1: 2 dilution with water), 1 ml of 20 % sodium carbonate, 2 ml of 0.1 N HCl and volume was made up with 0.1 N hydrochloric acid. The percentage drug entrapment was calculated as follows:
**Floating Behavior:**
Three hundred milligrams of the microspheres were placed in 900 ml of 0.1 N hydrochloric acid. The mixture was stirred at 100 rpm in a dissolution apparatus for 8 h. After 8 h, the layer of buoyant microspheres was pipetted and separated by filtration. Particles in the sinking particulate layer were separated by filtration. Particles of both types were dried in a dessicator until constant weight. Both the fractions of microspheres were weighed and buoyancy was determined by the weight ratio of floating particles to the sum of floating and sinking particles.

\[
\text{% Buoyancy} = \left[ \frac{W_f}{W_f + W_s} \right] \times 100;
\]

where \(W_f\) and \(W_s\) are the weights of the floating and settled microspheres, respectively. All the determinations were made in triplicate.

**In Vitro Drug Release:**
The release rate of CM from microspheres was determined in a USP XXIII paddle type dissolution apparatus (Electrolab Tablet Dissolution Tester USP TDT-06P). A weighed amount of microspheres equivalent to 100 mg of drug was filled into a hard gelatin capsule (#5) and placed in the dissolution apparatus. Nine hundred milliliters of 0.1 N hydrochloric acid containing 0.02% w/v Tween 20 was used as the dissolution medium. The dissolution medium was maintained at 37 ± 1 °C at a rotation speed of 100 rpm. Perfect sink conditions prevailed during the drug release study. Ten milliliter samples were withdrawn at each 1 hour interval and filtered. The initial volume of the dissolution medium was maintained by replacing 10 ml of fresh dissolution medium after each withdrawal. Samples were analyzed using a UV-visible spectrophotometer (Jasco Double Beam UV-VIS Spectrophotometer, Japan) at 760 nm. 1 ml of filtered withdrawn sample was mixed with 2 ml of Folin Ciocateau Phenol reagent, 2 ml of 20% w/v sodium carbonate solution and diluted to 10 ml using 0.1 N hydrochloric acid. The absorbance of these solutions was measured at 760 nm against reagent blank. All experiments were conducted in triplicate. The plots of cumulative % drug release verses time are shown in Fig 11 and 12.

**Residual Solvent:** Dichloromethane (Methylene chloride) is given in USP-NF VIII under class 2 residual solvent\(^{19}\). Eudragit S 100 microspheres were prepared by varying the stirring time (1 hr, 2 hr, 3 hr). 50 mg of dried microspheres were dissolved in acetone and subjected to Gas Chromatography using GC 17A Shimatzu Gas Chromatogram and a capillary column DB 5. The chromatographic peaks were analysed by Mass spectroscopy to find the presence of dichloromethane in the microspheres.

**Kinetic Study of Drug Release using various In Vitro Models:**
Two batches, made using Eudragit S 100 and Eudragit RS 100 (S2 and RS2), gave 72.28% and 77.14 % of drug release after 8 hours respectively. These data were subjected to various kinetic models to understand the kinetics of drug release from microspheres prepared using Eudragits. The graphical representations of these models are shown in figs. 13 to 17.

**RESULTS AND DISCUSSION**

i. **Characterization of Drug (Clarithromycin):**
The drug Clarithromycin was characterized by IR spectroscopy. The IR spectrum shown in fig. 2 was compared with the reference spectrum given in I. P. It showed the characteristic peaks for tertiary – N stretching, carbonyl stretching, aliphatic –CH stretching and –OH stretching as shown in Table 1.
ii. Drug-Excipients Compatibility Studies:
The drug excipients compatibility study carried out at room temperature revealed that combination of Clarithromycin with ES 100, E RL 100 and E RS 100 (IR spectra shown in figs. 3, 4 and 5 respectively) results into intact and broad characteristic groups such as tertiary amine, carbonyl and hydroxyl indicating that they are compatible with the drug, but there was intermolecular hydrogen bonding between polymeric groups and Clarithromycin that result in weak absorption frequency of hydroxyl groups in IR spectrum.

Combination of drug with E L 100 and E L 100 55 showed that the characteristic tertiary amine peak was weakened indicating the incompatibility of Clarithromycin with these two polymers. Also peaks of carbonyl and hydroxyl groups were not sharp showing incompatibility of the drug with these polymers.

![Fig 2](image)

**Fig 2**
*IR spectrum of Clarithromycin*

![Fig 3](image)

**Fig. 3**
*IR spectrum of Clarithromycin+ Eudragit S 100*
iii. Shape and Surface Morphology:
Photomicrograph (Fig. 6 and 7) taken using scanning electron microscope showed that the microspheres were spherical in shape. The porous surface was also clearly seen. The porosity is due to diffusion of solvents at 40°C from the microspheres formed for a period of one hour.
iv. Effect of Dispersing agent on particle size:
As the concentration of polyvinyl alcohol increased from 0.2 to 0.75% w/v, the particle size did not change appreciably but the increased concentration gave threading and film on drying, hence 0.2% w/v was determined as an optimum concentration of polyvinyl alcohol that gave more number of particles in the range of 100 to 150 micrometer. From the frequency distribution curves (Fig. 8, 9 and 10), it was observed that when the concentration of PVA was 0.2%, the highest frequency of particle size of 100-150 micron was observed. When concentration of PVA was increased from 0.2 to 0.5% the highest frequency decreased to 45 to 55 micron. On further increase in concentration to 0.75 % the particle size reduced to 20 to 30 micron.

The microspheres obtained were poly dispersed in nature. The particle size was found to be increased as the drug to polymer ratio increased.
v. Micromeritic Properties:
The Carr’s Index showed that the formulation S2 has very very poor flow properties (Carr’s Index = 39.42%) while formulation RS2 has poor flowability (Carr’s Index= 23.52%). The percent buoyancy of S2 was 74 % and that of RS2 was 83.2% after eight hours indicating better floatability of Eudragit RS 100 microspheres.

vi. In vitro Drug Release:
The drug release was found to be decreased as the polymer concentration increased as shown in figs. 11 and 12. 72.28% of cumulative drug release was given by S2 and 74.14 % by RS2 after eight hours in 0. 1 N hydrochloric acid indicating the potential of these two formulations to release the drug for 12 hours in the gastric medium.
vii. Kinetic Study of Drug Release using various In Vitro Models:
The in vitro release data of the two formulations S2 and RS2 was subjected to various kinetic models of drug release. The graph of % cumulative drug released Vs. Time was plotted (Fig. 13). The values were subjected to regression analysis. Batch S2 gave R\(^2\) value of 0.906 and RS2 gave 0.946 which were closer to one. So we can say that a somewhat zero order drug release pattern is shown by these two formulations. The plot of log of % cumulative drug released vs. time was plotted (Fig. 14) showing R\(^2\) values of 0.873 and 0.927. Higuchi’s Equation is used as a model for diffusion controlled drug release from insoluble polymeric matrix. The graph of % cumulative drug release Vs square root of time (Fig. 15) was plotted that gave regression values not near to one. Hixon Crowell Drug Release Model was used to find whether the drug release was dissolution dependent. A graph of Cube root of % Cumulative drug remaining Vs. Time (Fig. 16) was plotted that gave R\(^2\) values of only 0.678 for S2 and 0.864 for RS2. Korsemeyer Peppas Model was used to determine the drug release behaviour from eudragit polymeric system. The graph of log M\(_t\)/M\(_{\infty}\) Vs log time was plotted (Fig. 17) and the Slope (n) was calculated. The Korsemeyer Peppas model suggested that from batch S2 (Slope = 0.45 <0.465< 0.89) the drug release was non Fickian which is a combination of diffusion and erosion mechanism. The batch RS2 (Slope = 0.45 <0.583< 0.89) also showed non Fickian drug release.
**Zero Order Kinetic Model for Eudragit Microspheres of Clarithromycin**

![Zero Order Kinetic Model](image)

**First Order Kinetic Model for Eudragit Microspheres of Clarithromycin**

![First Order Kinetic Model](image)

**Higuchi’s Drug Release Model for Eudragit Microspheres of Clarithromycin**

![Higuchi's Model](image)
viii. Residual Solvent:
Dichloromethane that was used for preparation of microspheres has been listed as Type 2 Residual solvent in USP\textsuperscript{19}. The solvent should not be present in the final formulation. Batches of microspheres were prepared using the stirring time of one, two and three hours at 40°C. The gas chromatography and Mass spectroscopic results of these batches showed no detection of dichloromethane. This indicates that one hour stirring at 40°C was sufficient to remove dichloromethane and to form the hollow cavity in the microspheres.

CONCLUSION

The hollow microspheres of Clarithromycin using Eudragit S 100 and Eudragit RS 100 were successfully developed as gastroretentive drug delivery systems. The microspheres were round in shape with porous surface and having hollow cavity; suitable for floating. The bulk density and % compressibility indicated that they were having poor flow properties.
As the Drug: Eudragit ratio was increased from 1:1 to 1:3, particle size of microspheres increased and drug release decreased. Drug: polymer ratio of 1:2 gave better release among all formulations. After application of various kinetic models to the drug release data of two best batches S2 (with Eudragit S 100, 1:2 ratio) and RS2 (with Eudragit RS 100, 1:2 ratio), it was observed that the data was best fitting to the zero order drug release kinetics. The Korsemeyer Peppas model suggested that from batch S2 (Slope = 0.45 < 0.465 < 0.89) the drug release was non Fickian which is a combination of diffusion and erosion mechanism. The batch RS2 (Slope = 0.45 < 0.583 < 0.89) also showed non Fickian drug release.

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