Formulation and *In vitro* Characterization of Ramipril Microspheres

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**Abstract:** The purpose of the present investigation was to formulate and evaluate micro encapsulated ramipril produced by the non aqueous emulsification solvent evaporation method microspheres were prepared using polymethacrylate polymers (Eudragit® RS100 and RL 100) and hypromellose (HPMC) by solvent evaporation method. The impacts of different factors such as stirring rate, polymer concentration, and volume at processing medium on the characteristics of the micro spheres were investigated. Microspheres were characterized for their surface morphology, particle size, drug entrapment efficiency and *In vitro* release study the morphology of microspheres was studied using scanning electron microscopy and it was shown that microspheres were spherical in nature. The particle size of microspheres analyzed by optical microscopic method & was affected by stirring rate, concentration of polymers and volume of processing medium. *In vitro* drug release studies it was found that the controlled effect of microspheres depended on the polymer concentration and type of polymers used in the formulation. The mechanism of drug release from the microspheres was found to be non-fickian type.

**Key words:** Ramipril, Eudragit®, Hypromellase, microspheres, controlled release, non-aqueous solvent evaporation

**INTRODUCTION**

Microspheres are one of the particulate delivery systems and to achieve sustained or controlled drug delivery, improve bio availability and stability. Microspheres also offer advantages such as limiting fluctuation with a therapeutic range, reduction in side effects, decreased dose frequency and hence improved patient compliance. The popular, method for the encapsulation of drugs with in water in soluble polymer is the emulsion solvent evaporation method. This technique offers several advantages and is preferable to other preparation methods such as spray drying, sonication and homogenization because it requires only mild conditions such as ambient temperature and constant stirring. Thus, a stable emulsion can be formed with out compromising, the activity of drugs. In the present Investigation. Eudragit® Rs. 100- Eudrgit. RL100 Hypromellase (HPMC) were used as encapsulation materials. Eudragit® Rs. 100 and Eudrgit® RL 100 are referred to as ammnonio methacrylate Copolymers, with the former having 5% functional quaternary ammonium groups and low permeability and latter having 10% functional quaternary ammonium groups and high permeability. Hypromellase (HPMC) is used as dissolution enhancer. The drug of choice Ramipril is an effective antihypertension drug.

It is having low bio availability 28%, bitter taste and have short biological half life 2-4hrs. Which
make it more suitable to be designed as a controlled release formulation. The main purpose of the present research was to develop a controlled drug delivery system of ramipril for oral administration using biocompatible Eudragit® polymers in order to increase bioavailability, biological half life, mask the bitter taste of the drug and to determine the influence of formulation and preparation variables on micro particle characteristics, such as drug incorporation and in vitro drug release rate.

MATERIALS AND METHOD

Materials: Ramipril received from Bright Scientific Pvt Ltd Hyderabad, Eudragit® Rs 100, Eudragit® RL100HRMc received from Bright Scientific Pvt Ltd HYD, and acetone, ethanol, petroleum ether, liquid paraffin (light), span 80 received from S.d fine Chem. limited Mumbai.

Preparation of Microspheres

The ramipril microspheres were prepared by non-aqueous emulsion solvent evaporation technique. It was carried out by dispersion of organic phase which contains the drug and polymers (ramipril and Eudragit RS 100 and HPMC) were dissolved in mixed solvent system comprising of acetone and ethanol in equal volumes (1:1 ratio) to the processing medium of light liquid paraffin (100 ml) containing 1% of span 80. Which is being stirred at 1000 rpm using mechanical stirrer equipped with three bladed propeller. The stirring was continued for 4 hrs and the solvent was allowed to evaporate completely. The resulting microspheres were separated by filtration. Obtained microspheres were washed repeatedly with petroleum ether freed from oil (liquid paraffin). The collected microspheres were dried at room temperature and subsequently stored in desiccators.

### Table 1

**Batch Specifications of the Prepared Microspheres**

<table>
<thead>
<tr>
<th>FC</th>
<th>Ramipril</th>
<th>Eudragit Rs-100+HPMC</th>
<th>EudragitRL-100+HPMC</th>
<th>Liquid paraffin (ml)</th>
<th>Span-80 (%)</th>
<th>Stirringspeed (rpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>100</td>
<td>1</td>
<td>1000</td>
</tr>
<tr>
<td>F2</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>100</td>
<td>1</td>
<td>1000</td>
</tr>
<tr>
<td>F3</td>
<td>1</td>
<td>3</td>
<td>-</td>
<td>100</td>
<td>1</td>
<td>1000</td>
</tr>
<tr>
<td>F4</td>
<td>1</td>
<td>4</td>
<td>-</td>
<td>100</td>
<td>1</td>
<td>1000</td>
</tr>
<tr>
<td>F5</td>
<td>1</td>
<td>3</td>
<td>-</td>
<td>50</td>
<td>1</td>
<td>1000</td>
</tr>
<tr>
<td>F6</td>
<td>1</td>
<td>3</td>
<td>-</td>
<td>150</td>
<td>1</td>
<td>1000</td>
</tr>
<tr>
<td>F7</td>
<td>1</td>
<td>3</td>
<td>-</td>
<td>100</td>
<td>1</td>
<td>500</td>
</tr>
<tr>
<td>F8</td>
<td>1</td>
<td>3</td>
<td>-</td>
<td>100</td>
<td>1</td>
<td>1500</td>
</tr>
<tr>
<td>F9</td>
<td>1</td>
<td>-</td>
<td>3</td>
<td>100</td>
<td>1</td>
<td>1000</td>
</tr>
<tr>
<td>F10</td>
<td>1</td>
<td>-</td>
<td>4</td>
<td>100</td>
<td>1</td>
<td>1000</td>
</tr>
</tbody>
</table>

FC-Formulation Code

### Table 2

**Mean Particle Size, Entrapment Efficiency of Ramipril Microspheres**

<table>
<thead>
<tr>
<th>FC</th>
<th>Mean Particle size (µm) ± SD</th>
<th>Entrapment efficiency (%) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>F- 1</td>
<td>52±1.5</td>
<td>52.43±0.45</td>
</tr>
<tr>
<td>F- 2</td>
<td>68±1.04</td>
<td>66.21±0.3</td>
</tr>
<tr>
<td>F- 3</td>
<td>89±0.57</td>
<td>79.26±0.85</td>
</tr>
<tr>
<td>F- 4</td>
<td>100±0.52</td>
<td>83.16±0.66</td>
</tr>
<tr>
<td>F- 5</td>
<td>95±2.60</td>
<td>76.63±1.32</td>
</tr>
<tr>
<td>F- 6</td>
<td>83±3.02</td>
<td>73.4±1.04</td>
</tr>
<tr>
<td>F- 7</td>
<td>94±0.62</td>
<td>77.70±0.85</td>
</tr>
<tr>
<td>F- 8</td>
<td>82±2.08</td>
<td>74.5±1.05</td>
</tr>
<tr>
<td>F- 9</td>
<td>76±1.99</td>
<td>56.37±0.73</td>
</tr>
<tr>
<td>F- 10</td>
<td>93±1.1</td>
<td>62.35±0.45</td>
</tr>
</tbody>
</table>

FC-Formulation Code

**Scanning Electron Microscopy (SEM) Studies**

Figure 5

Figure 6

Figure: Scanning electron micrographs of (5) drug loaded microspheres and (6) surface morphology of drug loaded microspheres.
EVALUATION OF MICROSPHERES
The Microspheres are characterized by their morphology, particle size, drug entrapment efficiency, in vitro drug release studies.

MICROSPHERES MORPHOLOGY
The Morphology and surface characteristics of the microsphere were examined by a scanning electron microscopy (JMS-840-A, JEOL, JAPAN). The sample was mounted on to an aluminum stub and sputter – coated for 90 s with gold particles in an argon atmosphere.

PARTICLE SITE ANALYSIS
The Microsphere size distribution in a sample was determined by optical microscopy method using a calibrated stage micrometer and eyepiece calibration of the microscope was done prior to particle site measurement of the microspheres. The mean of 100 spheres was rotated as particle site.

DRUG ENTRAPMENT EFFICIENCY
To determine the drug entrapment efficiency a weighed quantity of microspheres were crushed into powder and transferred into 100 ml to volumetric flask. The contents was dissolved by using 6.8 PH phosphate buffer and made up to 100ml. The resulting mixture was kept for 24hrs at dark place then the solution was filtered through membrane filter of 0.45 µm pore size and 1 ml of this solution was diluted to 10 ml using phosphate buffer of PH 6.8. After further suitable dilution the samples were analyzed spectrophotometrically for the drug entrapment efficiency at 215nm. The drug entrapment efficiency was determined using the relationship.

\[
\text{Drug entrapment efficiency} = \frac{\text{Experimental drug content}}{\text{Theoretical drug content}} \times 100
\]

IN-VITRO DRUG RELEASE
Microspheres equivalent to 10mg of Ramipril was subjected for dissolution. Dissolution tests were carried out using USP basket type dissolution tests apparatus (LAB INDIA, Germany) the stirring rate was 100 rpm. The dissolution medium was 900ms of 6.8PH phosphate buffer and the temperature was maintained at 37±0.5 °C. A 5 ml quantity of the dissolution medium was sampled at predetermined time Intervals, and fresh dissolution medium was simultaneously used to replenish the dissolution medium on each occasion to keep the volume constant. The sample was filtered through filter disc and the filtrate was diluted with fresh dissolution medium if necessary. The samples were analyzed using UV double beam spectrophotometer at 215nm.
RESULT & DISCUSSION

Controlled released microsphere were prepared by the Non - Aqueous solvent evaporation method using polymethacrylate polymers (Eudragit® Rs100 and RL100) and Hypromellose (HPMC). To assess the effect of stirring rate, polymers concentration and volume of processing medium on the Characteristics of the microspheres. The batch specification were shown in table 1- formulations F1 to F8 prepared by using Eudragit Rs100 and HPMC combination. Formulatins F9 and F10 prepared by using Eudragit Rs100 and HPMC combination.

As Indicated In Table

The mean particle size of the formulations F1 to F4 was found to be increase from 52 µm ± 1.5, 68 µm ± 1.04, 89 µm ± 0.57 and 100 µm ± 0.52, respectively, when the drug to polymer ratio increased from 1:1, 1:2, 1:3, 1:4 respectively 3hrs.

The mean Particle size of the formulating F1 to F4 was found to be increased from 52 µm ± 1.5 to 100 µm ± 0.52. This increased mean Practical size could be due to the fact that as increasing polymer concentration increases frequency of collisions, resulting in fusion of semi formed particles and producing overall increase in the size of the microsphere. In formulation F5 & F6 the mean particle size was found to decrease 95 µm ± 2.60 ± 83 µm ± 3.02. This decrease in particle size could be due to when the volume of processing medium was increased the emulsion droplets could be moved freely in the liquid paraffin and they had less chance to collide with each other there by yielding small and uniform microspheres. Conversely when the volume is only 50ml, the emulsion droplets had more opportunities to collide with each other and fuse together to form larger microspheres. In formulations F7 & F8 the mean practical size was found to be decrease 94 µm ± 0.62 and 82 µm ± 2.08. This is due to when the stirring speed was increased, the mean particles size of the microspheres decreased and the practices size was uniform.

In formulations F9 & F10 the mean particle size was found to be increase 76 µm ± 1.99 and 93 µm ± 1.1. This is due to when the polymer concentration increased it increases the frequency of collisions and producing overall increase in the size at the microspheres.

The entrapment efficiency of the Formulations F1 to F4 was found to be increase from 52.43% ± 0.45 to 23.19% ± 0.66. This could be due to the increased viscosity in a fixed volume of solvent as the drug to polymer ratio increased.

In formulations F5 & F6 the drug, entrapment efficiency was found to decrease from 76.63± 1.32 and 73.4± 1.04, this could be due to increased volume of processing medium increases higher drug extraction into the processing medium resulting in lower entrapment efficiency. In formulations F7 & F8 the drug entrapment efficiency was found to decrease from 77.70± 0.85 and 74.5±
1.05. This could be due to the formation of larger and smaller emulsion droplets. When the stirring speed was increased it ensures the drug diffusion out of the microspheres be to they harden at low rpm.

In formulations F9 and F10 the drug entrapment efficiency was found to be increase from 56.37% ± 0.73 and 62.35% ± 0.45. This is due to increased viscosity in a fixed volume of solvent as the drug to polymer ratio increased. The drug entrapment efficiency which was found in formulations F9 to F10 was very low when compared to formulations F3 & F4, which could be due to the structural differences between polymer types i.e. the difference in the content of the quaternary ammonium group & high content of the ammonium group facilitates the diffusion of a part of entrapped drug to the surrounding medium during preparation of microspheres.

In vitro ramipril release studies were performed in PH 6.8 Phosphate butter. The results are given in fig (1), fig (2), fig (3), fig (4).

As illustrated in fig (1) in Formulated F1 to F4 the decrease in drug release rate from 10 to 24 hrs was observed. This is due to increasing polymers concentration decreases the amount drug present close to the surface and thereby retarding drug release. As illustrated in fig. (2) in formulations F5 & F6 the increase in drug release rate from 20th & 18th hrs was observed this is due to higher migration of drug to the surface at the microspheres during solvent evaporation from the freely moving emulsion droplets in large volume of processing medium. As the illustrated in fig. (3) in formulations F7 & F8 the increase in drug release from 20th & 18th hrs was observed this is due to increasing stirring speed decreases the practical size and increased the larger available surface area.

As illustrated in fig (4) the duration of drug release was shorter in formulations F9 and F10 when compared to formulations F3 & F4. This could be due to the difference in content of quaternary ammonium groups where the amount of quaternary ammonium group of Eudragit Rs100 is lower than that of Eudragit RL100 which less permeable - the Eudragit RL100 highly permeable polymer increases the porosity of the matrix and they accelerates the drug release.

Surface Morphology

Surface topography of the microspheres was investigated by scanning electron microscopy (SEM) seen in fig. (5). They are spherical in shape and exhibited porous surface and they suggest that the drug was released through pores and the mechanism of drug release was diffusion controlled. In fig. (6) the surface of the microspheres was rough and indicated the presence at drug particles on the surface.

CONCLUSION

The controlled release microspheres were successfully developed by solvent evaporation technique using Eudragit & HPMC polymer. From this study it is concluded that the drug to polymer ratio, stirring speed, volume of processing medium, were important for obtained desired spherical particles and also obtained desired encapsulation efficiency and drug release. The release volume of ramipril from the microspheres were depend upon the amount and type of polymers used. Microspheres containing Eudragit RL100 and HPMC combination gives very fast release of drug where as the desired controlled release rate is achieved by combination of Eudragit Rs. 100 and HPMC combination.

REFERENCES


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