

## SUSTAINED RELEASE OF RAMIPRIL FROM AMMONIO METHACRYLATE COPOLYMER MATRIX PREPARED BY HIGH PRESSURE HOMOGENIZER

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### ABSTRACT

**Objective:** Ramipril loaded nanoparticles were prepared by high pressure homogenization technique using ammonio methacrylate copolymers, Eudragit RSPO and Eudragit RLPO, as coating materials with different amount of polymers. Our objective was to apply this technique in order to develop poorly water soluble drugs loaded nanoparticles with both these copolymers.

**Method:** In the present work we have selected antihypertensive drug Ramipril due to its short half life and poor water solubility for the preparation of sustained release nanoparticles using modified O/W Emulsion Solvent Diffusion method. The sustained release nanoparticles of Ramipril with Eudragit in the different ratios of 1:1, 1:3, 1:5 and 1:7 (wt/wt) were prepared using surfactant like polyvinyl alcohol.

**Results:** The effect of surfactant concentration, stirring rate and polymer concentration on the surface morphology, encapsulation efficiency and drug release rate were studied. The obtained nanoparticles were subjected to FT-IR, X-Ray powder diffractometry, and scanning electron microscopy (SEM) as well as kinetic drug release study. The Eudragit loaded RSPO and RLPO nanoparticles were spherical with diameters in the range of 200-400 nm and 300-600 nm respectively by modified o/w method. The average Encapsulation efficiency (EE) was obtained  $76.67 \pm 0.45\%$  by using RSPO and  $89.47 \pm 0.30\%$  prepared by RLPO. Eudragit RLPO used singly produced nanoparticles with higher encapsulation efficiency as well as higher drug content more than nanoparticles formulated using RSPO polymer. *In vitro* drug release rate for nanoparticles was found to be sustained over 12 hours and a burst release of Ramipril loaded Eudragit RLPO nanoparticles was observed prepared by the modified o/w method.

**Conclusion:** It is concluded that nanoparticles containing both Eudragit RSPO and Eudragit RLPO biocompatible copolymers were effectively prepared and drug release rate can be controlled by choice of polymer type.

**Keywords:** Antihypertensive, High pressure Homogenizer; Eudragit, FE-SEM, Release kinetics.

### INTRODUCTION

Ramipril (RM) is a potent and long acting ACE inhibitor. In hypertensive patients, Ramipril leads to a reduction in supine and standing blood pressure without a compensatory increase in heart rate [1]. Blood pressure tends to be lower while asleep and elevated in the early morning. Similarly, the maximum death occurrence at morning from a heart attack is around 9 A.M., which coincides with peaks in platelet aggregation, plasma catecholamine levels, and blood pressure. Development of controlled drug delivery system is a better alternative for use of the multiple - dose regimen and for treatment of the heart diseases [2,3]. Ramipril is highly lipophilic, long acting angiotensin converting enzyme (ACE) inhibitor and chemically it is (2S,3aS,6aS) -1 [(S) -N- [(S) -1-carboxy-3-phenylpropyl] alanyl] octahydro cyclopenta [b] pyrrole-2-carboxylic acid-1-ethyl ester. Ramipril is an angiotensin-converting enzyme (ACE) inhibitor used to treat high blood pressure (hypertension), congestive heart failure and chronic renal failure [4,5]. Ramipril inhibits angiotensin-converting enzyme (ACE) and reduces the angiotensin II levels which leads to the reduction of aldosterone secretion thus lowering of blood pressure [6]. Peak plasma concentrations of ramiprilat are reached within 2 to 4 hours and the absolute bioavailability of ramipril and ramiprilat were 28% and 44%. Other than these the ramipril is a poorly aqueous soluble drug [7]. It is an official drug in Indian pharmacopoeia and British pharmacopoeia [8]. Eudragit RLPO and RSPO polymers belong to the class of poly (meth) acrylates that are insoluble but permeable in digestive fluids. Both are biocompatible polymers of acrylic and methacrylic acid ester having similar structures, different only in the extend of quaternary ammonium substitution on the molecule [9]. Eudragit RLPO is a polymer synthesized from acrylic and methacrylic acid esters, containing low-level quaternary ammonium groups and insoluble at physiological pH values and capable of swelling [10]. The purpose of the present investigation was to design and compare the release characteristics of sustained-release formulations of Ramipril naoparticles by using high pressure

homogenization techniques using Eudragit RSPO and RLPO. The physicochemical characteristics of Ramipril were studied by Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction analysis (XRD), and FE-SEM.

### MATERIALS AND METHODS

Ramipril was a kind gift from Dr. Reddy's Laboratories Ltd. (Hyderabad, India). Eudragit® RSPO and RLPO were gifted by Evonik Degussa India Pvt. Ltd. (Mumbai, India). Ethyl acetate, Acetone and n- Hexane were purchased from Merck (Mumbai, India). Polyvinyl alcohol (PVA, MW Approx. 1,25,000) from SD Fine Chem Ltd. (Mumbai, India). The experimental work was performed by using triple distilled water.

#### Preparation of Eudragit-Loaded Ramipril Nanoparticles

Ramipril (RM) loaded Eudragit RSPO and Eudragit RLPO polymeric nanoparticles were prepared by a modified o/w emulsion solvent evaporation method [11]. Drug and polymer (1:1, 1:3 1:5, 1:7) were dissolved in organic solvents 1:1 combination of Ethyl acetate and acetone by using a magnetic stirrer (Remi, India). This organic phase added drop by drop in external aqueous phase containing surfactant Polyvinyl alcohol (PVA 0.4% w/v) at 13,500 rpm (Omni GLH Homogenizer). During this homogenization external phase was held at cooling temperature. This dispersion was then processed in high pressure Homogenizer (Panda GEA Niro Soavi, Italy) for five cycles. Thereafter organic solvents from external aqueous phase were removed by evaporation. The formed RM-RSPO and RLPO polymeric nanoparticles were recovered by centrifugation (R243A, Remi, India ) at 18000 RPM for 20 min followed by washing thrice with distilled water and washed nanoparticles were subjected to freeze drying (Labosene, Scanvac coolsafe, Denmark).

Nanosuspension formula were established (table 1) with different ratios of polymers like Eudragit RSPO and RLPO and constant ratio of surfactant to obtain higher encapsulation efficiency, desired particle size and suitable drug release studies.

**Table 1: Composition of Ramipril loaded Eudragit RSPO and RLPO nanoparticles.**

	Drug/Polymer Ratio	Surfactant concentration	Drug (mg)	Polymer (mg)	Volume of solvent (ml)	External Phase(Water) (ml)
RSPO	1:1	0.4 %	100	100	25	100
	1:3		100	300	25	100
	1:5		100	500	30	100
	1:7		100	700	30	100
RLPO	1:1	0.4 %	100	100	25	100
	1:3		100	300	25	100
	1:5		100	500	30	100
	1:7		100	700	30	100

### Determination of Particle size, Zeta potential and Polydispersity index

To analyze particle size, zeta potential and polydispersity index nanosuspension were diluted with filtered (0.22µm) ultra pure water. The sample was analyzed using Master Sizer 2000 (Malvern instrument, UK) yielding the mean particle diameter of the suspension and polydispersity index [12].

### Calculation of Batch yield

The percentage yields of obtaining nanoparticles were calculated by using Eq. (1):

$$\text{Batch yield(\%)} = \frac{\text{Amount of nanoparticles obtained}}{\text{The total amount of drug, polymer and excipients}} \times 100 \dots\dots\dots(1)$$

### Determination of encapsulation efficiency and drug content

Accurately weighed freeze dried nanoparticles were dissolved in organic solvent. Then RM was extracted in 50 ml phosphate buffer (pH 7.4) solution. After the evaporation of organic solvent and removal of precipitated polymer by filtration, the amount of drug in phosphate buffer was measured using UV- visible spectrophotometer (UV- 1800 Shimadzu Co. Ltd., Japan). at 207 nm. Encapsulation efficiency (%) and drug content (% w/w) were represented by Eqs. (2) And (3) respectively [13,14].

$$\text{Encapsulation Efficiency (\%)} = \frac{\text{Amount of RM in nanoparticles}}{\text{Amount of RM used in the formulation}} \times 100 \dots\dots\dots(2)$$

$$\text{Drug content (\% w/w)} = \frac{\text{Amount of RM in nanoparticles}}{\text{Amount of nanoparticles recovered}} \times 100 \dots\dots\dots(3)$$

### Field Emission- Scanning Electron Microscopy

The external morphology of RM-Eudragit nanoparticles were investigated by using Field Emission- Scanning Electron Microscopy (FE- SEM) (S4800 Hitachi, Japan). The nanoparticles were mounted on stub, using double sided adhesive carbon tapes. Samples were coated with gold-palladium under argon atmosphere and observed for morphology, at an acceleration voltage of 10 KV.

### Studies of infrared spectroscopy

The crystalline samples RM incorporated nanoparticles were assessed by Fourier transform infrared spectrometry (FTIR) [15]. Sample of pure RM, Eudragit polymer and RM-Eudragit nanoparticles were homogeneously mixed with potassium bromide and infrared spectrums were recorded in the region of 4000-500 cm<sup>-1</sup> by using an infrared spectrophotometer (IR-8400, Shimadzu Co. Ltd., Singapore).

### X-ray diffraction

The diffraction patterns of samples were recorded using Model-D8 Advance, Bruker AXS GmbH, Germany diffractometer. An Cu Kα source operation with voltage of 40 KV and a current of 40 MA of the generator were used. The sample was exposed over a 2θ angular range from 3 to50° with a step size of 0.02° in 2θ and a 1 Sec counting per step at room temperature.

### Release study

The nanoparticles were suspended in 100 ml phosphate buffer saline (pH 7.4). The solution was stirred at 50 rpm with temperature adjusted to 37±1°C. At predetermined time intervals 5 ml samples were withdrawn and centrifuged at 20,000 rpm for 30 min. Aliquots of supernatant were analyzed by a UV spectrophotometer at 207 nm. The settled nanoparticles in centrifuge tube were redispersed in 5 ml fresh phosphate buffer saline (pH 7.4) and returned to the dissolution media [16,17].

### Release kinetic study

The dissolution data of each batch were fitted to various kinetic equations and mechanism of drug release investigated. Regression coefficient (r<sup>2</sup>) was determined from the slope of the following plots: Cumulative % drug release Vs Time (Zero order kinetic model), Log cumulative of % drug remaining Vs Time (First order kinetic model), Cumulative % of drug release Vs Square root of Time (Higuchi model), Log cumulative % drug release Vs Log time (Korsmeyer- Peppas model).

## RESULTS AND DISCUSSION

RM-RSPO and RM-RLPO nanoparticles were prepared by modifying solvent diffusion technique using 1:1 combination of ethyl acetate and acetone as an internal organic phase. Drug as well as Eudragit polymers both are completely soluble in the organic phase hence minimum chances of drug loss from polymer due to the homogenous nature of fused molecules [18].

Both drug and polymers were hydrophobic in nature thus maximum drug was encapsulated. At the time of adding a primary phase to external water phase containing polyvinyl alcohol as a surface active agent, RM-RSPO and RM-RLPO fusion start to precipitate due to insoluble nature and fast diffusion of ethyl acetate. Consequently matrix was going to in nano size by using high pressure Homogenizer. 0.4 % w/w Polyvinyl alcohol as a surfactant showed better results in terms of encapsulation efficiency, drug content and particle size of formulation (Table. 2). PVA has superior tendency to migrate toward the surface of nanoparticles prepared by RSPO and RLPO.

**Table 2: Particle size, Percentage yield, Encapsulation efficiency and drug content**

Polymer grade	Drug/Polymer Ratio	Yield (%)	Encapsulation Efficiency	Drug content	Size (nm) (mean±SD), n=3
RSPO	1:1	87.57±0.170	59.78±0.445	70.52±0.440	236.2±0.374
	1:3	95.52±0.302	62.76±0.325	73.71±0.420	317.0±0.427
	1:5	96.50±0.279	68.60±0.218	74.83±0.418	411.2±0.541
	1:7	97.17±0.257	76.67±0.451	79.35±0.456	756.8±0.320
RLPO	1:1	91.61±0.144	69.77±0.277	75.72±0.493	245.2±0.370
	1:3	91.53±0.179	72.71±0.288	79.21±0.442	341.9±0.449
	1:5	95.62±0.256	81.51±0.408	95.83±0.425	367.0±0.476
	1:7	90.66±0.236	89.47±0.309	96.62±0.111	527.2±0.720

The results concluded that formulation prepared by using Eudragit RLPO showed 10-14% higher Encapsulation efficiency than those prepared using Eudragit RSPO polymer. The drug holding capacity for maximum period was higher in RSPO nanoparticles than RLPO nanoparticles [19].

This is because of Eudragit RSPO has a less proportion of quaternary ammonium groups in their structure which is responsible for low water permeability and swellability (Eudragit data sheet). The average particle size of nanoparticles prepared by RSPO was less than nanoparticles prepared by RLPO. Particle size and encapsulation efficiency increases due to increasing the ratio of drug and polymer. Reason behind that stated the saturated concentration of organic phase increased with viscosity at higher ratio helps to maximize the size of particle with higher encapsulation [20].

Internal phase viscosity having ratio 1:7 shows higher than 1:5, 1:3 and 1:1 respectively. Other hand, lower drug polymer ratio getting small particle size due to diffusion of polymer solvent phase in external aqueous phase difficult to disperse due to resistance in higher mass transfer and resulted in larger globules give a maximum size of particle than a lower ratio of internal phase. Batch yield and encapsulation efficiency both are also affected by viscosity like higher concentration of polymer gives more binding capacity hence

maximum drug entrapped in polymeric core and gives more percentage yield as well as encapsulation efficiency. In this formulation 0.4% Polyvinyl alcohol was used to stabilize zeta potential. Obtained results showed formulations were stable. Polydispersity index (PDI) of selected higher ratio batches of the RSPO and RLPO showed 0.426 and 0.324 respectively. The obtained nanoparticles were uniform size, spherical shaped and having pores on the surface of nanoparticles it may be due to ethyl acetate diffused out from the organic phase before the stabilization of nanoparticles. (Fig. 1).

The average particle size, PDI and zeta potential of nanoparticles prepared by RSPO is higher than RLPO. There was also observed that the surfactant concentration directly proportional to the zeta potential. The positive charge on NPs was due to cationic quaternary ammonium group present in polymer structure and PVA surfactant on their surface. The obtained results were also exposed that the EE, % yield were increased like particle size and PDI results. As the ratio of polymer and surfactant concentration increased the EE was also increased. This may be due to difference in chemical structure makes Eudragit RLPO more hydrophilic compared to Eudragit RSPO, imparting higher water permeability and solubility to Eudragit RLPO than to Eudragit RSPO.

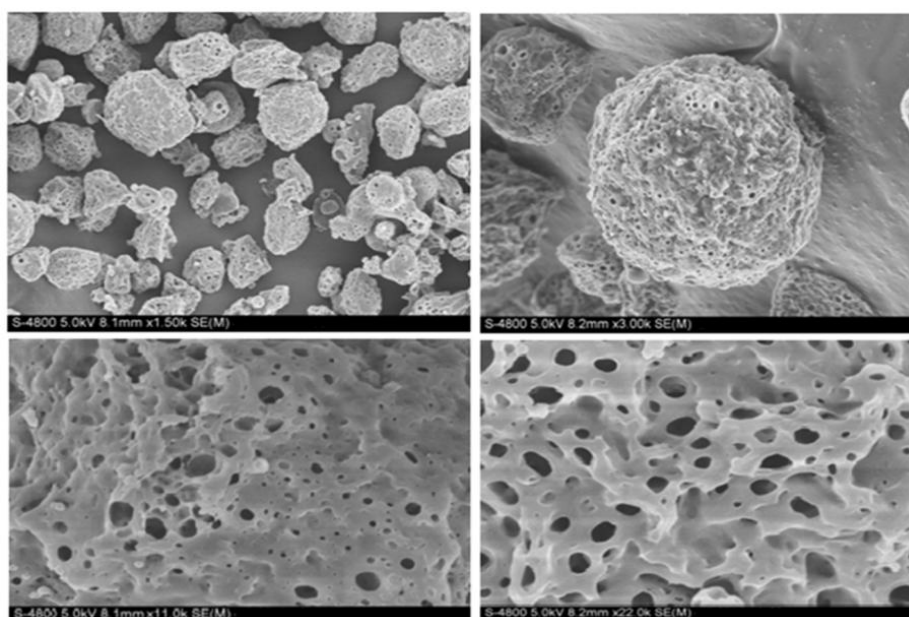


Fig. 1: Field Emission- Scanning Electron Microscopy

Infrared study was carried out to confirm compatibility between the polymers RSPO, RLPO, drug ramipril and the prepared nanoparticles. The spectra obtained from the IR studies are from 4000 $\text{cm}^{-1}$  to

500 $\text{cm}^{-1}$ . There were no major shifting and no loss of functional peaks between the spectra of pure drug and drug loaded nanoparticles. (Fig. 2)

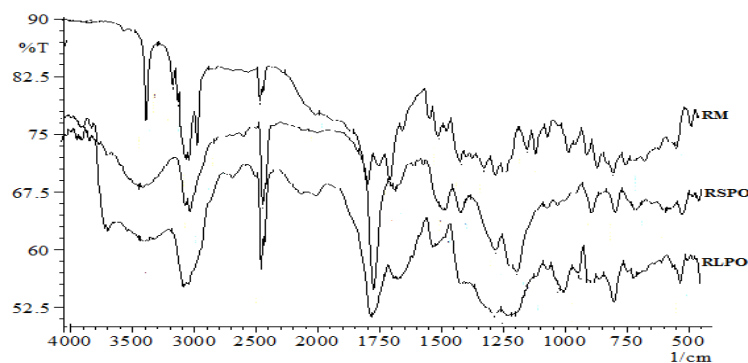


Fig. 2: FT-IR Spectra of Pure Drug Ramipril, RM-RSPO, and RM-RLPO.

Molecular arrangement of RM loaded RSPO and RLPO NPs was dissimilar than pure drug. (Fig. 3). The crystallinity of RM was 98.6 % and RSPO showed crystallinity 39.4% and crystallinity of highly encapsulated nanoparticles was 49.4%. The results concluded that the characteristic peak of RM may overlap by coated Eudragit polymers which show the drug is dispersed at the molecular level in polymer

matrix. This may be due to interference of RSPO molecule arrangement in ramipril molecules during solidification or precipitation.

All XRD graphs revealed that percentage crystallinity of RM was decreased after combinations with Eudragit polymer. This was due to the more amorphous nature of the Eudragit polymers.

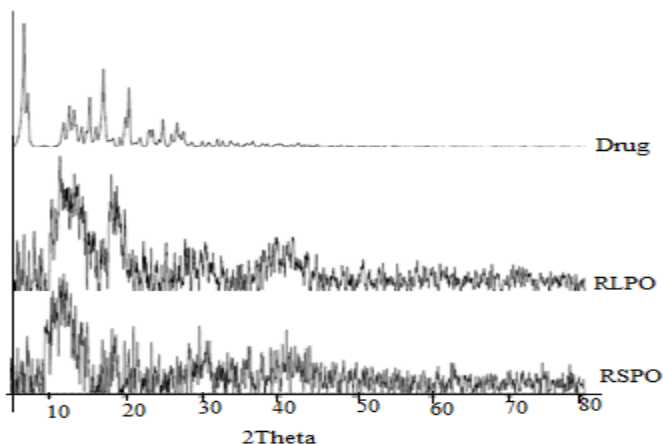


Fig. 3: X-ray diffraction spectra of Ramipril drug, Eudragit RM-RLPO and RM-RSPO.

In vitro dissolution study exposed that RSPO was efficiently sustained the release of RM at all four ratios (Fig. 4). Out of these 1:7 formulation was more efficiently sustained than other three formulations. Formulations prepared by RSPO having 1:7 ratio shows drug release  $40.28 \pm 0.147$  while 1:5, 1:3 and 1:1 shows  $43.40 \pm 0.188$ ,  $57.34 \pm 0.250$  and  $63.60 \pm 0.374$  respectively up to 12 hrs. In the other hand formulation prepared by RLPO having 1:7 ratio shows drug release  $59.79 \pm 0.385$  while 1:5, 1:3, 1:1 shows  $74.77 \pm 0.410$ ,  $83.99 \pm 0.220$ , and

$93.92 \pm 0.271$  percentage of drug release at the end of 12 hrs. As ratio increased drug holding capacity of polymer also increased. Formulation prepared using Eudragit RSPO released RM slower and less burst release than those prepared using Eudragit RLPO showing fast release and high burst release. This difference in the rate of Ramipril release of nanoparticles can be attributed to differences in the extend of hydrophilic quaternary ammonium substitution on Eudragit being 10 % in Eudragit RLPO and 5% in Eudragit RSPO.

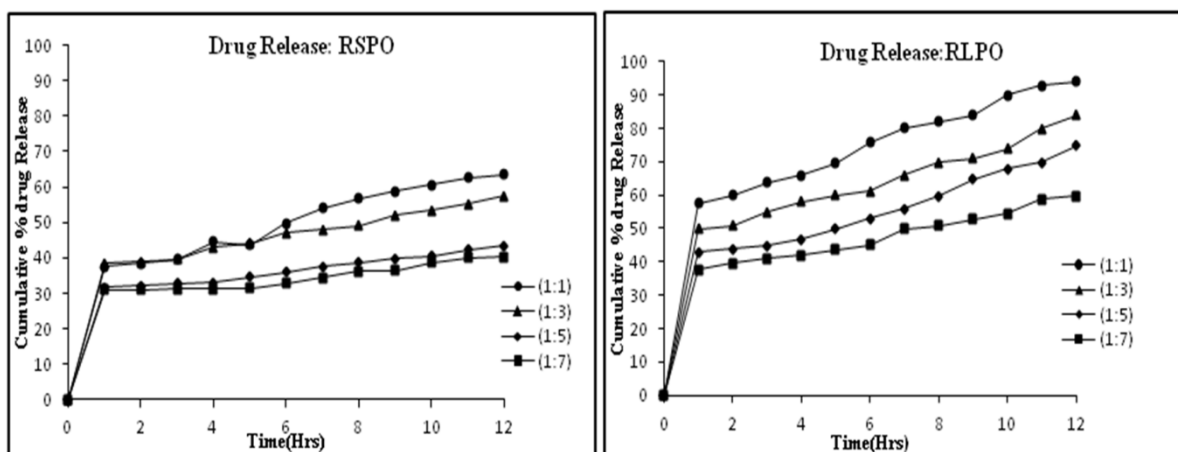


Fig. 4: Release profile of Ramipril from Eudragit RSPO and Eudragit RLPO

Table 3: Release kinetics model

Polymer grade	Drug/Polymer Ratio	Zero Order	First Order	Higuchi	Hixon Crowell	Korsmeyer
RSPO	1:1	0.9736	0.9767	0.9482	0.9704	0.8850
	1:3	0.9809	0.9855	0.9523	0.9875	0.8812
	1:5	0.9840	0.9808	0.9328	0.9820	0.8443
	1:7	0.9217	0.9170	0.8304	0.9186	0.7045
RLPO	1:1	0.9735	0.9827	0.9748	0.9833	0.9150
	1:3	0.9899	0.9854	0.9720	0.9893	0.8815
	1:5	0.9860	0.9810	0.9566	0.9835	0.8331
	1:7	0.9835	0.9707	0.9352	0.9760	0.8644

In vitro release kinetics (Table. 3) revealed that the drug released from 1:7 ratio formulation prepared by RSPO follow zero order. 1:5, 1:3 and 1:1 ratio fitted in the equation of zero order, hixon crowell and first order respectively. Other hand drug released from 1:7 ratio of formulation prepared by RLPO follow zero order. 1:5, 1:3 and 1:1 ratio fitted in the equation of zero order, zero order and Hixon-Crowell model respectively.

The first order describes the release from system where the release rate is concentration dependant. Zero order rates describe the system where the drug release rate is independent of its concentration [21].

## CONCLUSION

From obtained results it was concluded that Eudragit loaded nanoparticles of Ramipril was successfully prepared using high pressure homogenization method and sustained by Eudragit RSPO and RLPO polymers. The matrix with this polymer at nano size level can be produced reproducibly and reliably. The sustained release of drug from the ramipril nanoparticles suggest that the frequency of administration, dose and adverse effects of this molecule could be reduced. We can conclude that there is large scope for improving the use of ramipril in hypertensive treatments through nanoparticle as a drug delivery system.

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