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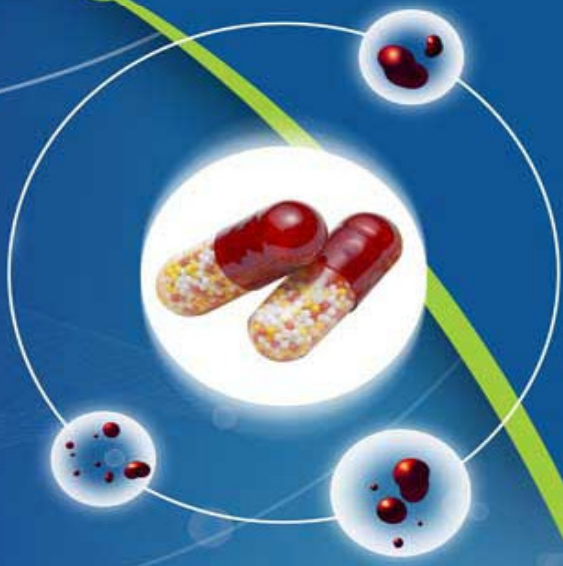

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Research Article

FORMULATION AND EVALUATION OF RIFAMPICIN MICROSPHERES FOR LUNG TARGETING

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ABSTRACT

The present investigation was aimed to develop lung targeting microspheres as drug carriers to reduce dose/dosing frequency in the management of tuberculosis. Microspheres of rifampicin were prepared by using Eudragit L100 and Eudragit RLPO by solvent evaporation technique and microspheres of rifampicin were prepared with natural polymer chitosan by emulsion polymerization technique. Rifampicin microspheres were prepared by using each polymer in drug to polymer ratio of 1:1, 1:2, 1:3 and 1:4 respectively. The microspheres were evaluated for surface morphology, SEM, percentage yield, average particle size, % drug entrapment efficiency and in vitro drug release studies. The particle sizes of the microspheres were found to be in the range of 18±3µm and 47±3µm. Rifampicin microspheres were found to be slightly rough surfaced but spherical and good appearance. The drug entrapment efficacy of Rifampicin microspheres was found to be in the range of 74.8% –95.5%. In vitro drug release after 8 hour was found to be in the range of 71.52%-97.1%. Rifampicin microspheres could be suitable and utilized for targeting rifampicin to the lung.

Key words*: Rifampicin microspheres, Lung targeting microspheres, Antitubercular drug microspheres and Eudragit*

INTRODUCTION

Treatment of tuberculosis is generally successful, except in the case of multiple drug-resistant strains of Mycobacterium tuberculosis. Tuberculosis(TB) remains a major cause of mortality. Approximately more than 2 billion people, equal to one-third of the world's population, are infected with TB bacilli, Mycobacterium[1] and have emerged as an occupational disease in the health care system. Oral therapy using the currently employed antitubercular drugs (ATDs) is very effective, but is still associated with a number of significant drawbacks. More than 80% of TB cases are of pulmonary TB alone and high drug doses are required to be administered because only a small fraction of the total dose reaches the lungs after oral administration[2]. Rifampicin is a major component in fixed dose combination therapy for the treatment of tuberculosis. Rifampicin has low solubility and high permeability with high dose and hence it is classified as class II drug in Biopharmaceutical Classification System (BCS). Rifampicin has poor and variable bioavailability because of its poor solubility, acid decomposition and, drug and food interaction[3]. This site specific or targeted delivery combined with delivery at an optimal rate would not only improve the efficacy

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of a drug but would also reduce the possibility of unwanted toxic side effects of the drug, thus improving the therapeutic index. The lung is an attractive target for drug delivery due to noninvasive administration via inhalation aerosols, avoidance of first-pass metabolism, direct delivery to the site of action for the treatment of respiratory diseases, and the availability of a huge surface area for local drug action and systemic absorption of drug[4].

In addition, the high frequency of pulmonary tuberculosis demands the development of novel drug delivery approaches that enhance the bioavailability of drugs. In recent years, one of the best ways to achieve higher drug levels in the plasma has been the development of new formulations (nanoparticles/microparticles-based) that are directly delivered to the desired site. The controlled delivery of antimycobacterial agents may be accomplished by employing various polymeric drug carriers[5]. The present study was aimed to prepare rifampicin microspheres using chitosan and eudragit polymers for lung targeting.

MATERIAL AND EQUIPMENT

Rifampicin was received as a gift sample from Aldoc, Pharmaceuticals, Ltd, Kota, Rajasthan. Eudragit L-100 and Eudragit RLPO were obtained as gift sample from Evonik Industries, AG, Mumbai, India. Chitosan was received from Alkem Lab., Mumbai, India. Span-80, Liquid-Paraffin, Methanol, Ethanol, n-Hexane, Petroleum Ether, Glutaraldehyde, Acetone, Chloroform, Sodium di-hydrogen ortho-phosphate and Potassium di-hydrogen phosphate were purchased from Central Drug House, New-Delhi, India. Mechanical stirrer (Instrument India, Mumbai, India), dissolution apparatus USP type II (Electro Lab., Mumbai, India), Magnetic stirrer(Instrument India, Mumbai, India) and UV-visible spectrophotometer (Shimadzu, Japan) were the equipments used in this study. All other chemicals and reagent were used in this study of analytical grade.

Preparation of Rifampicin microspheres using Eudragit polymer[6].

Microspheres were prepared using solvent evaporation method. Firstly, Polymer (Eudragit L-100 and Eudragit RLPO) was dissolved respectively in mixture of organic solvent, ethanol:dichloromethane (8:2) on a magnetic stirrer to obtain uniform mixing. To this mixture, magnesium stearate (5% w/v) was added which served as droplet stabilizer followed by addition of Rifampicin with continuous stirring. The drug-polymer ratio was taken as 1:1, 1:2, 1:3 and 1:4. The polymer drug solution was added dropwise into a mixture of liquid paraffin and n-Hexane containing Span 80 (2% w/v) maintained at 40 ºC. After complete evaporation of the solvent the microspheres were allowed to settle, supernatant was decanted and the microspheres were recovered by filtration through a whatmann filter paper (No. 41) followed by washing thoroughly with n-Hexane and petroleum ether. The microspheres were dried at room temperature in a desiccator and stored in desiccator till further use. Eight batches were prepared at each drug polymer ratios 1:1, 1:2, 1:3 and 1:4 respectively (Table No.1)

Preparation of Rifampicin microspheres using chitosan polymer[7].

Rifampicin microspheres were prepared using chitosan by emulsion polymerization method. Chitosan dissolved in 5% acetic acid solution was taken and to it the drug was added with continuous stirring. This solution was dispersed in 100 ml medium containing of liquid paraffin (1:1 mixture of light and heavy paraffin) and to it one ml of span 80 was added as an emulsifier. The suspension was stirred with mechanical stirrer (Instrument India, Mumbai, India) at a constant speed (1000 rpm) for 10 minutes and 1ml of glutaraldehyde was added into the medium and continued stirring for 3 hours. The microspheres were obtained, wash several times with petroleum ether, filtered and air drying. Four batches were prepared at drug polymer ratios 1:1, 1:2, 1:3 and 1:4 respectively.

Table No.1 Rifampicin microspheres formulation

Formulation code	Drug : Chitosan Ratio	Drug : Eudragit L-100 Ratio	Drug : Eudragit RLPO Ratio
RCHIT-1	1:1		
RCHIT-2	1:2		
RCHIT-3	1:3		
RCHIT-4	1:4		
REL100-1		1:1	
REL100-2		1:2	
REL100-3		1:3	
REL100-4		1:4	
RERLPO-1			1:1
RERLPO-2			1:2
RERLPO-3			1:3
RERLPO-4			1:4

λ_{max} of Rifampicin

Preparation of Phosphate buffer pH 6.8:

Dissolve 28.80gm sodium dihydrogen ortho-phosphate in sufficient amount of distilled water and 14.80gm Potassium dihydrogen phosphate in sufficient amount of distilled water and finally volume make up to 1000 ml with distilled water in beaker and properly shaking.

Make a dilution for λ_{max}:

Procedure: Accurately weighed 100 mg of drug was dissolved in 100 ml of Phosphate Buffer pH 6.8 in volumetric flask and prepare suitable dilution to make it to a concentration of 1000 µg/ml make adequate of sample with concentration range of 1-30 µg/ml. The spectrum of this solution was run in 225-400nm range in U.V spectrophotometer. The spectrum peak point graph of absorbance of rifampicin versus wave length was shown in Figure No.1.

Standard Curve of Rifampicin

Preparation of dilution for standard curve

100 mg of Rifampicin was accurately weighed and transferred to a 100 ml volumetric flask containing 100 ml of phosphate buffer pH 6.8 and shaken to dissolve. The solution resulted is ≈1000 µg/ml. Then 1 ml of this solution is transferred to another 10 ml volumetric flask to obtain solution of 100 µg/ml served as stock. From this stock solution, we further make suitable serial dilutions in conc.1, 5, 10, 15, 20, 25 and 30 µg/ml. in 10 ml volumetric flask by using phosphate buffer pH 6.8

The absorbance was taken on double beam U.V. spectrophotometer using λ_{max} at 236 nm. The absorbance values were plotted against concentration (µg/ml) to obtain the standard curve of rifampicin.

Scanning electron microscopy (surface morphology)

Scanning electron microscopy has been used to determine surface topography, texture, and to examine the morphology of fractured. SEM is probably the most commonly used method for characterizing of microspheres, SEM studies were carried out by using scanning electron microscope. Dry Rifampicin microspheres were placed on an electron microscope brass stub and coated with in an ion sputter. A since vacuum field is necessary for images generation SEM. Photomicrograph of Rifampicin microspheres were taken by random scanning of the stub. The SEM photomicrograph of Rifampicin microspheres are shown in Figure No.3-6

Determination of average particle size

Optical microscopy method

The size of the prepared microspheres was measured by the optical microscopy method using a calibrated stage micrometer (Labomed, Ambala, India). Randomly chosen microspheres were taken to measure their individual shape and size. Microspheres were visualized under 10× magnification. Optical microscope was used to determine the size of the particle that lies within a range from equal divisions and hence each division is equal to 10µm. The particles are measured along an arbitrarily chosen fixed line across the center of the particle. The average size is a factor to be considered important in formulation of microspheres. Determination of physical appearance and morphology of Rifampicin microspheres were observed by optical microscopy. The average sizes and image of rifampicin microsphere were shown in Table No.3 and Figure No. 7-15, respectively.

Determination of Percentage Yield of Rifampicin microspheres[8]

Percentage yield also known as process yield or recovery studies. The % yield was calculated as the ratio of the mass of microspheres obtained at the end of the process and the mass of initial substances added including drug and respective polymer. The % yield of the Rifampicin microsphere was calculated using the following equation.

$$\%\ Percentage\ Yeild = \frac{Weight\ of\ Recovered\ microshpere}{Total\ expected\ Weight\ of\ drug\ and\ polymer} \times 100$$

Drug Entrapment Efficiency [9]

Efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment as per the following formula:

$$\%\ Entrapment\ Efficiency = \frac{Estimated\,(practical)\ drug\ content}{Theoretical\ drug\ content)} \times 100$$

Theoretical drug content was determined by calculation assuming that the entire Rifampicin present in the polymer solution used gets entrapped in Rifampicin microspheres, and no loss occurs at any stage of preparation of microspheres. The rifampicin content in the microsphere was determined by a digestion method. The rifampicin microspheres (100 mg) were pulverized and incubated in 100 ml phosphate buffer (pH 6.8) at room temperature for 24 h. This solution was filtered and further diluted to make suitable dilution solution and assayed spectro-photometrically for rifampicin content at the wavelength of 236 nm. The absorbance of the solutions was measured using double beam UV-spectrophotometer against

phosphate buffer as blank and calculated for the percentage of drug present in the microspheres.

In-vitro Release Studies [10]

Dissolution studies were carried out by using USP type II (Electro Lab.) dissolution paddle assembly. A weighed amount of rifampicin microspheres equivalent to 100 mg drug were dispersed in 900 ml of Phosphate Buffer (pH 6.8) maintained at 37 ± 0.5°C and stirred at 100 rpm. 5 ml sample was withdrawn at predetermined intervals and filtered and equal volume of dissolution medium was replaced in the vessel after each withdrawal to maintain sink condition. The collected samples were suitably diluted with phosphate buffer pH 6.8. The absorbance of the samples were measured by using double beam UV-spectrophotometer at 236 wavelength. Total % drug release are shown in Figure No.18

RESULT AND DISCUSSION

λ_{max} of drug-

The rifampicin shows the absorbance maxima at 236.40 nm in Phosphate Buffer pH 6.8. Highest absorbance peak represented λ_{max} of drug.

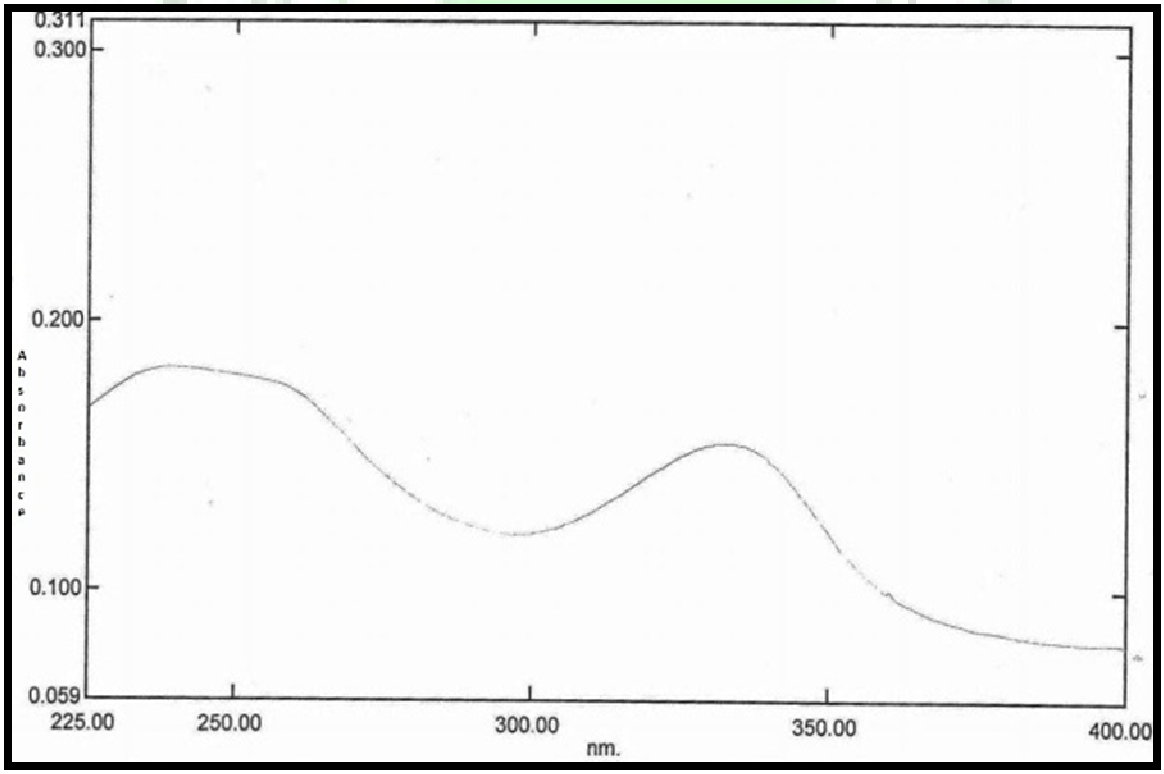


Figure No.1: λ_{max} graph of Rifampicin

Standard Curve of Rifampicin

Standard curve graph was found to be linear in the range of 1-30 µg/ml conc. at 236 nm wavelength by the double beam U.V spectrophotometer. The regression value was found to be 0.998 in phosphate buffer pH 6.8 are shown in Table No.2 and Figure No.2

Table No. 2. Standard Curve of Rifampicin

S.No	Concentration(µgm/ml)	Absorbance (nm)
1.	1	0.164
2.	5	0.198
3.	10	0.233
4.	15	0.269
5.	20	0.305
6.	25	0.338
7.	30	0.367

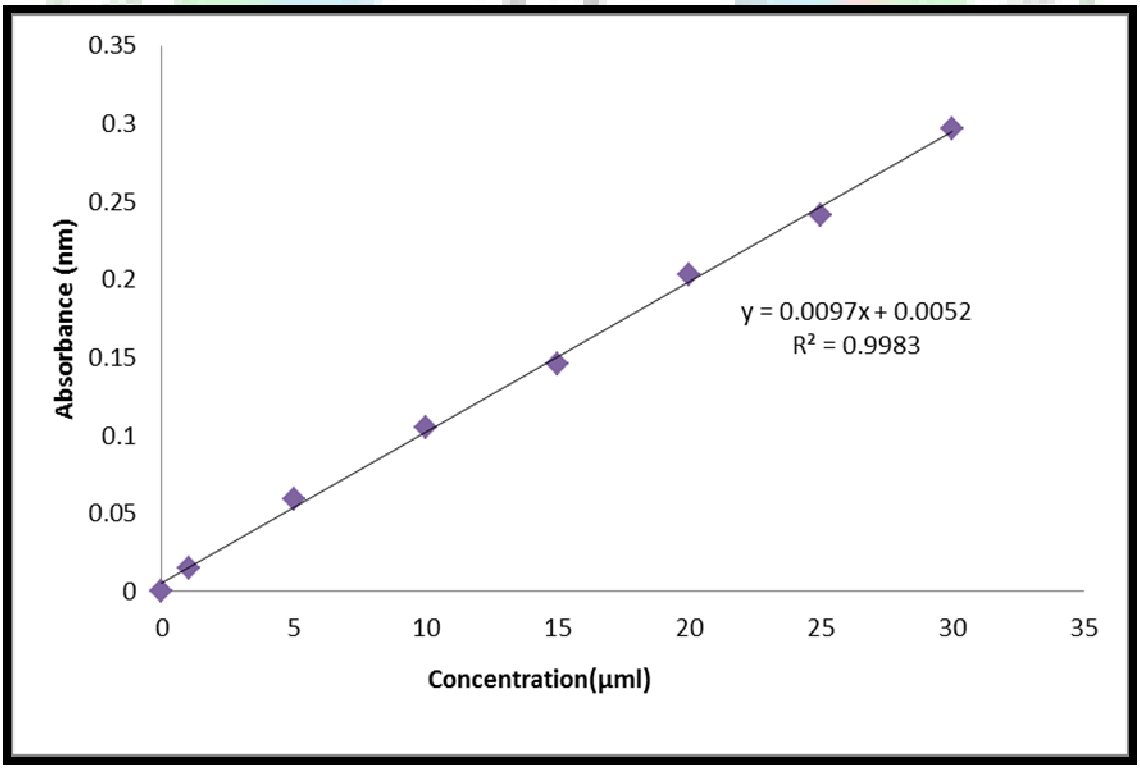


Figure No.2: Standard Curve of Rifampicin in phosphate buffer pH 6.8

Scanning electron microscope

The prepared Rifampicin microspheres were found to be slightly rough surface but spherical and good appearance. SEM photomicrograph of rifampicin microspheres are shown in Figure No.3-6.

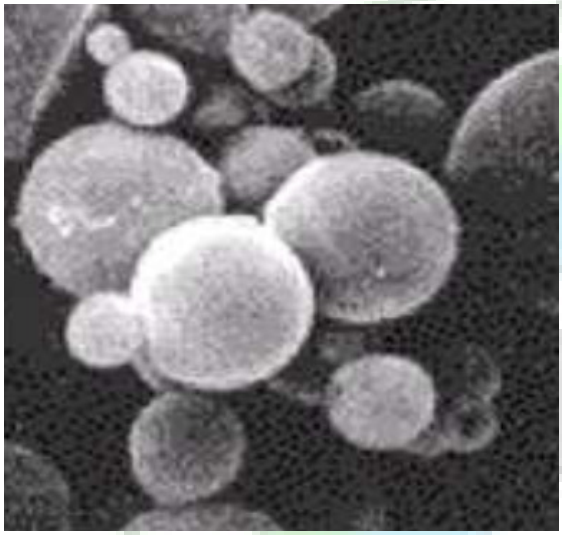


Fig.No.3 SEM of RCHIT-2

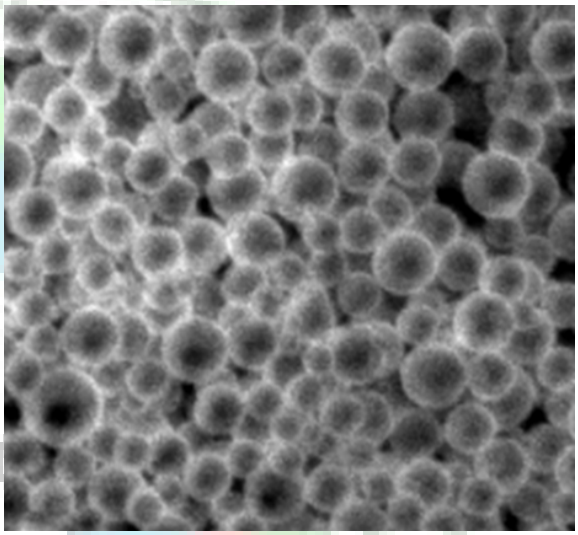


Fig. No.4: SEM of REL100-1

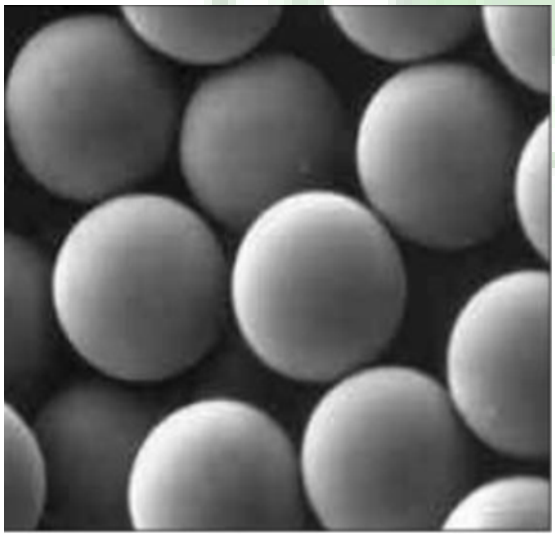


Fig.No.5: SEM of REL100-3

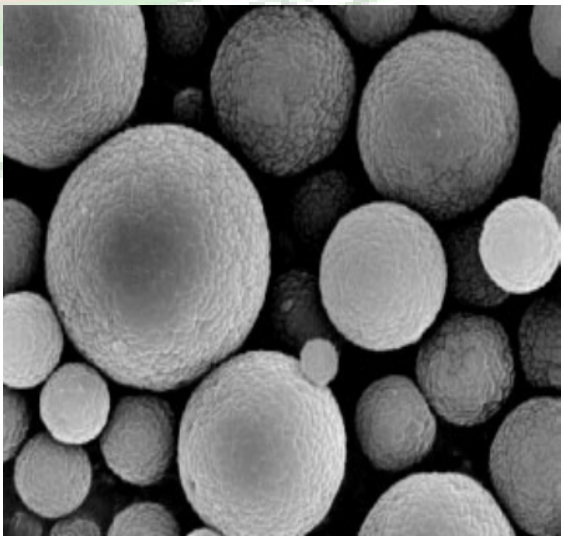


Fig. No.6: SEM of RERLPO-1

Average particle size

The average sizes of the prepared microspheres were determined by the optical microscopy method. The particle sizes of the microspheres were found to be in the range of 18µm-47µm. The average particle sizes and images of rifampicin microspheres are shown in Table No. 3 and Figure No.7-15, respectively.

Table No.3 Average size range of Rifampicin formulation

S.No.	Formulation code	Average size (µm)
1	RCHIT-1	47±3
2.	RCHIT-2	40±2
3.	RCHIT-3	29±4
4.	RCHIT-4	46± 2
5.	REL100-1	24±2
6.	REL100-2	30±3
7.	REL100-3	39±.2
8.	REL100-4	41± 2
9..	RERLPO-1	23±4
10.	RERLPO-2	18±3
11.	RERLPO-3	27±3
12.	RERLPO-4	36±2

Optical microscopic images of microspheres

Fig.No.7: RCHIT-1

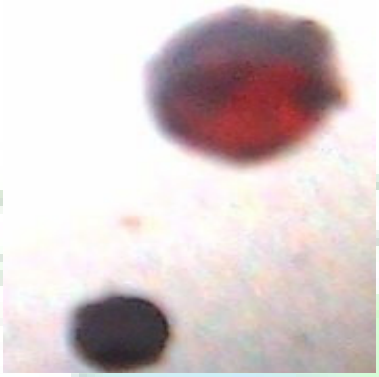


Fig.No.8: RCHIT-2

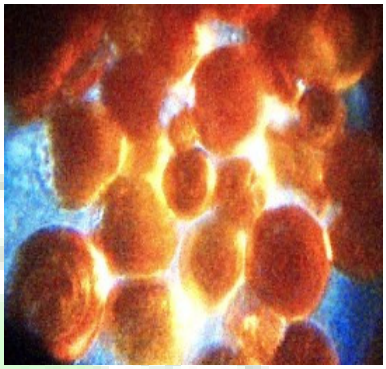


Fig.No.9 RCHIT-3

Fig.No.10: REL100-2

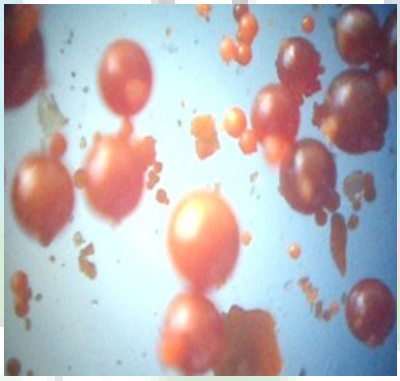
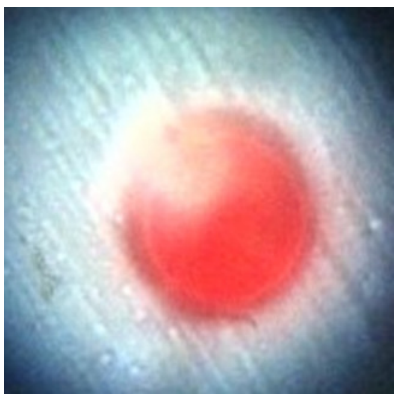


Fig.No.11: REL100-3

Fig.No.12: REL100-4



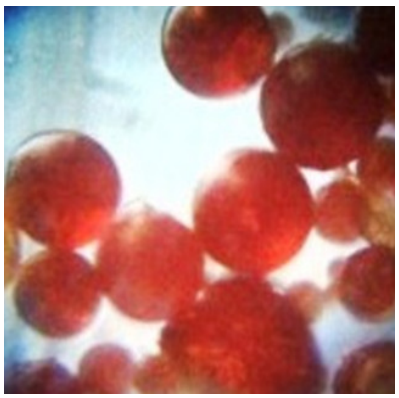


Fig.No.13: RERLPO-1 **Fig.No.14: RERLPO-2** **Fig.No.15: RERLPO-4**

Percentage yield

Percentage yield of Rifampicin formulations were found to be in the range of 73.92%-91.3%. The results indicated that rifampicin microspheres with eudragit polymer microspheres % yields better than chitosan polymer. Percentage yield are shown in Figure No.16

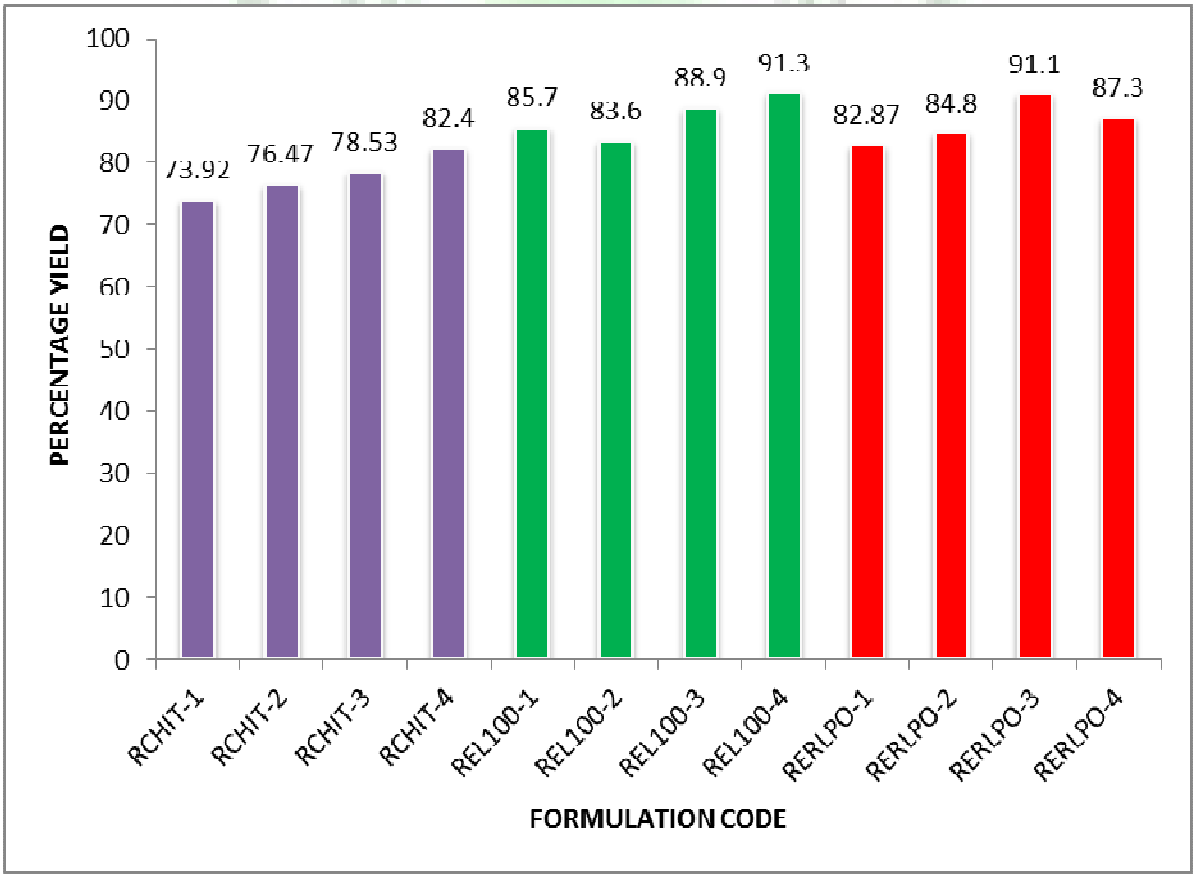


Figure No. 16: Graph of % yield of Rifampicin Microspheres

Drug Entrapment Efficiency

The Drug Entrapment Efficiency of rifampicin microspheres was found to be in the range of 74.8 %-95.5 % in RERLPO-1 and RCHIT-3, respectively. As the polymer concentration were increased from 1:1, 1:2, 1:3 and 1:4, the entrapment efficiency increased respectively. The results indicated the polymer concentration plays a major role in drug entrapment efficiency.

The % drug entrapment efficiency are showed in Figure No.17

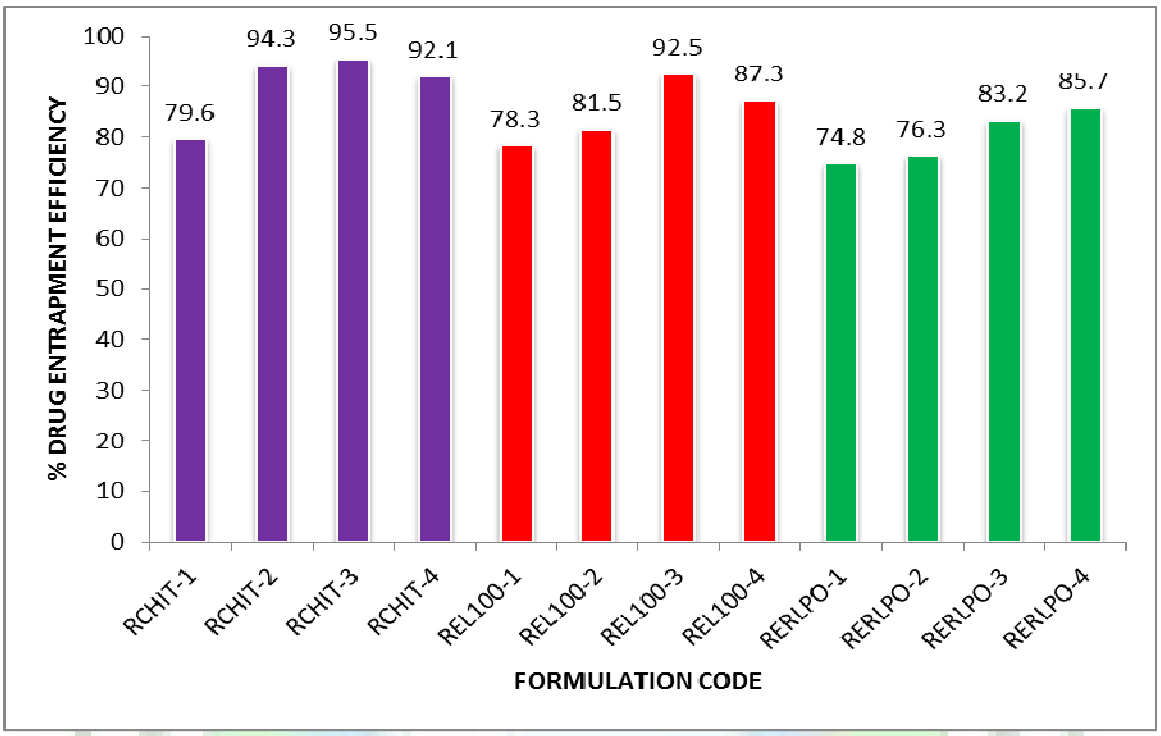


Figure No. 17: Graph of % Drug Entrapment of Rifampicin Microspheres

In- vitro drug release studies

In-vitro drug release study of rifampicin microspheres were done in USP dissolution apparatus using phosphate buffer pH 6.8 and maintain sink condition. The % cumulative drug release after 8 hours were found to be range of 71.52 % - 97.1 % in RCHIT-1 and RERLPO-3, respectively. Total % cumulative drug release of rifampicin microspheres are shown in Figure No.18

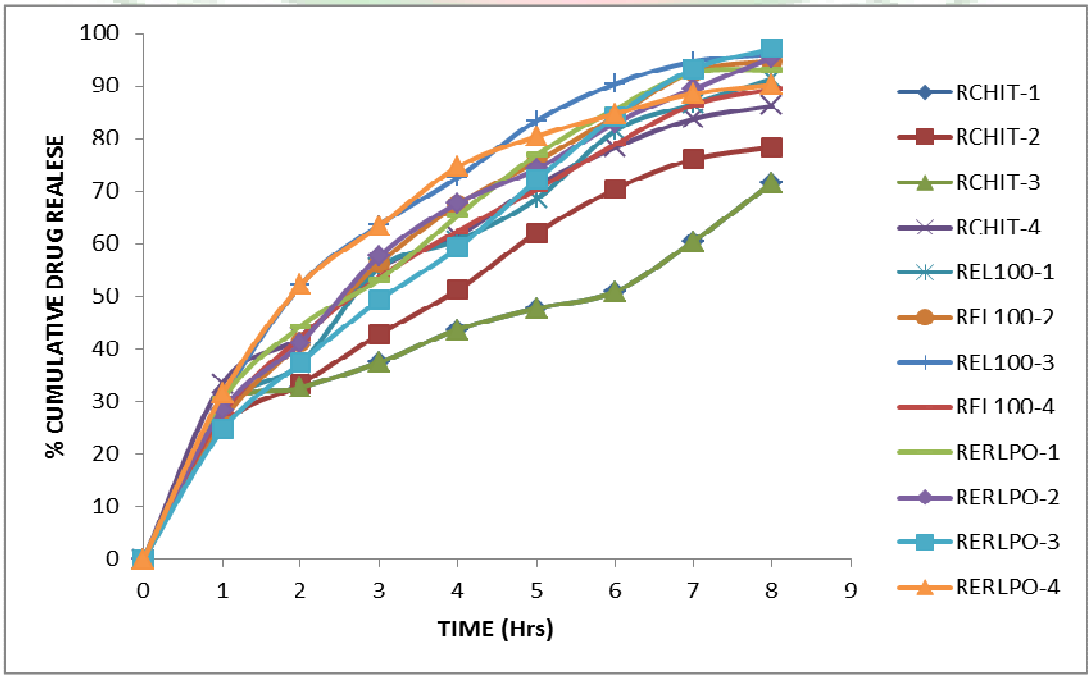


Figure No.18: Graph of In vitro Release of Rifampicin microspheres

CONCLUSION

From the present work, rifampicin microspheres were prepared using eudragit polymer by solvent evaporation method and using chitosan polymer by emulsion polymerization technique. The twelve batches of rifampicin microspheres were synthesized and evaluated for average particle size, shape, surface morphology, SEM, % yield, drug content, % entrapment efficiency and in-vitro drug release. The microspheres were found to be spherical, smooth surface. Average particle sizes of the microspheres were found to be in the range of 18µm -47µm by optical microscopy. Percentage yield were found to be in the range of 73.92%-91.3%. The Drug Entrapment Efficiency of rifampicin microspheres was found to be in the range of 74.8 % -95.5 % in RERLPO-1 and RCHIT-3, respectively. The in-vitro drug release studies were carried out for 8 hour in phosphate buffer pH 6.8. The maximum drug release (97.1 % in 8hrs) was shown by RERLPO-3. Drug content and in-vitro drug release studies on rifampicin microspheres were found to be good enough and feasible technique for the formulation. Thus it may be concluded that prepared microspheres could be utilized for the targeting of rifampicin to lung.

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