Research Article

Rifampicin As Microspheres By Non-Aqueous Solvent Evaporation Method

BV. Bhagat, Rohit P Mishra and Sameer Katiyar

Department of pharmaceutics, PDVVPF'S College of Pharmacy, Ahmednagar, Maharashtra, India-414111.

ABSTRACT

The aim of present study was to formulate and evaluate the Rifampicin loaded microspheres by non aqueous solvent evaporation technique. Ethyl cellulose and Carboxy methyl cellulose(CMC) are biocompatible polymers used as the retardant materials. The microspheres were characterized for Particle size measurement, percentage yield and encapsulation efficiency, percentage drug loading. The prepared microspheres were Red, free flowing and spherical in shape. It was observed the increase in concentration of the polymer, increases the mean particle size of the microspheres. The maximum yield of the microspheres was found to be 87.27% and the encapsulation efficiency was found to 27 %. *In-vitro* release studies were carried out in phosphate buffer at pH 7.4 . Formulation F3 selected as optimized formulation based on percent of drug entrapment and percent of drug loading.

Keywords: Rifampicin , microspheres, solvent evaporation and encapsulation efficiency.

1. INTRODUCTION

Despite tremendous advancements in drug delivery, oral route remains the preferred route for the administration of therapeutic agents, low cost of therapy and ease of administration leads to higher levels of patient compliance. Conventional oral dosage forms such as tablets, capsules provide specific drug concentration in systemic circulation without offering any control over drug delivery and also cause great fluctuations in plasma drug levels.

Carrier technology offers an intelligent approach for drug delivery by coupling the drug to a carrier particle such as microspheres, microparticles, liposomes, etc, which modulates the release and absorption characteristics of the drug. Microspheres constitute an important part of this particulate drug delivery system by virtue of their small size and efficient carrier characteristics.

Microspheres may be prepared by using several methods that are solvent evaporation method, phase separation method and spray drying method. The solvent evaporation technique may be a method of choice for the preparation of microspheres of water insoluble drugs. In the solvent evaporation method, the water insoluble drug and polymer are dissolved in an organic phase, evaporated the organic phase and the microspheres so formed are filtered and dried. Since Rifampicin is a water insoluble drug, solvent evaporation technique is selected for the preparation of microspheres. The main objective of this work was to investigate the possibility of obtaining a formulation of Rifampicin microspheres by using ethyl cellulose in various drugs, polymer concentrations. The various physicochemical characteristics and the *in-vitro* release rates from these microspheres were thus examined.

Rifampicin (Rifampin) is a semisynthetic derivative of rifamycin B obtained from stretomyces mediterannei. Rifampicin is bactericidal to M. tuberculosis and many other gram positive and gram negative bacteria like S.aureus, N.meningitidis, H influenza, E.coli, Klebsiella. Against TB bacilli it is as efficacious as INH and better than all other drugs. The bactericidal action covers all suboulations of TB bacilli, but acts slowly or intermittently(spurters) dividing bacilli as well as on many atypical mycobacteria. Both extra and intracellular organisms are affected. It has good sterlizing and resistance preventing actions.

Rifampicin inhibits DNA dependent RNA synthesis. Probably the basis of selective toxicity is that mammalian RNA polymerase does not avidly bind Rifampicin .

Mycobacteria and other organism develop resistance to Rifampicin rather rapidly. Rifampicin resistance is always due to mutation in repoB gene(the target of Rifampicin action)reducing it's affinity for the drug. No cross resistance with any other antitubercular drus has been noted.

2. MATERIALS AND METHODS

2.1. MATERIALS

Rifampicin was received as a gift sample from MSN laboratories Hyderabad, India.
Ethyl cellulose obtained from Himedia laboratories Ltd,CMC were obtained from and dichloromethane was obtained from poly vinylalcohol (PVA) were obtained from S.D. Fine chemicals. All reagents used were of analytical-reagent grade.

2.2. Preparation of Rifampicin microspheres by non aqueous solvent evaporation

Microspheres containing (Rifampicin) drug were prepared by using drug and polymers in different concentrations and dissolved in 2ml of solvent system (1:2 v/v of Acetone and dichloromethane respectively) with continuous agitation at room temperature. Then this was slowly introduced in to the 100ml dispersion medium (water) containing 7% poly vinyl alcohol (formed by continuous agitation at constant temperature of 90^{o}C, PVA is added slowly. The system was stirred at approx 800 rpm using a propeller type agitator at room temperature over a period of 8 hrs and the solvent was allowed to evaporate completely. After that microspheres were separated by filtration. Air dried for 24hrs and stored in desicator.

Table 1: Formulation of Rifampicin microspheres

Material	F1	F2	F3
Rifampicin (mg)	10	10	10
Ethyl cellulose (mg)	30	50	70
CMC(mg)	70	50	30
Polyvinyl alcohol(%v/v)	7	7	7
Dichloromethane:Acetone(ml)	3	3	3
Total	120	120	120

3.Evaluation parameters

3.1. Micromeritic studies

a). Tapped density

It is the volume of powder determined by tapping by using a measuring cylinder containing weighed amount of sample. The cylinder containing known amount of microspheres was tapped for about 1 minute on a tapped density apparatus until it gives constant volume.

$$\text{Tapped density} = \frac{\text{Mass of microspheres}}{\text{Volume of microspheres after tapping}}$$

b). Bulk density

It is defined as mass of powder divided by bulk volume.

$$\text{Bulk density} = \frac{\text{Weight of Microspheres in grams}}{\text{Bulk Volume of Microspheres}}$$

c). Carr's compressibility index

This is an important property in maintaining uniform weight Lower the compressibility values indicate better flow.

$$\text{Carr's Compressibility Index} = \frac{(TD - BD) \times 100}{TD}$$

Where, TD = Tapped density, & BD = Bulk density.

Table 2: Relationship between Carr's compressibility index and flowabiity

Carr's Compressibility Index	Flowability
5-15	Excellent
12-16	Good
18-21	Fair to passable
23-35	Poor
33-38	Very poor
>40	Extremely poor

d). Hausner's ratio

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

Values less than 1.25 indicate good flow (=20% Carr's index), where as greater than 1.25 indicates poor flow (= 33% Carr's index)

e). Angle of repose

Good flow properties are critical for the development of any pharmaceutical tablet, capsule or powder formulation. Angle of repose is defined as the maximum angle possible between the surface and the horizontal plane (Table 3). It was determined by glass funnel method. Powders were weighed accurately and passed freely through the funnel so as to form heap. The height of the funnel was so adjusted that the tip of the funnel touched the apex of the heap. The diameter of the powder cone was measured and the angle of repose was calculated using following equation:

$$\tan\Theta = h / r$$

where, = Angle of repose.
h = Height of the pile.
r = Radius of the powder cone respectively.

Table 3: Relationship between angle of repose and Flowability

Angle of repose (θ)	Flowablity
<20	Excellent
20-30	Good
30-34	Passable
>40	Poor

f). Particle size determination

Microsphere size was determined by using optical microscopic method with the help of ocular and stage micrometer. The sizes of around 100 particles were measured and their average particle size was determined.

g). Percentage yield

The prepared microspheres were collected and accurately weighed. The measured weight of prepared microspheres was divided by the total amount of all excipients and drug used in preparation of the microspheres, which gives the total percentage yield of microspheres.

h). Estimation of drug loading/ encapsulation efficiency

Microspheres weighing 200mg were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres and extracting the drug using pH 6.8 phosphate buffer (10ml). The extract was transferred to 100ml volumetric flask and volume was made up using pH 6.8 phosphate buffer. The solution was filtered and from the filtrate 10ml was taken and further diluted to 100ml and the absorbance was measured at 275nm against pH 6.8 phosphate buffer as blank. Drug loading and encapsulation efficiency was determined for all batches using the following formulas.

$$\%\ \text{Drug loading} = \frac{\text{Total weight of drug loaded in microspheres} \times 100}{\text{Total weight of microspheres}}$$

$$\% \text{ Drug entrapment efficiency} = \frac{\text{Actual drug content} \times 100}{\text{Theoretical drug content}}$$

5. RESULTS AND DISCUSSION

5.1 Characterization of microspheres

The prepared Rifampicin microspheres formulation (F1-F3) were evaluated for variable parameters such as bulk density, tapped density, Carr's compressibility index, Hausner's ratio and angle of repose and the evaluation parameters of six formulations (F1-F3) were in Table. 4. The Carr's compressibility index for formulations F1-F3 was found to be in the range of 13.0 ± 2.15 - 16.46 ± 1.22 which indicates good flow characteristics. The value of Hausner's ratio for the formulationsF1 to F3 was below 1.15 to 1.19 which indicates good flow property. The values of angle of repose for formulations F1 to F3, was found to be below 20º which indicates excellent flow of all the formulations.

Table 4: Micromeritc parameters of microspheres

Formulation	Bulk density (gm/cm3)	Tapped density (gm/cm3)	Carr's index (%)	Hausner's ratio (%)	Angle of repose (θ)
F1	0.1575	0.1818	13.36	1.15	14.07
F2	0.1812	0.2272	20.24	1.25	23.20
F3	0.2278	0.2727	16.46	1.19	20.80

5.2 Particle size determination

The mean particle size of the microspheres containing RIFAMPICIN was found to be in the range of 40.14 ± 1.17 to 53.88± 2.1 µ. The particle sizes of all formulations were shown in Table.5particle size comparisons of different formulations were shown in

Table 5: Particle size comparison of different batches

S. No	Formulation code	Particle size (µ)
1	F1	40.1435
2	F2	53.8820
3	F3	46.7941

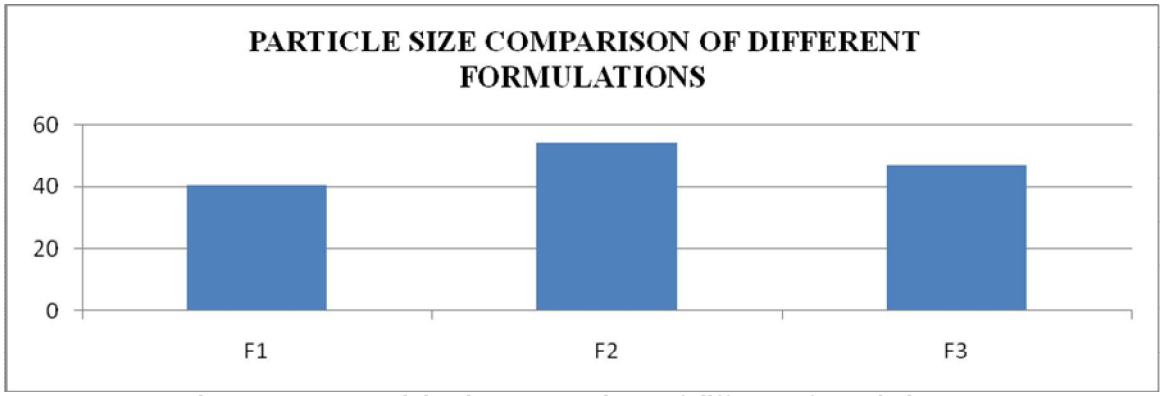

Fig. 1: Mean particle size comparison of different formulations

5.3 Percentage yield

The percentage yields of different formulations F1 to F3 were calculated and the yield was found to be in the range of 87.27 ±3.06 to 90.90±1.02%. The loss of material during preparation of microspheres may be due to process parameters as well as during filtration of microspheres. Percentage yield of all the batches is shown in Table 6. Percentage yield comparisons of different formulation were shown in Fig.2.

Table 6: Percentage yield comparison of microspheres

Formulation code	Practical yield	Percentage Yield
F1	100	90.90
F2	85	77.27
F3	96	87.27

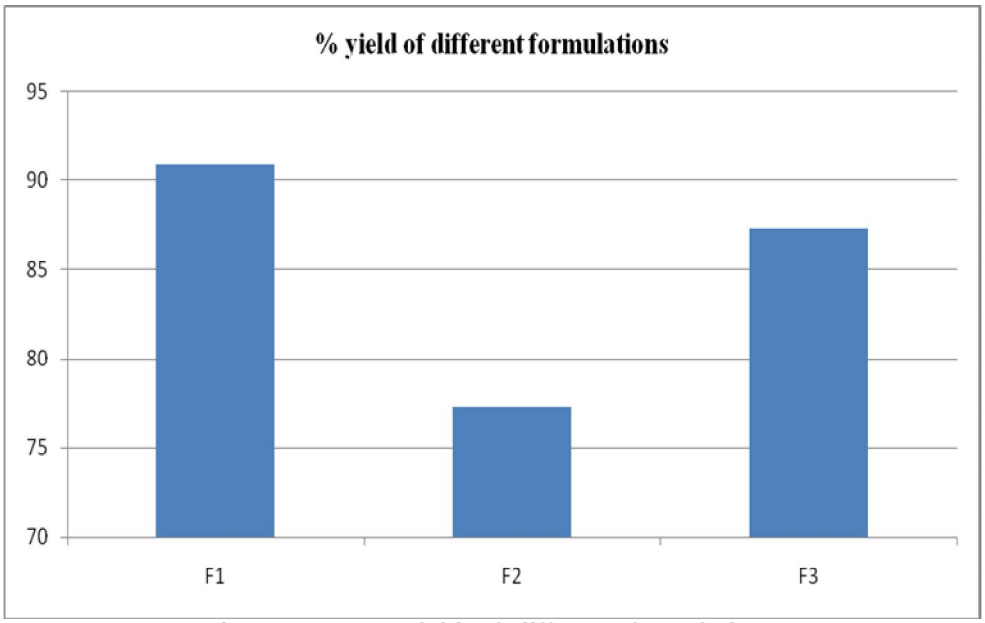

Fig. 2: Percent yield of different formulations

5.3 Estimation of drug loading and encapsulation efficiency:

The drug loading was found to be in the range of 80 ± 1.10% to 93.5 ± 2.31% for formulations F1 to F3. The percentage encapsulation efficiency of Rifampicin microspheres in all the formulations was found to be in the range of 42.23 ± 2.23 to 84.34 ± 2.18 %. The microspheres of batch F3 showed maximum drug encapsulation of 84.34 ± 2.18 %. The F1 batch microspheres showed lowest drug encapsulation of 42.23 ± 2.23 %. From the results it was seen that as the polymer concentration increased, viscosity of the dispersed phase increased, encapsulation efficiency increased. The percentage encapsulation efficiency, percentage drug loading were shown in Table 7. Fig.4. shows the comparison of % drug loading of different formulations, Fig.3. Show the comparison of % encapsulation efficiency of different formulations.

Table 7: % Drug loading and % Drug encapsulation of different

Formulation	%Drug loading	%Encapsulation efficiency
F1	80.0	42.23
F2	85.0	51.30
F3	93.5	84.34

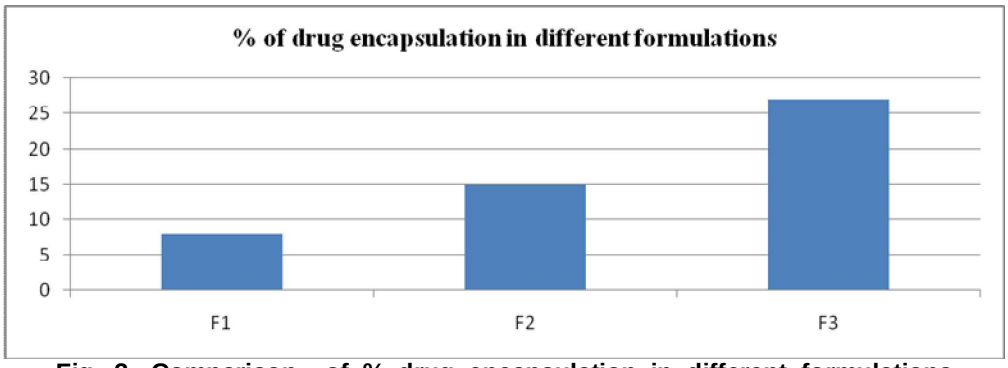

Fig. 3: Comparison of % drug encapsulation in different formulations

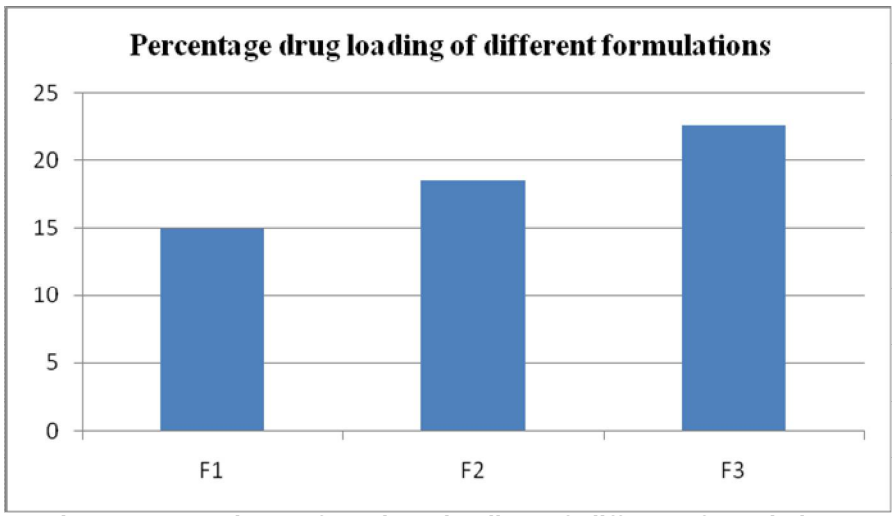

Fig. 4: Comparison of % drug loading of different formulations

5.5. In-Vitro drug release

The *in vitro* drug release characteristic were studied in pH 6.8 phosphate buffer for a period of 12 hrs using USP XXXIII dissolution apparatus, type-II. The microsphere containing Rifampicin (F1-F3) were prepared. The results of the dissolution studies indicated that the formulations F1, F2, and F3 released 80.567%, 68.237%, 59.368%, of Rifampicin at the end of 12hrs (Fig.5). The change in polymer concentration may also affect the *in vitro* drug release mechanism of drug from the microspheres. By increasing the polymer concentrations the rate of drug release was increased. Further, by increasing the concentration of polymer in the microsphere formulation, a point will be reached where the pores or channels formed by the drug particles within the polymer matrix were diminished. In other words, decreased polymer concentration affects the drug leaching and diffusion process from the matrix, by making it less porous and slower drug release rate occurs.

Table 8: Dissolution studies for optimized batch F3

Sr.No.	Time	Avg. %R	Amt. (mg)
1	0	0.000	0.00
2	2	14.126	1.41
3	4	29.277	2.93
4	6	51.844	5.18
5	8	67.058	6.71
6	10	80.561	8.06

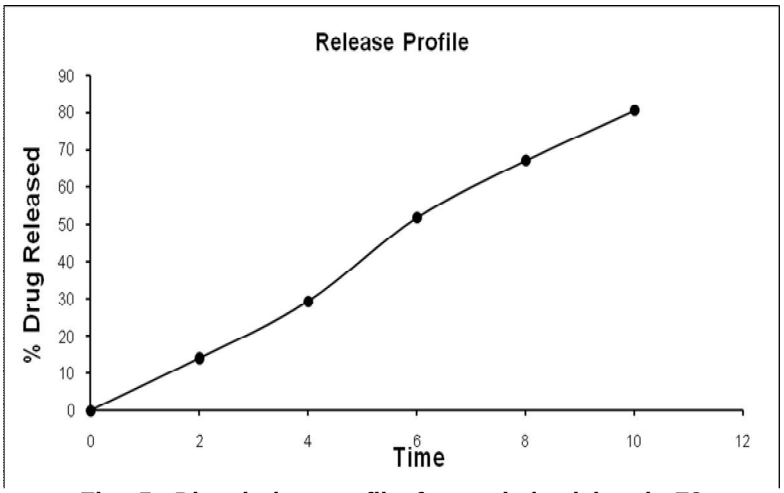

Fig. 5: Dissolution profile for optimized batch F3

CONCLUSION

Rifampicin (Rifampin) is a semisynthetic derivative of Rifamycin B obtained from the streptomyces mediterannei. Rifampicin is bactericidal to M. tuberculosis and many other gram positive and gram negative bacteria like S. aureus, N.meningitis, H-influenza, E-coli, etc. Because of its poor aqueous solubility and short elimination half life, it usually requires multiple dosing to achieve and maintain the therapeutic levels. The present study has been a satisfactory attempt to formulate Rifampicin microspheres, an orally administered anti-tubercular drug with a view of improving its oral bioavailability and giving a prolonged drug release of drug. Sustained release microspheres with biocompatible polymer such as cellulose polymer, CMC, ethyl cellulose were successfully prepared by non-aqueous solvent evaporation method . Microspheres of different sizes and improved drug entrapment efficiency could be obtained by varying the drug to polymer concentrations. The formulation shoe good flow properties, suggesting that, in future they could be easily and successfully packed into a capsule dosage form. Thus the prepared microspheres proved to be a potential candidate as a microparticulate drug deliveryin this area of patent novel.

ACKNOWLEDGEMENT

The author wish to thanks P.D.V.V.P.F;s COP, Ahmednagar, Maharashtra state, India for providing Rifampicin as a gift sample and also to the principal, Dr. P.Y. Pawar for providing necessary facilities to carry out the research work and Prof. B.V. Bhagat and Mr. Sameer Katiyar for guiding during the work.

REFERENCES

1. Lachman leon, Liberman HA and Laig JL. The Theory and practice of industrial pharmacy" 3rd edition, Varghese Publishing House Bombay. 171-195.
2. Alfred Martine. Physical Pharmacy, 4th edition, BI Waverlypvt Ltd,New Delhi, 1996;423-431.
3. Thompsona CJ, Hansford D, Higgins S, Roston C, Hutcheon GA and Mundaya DL. Evaluation of Ibuprofen loaded Microspherespreparedfrom novel copolysters. Int J Pharma. 2007;329:53-61.
4. Ethylcellulose (10-2013) A Technical Review, A Portfolio of Versatile Solutions to help Address a Variety of Formulation abd Processing Needs, Ethylcellulose-Technical Bulletin.
5. Harshad P, Sunil B and Nayan G. Different Method of Formulation and Evaluation of Mucoadhesive Microspheres. 2010;1(3).
6. Naik JB and Lokhande AB. Development of Sustained Release Micro Particles Using Different Solvent Emulsification Method Techniques: A Review. International Journal of Pharma and Bio Sciences. 2012; 3(4): 573-590.

7. Prasanth VV, Akash C and Sam TM. Microspheres - An Overview. International Journal of Research in Pharmaceutical and Biomedical Sciences. 2011; 2(2): ISSN 2229-3701.
8. Gaurav Tiwari. Biodegradable Microspheres For Controlled Delivery Of Metronidazole In The Treatment of Periodontal Diseases: Formulation Consideration. International Journal of Pharma and Boisciences. 2010;1(2).
9. Dinesh Babu G, Vishnu VR and Rajeev S. Preparation and Evaluation of Ethyl Cellulose and Eudragit based Microspheres of Diclofenac Potassium using Double Emulsion-Solvent Evaporation Method. International Journal of Current Pharmaceutical Research. 2011;3(4):ISSN 0975-7066.
10. Garud N, Akansha G and Neetesh J. Formulation design and in-vitro evaluation of metformin microspheres using ionotropic gelation technique. Journal of Pharmacy Research. 2011;4(7): ISSN 0974-6943.
11. Naveen C, Kiran Y and Rashmi V. Preparation and Evaluation of Ethyl Cellulose Microspheres Containing Diclofenac Sodium by Novel W/O/O Emulsion Method. Journal of Pharmaceutical Science and Research. 2010;2(12):884-888.
12. Yeamin H, Hyun-Jong C and In-Soo Y. Preparation and Evaluation of spray-dried hyaluronic acid microspheres for intranasal delivery of fexofenadine hydrochloride. European Journal of Pharmaceutical Sciences. 2010;40:9-15.
13. Perumal D, Dangor CM, Alcock RS, Hurbons N, Moopanar KR. J MIcroencapsul. 1996;16:475-487.
14. Shivarani E and Kumara Swamy. Formulation and Evaluation of Sustained Release Acelofenac Microspheres. Journal of Advanced Pharmaceutical Sciences. eISSN 2249-5979.