Volume 2, Issue3, July 2011
Available Online at www.ijppronline.com
International Journal Of Pharma Professional's Research
Research Article

MICROPARTICULATE ORAL DRUG DELIVERY OF RIFAMPICIN FOR TUBERCULOSIS TREATMENT USING 3^2 FULL FACTORIAL DESIGN

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ISSN NO:0976-6723

Abstract
The present work deals with formulation of the ethyl cellulose microparticles of rifampicin in order to improve rifampicin stability by avoiding its direct contact with acidic environmental of stomach. The microparticles were developed as sustained delivery carriers for rifampicin in order to improve patient compliance in tuberculosis treatment in terms of reducing the dosing frequency. The formulation of rifampicin microparticles was prepared by solvent evaporation method using ethyl cellulose as wall material. Rifampicin is a major component in fixed dose combination therapy for the treatment of tuberculosis. Rifampicin has variable bioavailability due to its poor poor solubility, acid decomposition and food interaction. The various formulation variables which effect the physical characteristic and microsphere stability were investigated. Microparticles were found to be small, free flowing, discrete and irregular shaped. The drug loaded microparticles were evaluated for in-vitro drug released profiles. These drug loaded micro particles exhibited sustained released for 24 hrs in physiological media (pH 7.4 Phosphate buffer solutions without enzymes). The in-vitro release profile of drug loaded microparticles was 90.6 percent after 24 hrs, where as pure drug sample exhibited 87 percent of release within 3 hrs.

Keywords: - Microparticles, Rifampicin, Ethyl cellulose,stability

Introduction
The concept of microencapsulating technology began as an alternative means of delivering drugs. It continued guest for more refined system, in 1980s polymer membrane technology came to be known at forefront. Further, the process of targeting and site specific delivery with absolute accuracy has been shown to be achieved by attaching bioactive molecule to liposome's, bio-erodible polymer, implants, monoclonal antibodies and various particulate carriers. The micro particulates delivery system are considered and accepted as a reliable means to deliver the drug to the target site with specificity and to maintain the desired concentration at the site of interest without untoward effects.[1] A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target tissue in the optimal amount in the right period of time there by causing little toxicity and minimal side effects.[2]

Tuberculosis, caused by Mycobacterium tuberculosis, is a major infectious burden worldwide and statistical estimates continue to worsen with each passing year.[3] Mycobacterium tuberculosis, the bacteria responsible for TB, invades and replicates in alveolar macrophages. In order to improve tuberculosis treatment using available drugs, Rif has been investigated with new drug delivery methods to increase targeted delivery to alveolar macrophages. The rationale for targeted Rif delivery is to achieve enhanced uptake of Rif into target cells to reduce drug dosage and possibly the duration of treatment. This approach also has the potential to reduce antibiotic resistance and Rif adverse effects, such as hepatotoxicity. New drug delivery methods that have been evaluated with Rif include; liposome's, Ethyl

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cellulose microparticles, nanoparticles, and dendrimers .

M. tuberculosis is carried in airborne particles, or droplet nuclei, that can be generated when persons who have pulmonary or laryngeal TB sneeze, cough, speak, or sing. The particles are an estimated 1–5 um in size, and normal air currents can keep them airborne for prolonged time periods and spread them throughout a room or building. Infection occurs when a susceptible person inhales droplet nuclei containing M. tuberculosis, and these droplet nuclei traverse the mouth or nasal passages, upper respiratory tract, and bronchi to reach the alveoli of the lungs. Once in the alveoli, the organisms are taken up by alveolar macrophages and spread throughout the body. Usually within 2–10 weeks after initial infection with M. tuberculosis, the immune response limits further multiplication and spread of the tubercle bacilli; however, some of the bacilli remain dormant and viable for many years. This condition is referred to as latent TB infection. Persons with latent TB infection usually have positive purified protein derivative (PPD)-tuberculin skin-test results, but they do not have symptoms of active TB, and they are not infectious.[4,5]

The four major drugs rifampicin (RIF), isoniazid (INH), pyrazinamide (Z) and ethambutol (E) are used widely for the treatment of TB [2]. Among these, INH, Z and E belong to the class I (highly soluble/highly permeable) of biopharmaceutical classification system whereas RIF is a class II drug (low soluble/highly permeable), which has high permeability and low solubility[3]. Due to the low solubility, RIF (solubility 2.5 mg/ml, log *p* 1.086, p*K*a 4.96±0.7) has poor bioavailability in both fixed and single dose formulations [4]. The reason for low bioavailability of RIF are changes in the crystalline nature, interaction with excipients, degradation in gastro-intestinal tract, variability in absorption and metabolism, its pH dependent solubility, etc. [5, 6]. Bioavailability problem of RIF is cause for the poor therapeutic outcomes including failure of therapy and drug resistance. Chemotherapy of TB is complicated by the need of multi-drug regimens given over long periods, patient noncompliance and the development of multi-drug resistant strains (MDR).[6,7,8,9] Rifampicin or rifampin (Rif) is an antibiotic that has been used in the treatment of tuberculosis for the past four decades. Tuberculosis (TB) is still a common infectious disease with high mortality and morbidity rates in developing countries, and in the developed compromised by substance addictions and immunosuppressive drugs. There is also a growing problem of tuberculosis exhibiting resistance to TB drugs, including Rifampicin.[10] Poor patient compliance is another single most common reason for failure of TB therapy.[11]

Development of carrier delivery systems that release drugs in a sustained manner at therapeutic concentration over a period of time can ensure patient compliance in terms of reducing dosing frequency, and may also minimize the risk of emergence of drug resistant mutants and potential toxicity[12]. Various other carrier systems such as liposomes and microspheres have been developed for the sustained delivery of anti tubercular drugs in mice with better chemotherapeutic efficacy.[13]

EXPRIMENTAL METHODS:

MATERIALS:

Rifampicin, ethyl cellulose was purchased from r.k. Enterprises meerut India, PVA was purchased from Deepak Enterprises Barelly, India. All other reagents and chemicals used in this study were of analytical grade.

METHODS:

FORMULATION OF MICROPARTICLES:

Ethyl cellulose microparticles were prepared by solvent evaporation method. Briefly, 250mg of ethyl cellulose and rifampicin was completely dissolved in 20ml ethanol and dichloromethane (1:1) by stiring. This solution was poured into water containing 1% PVA as hydrophilic surfactant under moderate stirring. Finally, the organic solvents were evaporated under reduced pressure at 58^0 C. the process variables involved in microparticle preparation is present in table no.1. All other formulations were prepared in the similar manner.

Table 1:Formulation of different batches.

Formulation code	Drug(mg)	Polymer(mg)
F_1	250	250
F_2	250	500
F_3	250	750
F_4	250	1000
F_5	250	1250

EVALUATION OF MICROPARTICLES:

1.Percentage yield (% yield):

The yield was calculated as the weight of the microparticles recovered from each batch
divided by total weight of drug and polymer used to prepare that batch multiplied by 100.

2. Entrapment Efficiency:

The drug loading capacity of ethyl cellulose microparticles was detected by soaking them in phosphate buffer solution after filtering through nylon disc filter. The concentration of drug was analyzed by UV-spectrophotometer at _ 475 nm. Drug entrapment efficiency capacity was calculated using the formula

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

3. Particle size analysis:

The microparticles size distribution was determined by the optical microscopy method using a calibrated stage micrometer (μm) was calculated by using equation,

$$Xg = 10 \times [(ni \times \log Xi) / N]$$

Xg is geometric mean diameter, ni is number of particle in range, xi is the midpoint of range and
N is the total number of particles. All the experimental units were analyzed in triplicate (n=3).

4.Percentage of moisture loss:

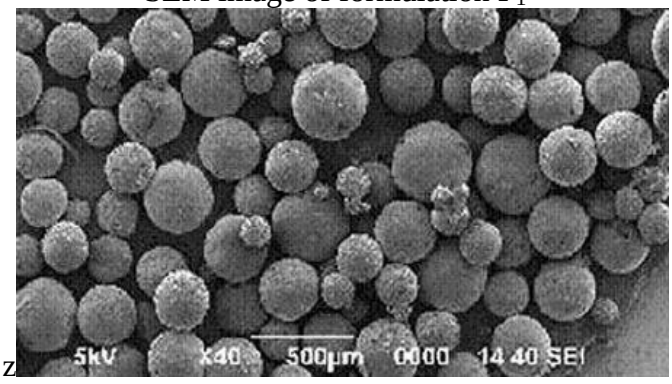
The Losartan loaded microparticles of different polymers were evaluated for percentage of moisture loss which sharing an idea about its hydrophilic nature. The microparticles weighed initially and kept in desiccator containing calcium chloride at 37 °C for 24 hours. The final weight was noted when no further change in weight of sample.

$$\textbf{\% of moisture loss} = \frac{Initial\ weight - final\ weight}{Initial\ weight} \times 100$$

5. Scanning electron microscopy (SEM) of microparticles.

SEM of microparticles was recorded using Scanning Electron Microscope with a 10 kV accelerating voltage.

SEM image of formulation F_1



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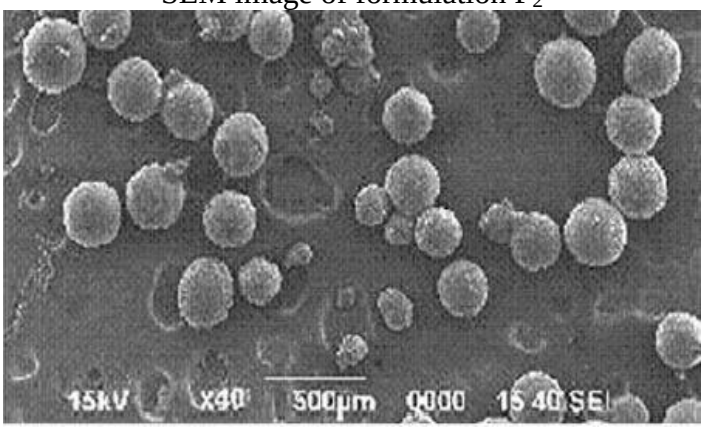
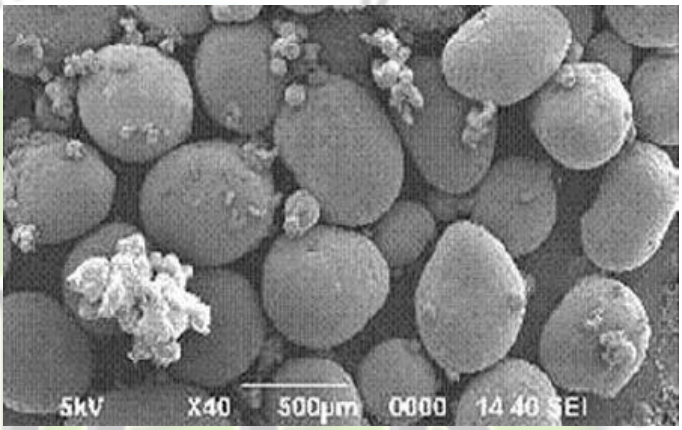
SEM image of formulation F_2



SEM image of formulation F_3



Dissolution studies.

In-vitro release study was carried out by USP Type 2 (rotary basket) method for 24 hrs in the physiological fluid (pH 7.4 phosphate buffer solution) samples were withdrawn at appropriate intervals and fresh sample were replaced. The withdrawn samples were diluted and absorbance was analyzed by an UV spectrophotometer (Shimadzu) at 475 nm.

Factorial Design

A 3^2 full factorial design was used in the present study. In this design 2 factors namely amount of PVA (X1) and the Ethylcellulose (X2) are evaluated, each at 3 levels, and experimental trials were performed at all 9 possible combinations. The amount of PVA Solution(X1) and the Ethylcellulose (X2) were chosen as independent variables.

Multiple regression analysis for 32 factorial design

The responses obtained from 3^2 factorial design analyses were subjected to multiple regression analysis. The polynomial equations determined using the form:

$$Y = b0 + b1X1 + b2X2 + b12X1X2 + b11X1^2 + b_{22}X_2^2$$

Where Y are the dependent variables, namely,% Yield (Y1), % entrapment efficiency (Y2) and Particle size (nm) (Y3). The main effects (X1 and X2) represent the average result of changing one factor at a time from its

low to high value. The interaction term (X1X2) shows how the response changes when 2 factors are simultaneously changed. The polynomial terms (X12 and X22) are included to investigate nonlinearity. The simplified models were then utilized to produce three dimensional response surface plots and contour plots to analyze the influence of independent variables.

RESULT AND DISCUSSION :

The microparticles were prepared by solvent evaporation method and characterized for % entrapment, particle size and % Yield of microspheres was high in ethyl cellulose in ratio of 1:3(drug : ethyl cellulose) in 3rd batch. The particle sizes of microparticle were found to increase by increasing the polymer concentration(as in table 2).

Table 2:Result of evaluation of parameters.

Formulation code	Yield(%)	Drug Entrapment Efficiency(%)	Particle Size (µm)	(%) Moisture loss
F_1	72.21	77.42	43.54	4.93
F_2	85.54	85.95	45.22	3.12
F_3	89.61	86.861	48.65	2.97
F_4	83.78	78.842	49.54	3.850
F_5	86.88	80.562	52.80	3.260

Graph 1:Drug release profile of rifampicin microparticles:

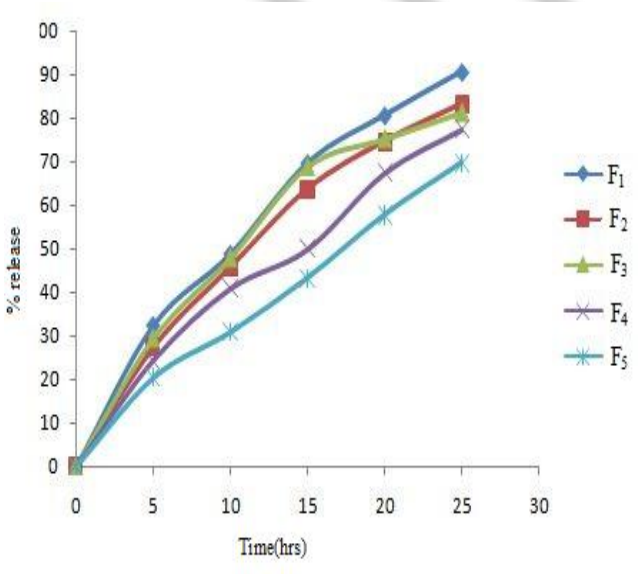


Table 3: Experimental design and Parameters for 3^2 Full Factorial Design Batches

Formulation Code	Variables in Coded Form		(%) Production Yield	Mean Particle Size	(%) Entrapment Efficiency
	X_1	X_2			
F_1	-1	-1	63.3	46.25	47.87
F_2	-1	0	95.14	50	41.75
F_3	-1	+1	96.84	43.75	46.83
F_4	0	-1	98.37	31.25	51.75
F_5	0	0	97.36	53.75	38.4
F_6	0	+1	66.6	53.75	41.7
F_7	+1	-1	60	60	61.8
F_8	+1	0	41.02	38.75	44.75
F_9	+1	+1	63	46.25	46.46

Table 4: Parameters of check point formulation

Variables	Levels		
	Lower (-1)	Middle (0)	Upper (+1)
X_1 (Conc. Of PVA)	1	2	3
X_2 (Amount of EC)	250	500	750

From the preliminary study, the best batch as a reference was selected.On the basis of this study a 3^2 full factorial design was employed to study the effect of independent variables (i.e. amt PVA and amt of ethyl cellulose [X2]) on dependant variables % production yield, particle size, drug entrapment efficiency of different batches were studied. From the factorial batches it was found that the production yield of BD 4 and BD 8 found to be 98.37% and 41.02% respectively (As shown in table 3). Factorial equations for four responses as per the coefficients obtained were as follows:

$Y\ 1 = 89.55 - 15.21\ X1 + 0.80\ X2 - 7.64\ X1X1 - 17.56\ X_1^2 - 3.15\ X_2^2$

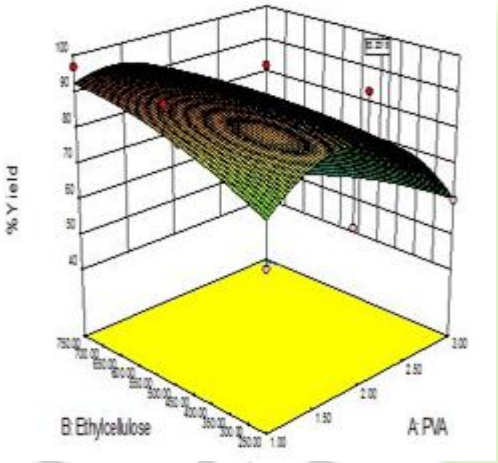
$Y\ 2 = 46.67 - 0.83\ X1 - 1.04\ X2 + 2.81\ X1X2 + 1.25\ X_1^2 - 0.62\ X_2^2$

$Y\ 3 = 40.45 + 2.76\ X1 - 4.52\ X2 - 3.58\ X1\ X2 + 3.43\ X_1^2 + 6.55X2^2$

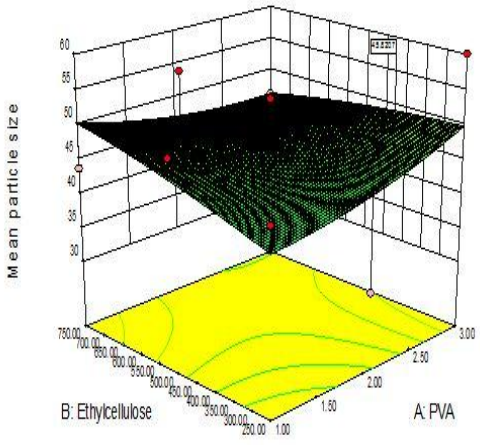
The RSM obtained for the relationship between independent variables PVA (X1), EC (X2) and the responses Y1, Y2, Y3 support and substantiate earlier discussions.

The various graph of factorial design between dependent and independent variables are:

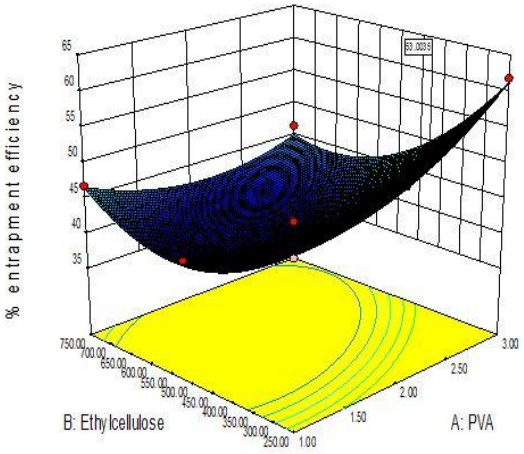
Graph 1: Graph between % Yield and independent variables



Graph 2: Graph between mean particle size and independent variables



Graph 3: Graph between % entrapment efficiency and independent variables



Conclusion:

From the above research we conclude that rifampicin microparticle was prepared by solvent evaporation method. In this f3 batch was found to be better formulation then other four batches.In –vitro release studies were performed for 24 hours.

References:

1).Vyas, S. P., Khar, R. K. 2002. (Eds.); In Targeted and Controlled Drug Delivery, I Edn. Section II: 417-457.

2).N.K.Jain, Controlled and Novel drug delivery, 04 Edition, 236-237, 21.

3).Nanotechnology based drug delievery system for the management of tuberculosis, review aticle, Indian journal of Experimental Biology, vol,44, May 2006, p.p. 357-366.

4).American Thoracic Society/CDC. Diagnostic standards and classification of tuberculosis. Am Rev Respir Dis 1990;142:725–35.

5).Wells WF. Aerodynamics of droplet nuclei. In: Airborne contagion and air hygiene. Cambridge: Harvard University Press, 1955:13–9.

6).Kasim NA, Whitehouse M, Ramachandran C, Bermejo M.Molecular properties of WHO and provisional biopharmaceutical classification. Mol Pharm. 2004; 1: 85–96. doi:10.1021/mp034006h

7).Panchagnula R, Agrawal S. Biopharmaceutic and pharmacokinetic aspects of variable bioavailability of rifampicin. Int J Pharm. 2004; 271: 1-4. doi:10.1016/j.ijpharm.2003.11.031

8).Agrawal S, Kaur KJ, Singh I, Bhade SR, Kaul CL, Panchagnula R. Assessment of bioequivalence of rifampicin, isoniazid and pyrazinamide in a four drug

fixed dose combination with separate formulations at the same dose levels.Int J Pharm. 2002; 233: 169–177.
doi:10.1016/S0378-5173(01)00939-5

9).McIlleron H, Wash P, Burg rma S, Khuller GK, Prasad B.Poly (DL-lactide-co-glycolide) nanoparticle-based inhalable sustained drug delivery system for experimental tuberculosis. J Antimicrob Chemother.2003;52:981–986.

10).Pandey R, Khuller GK. Solid lipid particle-based inhalable sustained drug delivery system against experimental tuberculosis. Tuberculosis. 2005; 85: 227–234.

12).Quenelle DC, Winchester GA, Staas JK, Barrow ELW, Barrow WW. Treatment of tuberculosis using a combination of sustained-release rifampin-loaded microspheres and oral dosing with isoniazid. Antimicrob Agents Chemother. 2001; 45: 1637–1644.

13).Dutt M, Khuller GK. Chemotherapy of Mycobacterium tuberculosis infections in mice with a combination of isoniazid and rifampicin entrapped in Poly (DL-lactide-co-glycolide) microparticles. J Antimicrob Chemother. 2001; 47: 829–835.

14).V. Kusum Devi U.V. Bhosale , Formulation and optimization of polymeric nano drug delivery system of acyclovir using 3^2 full factorial design, International Journal of PharmTech Research, Vol.1, No.3, pp 644-653 , July-Sept 2009

15).Sanford Boltan,Charles Bon, Pharmaceutical Statisiics,practical and clinical applications fifth edition, Drug and the pharmaceutical sciences volume 203.