An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

DESIGN AND CHARECTERIZATION OF NANOPARTICLES FOR AN ANTI-TUBERCULAR DRUG

Anisha Das[1], K.Mohan Kumar[2], Govinda Raju. N[3], Raghuveera H.G[4], Shishir Ojha[5]

Srihari Kalyanam[6], Anjaneyulu Vinukonda*[7]

[1, 2]Department of Pharmaceutics; Swamy Vivekananda College of Pharmacy Elayampalayam tiruchengode, Tamilnadu. India.

[3, 4, 5, 6, 7] Alembic Pharmaceuticals Ltd, Vadodara, India

*Corresponding author E-mail: **anji002@gmail.com**

ARTICLE INFO

Key Words

Rifampicin, Nano-Particles, Ascorbic acid, PGLA, Poly dispersity index

ABSTRACT

The drug Rifampicin is used as anti-tubercular and the bioavailability of is reduced owing to degradation of the drug in the gastric fluids[3]. The current investigation was designed to enhance the drug stability in stomach and to improve the therapeutic efficacy of the drug. This was carried out by preparing Rifampicin loaded PLGA nanoparticles using ascorbic acid as an antioxidant. Dug loaded nanoparticles were fabricated by a multistep emulsion procedure and evaluations of the prepared nanoparticles were then carried out by various methods. In this study four types of formulations were prepared. Formulation 1 (F1) is rifampicin alone loaded PLGA nanoparticles, formulation II (F2) is rifampicin – ascorbic acid (1:1) loaded PLGA nanoparticles, formulation III (F3) is rifampicin - ascorbic acid (1:2) loaded PLGA nanoparticles and formulation IV (F4) is rifampicin – ascorbic acid (1:3) loaded PLGA nanoparticles. As per the study outcome it was concluded that ascorbic acid can minimize the degradation of rifampicin in acidic pH condition and thus improves the stability and bioavailability of rifampicin. The results also demonstrate that there is a statistically significant change in the percentage drug degradation profile when the concentration of ascorbic acid was increased.

INTRODUCTION

Rifampicin is a first line anti-tubercular drug, administered orally in fixed dose combination with Isoniazid, Pyrazinamide and Ethambutol in order to overcome drug resistance to tuberculosis arising from administration of these drugs separately. However bioavailability of rifampicin is reduced owing to degradation of the drug in the stomach. Rifampicin degrades in acidic condition of the stomach and the degradation of rifampicin is pH dependent.[1]some of the reportsdescribes that the problem of poor absorption of rifampicin from combination products is perhaps due to Increased decomposition in stomach conditions and the decomposition of rifampicin is enhanced in the presence of INH.[2] Rifampicin is well absorbed in the pH range of 1-2 even though it undergoes degradation in the acidic medium. Rifampicin hydrolyses to 3 formyl rifampicin SV (3-FRSV)in acidic condition and it undergoes air oxidation in alkaline medium to form in active quinone derivative rifampicin quinine.[3]Hence, the development of alternative formulation is essential, that can prevent or minimize degradation of rifampicin in the stomach either as a single drug or in combination of

other anti-tubercular drug is therapeutically beneficial and can achieve effective control of tuberculosis with improved bioavailability of rifampicin.

MATERIALS AND METHODS

Materials

Rifampicin APIis the gift sample of MSN Labs, Hyderabad, and Telangana, India. Ascorbic acid and PLGA poly (lactic-*co*-glycolic acid) were procured as gift sample of Alphamed formulations Labs, Hyderabad, Telangana, India.

Preparation of Nanoparticles: Rifampicin and ascorbic acid loaded PLGA nanoparticles were fabricated by an Emulsification/solvent evaporation method, which involved the formation of stable emulsion and evaporation of organic solvent by continuous stirring. The study is carried out by preparing four types of formulations[8].

Procedure: Drug loaded PLGA nanoparticles were prepared by a multistep emulsion procedure.50 mg of rifampicin and required quantity of ascorbic acid were accurately weighed and added to 10 mL of dichloromethane containing the polymer [drug : polymer ratio was taken as (1:1)]. Distilled water was emulsified in the DCM containing drug and polymer to form w/o primary emulsion. It was then emulsified by sonication for 15 min. The primary emulsion was then poured into 8mL of 1%w/v aqueous Poly Vinyl Alcohol solution and stirred using a magnetic stirrer to form the second w/o/w multiple emulsion. The latter was then stirred continuously overnight for the complete removal DCM. The nanoparticles were then recovered by centrifugation (9000 -10,000 rpm for 15 minutes), washed thrice with distilled water andvaccum dried[6].

Evaluation of the Prepared Nanoparticles[6]:

It includes determination of particle size, size distribution, shape, surface morphology, Poly disparity Index and zeta potential.

Shape and surface morphology of nanoparticles[4]:

The morphology of Rifampicin –ascorbic acid loaded PLGA nanoparticles were analyzed using a scanning electron microscope. Samples were prepared from dilutions in distilled water of particle suspensions and dropped onto stubs using double sided sticking tape. After air drying, particle were coated with a thin layer of platinum film and then examined by scanning electron microscopy.

Particle size characterization of the Nanoparticles: The particle size, size distribution and poly dispersity index of the nanoparticles were measured by a laser particle size analyzer.

Zeta Potential Study: The surface charge of nanoparticles was determined by the electrophoretic mobility of nanoparticles in a U type tube at 25˚C, using a zetasizer.

***In vitro* Release Study[4]:**

A solution of 0.1N HCL was placed in the vessel of USP dissolution apparatus type 2 (US Pharmacopoeia XXIII, 1995) with rotating paddle at 100rpm and the temperature was maintained at 37±0.2^{o}c.RIF loaded PLGA nanoparticles with ascorbic acid of different ratios were accurately weighed, dissolved in and diluted to 100ml with 0.1N HCL. The resulting solution was transferred immediately to the dissolution bath. Specimens were withdrawn at 15 min, 30min and 60min. An aliquots of 1mL, 2 mL,3 mL,4 mL and 5 mL were extracted immediately with 100 mL of pH 1.2 using a cyclomixer. Samples were analyzed spectrophotometrically at 475nm and the percentage degradation[4] was calculated by using the given formula

$$\% \text{ Degradation loss} = \frac{\text{Initial concentration - Final concentration}}{\text{Initial Concentration}} \times 100$$

Preparation of standard stock solution:

100mg Rifampicin PLGA nanoparticles were dissolved in 100ml pH 1.2 solution. From this required quantities were taken for further dilution process.

Standard calibration curve for Rifampicin + Ascorbic acid (1:1) nanoparticles at pH 1.2: 100 mg of rifampicin - ascorbic acid nanoparticles (1:1) were accurately weighed and dissolved in 100ml of pH 1.2 buffers.

RESULTS AND DISCUSSION

Shape and Surface Characterization of the Prepared Nanoparticles: Scanning electron micrograph of the prepared nanoparticles are revealed that the nanoparticles were spherical with smooth surface and they were relatively mono dispersed.

Table 1: Composition of formulations

Formulation Code	Ingredients
F0	PURE RIFAMPICIN
F1	RIFAMPICIN+PLGA(1:1)
F2	RIF+PLGA+ASC(1:1:1)
F3	RIF+PLGA+ASC(1:1:2)
F4	RIF+PLGA+ASC(1:1:3)

Table 2: Particle Size Characterization of the Nanoparticles

S.No	Formulations	Mean Diameter(Nm)±SD	PdI
1	RIFAMPICIN+PLGA(1:1)	375± 20	0.312
2	RIF+PLGA+ASC(1:1)	378 ± 22	0.316
3	RIF+PLGA+ASC(1:2)	374 ± 18	0.308
4	RIF+PLGA+ASC(1:3)	380 ± 23	0.317

Table 3: Standard curves of rifampicin alone and in combination with ascorbic acid in different ratios at pH 1.2 buffer

Conc.(µg/mL)	**RIF**	**RIF + PLGA NPS**	**RIF+ ASC (1:1) NPS**	**RIF + ASC (1:2) NPS**	**RIF+ASC(1:3) NPS**
0	0	0	0	0	0
5	0.082	0.084	0.090	0.052	0.054
10	0.16	0.162	0.140	0.103	0.093
20	0.305	0.320	0.280	0.192	0.181
30	0.456	0.473	0.422	0.280	0.273
40	0.607	0.633	0.561	0.381	0.352
50	0.759	0.801	0.701	0.475	0.446

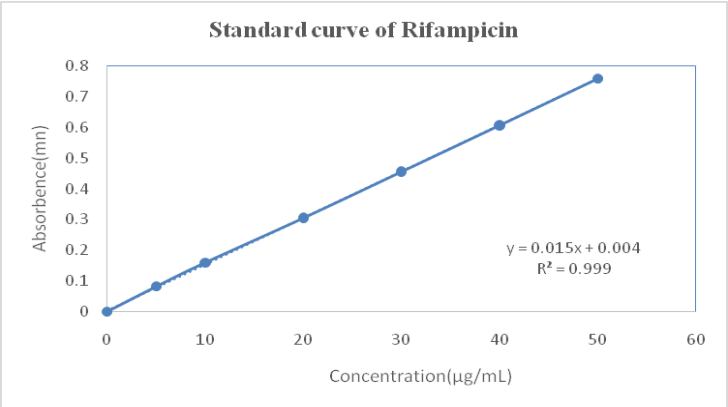

Fig. 4: Standard curve of Rifampicin

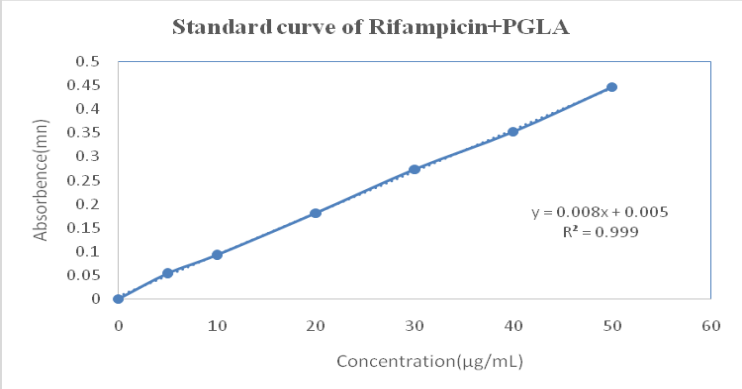

Fig. 4: Standard curve of Rifampicin+ PLGA

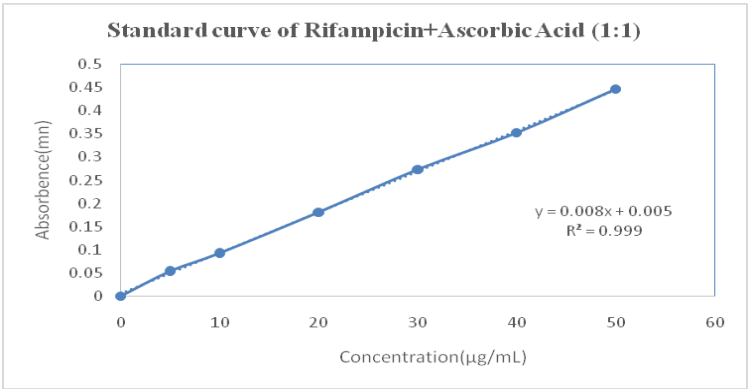

Fig. 5: Standard curve of Rifampicin+ Ascorbic acid (1:1)

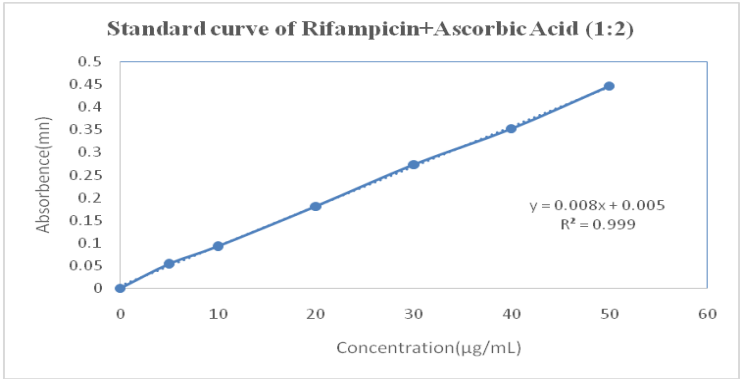

Fig. 6: Standard curve of Rifampicin+ Ascorbic acid (1:2)

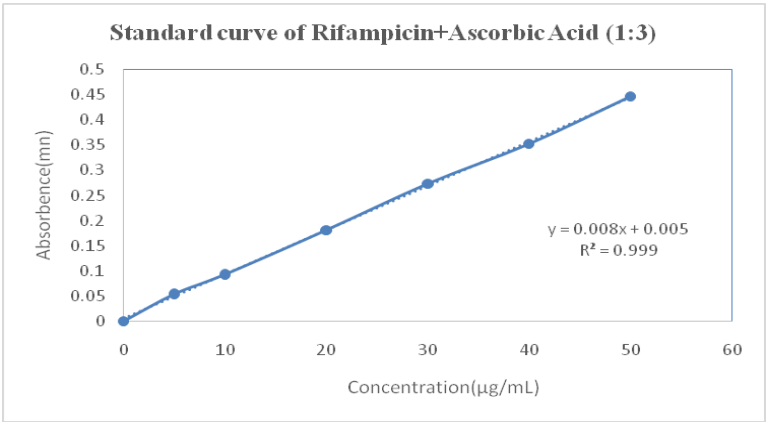

Fig. 7: Standard curve of Rifampicin+ Ascorbic acid (1:3)

Table 4: Pure Rifampicin

Time (mins)	Absorbance			Concentration			% drug release			Mean % drug release±SD
	T1	T2	T3	T1	T2	T3	T1	T2	T3	
0	0	0	0	0	0	0	0	0	0	0
15	0.620	0.621	0.619	43.20	43.21	43.19	38.8	38.81	38.79	38.80±0.010 %
30	0.861	0.860	0.859	56.41	56.40	56.39	50.77	50.76	50.75	50.76±0.010 %
60	0.980	0.980	0.981	71.20	71.20	71.21	64.08	64.08	64.09	64.08±0.005 %
P value										*0.0197

T- Trial

Table 5: Rifampicin + PLGA Nanoparticles

Time (mins)	Absorbance			Concentration			% drug release			Mean % drug release±SD
	T1	T2	T3	T1	T2	T3	T1	T2	T3	
0	0	0	0	0	0	0	0	0	0	0
15	0.190	0.202	0.201	18.1	18.2	18.2	16.39	16.42	16.42	16.38± 0.07 %
30	0.335	0.334	0.335	21.90	21.8	21.9	19.71	19.70	19.71	19.71±0.005 %
60	0.420	0.421	0.422	27.20	27.20	27.21	24.48	24.48	24.50	24.48 ±0.011%
P value										*0.0133

Table 6:Rifampicin + Ascorbic Acid (1:1)

Time (mins)	Absorbance			Concentration			% drug release			Mean % drug release±SD
	T1	T2	T3	T1	T2	T3	T1	T2	T3	
0	0	0	0	0	0	0	0	0	0	0
15	0.410	0.413	0.412	22.5	29.4	30.0	26.46	26.48	26.48	26.46±0.011%
30	0.472	0.471	0.472	34.20	34.11	34.20	30.61	30.59	30.61	30.60±0.010 %
60	0.540	0.542	0.541	39.80	39.82	39.82	35.82	35.83	35.83	35.82±0.005 %
Pvalue										**0.0076

Table 7: Rifampicin + Ascorbic Acid (1:2)

Time (mins)	Absorbance			Concentration			% drug release			Mean % drug release±SD
	T1	T2	T3	T1	T2	T3	T1	T2	T3	
0	0	0	0	0	0	0	0	0	0	0
15	0.360	0.359	0.361	37.00	36.90	37.10	33.20	33.19	33.21	33.20±0.010%
30	0.391	0.390	0.389	43.21	43.20	43.14	38.81	38.80	38.79	38.80±0.010 %
60	0.442	0.440	0.440	45.73	45.71	45.71	41.15	41.13	41.13	41.13±0.011%
P value										**0.0039

Table 8: Rifampicin + Ascorbic Acid (1:3)

Time (mins)	Absorbance			Concentration			% drug release			Mean % drug release±SD
	T1	T2	T3	T1	T2	T3	T1	T2	T3	
0	0	0	0	0	0	0	0	0	0	0
15	0.351	0.349	0.350	44.71	44.69	44.7	40.21	40.19	40.20	40.20±0.010 %
30	0.442	0.440	0.444	49.20	49.00	49.22	44.20	44.19	44.22	44.20±0.015 %
60	0.446	0.445	0.447	49.90	49.89	49.90	44.89	44.88	44.90	44.89±0.010 %
P value										**0.0011

Table 9: Comparative % Drug Release Profile of the Formulations

Time (min)	F0	F1	F2	F3	F4
0	0	0	0	0	0
15	38.80±0.010 %	16.38±0.07%	26.46±0.011%	33.20±0.010%	40.20 ±0.010 %
30	50.76±0.010 %	19.71±0.005%	30.60±0.010%	38.80±0.010%	44.20 ±0.015%
60	64.08± 0.005%	24.48±0.011 %	35.82±0.005%	41.13±0.011%	44.89 ±0.010%

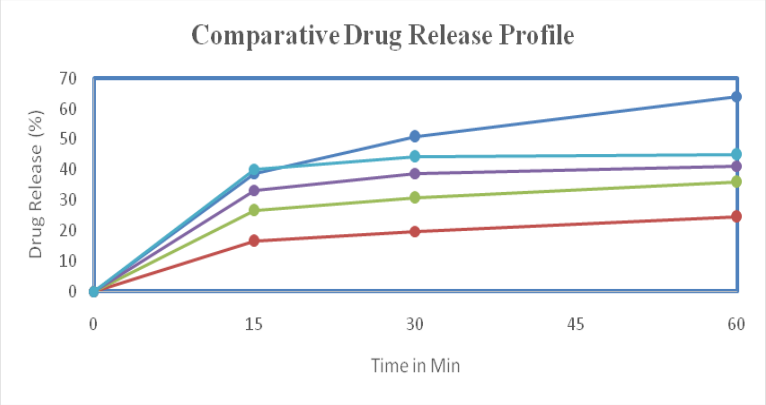

Fig 8: Comparative % Drug Release Profile of the Formulations

Scanning electron micrograph of the prepared nanoparticles are shown in Figure 3.SEM images

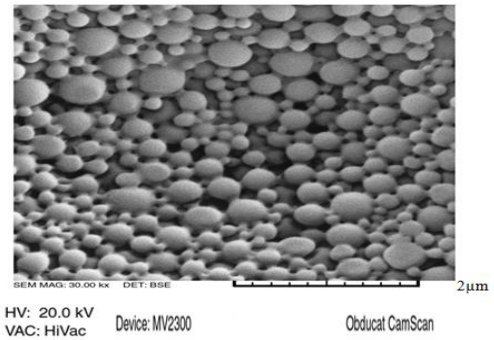

Fig. 3: Scanning electron micrograph of prepared nanoparticles

Particle Size Characterization of the Nanoparticles: Laser particle size analyzer yields the diameter of the bulk population. Particles were in the size range of 369-380 ± 14-23 (SD) nm. Polydispersity index is a measure of the distribution of particles in a given polymer sample. It gives the distribution range from 0.000 to 0.500.Polydispersity index greater than 0.5 indicates aggregation of particles. Here it is in the range of 0.304 - 0.317. The mean particle size and polydispersity Index of all the samples were determined and explained in Table 2.

Zeta potential study: Zeta potential is a term related to the stability of samples. For molecules and particles that are small enough, high zeta potential will confer stability i.e. it resist aggregation. . Here zeta potential of the prepared nanoparticles was found to be -46.6, which would not allow aggregation.

***In-vitro* Release Study:** *In-vitro* Stability study of rifampicin PLGA nanoparticles and rifampicin in combination with ascorbic acid in different ratios at pH 1.2 buffer. Results are tabulated in 4-7.

RESULTS & DISCUSSION:

The results of the *in-vitro* drug dissolution study indicates that the percentage drug release of the formulation F0, F1, F2, F3 and F4 at 60 mins was found to be 64.08± 0.005%, 24.48±0.011 %, 35.82±0.005%, 41.13±0.011% and 44.89 ±0.010% respectively and the percentage drug degradation of the formulation F0, F1, F2, F3 and F4 was found to be 64.81%, 49.77 %, 40.17 %, 23.54 % and 11.61 % respectively.

CONCLUSION:

The results of the investigation demonstrate that the ascorbic acid can minimize the degradation of Rifampicin in gastric pH condition and thus enhances the stability and therapeutic efficacy. It can be concluded that there is a significant change in the drug degradation profile when the concentration of ascorbic acid was increased. Further in-vivo studies are recommended to address the therapeutic efficacy of rifampicin – ascorbic acid loaded PLGA nanoparticles

REFERENCES:

1. Seifart HI, Parkin DP, Donald PR. Stability of isoniazid, rifampicin and pyrazinamide in suspensions used for the treatment of tuberculosis in children. Pediatr Infect Dis 1991; 10:827–83.
2. Maggi N, Pasqualucci CR, Ballotta R, Sensi P. Rifampicin: A new orally active Rifamycin. Chemotherapia 1966; 11:285–92

3. Richard JP, John MW, Joel HP. Kinetics for degradation of rifampicin, an azomethine-containing drug which exhibits reversible hydrolysis in acidic solutions. Int J Pharm 1992; 78:59-67.
4. Akansha Tripathi, Ranjana Gupta, Shubhini A.Saraf. PLGA nanoparticles of anti-tubercular drug: Drug loading and release studies of a water in-soluble drug. Int J Pharm Tech Res 2010; 2: 2116-2123.
5. Bhavika Mohan, Nishi Sharda, Saranjith Singh. Evaluation of the recently reported USP gradient HPLC method for analysis of anti-tuberculosis drugs for its ability to resolve degradation products of rifampicin. J of Pharma Biomed anal 2003; 31:607-612.
6. Gambhire Vaishali, Bhalekar Mangesh, Gambhire Makarand. Development of rifampicin nanoparticles by 3^2 factorial design. Int J Pharm Sci nanotech 2010; 3(3) : 1085-1091.
7. Lisa Claire du Toit, VinessPillay and Michael Paul Danckwerts.Tuberculosis chemotherapy: current drug delivery approaches. Respiratory Research2006;7:1-18.
8. L. Zhang, D. Pornpattananangkul, C. M.J. Hu and C.M. Huang. Development of Nanoparticles for Antimicrobial Drug Delivery .Current MediChem2010;17: 585-594.