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RESEARCH ARTICLE

FORMULATION AND EVALUATION OF RIFAMPICIN SOLID LIPID NANOPARTICLES

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ABSTRACT

The solid lipid nanoparticles (SLNs) are sub-micron sized colloidal carriers composed of physiological lipid dispersed either in water or in an aqueous surfactant solution. SLNs as colloidal drug carrier combine the advantages of polymeric nanoparticles, fat emulsions and liposomes. Rifampicin is bactericidal to *M.tuberculosis*and many other gram positive and negative bacteria. The solid lipid nanoparticles of rifampicin were prepared by 'emulsion solvent diffusion method'. PVA (cold aqueous soluble) was used as shell material, Stearic acid as lipid core and Methanol and Chloroform as organic solvents. Processing parameters were optimized to achieve maximum yield and high quality of solid lipid nanoparticles of Rifampicin.

KEYWORDS: Solid lipid nanoparticles, SLN, Rifampicin.

INTRODUCTION:

Solid lipid nanoparticles have gained much attraction in delivery of drugs with poor water solubility. SLN has combined advantages of fat emulsions, polymeric nanoparticles and liposomes. SLN is a colloidal carrier system wherein drug is entrapped in a biocompatible lipid core and surfactant at the outer shell. SLN thus offers advantages like lower toxicity, improved drug stability, ease in modulation of production process to achieve desired drug release and avoidance of organic solvents.

Rifampicin is a semi synthetic derivative of rifamycin B obtained from Streptomyces mediterranei. Rifampicin is bactericidal to M.tuberculosis and many other gram positive and negative bacteria. It is as efficacious as INH and better than all other drugs against tuberculosis. Though the bactericidal action covers all subpopulations of TB bacilli, it acts best on slowly or intermittently dividing bacilli as well as on many atypical mycobacteriea. Both extracellular and intracellular organisms are affected. It has good sterilizing and resistance preventing actions. Rifamycin inhibits DNA dependent RNA synthesis. Probably the basis of selective toxicity is that mammalian RNA polymerase does not avid bind Rifampicin.

The aim of this study was to prepare solid lipid nanoparticles of rifampicin in order to have a sustained release of the encapsulated drug so that the dosing frequency can be reduced, reduce the dose dependent side effects of the drug, minimize the incidence of drug resistance in the body; this normally occurs in the normal therapy when a single dose of the drug is missed and to make the patient comfortable with the therapy.

MATERIALS AND METHOD:

MATERIALS:

Rifampicin was received as a gift sample from Ronak Pharmaceuticals pvt Ltd., Patan. Stearic acid, PVA aqueous soluble (cold), Methanol and Chloroform were purchased from Central Drug House (P) Ltd., New Delhi.

PREPARATION OF RIFAMPICIN SLN:

Solid lipid nanoparticles of rifampicin were prepared by 'emulsion solvent diffusion method'. Here, lipid was dissolved in the organic phase in water bath at 50^{o}C. Stearic acid and rifampicin were accurately weighed to the required amount and dissolved completely in a mixture of chloroform and methanol in a water bath at 50^{o}C. The resulting organic solution was poured into poly vinyl alcohol at 4-8^{o}C. The mixture was then homogenized for 15 minutes. Then the organic solvents were removed through rotary flash evaporator at 50^{o}C for 15minutes. The SLNs formed were recovered by centrifugation at 13,000 rpm for 30 min at 4^{o}C. The recovered SLNs were washed thrice with distilled water to remove any excess of PVA remain intact with the formulation and were lyophilized. Twelve formulations were prepared and various parameters considered for optimization were shown in table1.

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EVALUATION:

DETERMINATION OF PARTICLE SIZE:

The particle size distribution of the nanoparticles was determined by laser diffraction method using Microtrac Particle Size Analyzer. The samples were scanned by the refractive index at 30 s run time *Determination of entrapment efficiency* the entrapment efficiency of the drug was determined by measuring the concentration of free drug present in the supernatant after centrifugation at 13,000 RPM for 30 min at 4oC. Theconcentration of drug present in the supernatant was determined through UV spectrophotometer (Schimadzu 1800, Japan) at 333nm. The percentage drugentrapment was determined by the following formula.

$$\%EP = \frac{\text{Amount of drug added initially} - \text{Amount of drug present in the supernatant}}{\text{Amount of drug added initially}} \times 100$$

Stability studies

The physical and chemical stability of prepared formulations was performed at room temperature and at refrigeration temperature. Sampling interval was one month. Samples were evaluated for clarity and % entrapment efficiency.

In-vitro release studies

The release study was performed using a dialysis membrane of pore size 2.5nm membrane was soaked in double distilled water for 12 hours before use. Simulated gastric fluid was used as a medium. 1 ml of the formulation and 50 ml of simulated gastric fluid were taken in dialysis membrane. 5 ml samples were withdrawn at regular intervals and replaced with fresh media.

RESULTS & DISCUSSION:

Table 1: formulation optimization of rifampicin SLN

Formulation code	Formulation ratio Rifampicin: stearic acid	Concentration of PVA (%)
F1	1:1	1
F2	1:2	1
F3	1:3	1
F4	1:5	1
F5	1:1	2
F6	1:2	2
F7	1:3	2
F8	1:5	2
F9	1:1	3
F10	1:2	3
F11	1:3	3
F12	1:5	3

Table 2: percentage entrapment efficiency

Formulation code	Drug:lipid ratio	Concentration of PVA (%)	% EP
F1	1:1	1	29.3±0.9%
F2	1:2	1	28.07±0.5%
F3	1:3	1	33.87±0.8%
F4	1:5	1	20.48±0.9%
F5	1:1	2	48.09±0.1%
F6	1:2	2	77.57±0.5%
F7	1:3	2	57.9±0.7%
F8	1:5	2	36.9±0.9%
F9	1:1	3	33.94±0.4%
F10	1:2	3	50.8±0.7%
F11	1:3	3	69.4±0.8%
F12	1:5	3	40.7±0.9%

Table 3: stability studies of SLN of Rifampicin (sampling after one month)

Formulation code	Storage conditions			
	Room temperature (28ºC)		Refrigeration temperature(4ºC)	
	Visual inspection	% EP	Visual inspection	% EP
F1	Emulsion was clear and SLN of rifampicin were visible under microscope	28.7%	Emulsion was clear and SLN of rifampicin were visible under microscope	28.9%
F2		26.4%		26.1%
F3		35.9%		36.1%
F4		21.3%		21.5%
F5		45.8%		45.9%
F6		78.4%		78.3%
F7		53.9%		53.4%
F8		34.8%		34.9%
F9		35.7%		36.1%
F10		48.9%		47.1%
F11		69.2%		69.8%
F12		44.7%		43.9%

Table 4: stability studies of SLN of Rifampicin (sampling after three months)

Formulation code	Storage conditions			
	Room temperature (28ºC)		Refrigeration temperature(4ºC)	
	Visual inspection	% EP	Visual inspection	% EP
F1	Emulsion was clear and SLN of rifampicin were visible under microscope	Fungal growth was observed and hence % EP could not be determined	Emulsion was clear and SLN of rifampicin were visible under microscope	28.4%
F2				25.9
F3				35.7%
F4				21.4%
F5				45.2%
F6				78.3%
F7				52.6%
F8				34.3%
F9				36.4%
F10				46.5%
F11				69.7%
F12				43.4%

Table 5: *in-vitro* release studies

T(MIN)	DRUG	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0	0
15	14.6±0.5	5.5±0.8	5.6±0.3	4.6±0.2	7.4±0.6	8.14±0.7	2.194±0.4
30	38.4±0.3	7.8±0.7	8.1±0.1	7.4±0.3	9.2±0.7	10.72±0.4	2.48±0.7
45	46.7±0.9	11.6±0.4	11.8±0.2	11.7±0.8	11.7±0.9	11.95±0.5	2.57±0.4
60	65.8±0.4	15.7±0.1	16.3±0.4	13.6±0.7	13.5±0.3	13.9±0.4	2.57±0.2
90	79.2±0.2	21.8±0.5	22.6±0.1	20.1±0.5	19.6±0.8	22.77±0.4	3.91±0.6
120	89.9±0.1	27.7±0.4	29.7±0.1	25.6±0.2	25.6±0.7	28.24±0.6	4.7±0.8
180	93.8±0.4	35.4±0.6	35.7±0.4	30.8±0.3	37.4±0.6	31.09±0.6	6.36±0.4
240	-	39.7±0.3	40.6±0.7	35.7±0.6	43.6±0.5	31.98±0.3	8.15±0.5
300	-	41.1±0.1	42.2±0.5	38.9±0.5	51.8±0.5	33.08±0.7	8.97±0.7
360	-	45.7±0.2	47.5±0.2	43.2±0.6	66.7±0.5	35.51±0.3	9.67±0.3
420	-	53.6±0.3	55.5±0.6	51.1±0.3	68.6±0.5	37.62±0.7	10.36±0.4
480	-	57.7±0.5	58.6±0.8	55.7±0.6	75.4±0.6	39.24±0.2	10.67±0.7

540	-	63.3±0.7	64.1±0.2	62.6±0.4	78.3±0.6	41.18±0.4	10.74±0.4
600	-	70.2±0.8	71.2±0.3	65.6±0.1	90.6±0.1	45.89±0.8	11.73±0.3
660	-	75.2±0.4	76.5±0.5	72.5±0.3	100.2±0.4	48.87±0.7	12.3±0.1
720	-	80.4±0.3	82.2±0.6	77.6±0.8		53.68±0.2	12.79±0.3

Table 6: *in-vitro* release studies

T(MIN)	F7	F8	F9	F10	F11	F12
0	0	0	0	0	0	0
15	4.4±0.8	7.1±0.3	5.2±0.8	6.54±0.7	2.9±0.6	7.1±0.3
30	6.3±0.6	8.5±0.8	9.1±0.6	8.7±0.2	2.4±0.8	8.5±0.8
45	9.8±0.5	10.6±0.3	12.1±0.8	10.8±0.4	3.2±0.4	11.98±0.6
60	10.3±0.7	13.58±0.5	14.3±0.6	11.1±0.7	3.4±0.8	13.58±0.5
90	11.42±0.6	19.7±0.2	21.7±0.3	18.7±0.4	4.1±0.8	22.76±0.8
120	14.79±0.8	24.5±0.3	25.3±0.2	23.3±0.7	4.3±0.7	28.24±0.1
180	15.45±0.4	29.8±0.4	32.4±0.2	25.9±0.3	6.4±0.4	31.05±0.4
240	16.34±0.1	34.77±0.6	35.6±0.9	29.7±0.6	7.2±0.3	31.85±0.7
300	17.45±0.8	39.34±0.5	42.8±0.8	30.4±0.7	7.3±0.2	33.07±0.6
360	19.24±0.3	43.59±0.7	44.4±0.8	33.1±0.5	9.3±0.2	35.44±0.7
420	18.57±0.5	50.53±0.3	52.3±0.8	35.6±0.5	9.4±0.1	37.47±0.7
480	20.12±0.7	54.7±0.7	55.4±0.6	39.7±0.6	9.9±0.6	39.24±0.1
540	21.810.4	59.8±0.1	62.5±0.5	41.2±0.7	10.4±0.5	42.28±0.4
600	23.78±0.3	66.7±0.3	66.8±0.4	43.4±0.7	11.4±0.3	45.77±0.8
660	25.86±0.3	70.7±0.8	71.3±0.4	45.7±0.8	13.2±0.8	48.87±0.4
720	28.87±0.3	74.7±0.3	77.9±0.7	50.8±0.3	13.4±0.6	53.87±0.5

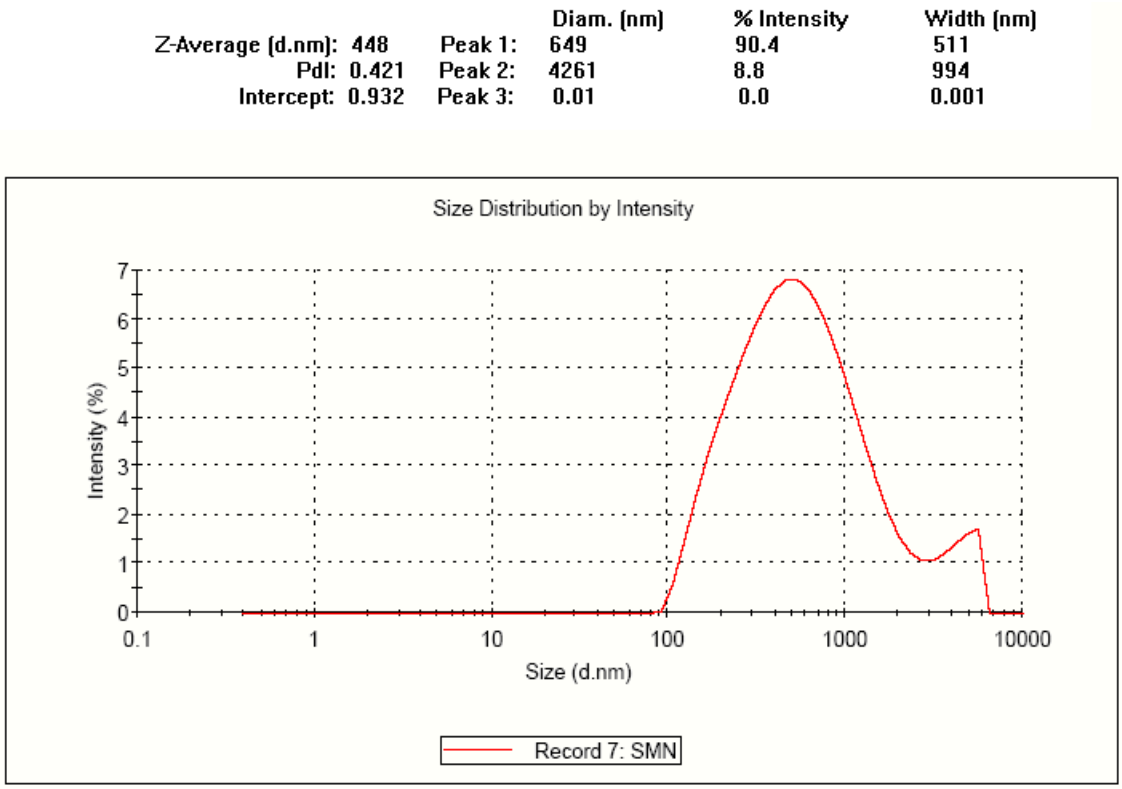

Figure 1: particle size analysis

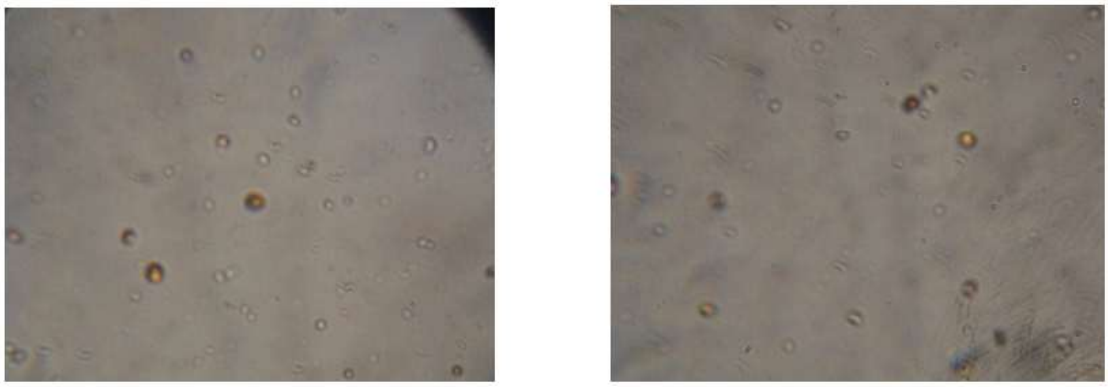

Figure 2: photos of SLN of Rifampicin

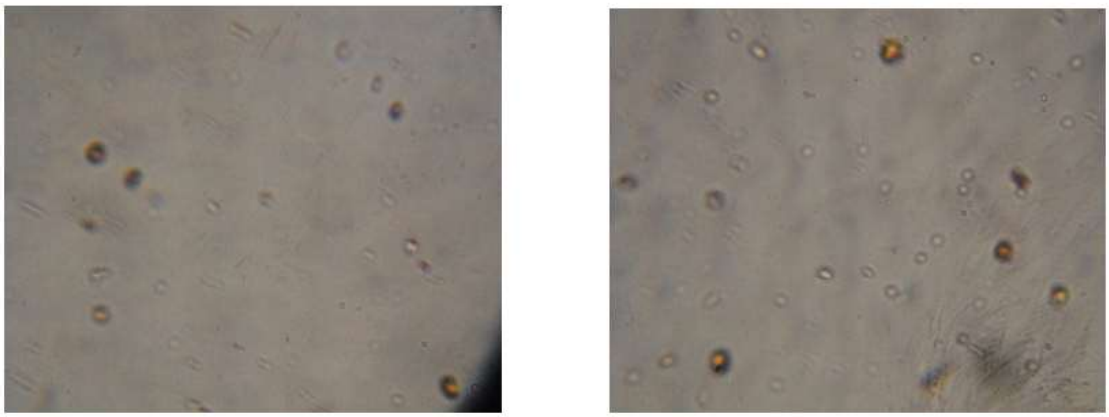

Figure 3: photos of SLN of rifampicin

CONCLUSION:

Emulsion solvent evaporation technique was found to be suitable for preparation of solid lipid nanoparticles of rifampicin. Rifampicin was successfully loaded in SLN of stearic acid by this method. *In vitro* release studies shows that this system was most suitable for the treatment of tuberculosis.

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