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Formulation, Optimization and *in vitro* Evaluation of Rifampicin Nanoemulsions

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ABSTRACT

The aim of the present investigation was to develop, optimize and evaluate nanoemulsion of rifampicin to improve the solubility, stability and oral bioavailability. Rifampicin nanoemulsions were prepared by o/w nanoemulsion region of the phase diagrams, which were subjected to physical stability and phase separation tests. Prepared rifampicin nanoemulsions were evaluated for particle size, zeta potential, PDI and drug content. *In vitro* dissolution was performed by using dialysis bag method and morphology by transmission electron microscopy (TEM). Best results were obtained with the formulation which consisted of 10 mg of rifampicin, 15% w/w of sefsol 218, 25% w/w of tween 80, 15% w/w of tween 20 and 45% w/w of water. *In vitro* release studies revealed that 99.85 ± 1.85% was observed in 12h. TEM studies shows the globules are in spherical shape with dark surroundings. DSC studies revealed that no interaction between the drug and excipients. The optimized formulation was also subjected to stability studies according and was found to be stable for one month with no phase separation. These results indicated the potential of nanoemulsions of rifampicin could be promising to improve solubility, stability and oral bioavailability.

Keywords: Rifampicin, nanoemulsions, phase diagram, size, TEM, stability.

INTRODUCTION

Nanoemulsions are colloidal dispersions with a size range of 20-400nm [1] and composed of an oil phase, aqueous phase, surfactant and co surfactant at appropriate ratios. Unlike coarse emulsions micronized with external energy. Nanoemulsions are based on low interfacial tension. This is achieved by adding a co-surfactant, which leads to spontaneous formation of a thermodynamically stable nanoemulsion. [2] The term 'Nanoemulsions' is often used to designate emulsions with the internal phase droplets smaller than 1000nm.[3] The Nanoemulsions are also referred as mini emulsions, ultrafine emulsions and submicron emulsions. [4] Phase behavior studies have shown that the size of the droplets is governed by the surfactant phase structure (bicontinuous microemulsion or lamellar) at the inversion point induced by either temperature or composition.

Rifampicin (RF) is a semisynthetic antibiotic produced from *Streptomyces mediterranei*, acts against several forms of Mycobacterium. [5] It inhibits DNA-dependent RNA polymerase activity by forming a stable complex with the enzyme. Rifampicin is bactericidal, and acts on both intracellular and extracellular organisms. Rifampicin is well absorbed when taken orally and is distributed widely in body tissues and fluids, including the CSF. It is metabolized in the liver and eliminated in bile and, to a much lesser extent, in urine, but dose adjustments are unnecessary

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with renal insufficiency. Protein binding of the drug was reported as 89% and less than 30% of the dose is excreted in the urine as rifampin or metabolites and its half life is 3-4 hours. Rifampicin is soluble in DMSO, DMF, methanol, chloroform, ethyl acetate, acetone and is slightly soluble in water and in 95% ethanol. Previously, Barrow et al., reported targeted delivery of tuberculosis by rifampicin in infected macrophages. [6]

The aim of this study was to assess the feasibility of preparing rifampicin nanoemulsion by self emulsification method, and the physicochemical properties of obtained rifampicin loaded nanoemulsions, such as particle size, zeta potential, drug entrapment efficiency, *in vitro* drug release behavior. Differential scanning calorimetry (DSC) analyses were performed to investigate the drug and excipient compatibility and Shape and surface morphology of the finished formulation was determined by TEM.

Table 1: Solubility of Rifampicin in different oils (Mean±SD, n=3)

Oil	Solubility (mg/mL)
Sefsol	88.43 ± 2.73
Sunflower oil	71.73 ± 1.74
Oleic acid	60.42 ± 2.05
Capryol 90	63.84 ± 1.93
Olive oil	70.67 ± 2.00
Linseed oil	45.86 ± 3.04
Transcutol P	80.46 ± 2.93
PEG 400	78.64 ± 1.83
Propylene glycol	73.75 ± 1.75
Tween 80	86.84 ± 1.84
Tween 20	85.73 ± 3.01

Table 2: Composition of selected nanoemulsion formulations of Rifampicin

Code	Oil	S_{mix}		Water
		Surfactant	Cosurfactant	
A1	10	30	10	50
A2	15	30	10	45
A3	15	25	15	50
A4	20	30	10	40

MATERIALS AND METHODS

Materials

Rifampicin was purchased from Sigma (Hyderabad, India). Diethylene glycol monoethyl ether (Transcutol P), Sefsol and propylene glycol monocaprylate (Capryol 90) were gift samples from Gattefosse (Cedex, France). Isopropyl myristate, Oleic acid, Propylene glycol, Tween 20, Tween 80, PEG 400 and Glycerol were purchased from SD fine chemicals Pvt. Ltd., (Mumbai, India).

Methods

Solubility studies

The solubility of Rifampicin in various oils (Sefsol, Sunflower oil, Oleic acid, Capryol 90, Olive oil and Linseed oil), surfactants (Tween 80 and Tween 20) and co-surfactants (Transcutol P, Glycerol, PEG 400 and Propylene glycol) was determined by adding an excess quantity of rifampicin to oils, surfactants and co-surfactants separately in stopper vials, and mixed for five minutes using a cyclic mixer and resulting vials were then kept in an Orbital shaker for 72 h to reach equilibrium. The samples were removed after achieving equilibrium and centrifuged at 3000 rpm for 15 min. The supernatant was taken and filtered through a 0.45µm membrane filter. The filtrate was solubilized in suitable solvent, diluted with the pH 7.4 buffer and the concentration of Rifampicin was determined using UV-Visible spectrophotometer at 255nm.

Construction of phase diagrams

The pseudo ternary phase diagrams were constructed by aqueous titration method using Sefsol as oil, Tween 80 and Tween 20 as surfactants and Propylene glycol and Transcutol P as co-surfactants. Surfactant and co-surfactant (Smix) were mixed in three different weight ratios (1:1, 1:2 and 1:3). These Smix ratios were chosen by increasing co-surfactant concentration with respect to surfactant concentration for the study of phase diagrams needed for nanoemulsion formation. For each phase diagram, oil and Smix were combined in different weight ratios from 1:9 to 9:1 in different glass vials. Slow titration with the aqueous phase was done to each weight ratio of oil and Smix, and visual observations were made for transparent and easily flowable nanoemulsions. The pseudo ternary phase diagrams were constructed using Triplot V4 software (4.1.2. version).

Preparation of nanoemulsions

Nanoemulsions were prepared by aqueous phase titration method. The composition of the nanoemulsions was chosen according to the pseudo ternary phase diagram. The drug was dissolved in the oil, surfactant and co-surfactant mixture was added in the chosen concentration, and water was added drop wise with continuous stirring until clear nanoemulsion was formed. Resulting rifampicin containing nanoemulsion was subjected to homogenization for 10 minutes using ultra turrex at 8000 rpm to get uniform and stable nanoemulsion.

Drug - Excipient compatibility studies

The DSC thermal analysis of RF and optimized formulation was performed using Perkin Elmer, USA. The instrument was calibrated with indium. All the samples (~10mg) were heated in aluminium pans using dry nitrogen as the effluent gas. The thermograms obtained in the heating range of 20–200°C and at a rate of 20°C/min.

Particle size, PDI and zeta potential measurement

About 0.1mL of the NE formulation was diluted 50 times with double distilled water. Resulting diluted nanoemulsion was measured for particle size, polydispersile index and zeta sizer. The size and PDI of nanoemulsion was measured by photon correlation spectroscopy using a Zetasizer 3000 HSA (Malvern, UK). Zeta potential measurements were done at 25°C, and the electric field strength was around 23.2 V/cm. [7]

Drug content

Drug content of RF in nanoemulsion was measured by using UV visible spectrophotometer at 255 nm. About 0.1 mL of the formulation was suitably diluted with 5

mL in pH 7.4 phosphate buffer and analyzed for drug content.

***In vitro* drug release studies**

The *in vitro* drug release of Rifampicin from the nanoemulsion formulation was determined by dialysis bag method. In this method 0.1N HCl and pH 7.4 phosphate buffer was selected as *in vitro* release medium. About 1 mL of formulation was placed in the dialysis bag (single dose containing 10 mg of Rifampicin), which was immersed in 50 mL of 0.1 N HCl for 2 h and replaced with pH 7.4 phosphate buffer maintained at 37°C and stirred with a magnetic stirrer. Samples (2 mL) were withdrawn at predetermined time intervals and replenished with equal volume of fresh medium. The samples were analyzed by the UV-Visible spectrophotometer at 255 nm to determine the rifampicin content.

Transmission electron microscopy (TEM)

Morphology and structure of the nanoemulsion were studied using transmission electron microscopy TOPCON 002B operating at 200 KV (Topcon, USA) and capable of point to point resolution. Combination of bright field imaging at increasing magnification and of diffraction modes was used to reveal the form and size of nanoemulsion droplets. In order to perform the TEM observations, a drop of the nanoemulsion was directly deposited on the holey film grid and observed after drying.

Stability studies

The stability of optimized nanoemulsion was tested by subjecting them to room temperature for one month and checked for size, PDI and ZP after 7, 15 and 30 days. Simultaneously also observed for phase separation of formulation.

Table 3: Size, PDI, zeta potential of nanoemulsion formulations of Rifampicin (mean ± SD, n=3)

Parameter	Size (nm)	PDI	ZP (mV)	Assay (% w/w)
A1	234.9 ± 2.7	0.273 ± 0.083	-20.36 ± 2.64	98.5
A2	259.6 ± 2.0	0.302 ± 0.074	-19.84 ± 2.06	99.1
A3	204.0 ± 3.8	0.124 ± 0.062	-26.83 ± 3.91	99.8
A4	285.8 ± 2.9	0.294 ± 0.082	-20.28 ± 1.93	99.3

Table 4: Stability studies of optimized Rifampicin nanoemulsion formulation (mean ± SD, n=3)

Time (days)	Size (nm)	PDI	ZP (mV)
Initial	204.0 ± 3.8	0.124 ± 0.062	-26.83 ± 3.91
7	208.7 ± 7.5	0.142 ± 0.073	-26.73 ± 3.05
15	209.7 ± 5.9	0.139 ± 0.063	-26.12 ± 2.00
30	211.7 ± 6.2	0.144 ± 0.092	-26.04 ± 1.95

RESULTS AND DISCUSSION

Solubility studies

The most important criterion for screening of excipients is the solubility of the poorly soluble drug in oil, surfactants, and co-surfactants. The solubility of Rifampicin in different oils/ surfactants/co-surfactants was determined. As shown in Table 1, the solubility of Rifampicin was found to be highest in Sefsol as compared to other oils. Hence, Sefsol was selected as the oil phase. High drug solubility was found in Tween 20 and Tween 80 among surfactants and in Transcutol P and Propylene glycol among co-surfactants. Therefore, Tween-80 and Tween20 were selected as surfactants and Transcutol P and Propylene glycol were selected as co-surfactants, respectively, for the Pseudo ternary phase diagrams study. From the phase solubility diagram, different formulations of nanoemulsions of RF were prepared as per Table 2.

Construction of phase diagrams

Pseudo ternary phase diagrams were constructed for 1:1, 1:2 and 1:3 surfactant to cosurfactant ratios (Smix). So that nanoemulsion regions could be identified. In construction of phase diagrams Capryol 90 was used as oil, Tween 20 and Tween 80 were used as surfactants and Transcutol P was used as cosurfactant.

Initial pseudo ternary phase trial was performed utilizing Capryol 90 as oil, Tween 20 as surfactant and Transcutol P as cosurfactant. Maximum amount of oil emulsified was found to be 60% (w/w) using 33% (w/w) of Smix when the Smix ratio was 1:1, where as the proportion of cosurfactant was doubled (Smix 1:2) the nanoemulsion region decreased slightly when compared to Smix 1:1 and the maximum amount of oil that could be emulsified was found to be 64% (w/w) using 38% (w/w) of Smix. Furthur increasing in Smix proportion by three times (Smix 1:3) the nanoemulsion region was further decreased and the maximum amount that could be emulsified was found to be 58% (w/w) using 32% (w/w) of Smix (Figure 1).

In the subsequent trial was performed utilizing Capryol 90 as oil, Tween 80 as surfactant and Transcutol P as cosurfactant (Figure 2). Maximum amount of oil that could be emulsified was found to be 59% (w/w) using 33% (w/w) of Smix in case of Smix ratio was 1:1, when the proportion of cosurfactant was doubled (Smix 1:2) and increased by three times (Smix 1:3) the nanoemulsion region was increased when compared to Smix 1:1 and the maximum amount of oil that could be emulsified was found to be 63% (w/w) using 30% (w/w) of Smix and 62% (w/w) using 28% (w/w) of Smix respectively.

DSC studies

DSC thermogram of pure drug and optimized nano emulsion is shown in Figure 3. DSC thermogram of pure rifampicin showed sharp endothermic peak at 185^0C and which is corresponding to its reported melting point. In case of optimized formulation, drug peak was preserved but changes in the peak intensity were observed this might be due to the mixing of excipients for analysis. From the results, there was on interaction between the drug and other excipients in the formulation.

Size, PDI, ZP and drug content measurement

All the prepared formulations were evaluated for size, PDI and zeta potential. The size of the formulations ranging from 204 ± 3.8 nm to 285.8 ± 2.9 nm, PDI from 0.302 ± 0.074 to 0.124 ± 0.062 and ZP ranging from -19.84 ± 2.06 to -26.83 ± 3.91 mV (Table 3). As the oil ratio changes the size will be predominantly increased.

From all these formulations A3 showed better size with narrow size distribution and zeta potential. The drug content of all the formulations was 9.85 ± 1.03 to 9.98 ± 0.83 mg.

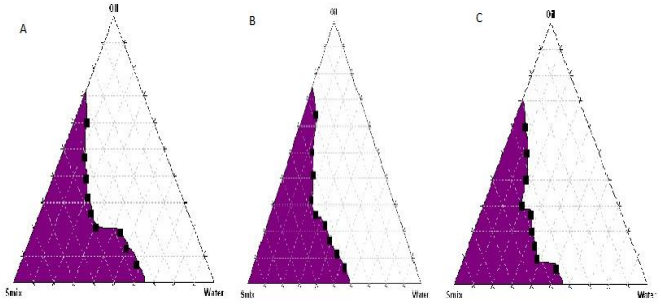

Fig. 1: Pseudo Ternary phase diagram of capryol 90, tween 20, transcutol P and water A). (Smix 1:1), B) (Smix 1:2), & C) (Smix 1:3)

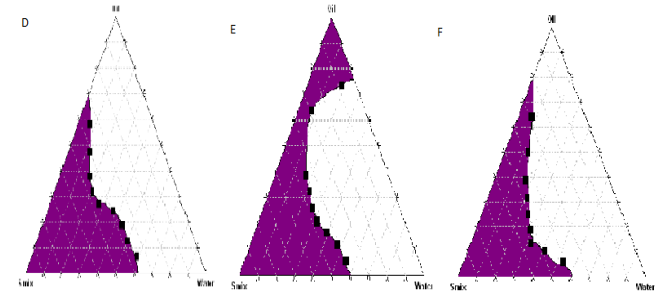

Fig. 2: Pseudo Ternary phase diagram of capryol 90, tween 80, transcutol P and water D). (Smix 1:1), E) (Smix 1:2), & F) (Smix 1:3)

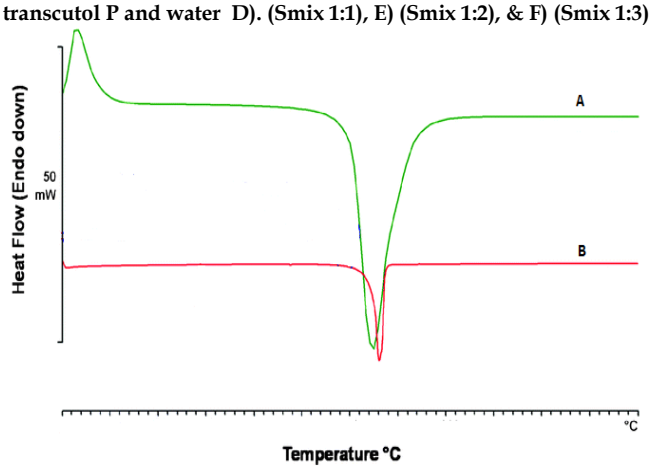

Fig. 3: DSC thermogram of Pure drug and Optimized nano-emulsion Formulation (A3)

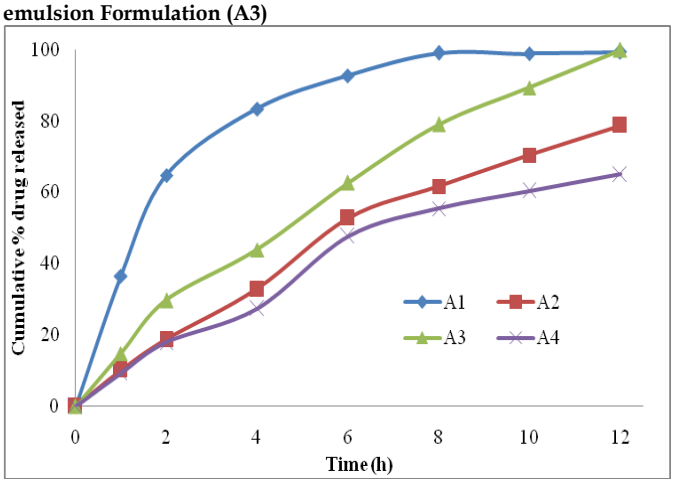

Fig. 4: *In vitro* dissolution profiles of Rifampicin nanoemulsion formulations

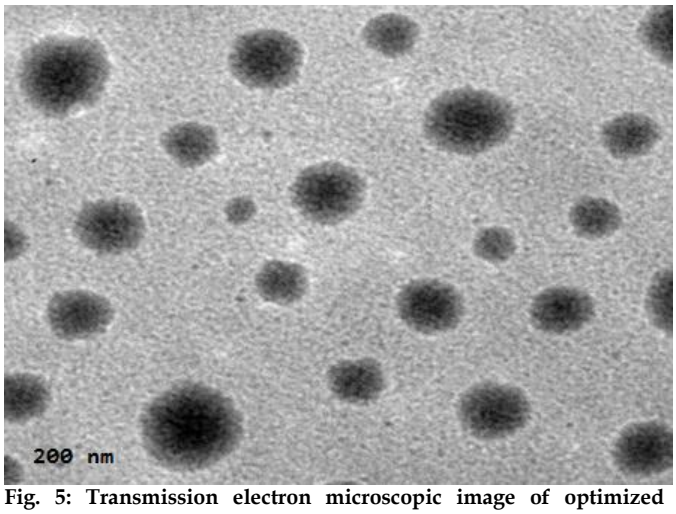

Fig. 5: Transmission electron microscopic image of optimized Rifampicin nanoemulsion (A3)

In vitro drug release studies

Drug release studies were performed for the nanoemulsions using dialys is bag method and are shown in Figure 4. The dissolution study was performed for 12 h. Drug dissolution from formulation A was very fast as 98.94 ± 2.83% of drug released in 8 h; while formulations A2, A3 and A4 showed comparatively slow release i.e. 78.73 ± 2.04, 99.85 ± 1.85 and 64.93 ± 2.85 in 12 h. In contrast to this drug released from API suspension was found to be very low i.e. 40.75 ± 1.94% in 12 h. This result was attributed to the fact that formulation A3 is having comparatively smaller size (204nm) of oil droplets and hence the larger surface area for dissolution as justified by droplet size distribution. Another possible reason was the oil concentration in formulation A1 (10%) as compared to the A3 (15%), although having the same concentrations of the Smix, which was not sufficient to emulsify the increased amount of oil in A2 & A4.

Transmission electron microscopy

Morphology of the rifampicin nanoemulsion optimized formulation (A3) was studied using transmission electron microscopy and results revealed that the nanoemulsion appeared dark with spherical size globules and with bright surroundings (Figure 5). The droplet size ranged between 200 nm and was in agreement with the droplet size distribution measured using photon correlation spectroscopy. [8]

Stability studies

Nanoemulsions are physically stable systems and are formed at a particular concentration of oil, surfactant and water, making them stable to phase separation, creaming or cracking. [9-10] It is the thermostability that differentiates nanoemulsion from emulsions with kinetic stability and eventually phase separation. [11- 12] Thus, the formulations were tested for their physical (dispersion) stability by measuring the size, PDI and ZP after storage at room temperature for one month time period. There was no statistically significant changes were noticed in these parameters (Table 4) and there was no phase separation also observed.

The formulation was found to be the optimized formulation based on the results of pseudo ternary phase diagram, in vitro drug release, droplet size and morphology parameters. Nanoemulsions prepared by aqueous phase titration method showed around 200 nm in particle size. TEM results revealed that the nanoemulsion appeared dark with spherical size globules and with bright surroundings with droplet size ranged between 200 nm. The present study was clearly indicated that the usefulness of nanoemulsion in the improvement of the solubility, dissolution rate and there by oral bioavailability of poorly water soluble drugs like rifampicin without incompatibility between the selected ingredients.

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