Characterization of Nanoemulsion Prepared from Self-emulsifying Rifampicin and its Antibacterial Effect on *Staphylococcus aureus* and *Stap. epidermidis* Isolated from Acne

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ABSTRACT: In this study, three different self emulsifying drug delivery systems of rifampicin (SEDD-R) were made using oleic acid and different surfactants such as Tween 80, Chremophor RH 40 and Chremophor EL designated as RN-TW, RN-CRH and RN- CEL. These self-emulsifying systems were converted to rifampicin nanoemulsion by adding water under sonication. The resulting particle sizes were found to be 192.7 nm, 183.4 nm and 179.2 nm for RN- CEL, RN-CRH and RN-TW, respectively using Zetasizer. Drug content, entrapment efficiency, *in vitro* drug release and pH stability tests was performed. Drug content and entrapment efficiency for RN-TW, RN-CRH and RN- CEL were found as 0.9945% & 0.9835%, 0.9720% & 84.31% and 74.58 % & 56.89%, respectively. The release of drug from RN-TW, RN-CRH and RN-CEL occurred for 450⁻, 330⁻ and 240⁻ minutes, respectively. The pH stability study demonstrated that all formulations retained maximum drug at pH 5.8. Antibacterial effect of the preparations was evaluated against *S. epidermidis* and *S. aureus* isolated from acne and showed effective results.

Key words: Rifampicin nanoemulsion, entrapment efficiency, *in vitro* drug release, pH stability

INTRODUCTION

Nanoemulsions are isotropic, thermodynamically stable, transparent or translucent dispersions of oil and water stabilized by an interfacial film of surfactant molecules having the droplet size 20-500 nm.[1] Selection of an appropriate oily phase is very important as it influences the selection of other ingredients of nanoemulsions, mainly in case of o/w nanoemulsions which also helps to achieve maximum drug loading in the nanoemulsions.[2] However, aqueous phase, pH and ionic content of aqueous phase also influence stability and droplet size of nanoemulsions.[3] Nanoemulsions are used to deliver drugs to the patients via several routes, but the topical application of nanoemulsions is given special interest.[4-6] Nanoemulsions improve the transdermal delivery of several drugs over the conventional topical preparations of emulsions and gels.[7] Acne primarily affects the face, chest and back because these areas contain the highest concentration pilosebaceous glands.[8] It is the most common skin disorder in the world with prevalence about 70-87% and moderate to severe acne affects around 20% of young people.[9,10] *Staphylococcus epidermidis* and *Staph. aureus* is the causative agent for acne.[11-13]

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Bacterial resistance to conventional antibiotics such as clindamycin, erythromycin and tetracycline have been reported.[14] Rifampicin and combination of rifampicin with other antibiotics were found to be the most effective antibiotic for treatment of acne vulgaris.[15-17] In this study, three different self emulsifying drug delivery systems of rifampicin (SEDD-R) were made using organic acid and different surfactants for topical delivery of drug at the target sites on skin for the treatment of acne.

MATERIALS AND METHODS

Materials. Rifampicin was procured from Cipla (India). Oleic Acid, Tween 80, Cremophor RH 40, Cremophor EL), methanol, n-propanol and EDTA were procured from Merck (Germany). All chemicals were of analytical grade. Instrument including analytical balance (Boeco, Germany), Ultra sonic bath (Sonoswiss, Switzerland), Zetarsizer Ver 7.01 (Marlvern UK), Ultraviolet spectrophotometer (Shimadzu, Japan), microcentrifuge machine (M-244, Boeco, Germany) were utilized.

Formulation and preparation of rifampicin nanoemulsion. Three rifampicin nanoemulsion products were formulated and designated as RN-TW, RN-CRH and RN-CEL. Ingredients and amounts are given in table 1.

Table 1. Formulation of rifampicin nanoemulsions.

	RN-TW	RN-CRH	RN-CEL
Ingredients	Weight in g	Weight in g	Weight in g
Rifampicin	1.0	1.0	1.0
Oleic Acid	5.0	5.0	5.0
Tween 80	40.0	-	-
Cremophor RH 40	-	40.0	-
Cremophor EL	-	-	40.0
Distilled Water	54.0	54.0	54.0
Total	100	100	100

Preparation RN-TW, RN-CRH and RN-CEL. Preparation of RN-TW was done according to the sonication method with some modification.[18] For this 1.0 gm of rifampicin was dissolved in 5 gm of oleic acid (oil phase) in 20 ml screw cap glass vial and sonicated at 70°C to make a clear solution. Tween 80 (40 gm) was taken in 100 ml glass bottle and heated up to 70°C with sonication. Then rifampicin solution in oleic acid was added into Tween 80 in 100 ml glass bottle. On the other hand, 54 g of water was taken in 100 ml glass bottle and heated at 70°C in heating bath. This hot water was slowly added to previously prepared mixture containing rifampicin, oleic acid and Tween 80 with occasional shaking with a vortex. Cloudy nanoemulsion was formed. Then namoemulsion preparation was cooled at room temperature. Same procedures were followed for RN-CRH and RN-CEL nanoemulsion preparation (Table 1).

Determination of nanoemulsions. Drop dilution test method was used to determine the type of nanoemulsion. 1 ml sample from each formulation was taken into three different test tubes and equivalent amount of distilled water was added to the test tubes. Then miscibility was observed visually.

Determination of droplet size. 250 μl of nanoemulsion was added to 300 ml of distilled water in a 500 ml beaker. A glass rod was used to induce gentle agitation in the mixture. The average droplet size of the nanoemulsions were determined by dynamic light scattering using Zetasizer (Malvern Instruments, Worcestershire, UK) which measures the Brownian motion of the droplet and its relation to the droplet size based on the principle that larger droplets have slower motion.

Determination of drug content. A calibration curve was plotted using the absorbance data against drug concentration. 10 mg rifampicin was taken in 100 ml volumetric flask and methanol was added up

to the mark to obtain concentration of 100 mg/l. Dilution was made to obtain solutions of 5 different concentrations (10 mg/l, 20 mg/l, 30 mg/l, 40 mg/l and 50 mg/l). The absorbance of each solution was measured at 475 nm using ultraviolet spectrophotometer and standard curve (Figure 1) was constructed. The calibration curve was used to estimate drug content of rifampicin nanoemulsions (RN-TW, RN-CRH and RN-CEL).
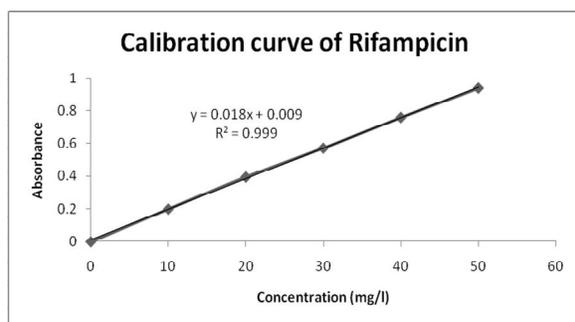

Figure 1. Calibration curve of rifampicin.

Determination of entrapment efficiency. Nanoemulsions containing drug were separated from unentrapped drug by centrifugation at 14000 rpm for 30 minutes and decanted fluid was separated from precipitation. The entrapped drug (1 ml) was disrupted using 3 ml 50% propanol for 5 minutes which was analyzed spectrophotometrically for drug content at 475 nm to calculate the concentration of entrapped drug against 50% propanol as blank. Percentage of entrapped drug was calculated by applying equation (1).[19]

% of entrapment = Ae × 100/ Ai -------------- (1)

Where, Ae is the amount of entrapped drug and Ai is the initial amount of drug in oil phase.

Determination *in vitro* drug release. The release of drug from nanoemulsion formulations was determined by dialysis method.[19] After separating the unentrapped drug, nanoemulsions particles were pipetted into dialysis bag (Visking, DTVO3500). This was placed in 100 ml of phosphate buffer pH 7.4 and kept with constant agitation with magnetic stirrer maintained at 37°C. 2 ml sample was withdrawn every 30 minutes and the same volume of buffer was replaced. Then samples were assayed spectrophotometrically at 475 nm using buffer medium as blank. The release was compared with pure drug solution.

pH stability test. 1 mg entrapped nanoemulsion in 5 ml of phosphate buffer (pH 5.8, 7.00, 8.00) was kept in screw cap test tube for 2 hours at 25 °C and the samples were withdrawn. Samples were analyzed spectrophotometrically at 475 nm.[19]

Antibiotic sensitivity test. Antibiotic sensitivity test was performed by cup diffusion using Mueller Hinton agar plates. Bacterial strains such as *S. epidermidis* and *S. aureus* were obtained from laboratory stock which was identified in our previous study.[20] 50 µg/ 100 µl of rifampicin and nanoemulsion preparations (RN-TW, RN-CRH, and RN-CEL) were applied in different cup. The plates were incubated at 37 °C for 24 hours. Diameter of zone of inhibition was recorded in millimeters and the average diameter of zone of inhibition was taken from duplicate test to determine its sensitivity.[21]

RESULTS AND DISCUSSION

Nanoemulsion type. Each test tube was visually observed after shaking. Water was mixed well and no phase separation could be observed for each test tube which suggested that the outer layer was water indicating formulations belonged to o/w type emulsion.

Droplet size. Droplet size was analyzed by using Zetarsizer and droplet size of different nanoemulsions were found as 192.7 nm, 183.4 nm and 179.2 nm for RN- CEL, RN-CRH and RN-TW, respectively (Figure 2, Table 2). Droplet size of all formulations was within the range and variation occurred due to different composition of each formulation.

Table 2. Droplet size of rifampicin nanoemulsions.

Formulation codes	Average droplet size (nm)
RN-CEL	192.7
RN-CRH	183.4
RN-TW	179.2

Size Distribution Report by Intensity

v2.2   Zetasizer Ver. 7.01
Serial Number : MAL1076973

Malvern

Results   Sample: RN-TW

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	179.2	Peak 1:	256.2	94.7	141.0
Pdl:	0.442	Peak 2:	4406	5.3	919.5
Intercept:	0.960	Peak 3:	0.000	0.0	0.000

Result quality :   Refer to quality report

Size Distribution by Intensity

Results   Sample: RN-CEL

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	192.7	Peak 1:	283.2	86.6	98.84
Pdl:	0.383	Peak 2:	60.16	11.6	13.19
Intercept:	0.961	Peak 3:	5245	1.8	445.1

Result quality :   Refer to quality report

Size Distribution by Intensity

Results   Sample: RN-CRH

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	183.4	Peak 1:	267.3	97.4	145.5
Pdl:	0.433	Peak 2:	4853	2.6	698.9
Intercept:	0.957	Peak 3:	0.000	0.0	0.000

Result quality :   Refer to quality report
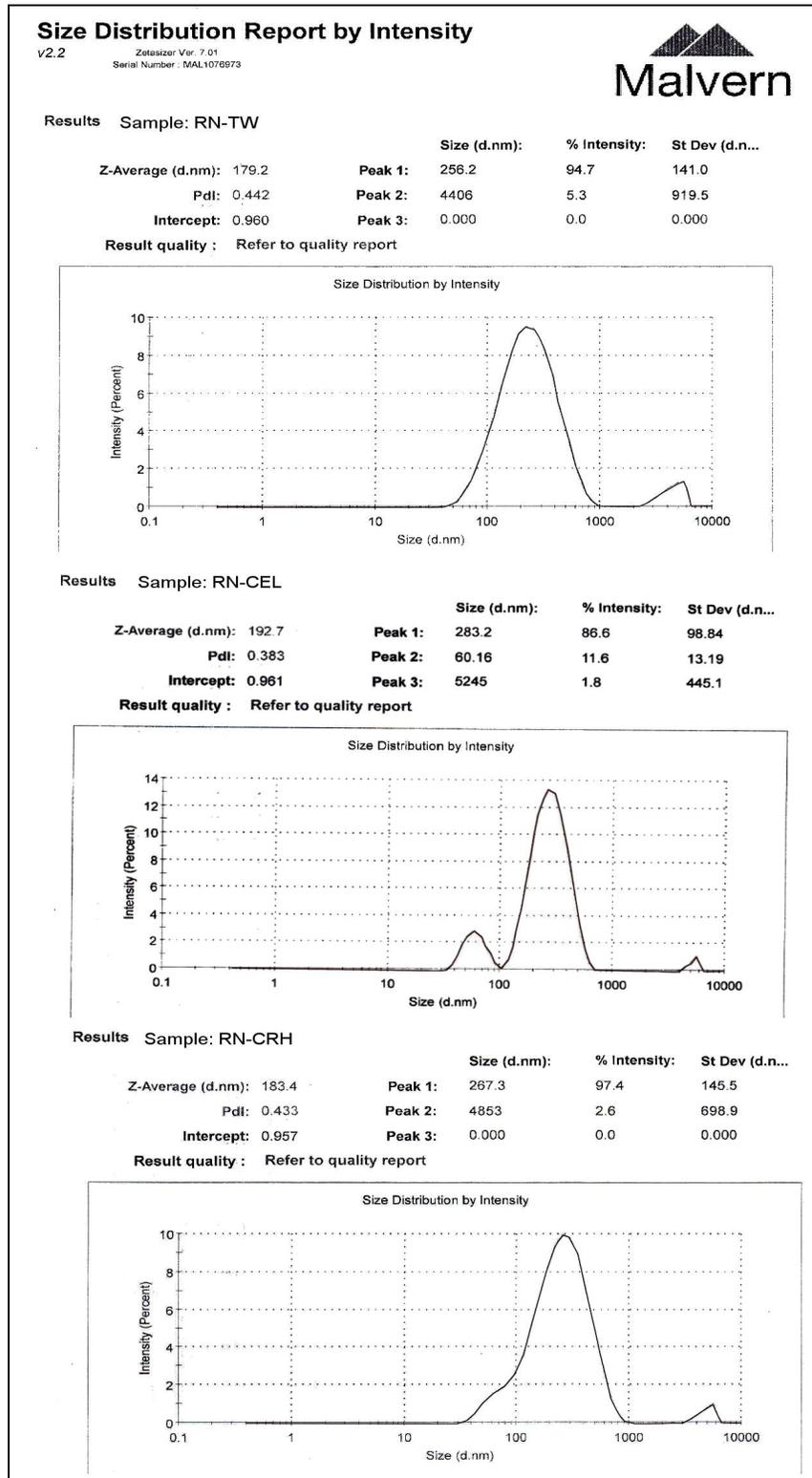
Size Distribution by Intensity

Figure 2. Zetasizer plot of nanoemulsion droplet sizes of RN-TW, RN-CEL and RN-CRH formulaitons.

Drug content. Drug content of each formulation was calculated by using standard curve. Drug contents of RN-TW, RN-CRH and RN- CEL were found as 0.9945%, 0.9835% and 0.9720%, respectively. Drug content for all nanoemulsions was found to be satisfactory (Table 3).

Table 3. Drug content of rifampicin in formulations.

Formulations code	Concentration (mg/l)	Drug content (%w/w)
RN-CEL	19.44	0.9720
RN-CRH	19.67	0.9835
RN-TW	19.89	0.9945

Entrapment efficiency. Results of entrapment efficiency showed that, the highest entrapment efficiency was found in Tween-80 surfactant containing nanoemulsions (84.31%), then Cremophor RH 40 (74.58%) and moderately entrapped when Cremophor EL (56.89%) was used (Figure 3). Becher[22] reported that, o/w nanoemulsion was required emulsifying agents having HLB value ranging from 8-18 and higher HLB values offered greater entrapment clarity efficiency. Skin penetration as well as skin retention of drug depend on percentage of entrapped drugs in nanoemulsion system.[23] Nanoemulsion has been explored extensively for topical application to enhance skin penetration as well as to improve skin retention of drugs.
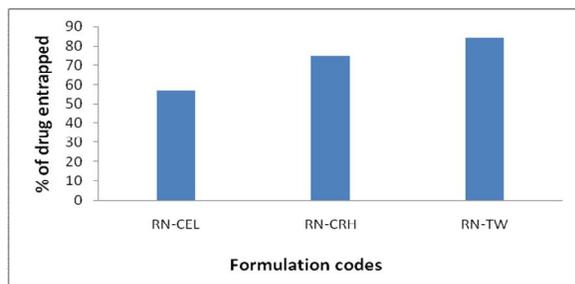

Figure 3. Entrapment efficiency of rifampicin nanoemulsions.

In vitro drug release. Results of _in vitro_ drug release revealed that Tween 80 surfactant containing nanoemulsion (RN-TW) was released drugs for longer time (450 minutes) at a controlled rate (0.33 mg/l per 30 minutes) due to high entrapment efficiency of drugs as compared to other formulations. It was found that 3.3 mg/l standard drug released within 90 minutes whereas RN-CEL, RN-CRH and RN-TW required 150, 240 and 360, minutes respectively. Finally, all standard drugs were released within 150 minutes but equivalent amount of drug in RN-CRH, RN-TW could be released in 300 minutes and 450 minutes, respectively (Figure 4). From this study, it was suggested that Tween 80 surfactant containing nanoemulsions offered better extended release of drugs at controlled rate when compared with other formulations.
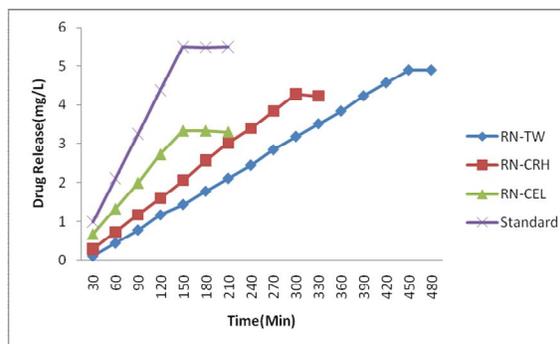

Figure 4. _In vitro_ drug release of rifampicin standard and different rifampicin nanoemulsions.

pH stability test. In this study, all preparations retained maximum drugs at pH 5.8 which indicated that formulations were stabled at pH 5.8 (Figure 5). Barry[24] reported that, delivery of drug at skin highly depends on pH. Therefore, the preparations were suitable for application in skin and will easily penetrate through the skin to reach the target site.
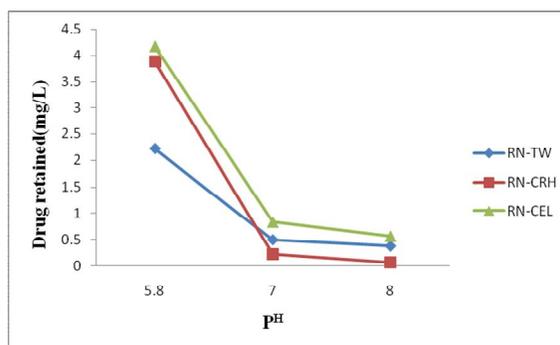

Figure 5. pH stability test for rifampicin nanoemulsions.

Antibacterial sensitivity test. All rifampicin preparations showed strong antibacterial effect against *S. epidermidis* and *S. aureus* isolated from acne when compared with standard rifampicin (Figure 6). The results of our *in vitro* studies of this work are encouraging.
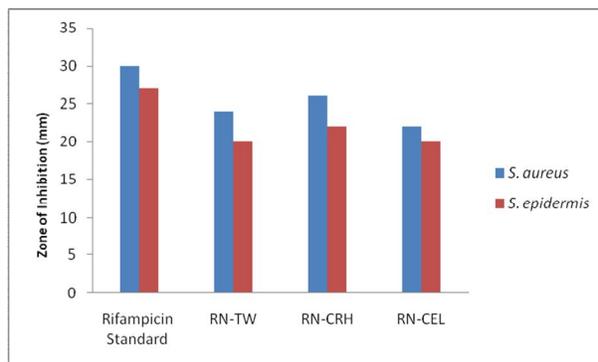

Figure 6. Zone of inhibition for standard rifampicin and rifampicin nanoemulsions.

CONCLUSION

Rifampicin nanoemulsion preparations were found to be effective against *S. epidermidis* and *S. aureus* isolated from acne. Our study suggests that the rifampicin nanoemulsion formulation has a greater potential in acne. The formulations have shown satisfactory results of particle size, entrapment efficiency, *in vitro* drug release and pH stability. This study revealed that nanoemulsion preparetions provide consistent and prolonged release of rifampicin. However, extensive research is required for optimizing its future applications.

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