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**ORIGINAL ARTICLE** **OPEN ACCESS**

# Design and Characterization of Pulsatile Drug Delivery of S-SNEDDS of Rifaximin

**Siddhi Bhairavkar[1], Madan Pomaje[1*], Ashwini Patil[1], Viraj Kamat[1]**
[1]Govindrao Nikam College of Pharmacy, Sawarde, Ratnagiri, Maharashtra 415606, India
*Corresponding author email: madanpomaje@gmail.com

**ABSTRACT**

*Rifaximin is a gastrointestinal-selective antibiotic, used in the treatment of inflammatory bowel disease (IBD). Due to their ability to alter the dysbiotic colonic microbiota and reduce immunogenic and inflammatory responses in patients, antibiotics are showing potential in the treatment of IBD. Pulse-in-cap system containing SNEDDS provide the colon targeted delivery and enhanced the antibacterial action of the drug. SNEDDS made Rifaximin available in soluble form and enhance its solubility in region where it has very poor solubility and enhance its antibacterial effect at lower doses. The objective was to formulate and evaluate SNEDDS of Rifaximin for colon specific drug delivery and study its effectivity. The study includes preparation and characterization of drug excipient compatibility by FTIR. The prepared L-SNEDDS were characterized for the emulsification time, antibacterial activity, particle size and zeta potential by photon correlation spectroscopy and also analyzed by SEM. Selected best L-SNEDDS formulation was then converted into S-SNEDDS by using Aerosil 200 as a solid carrier and targeted to the colon by using pulsincap system. Prepared pulsincap system containing S-SNEDDS formulation was then subjected to the in vitro drug release study. The study demonstrated that the F6 formulation is the optimum formulation in terms of SNEDDS characterization and hydrodynamic size with a hydrodynamic size of 74.7 nm and the zeta potential of –38.2 mV. Additional parameters, including emulsification time, invitro drug release, antibacterial activity and SEM, were also reported. The study concluded that pulsincap system containing SNEDDS of Rifaximin (F6) has potential for the colon specific delivery which enhanced the activity of Rifaximin and reduced the inflammatory condition in IBD by inhibiting the bacterial overgrowth.*

***Keywords:*** *Solid-Self-nanoemulsifying drug delivery system (S-SNEDDS), Rifaximin (RFX), Pulsincap delivery, HPMC K100M, Eudragit S 100, Anti-bacterial activity, Inflammatory Bowel Disease.*

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## INTRODUCTION

Many different bacterial infections frequently infect people with their diseases in the colon. A number of diseases, including inflammatory bowel disease, are significantly influenced by the microbiota. The immune reactions to bacteria in the gastrointestinal tract leads to the development of inflammatory bowel disease (IBD) [1, 2] which causes gastrointestinal inflammation and digestive problems. Weight loss, rectal bleeding, consistent diarrhoea and stomach discomfort are a few of the signs and symptoms of CD and UC. They are mostly characterized by inflammation[3]. The most common and distressing symptom of IBD is recurrent, bloody diarrhoea [4, 5]. Inflamed mucosa has a decreased variety in mucosa-associated bacteria with a significant decrease in Bacteroides and Firmicutes, which are linked to anti-inflammatory characteristics and an increase in Enterobacteriaceae, Escherichia coli and Fusobacterium[6-9]. These alterations in the microbiota refers to "Dysbiosis".

Remission and maintenance of remission are the primary goals of IBD therapy, however finding an effective cure is still difficult. Although there are basic treatment options available, they do not successfully maintain remission, hence innovative medication therapies delivered by means of drug delivery systems are strongly advised [10]. By supporting beneficial bacteria, antibiotics have the ability to change the composition of the gut microflora [11]. Antibiotics are crucial in IBD because they can penetrate intracellular sites where bacteria are lodged and lessen their negative effects [12]. For the treatment of IBD, rifaximin recently received FDA approval [13].

Numerous studies have shown that rifaximin has the ability to treat IBD [14]. It is becoming more significant in IBD situations because it has broad range effectiveness and few adverse effects [15]. However, rifaximin demonstrates relatively little antibacterial efficacy against intracellular infections because of several inherent features including low permeability and low solubility. Therefore, SNEDDS have been developed with the goal of improving solubility and therapeutic effectiveness of rifaximin at the inflamed

region. The use of SNEDDSs are a critical strategy which combines the advantages of LBDDS with nanotechnology [16]. SNEDDS are widely recognized for their ability to improve the solubility and absorption of lipophilic medicines by increasing the surface area and lowering the size of oil droplets that are easily digested and integrated into mixed micelles that may cross the intestinal lumen [17]. Additionally, SNEDDS have been linked to an increase in trans-cellular permeability due to their capacity to improve the lipid fluidity of enterocyte membranes and block efflux pumps, both of which increased oral bioavailability. However, there are a few issues with liquid SNEDDS when they are placed inside capsules [18]. Therefore L-SNEDDS are then converted into the S-SNEDDS by using adsorption on solid carrier method. S-SNEDDS is the perfect stable formulation to put inside of hard gelatin capsules to serve as a dosage unit container [19].

In this research work, the colon-specific pulsincap containing S-SNEDDS of rifaximin for effective treatment of inflammatory bowel disease were prepared. This system is based on an impermeable capsule containing a rifaximin-loaded S-SNEDDS inside it and HPMC K100M plugs in the capsule mouth. An in vitro release study of the pulsincap formulation is showing a release profile with a specific lag time. The lag time determines how effective colon-specific delivery is and results showing that the pulsincap with SNEDNDS has potential for the colon-specific delivery of Rifaximin.

## Material and Methods

### Material

Rifaximin was obtained as a gift sample from Lupin Ltd, Aurangabad, India. All other chemicals used in the research work are provided by the institution which were purchased from Loba Chemie Pvt. Ltd., Mumbai and buffer solutions (phosphate buffer pH 6.8, phosphate buffer pH 7.4 and 0.1N HCl) were freshly prepared.

### Methods

### Preformulation Studies

### UV spectroscopy: [20]

10 mg standard Rifaximin was correctly weighed and transferred to a 100 ml volumetric flask, where it was dissolved and diluted up to the mark using phosphate buffer pH 6.8 to produce a standard solution with a concentration of Rifaximin (100 µg/ml). After proper dilution the solution was scanned in the range of 200-800 nm and absorption maximum was determined.

### Calibration curve of Rifaximin in different buffer solutions:[21, 22]

About 10 mg of Rifaximin was accurately weighed and dissolved in 100 ml of 0.1 N HCl to give stock solution of 100 µg/ml. From this standard stock solution, different concentration ranging from 2-14 µg/ml were prepared by transferring required aliquots of the solution to 10ml volumetric flask and the volume was made up with 0.1 N HCl. These were sonicated for 10 mins and then filtered out. One of the above solution was scanned in UV range using 0.1 N HCl as a blank and wavelength of maximum absorption was found to about 440 nm. The absorbances of different concentrations were measured at 440 nm using 0.1 N HCl as a blank. Calibration curve was plotted between absorbance vs. concentration. The same procedure was carried out using phosphate buffer pH 6.8 and phosphate buffer pH 7.4 as the SNEDDS formulation designed for colon targeting.

### Drug-Excipients Compatibility studies:

Compatibility of the drug with different excipients was tested using FTIR and DSC methods.

### A. Fourier Transmittance Infra-Red (FTIR)

FTIR spectrum analysis was performed to confirm the compatibility of the pure drug rifaximin with the different excipients used in development of rifaximin loaded SNEDDS.

### B. Differential scanning Calorimetry (DSC)

The difference in the amount of heat required to raise the temperature of a sample and reference is measured as a function of temperature using a thermal analytical technique known as a differential scanning calorimeter or DSC. Throughout the experiment, both the sample and reference were mentioned at temperatures close to sample temperature.

### Formulation Studies

### Determination of solubility of drug in different oils, surfactants and co-surfactants:

### A. Solubility of drug in different oils: [19]

Solubility of rifaximin in different oils, surfactants and co-surfactants was measured using the Shake Flask method. An excess amount of rifaximin was introduced into 2 ml of each excipient. The vials containing mixture were tightly stoppered and stirred for 10 minutes in a vortex mixer. After a thorough mixing, the samples were kept in a mechanical shaker (3000 rpm) for 48 hr at room temperature. Each vial was then centrifuged at 15,000 rpm for 10 min followed by removal of the undissolved drug by filtering with a membrane filter (0.45µm). The filtered supernatant liquid was diluted suitably with methanol and the

absorbance was determined by using a double-beam UV visible spectrophotometer at λmax 440nm to determine the amount of drug dissolved in the selected solvents. Solubility of rifaximin in selected oils, surfactant and cosurfactant was studied and expressed as mg/ml.

**B. Screening of surfactant and Co-surfactant: [23]**

**Surfactant (emulsification study):** Surfactant selection was performed on the basis of %transparency and ease of emulsification. 300 mg of the surfactant was added to 300 mg of the selected oily phase. The mixtures were gently heated at 50°C for homogenization of the components. From each mixture, 50 mg was taken and diluted with distilled water to 50 ml in a stoppered conical flask. Ease of emulsification was observed visually by the number of flask inversions required to yield a homogenous emulsion. The emulsions were allowed to stand for 2 hrs and their % transparency was evaluated at 440 nm by a double-beam UV spectrophotometer using distilled water as a blank. The surfactant which forms a clear emulsion with lesser number of inversions and with more transmittance was selected.

**Co-surfactant (emulsification study):** Mixtures of 100 mg of the co-surfactant, 200 mg of the selected surfactant and 300 mg of the selected oil were prepared and evaluated in a similar fashion as described in the above section of surfactant selection.

**Construction of pseudo-ternary phase diagrams with different ratios of surfactant and co-surfactant: [24, 25]**

Pseudo-ternary phase diagrams were constructed to identify the self-nanoemulsifying regions and to optimize surfactant to co-surfactant ratio and the concentration of oil. Pseudo-ternary phase diagrams of oil, surfactant/co-surfactant and water were developed using the water titration method. The procedure consisted of preparing solutions of different ratio of surfactant to co-surfactant by weight such as 1:1, 2:1, 1:2, etc. these solutions then vortexed for 5 min. and placed at 50°C for one hour, so that an isotropic mixture was obtained. Each of these solutions were used for preparing a mixture containing oil and Smix in the following ratios by weight, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 and after preparation vortexed for 5 mins. Then each mixture of oil and S/CoS was titrated with water and visually observed for phase clarity and flowability. The concentration of water at which transparency-to-turbidity and turbidity-to-transparency transitions occurred was derived from the weight measurements. These values were then used to determine the boundaries of the self-emulsifying domain corresponding to the chosen values of oils, as well as the S/CoS mixing ratio. The size of nanoemulsion region in the phase diagrams was compared; the larger the size the better the self-nanoemulsifying property.

**Preparation of Rifaximin containing L-SNEDDS:**[23]

From the pseudoternary phase diagram ratio of surfactant to co-surfactant was optimized. Then by varying amount of oil to Smix, different formulations were prepared. Formulations were prepared by preparing optimized ratio of Smix first, for this surfactant (Tween 80) and co-surfactant (Propylene glycol) were accurately weighed and then vortexed for 5-10 mins. After that Smix was placed in oven at 50°C for one hour. Rifaximin (10 mg) was added in accurately weighed amount of oil (Clove oil) into a screw-capped glass vial and melted in a water bath at 37°C. Then Smix was added to the oily mix using a positive displacement pipette and stirred with a magnetic stirrer. The formulations were further sonicated for 15 min to obtain a clear solution and stored at room temperature until their use.

**Characterization of L-SNEDDS: [26, 27]**

**A. Visual assessment, Self-emulsification time and Dispersibility test**

The self-emulsification efficiency of SNEDDS was evaluated using a standard USP dissolution apparatus type II. 1 mL of each formula was added to 500 mL of distilled water maintained at 37±0.5°C. Gentle agitation was provided by a standard stainless steel dissolution paddle rotating at 50 rpm. The prepared formulae were assessed visually according to the rate of emulsification and final appearance of the nanoemulsion. The in vitro performance of the formulation was visually evaluated using the following grading system.

**Grade A:** Rapidly forming (within 1 min) Nano emulsion, having a clear or bluish appearance.

**Grade B:** Rapidly forming, slightly less clear emulsion, having a bluish white appearance.

**Grade C:** Fine milky emulsion that formed within 2 min

**Grade D:** Dull, greyish white emulsion having slightly oily appearance that is slow to emulsify (longer than 2 min).

**Grade E:** Formulation, exhibiting either poor or minimal emulsification with large oil globules present on the surface.

**B. Robustness to dilution**

This test was performed by diluting 1 mL of each formula to 10, 100 and 1000 times with distilled water, phosphate buffer pH 6.8 and phosphate buffer pH 7.4. The diluted systems were mixed using a magnetic stirrer at 100 rpm and 37°C to simulate body temperature to complete homogeneity. These systems were

stored at an ambient temperature for 24 hr then visually observed for any signs of phase separation or drug precipitation.

**C. Absolute drug content in L-SNEDDS**

Liquid SNEDDS containing Rifaximin, equivalent to 2 mg was diluted in suitable quantity of methanol. The sample was mixed thoroughly to dissolve the drug in methanol by stirring and filtered through whatman filter paper. The above solution was analyzed in UV Spectrophotometer at λmax 440nm by keeping blank nanoemulsion.

**D. Determination of Globule size and Polydispersibility Index**

The globule size and polydispersibility index of L-SNEDDS were determined by dynamic light scattering (DLS) using a photon correlation spectroscopy (which analyse the fluctuations in light scattering due to Brownian motion of the particles) with a Nano sizer SZ-100 (Horiba nano size analyzer, Japan) able to measure size between 10-3000 nm.

**E. Determination of Zeta potential**

Zeta potential of Rifaximin SNEDDS formulation was determined by dynamic light scattering technique using a zeta size analyser.

**Conversion of liquid SNEDDS into solid SNEDDS:[28]**

Solid SNEDDS were prepared by mixing L-SNEDDS containing Rifaximin with Aerosil 200 in 1:1 proportion. Liquid SNEDDS was added drop wise over Aerosil 200 and homogenized using glass rod to ensure uniform distribution of formulation in a china dish. Resultant damp mass was passed through sieve no. 120 and dried at ambient temperature and stored until further use.

**Evaluation of S-SNEDDS of Optimised Formulation F6:[27, 29]**

**Micromeritic properties:**

Various micromeritic properties (bulk density, tapped density, angle of repose, Carr's index, and Hausner's ratio) of the prepared S-SNEDDS were determined.

**Infrared (IR) spectroscopic studies:**

Infrared (IR) spectrum analysis is carried out for the selected formulation of S-SNEDDS to find out the compatibility between the drug and excipients by using FTIR spectrophotometer.

**Morphological studies of S-SNEDDS by using Scanning Electron Microscopy (SEM)**

Morphological evaluation of the selected rifaximin loaded S-SNEDDS formulation was carried out in scanning electron microscope (SEM).

**Differential Scanning Calorimetry:**

Physical state of rifaximin in S-SNEDDS was characterized using differential scanning calorimeter.

**Anti-bacterial activity of Pure drug and S-SNEDDS of Rifaximin:**

The anti-bacterial activity of pure drug and S-SNEDDS of Rifaximin was performed by using cup plate method. Nutrient agar medium in distilled water was prepared and sterilise that media in autoclave at $121^0$C for 15 min. Then transferred that media aseptically in sterile Petri plate, allow the media to solidify for few minutes. After solidification, add test micro-organism i.e., *E. Coli* (100µl) in each plate and culture was spread with the help of spreader. Then wells were prepared in plate with the help of borer. Add the test sample (pure drug and S-SNEDDS) 200µl in each well aseptically. Plates were kept in an incubator at $37^0$C for 24 hrs and then results were observed for the zone of inhibition around the well.

**Comparative in vitro release profile of pure drug and S-SNEDDS of F6**

Drug release study of pure drug and S-SNEDDS was performed using USP Type II apparatus with 900 ml pH 6.8 phosphate buffer as a medium at 37±0.5°C. Rotating speed of the paddle was adjusted to 50 rpm. Rifaximin loaded S-SNEDDS (equivalent to 10 mg Rifaximin) and Pure drug Rifaximin 10 mg loaded capsules were placed in dissolution apparatus and at predetermined time intervals of 1h, 2h,3h and 4 h; an aliquot 5 ml of sample was collected. The withdrawn sample was replaced by equal volumes of dissolution medium to maintain the volume and sink conditions constant. The sample was then filtered and analysed for the % drug release by UV Spectroscopic method.

**2.2.7 Preparation of polymer plugs:[30, 31]**

The polymer plugs which were used for plugging the opening of capsule bodies were selected by swelling index (hydroxyl ethyl cellulose, hydroxypropyl methylcellulose K 100 M, chitosan, MCC, hydroxypropyl methylcellulose K 4 M, hydroxypropyl methylcellulose E50).

**Swelling Index of Polymers**

Different polymer of 10 gm was weighed, the initial weight of the polymer was recorded ($W_0$) and then it was kept in a 100 ml measuring cylinder containing 100 ml water. Kept it for 12 hrs and excess of water was carefully removed and swollen films were re-weighed (Wt). Then the percentage swelling was calculated by following formula,

**% Swelling Index =Wt-Wo / Wo x 100**

Where,
Wt - Weight of swollen matrix at time t;
Wo- Initial weight of matrix

**Lag Time**

Lag time was determined visually using 0.1N HCL, phosphate buffer pH 6.8 and pH 7.4. For lag time determinations USP Type II (paddle) apparatus was used.

**2.2.8 Preparation of water insoluble hard gelatine cap body and enteric coated cap of hard gelatine capsule**

**Preparation of water-insoluble hard gelatin cap body:[30]**

Weighed required quantity of ethyl cellulose dissolved in sufficient qty of acetone (1:10 proportion). Dipped body of hard gelatin capsule in the above solution, took out, dried, again dipped. Repeated this procedure of coating up to 50% weight gain.

**Preparation of enteric coated cap of hard gelatine capsule: [32]**

The enteric coating solution was composed of Eudragit S100 in acetone (12% w/v). Dipped cap of hard gelatin capsule in the above solution, took out, dried, again dipped. Repeated this procedure of coating up to 20 % weight gain.

**Development of Pulsincap dosage form containing S-SNEDDS of Rifaximin:[33-36]**

The capsule body and cap were completely coated with water-insoluble ethyl cellulose and Eudragit S100 respectively. Dip coating method was used for coating the capsule body and cap. This coating was given in order to prevent variable gastric emptying. SNEDDS of Rifaximin were filled manually into the ethyl cellulose treated capsule bodies. The opening of capsule bodies containing SNEDDS was then closed by plugging it with selected polymer. Further, a small amount of 5% ethanolic solution of ethyl cellulose was used to seal the joint between the capsule body and cap.

***In vitro* study of pulsincap formulation**

The pulsincap formulations were evaluated for dissolution testing in three dissolution media i.e., pH 1.2, pH 7.4 and pH 6.8 for a period of 2, 3 and further 4 hours respectively. These three-dissolution media are used to match the pH change criteria of the GI tract. The apparatus used for dissolution testing is USP type I apparatus (i.e., basket type). Capsules were placed in a basket so that the capsule should be immersed completely in dissolution media but do not float. In the first 2 hours, 900 ml of 0.1 N HCl of pH 1.2 dissolution medium was used and then test was subsequently continued for next 3 hrs. in 900ml of buffer solution of pH 7.4. After 3 hrs (average small intestinal transit time is 3 hrs) the medium was removed and further study was continued for next 4 hrs. in 900 ml of freshly prepared buffer solution of pH 6.8. Rotating speed of the basket in dissolution media was maintained at 50rpm and the temperature was maintained at 37±0.5$^0$C. At predetermined intervals of time, 5ml of dissolution media was taken and replaced with fresh dissolution media to maintain the sink conditions. The withdrawn samples were analyzed at 440nm to determine drug release in 1.2, 7.4 and 6.8 pH buffers respectively by using UV spectroscopic method.

## RESULT AND DISCUSSION

**Preformulation Studies:**

**Determination of λmax:**

The absorption maximum of Rifaximin was found to be 440 nm in phosphate buffer pH 6.8 and this wavelength was selected and utilized for further studies (Figure 1).

**Calibration curve of Rifaximin:**

The absorbance data of the standard solutions are shown in table 1.

The values of Abs. (absorbance) were plotted against respective concentrations (Figure 2). The conc. showed linearity when the curve was plotted indicating it obeyed Beers lambert law. The regression coefficient value was found to be 0.9923 in 0.1N HCl, 0.9922 in phosphate buffer pH 6.8 and 0.9855 in phosphate buffer pH 7.4.

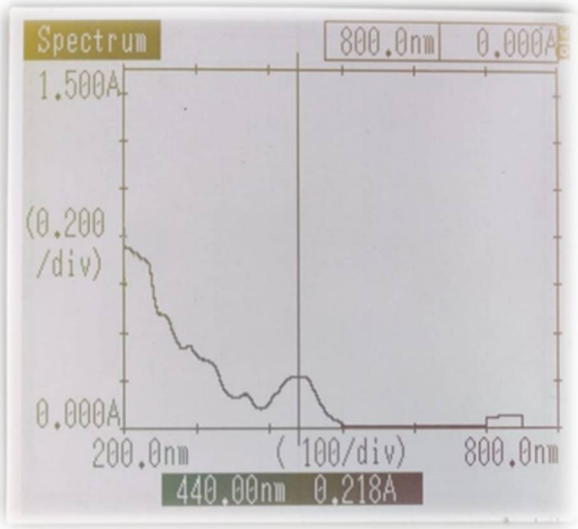



**Figure 1: UV Spectra of Rifaximin**

**Table 1: Calibration curve of Rifaximin in different buffer solution**

| Conc. (mcg/ml) | Abs. (0.1N HCl pH 1.2) | Abs. (pH 6.8 Buffer solution) | Abs. (pH 7.4 buffer solution) |
|---|---|---|---|
| 0 | 0 | 0 | 0 |
| 2 | 0.11 | 0.107 | 0.111 |
| 4 | 0.154 | 0.182 | 0.16 |
| 6 | 0.221 | 0.205 | 0.231 |
| 8 | 0.282 | 0.275 | 0.318 |
| 10 | 0.334 | 0.355 | 0.349 |
| 12 | 0.422 | 0.416 | 0.402 |
| 14 | 0.499 | 0.486 | 0.492 |

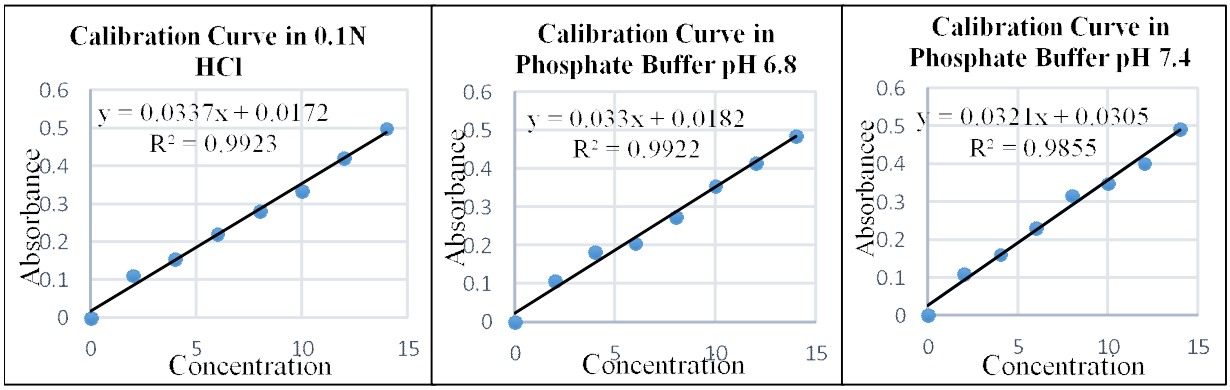


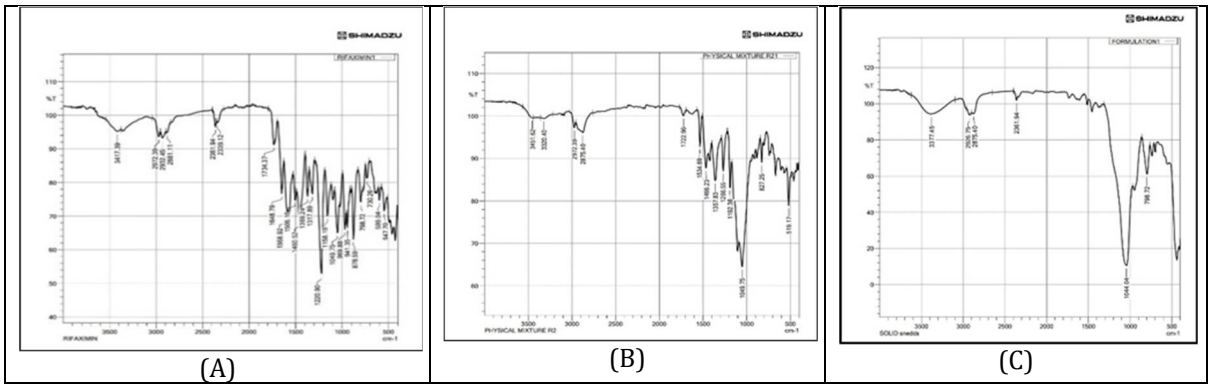

**Figure 2. Calibration curve of Rifaximin in 0.1N HCl, Phosphate buffer pH 6.8 and buffer pH 7.4**

**Drug-Excipients Compatibility Studies**

**A. Fourier Transformed Infrared (FT-IR) Spectroscopic Analysis**

The drug rifaximin showed the significant bands at 3417.39 cm-1 for OH stretch. C-N stretching was observed at 1158.15 cm-1. The peak for ester linkages was observed at 1734.37 cm-1 and C-H stretching was marked at 2972.39 cm-1 and 2881.11 cm-1. The O-C stretching and CH bending was observed at 1049.75 cm-1 and 798.72 cm-1 respectively. The physical mixture demonstrated the distinct peak representing -OH stretching at 3451.62 cm-1, ester linkage at 1722.96 cm-1, C-N stretch and C-H bending was observed at 1192.38 cm–1 and 827.25 cm–1 respectively. The peak for C-H stretching was marked at 2972.39 cm-1 and 2875.40 cm-1. The O-C stretching was also found at 1049.75 cm-1. These results indicated that excipients were chemically compatible with the rifaximin drug. The final formulation of S-SNEDDS containing Rifaximin, Clove oil, Tween 80, Propylene glycol and Aerosil 200 showed significant peaks at 3377.45 cm-1 for OH stretching, 2926.75 cm-1 and 2875.40 cm-1 for C-H stretching, 1044.04 cm-1 for O-C stretching and 798.72 cm-1 for CH bending which rules out any interaction between the drug and

excipients used in the S-SNEDDS formulation. The FTIR spectrum of the drug with different excipients mixture showed the drug is in its intact form in physical mixture as well as in the final formulation. The results are shown in figure 3.

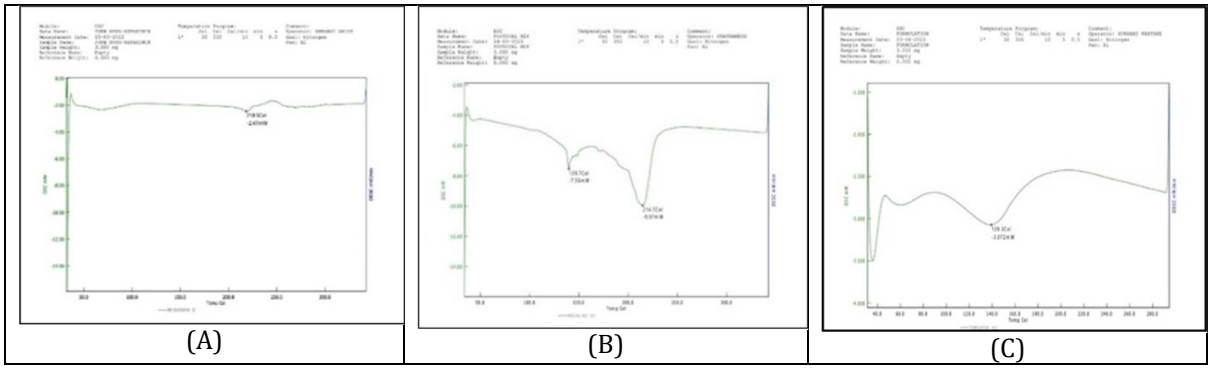


(A) (B) (C)

**Figure 3: FTIR spectra of (A) Rifaximin pure drug, (B) Physical mixture, and (C) Final formulation**

**B. Differential Scanning Calorimetry**

The thermal behaviours of (A)pure drug, (B)Physical mixture and (C)final formulation are shown in figure 4. The DSC thermogram of Rifaximin showed a characteristic endothermic peak at 218.5$^0$C. The DSC thermogram of physical mixture containing Clove oil, Tween 80, Propylene glycol and Aerosil200 showed the characteristic peak at 214.7$^0$C. The DSC analysis of pure drug and physical mixture revealed that there was negligible change in the melting point of Rifaximin when mixed with other excipients, indicating no interaction between the drug and excipients. The DSC thermogram of prepared SNEDDS formulation showed a characteristic endothermic peak at 139.3$^o$C which revealed that there was moderate change in the melting point of rifaximin when mixed with other excipients, indicating some modification or interaction between the drug and excipients in the final formulation.



(A) (B) (C)

**Figure 4: DSC of Rifaximin drug, Physical Mixture and Final formulation**

**Formulation Studies**

**Determination of solubility of drug in different oils, surfactants and co-surfactants**:

Solubility of rifaximin in various vehicles was screened and the results are presented in figure 5. Rifaximin had significantly higher solubility in Clove oil 475.00 mg/ml, Cinnamon oil 425.25 mg/ml and Mentha oil 201.50 mg/ml than other oils like Oleic acid, Anise oil, Peppermint oil, Lavender oil, Isopropyl myristate etc. Among surfactant and co-surfactants, Tween 80 (56.50mg/ml) and Propylene glycol (71.36mg/ml) showed highest solubility. Therefore, Clove oil, Cinnamon oil and Mentha oil were selected as oil phase based on solubility studies.

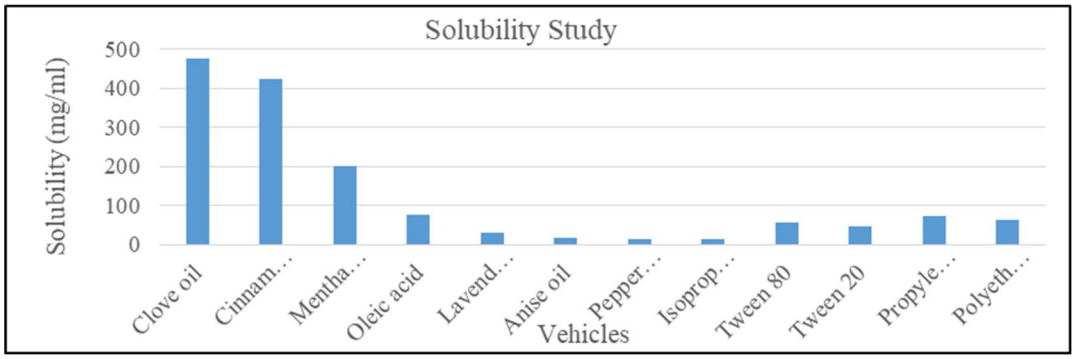



**Figure 5: Graph of Solubility Study**

**Screening of Surfactant and Co-Surfactant:**

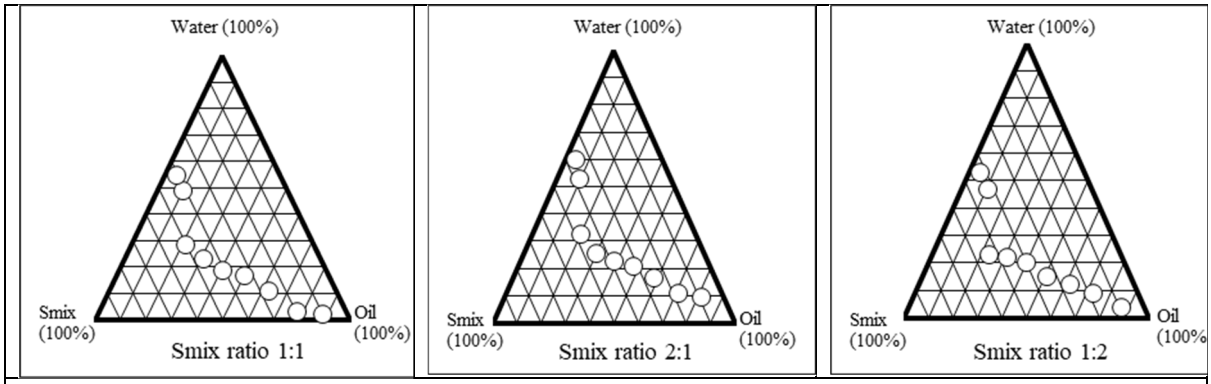
Surfactants and co-surfactants are selected based on the % Transmittance. Out of various surfactants and co-surfactant screened Tween 80 and propylene glycol showed the highest value amongst all. Hence Tween 80 was selected as a surfactant to form a stable nanoemulsion and Propylene glycol was selected as a co-surfactant for the development of the formulation. The results were shown in Table 2.

**Table 2: % Transmittance values of Surfactants and Co-Surfactants**

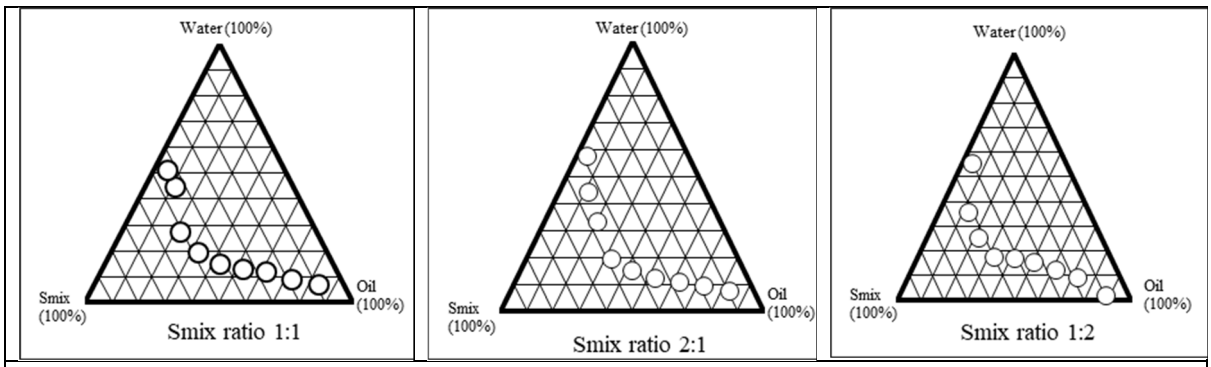
| Sr. no. | Components | % Transmittance Value | | |
|---|---|---|---|---|
| | | Clove oil | Cinnamon oil | Mentha oil |
| 1. | Tween 80 | 95.49 | 82.98 | 70.02 |
| 2. | Tween 20 | 92.20 | 80.89 | 42.36 |
| 3. | Propylene glycol | 97.05 | 87.70 | 81.84 |
| 4. | Polyethylene glycol 200 | 95.27 | 85.31 | 77.80 |

**Construction of Pseudo ternary phase diagram:**

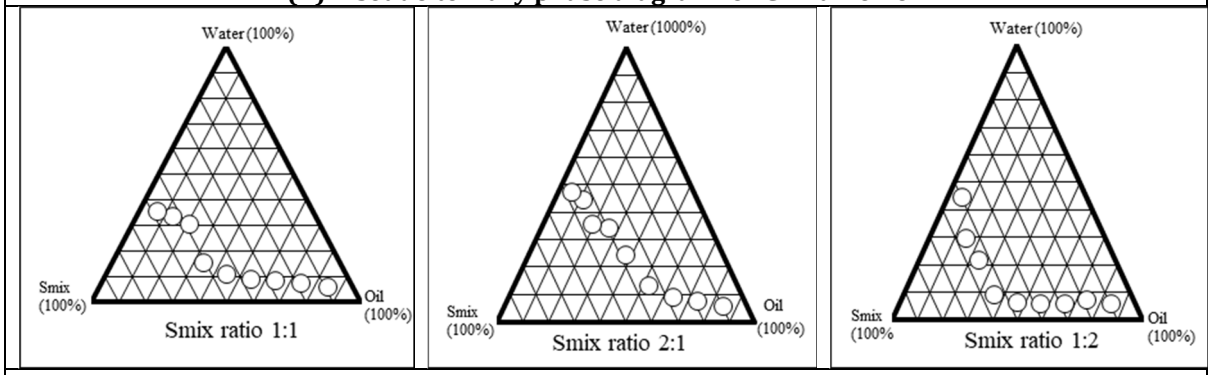
The nanoemulsion region was determined by plotting data in pseudo ternary phase diagram. The selected oils, surfactants and co-surfactants were used to formulate nanoemulsion. Nine different combination of oils and Smix were selected to construct phase diagram for formulation. The ratio of Smix was selected as 1:1, 2:1, 1:2. The diagrams are depicted in figures 6(A), 6(B) and 6(C). From the figures Clove oil showed that Smix ratio of 2:1 (Figure 6A) has more emulsification area. Hence for the formulation of SNEDDS, Clove oil was selected with the ratio of surfactant mixture 2:1.




**(A): Pseudo ternary phase diagram for Clove oil**




**(B): Pseudo ternary phase diagram for Cinnamon oil**




**(C): Pseudo ternary phase diagram for Mentha oil**

**Figure 6. Pseudo ternary phase diagrams**

**Formulation of Liquid SNEDDS**

Based on the pseudo ternary phase diagrams, the formulation with the best self-nanoemulsifying properties, containing Clove oil with Smix of Tween 80 and Propylene glycol with ratio (2:1) were formulated with varying amount of oil, surfactant and co-surfactant as shown in table 3.

**Table 3: Formulation of Liquid SNEDDS**

| Formulation | Drug (mg) | Oil (mg) | Smix 2:1 (mg) |
|---|---|---|---|
| F1 | 10 | 15 | 35 |
| F2 | 10 | 15 | 58.33 |
| F3 | 10 | 15 | 81.66 |
| F4 | 10 | 25 | 35 |
| F5 | 10 | 25 | 58.33 |
| F6 | 10 | 25 | 81.66 |
| F7 | 10 | 35 | 35 |
| F8 | 10 | 35 | 58.33 |
| F9 | 10 | 35 | 81.66 |

**Characterization of Liquid SNEDDS**

**A. Visual assessment and Dispersibility test and Self emulsification time**

The formulation F1, F2, F4, F6 and F8 emulsifies rapidly (in less than a minute) having a clear appearance. Therefore, they belong to grade A category. While formulation F3, F5, F7 and F9 emulsifies in less than 2 min having rapidly forming, slightly less clear emulsion, belong to grade B category.

**B. Robustness to dilution**

After diluting L-SNEDDS to 50, 100 and 1000 times with water, buffer pH 7.4 and pH 6.8 and storing for 12hr, it was observed that there was no sign of phase separation or drug precipitation in formulations.

**C. Absolute drug content in L-SNEDDS**

Drug content was determined by measuring the absorbance in UV spectrophotometer. The results are in the range of 94.83% - 98.37%. The results are represented in Table 4. From the results, formulation F6 (98.37%) show the highest drug content amongst all the other formulations.

**D. Determination of Globule size and Polydispersibility Index**

The result of Globule size and Polydispersibility index of the optimized formulation was shown in Table 4 and Figure 7. The Globule size of the L-SNEDDS was found in range of 74.7 nm to 202.0 nm and polydispersibility index in range of 0.356-0.750. The formulation F6 shows lowest globule size which is 74.7 nm with polydispersibility index of 0.356. Since the value of polydispersibility index is less than 1 indicates uniform distribution of droplets throughout the formulation.

**Table 4: Globule size, Polydispersibility Index and Absolute drug content in L-SNEDDS**

| Sr. no. | Formulation | Droplet Size (nm) | PDI | Drug content (%) |
|---|---|---|---|---|
| **1.** | **F1** | 184.0 | 0.750 | 95.70 |
| **2.** | **F2** | 119.2 | 0.497 | 94.93 |
| **3.** | **F3** | 160.2 | 0.540 | 96.30 |
| **4.** | **F4** | 202.0 | 0.465 | 94.83 |
| **5.** | **F5** | 164.1 | 0.402 | 96.85 |
| **6.** | **F6** | 74.7 | 0.356 | 98.37 |
| **7.** | **F7** | 199.6 | 0.425 | 97.40 |
| **8.** | **F8** | 197.3 | 0.360 | 96.30 |
| **9.** | **F9** | 116.6 | 0.370 | 97.60 |

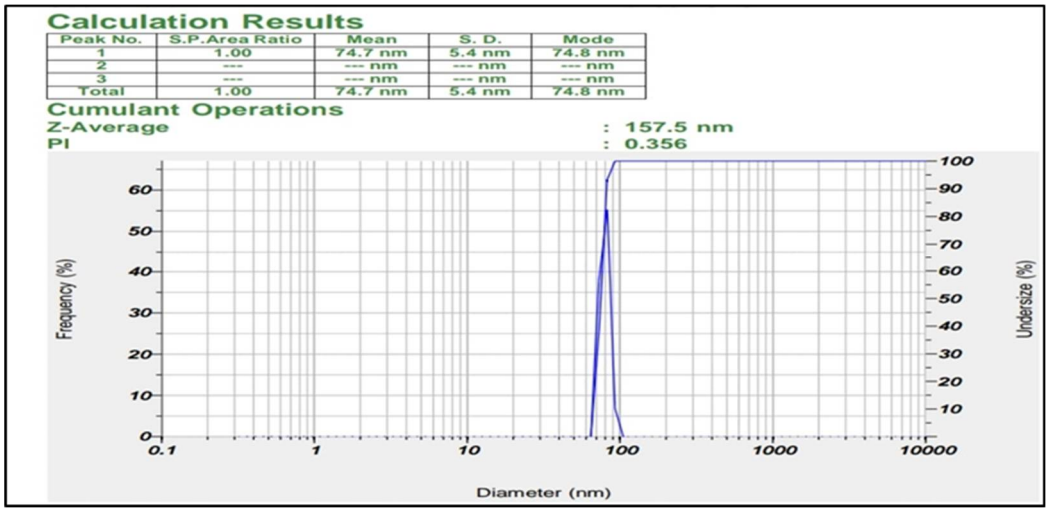



**Figure 7: Globule size and Polydispersibility Index of Formulation F6**

**Selection of best L-SNEDDS formulation to convert into S-SNEDDS**

From all above result, formulation F6 was selected as best formulation as it emulsifies rapidly (in less than a minute) having a clear appearance and there was no sign of phase separation or drug precipitation in formulations also formulation F6 show the highest drug content (98.37%) from all the other formulations as well as the globule size of the F6 was found to be 74.7 nm with polydispersibility index of 0.356. hence formulation F6 was selected as optimized formulation which was converted into S-SNEDDS.

**Evaluation of S-SNEDDS of Optimised Formulation F6**

**A. Micromeritic properties**

The angle of repose of the optimized formulation F6 was found to be 29.03. The compressibility index was 15.80%, bulk density 0.245g/ml and tapped density was 0.291g/ml. The angle of repose for formulation was <30 indicating good flow properties of S-SNEDDS. This was further supported by lower compressibility index values. Compressibility index values up to 16% results in good flow properties.

**B. Morphological studies of SNEDDS by using Scanning Electron Microscopy (SEM)**

SEM studies of L-SNEDDS and S-SNEDDS suggest that rifaximin is present in a completely dissolved state in the SNEDDS. The SEM image of L-SNEDDS helps in concluding that the particles are globular and well separated. However, the size was not uniform (Figure 8A). The image of S-SNEDDS illustrates that particles have a narrow particle size distribution with an uneven surface area shown in figure 8B.

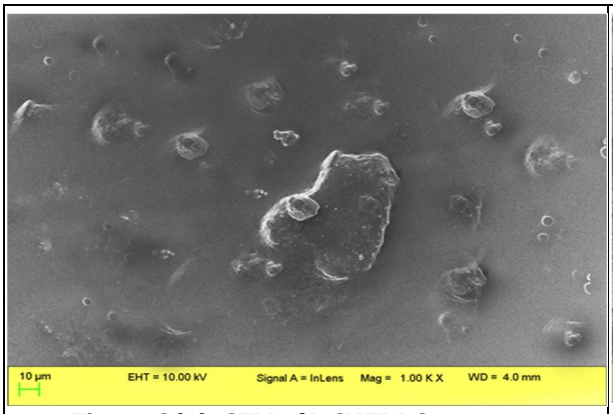



**Figure 8(A):** SEM of L-SNEDDS

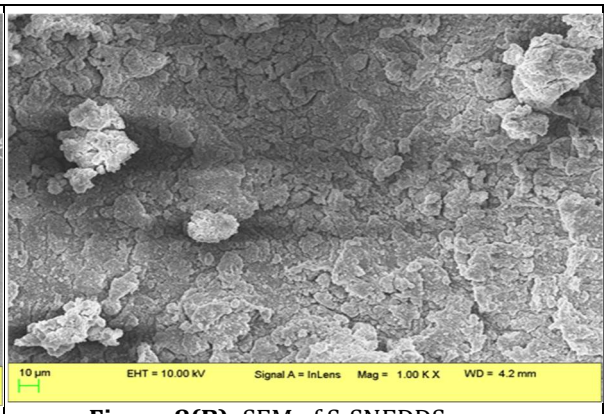



**Figure 8(B):** SEM of S-SNEDDS

**C. Determination of Zeta potential**

The zeta potential value of the best formulation F6 was found to be -38.2 mV. The negative zeta potential value indicates the stable formulation. The result shown in figure 9.

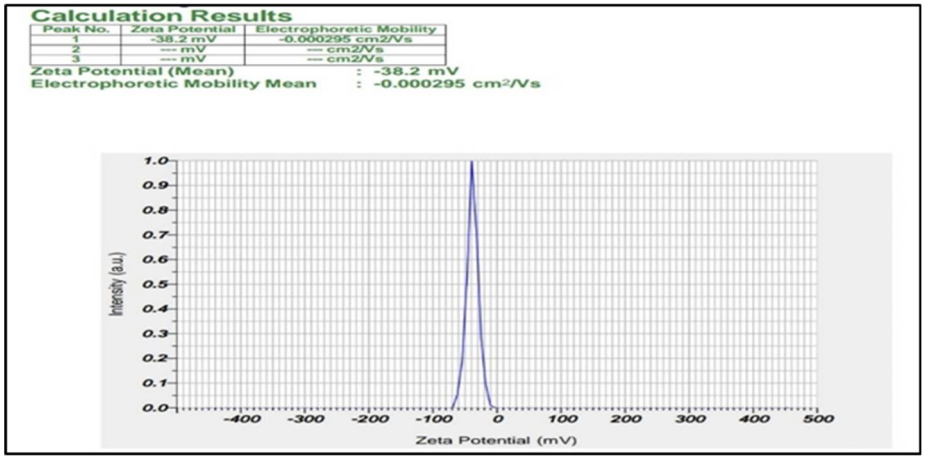
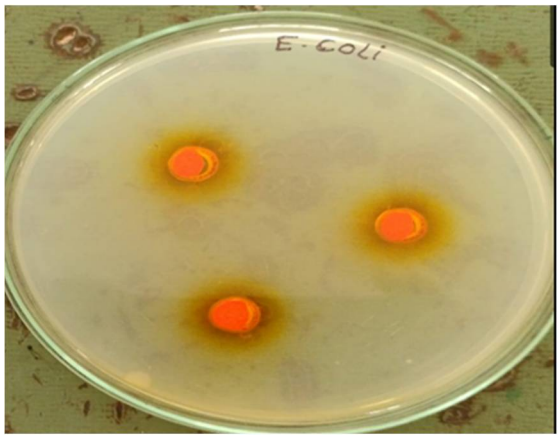


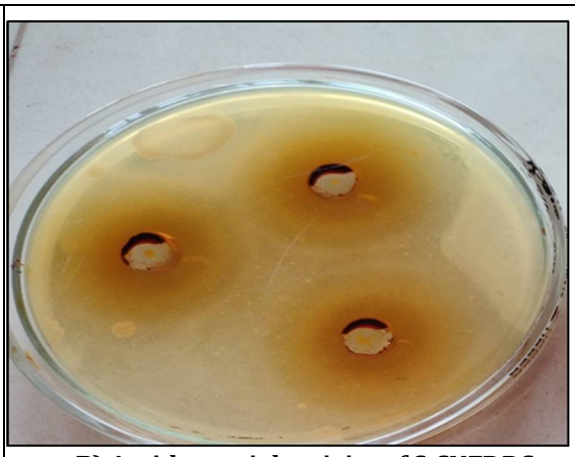

**Figure 9: Zeta potential of optimized formulation**

**D. Anti-bacterial activity of Pure drug and S-SNEDDS of Rifaximin:**

The result of anti-bacterial activity of pure drug was shown in fig.10A and of S-SNEDDS was shown in fig.10B. The results showed that pure drug rifaximin after 24hrs showed very negligible area (13mm) of zone of inhibition whereas S-SNEDDS showed area of zone of inhibition 39mm which is greater than that of pure rifaximin drug.




**A) Anti-bacterial activity of pure drug**



**B) Anti-bacterial activity of S-SNEDDS**

**Figure 10. Antibacterial activity**

**E. Comparative in vitro release profile of pure drug and S-SNEDDS of F6**

The percent cumulative drug release of S-SNEDDS formulation was found to be 92.01% after 4 hrs. Compared with drug release of pure drug rifaximin was found to be 61.53%. Drug release of rifaximin from S-SNEDDS was significantly improved shown in figure 11. As rifaximin is BCS Class IV drug by increasing solubility, bioavailability of drug can be increased. So, dissolution study shows that formulation of SNEDDS showed early onset of action as compared with the pure drug.

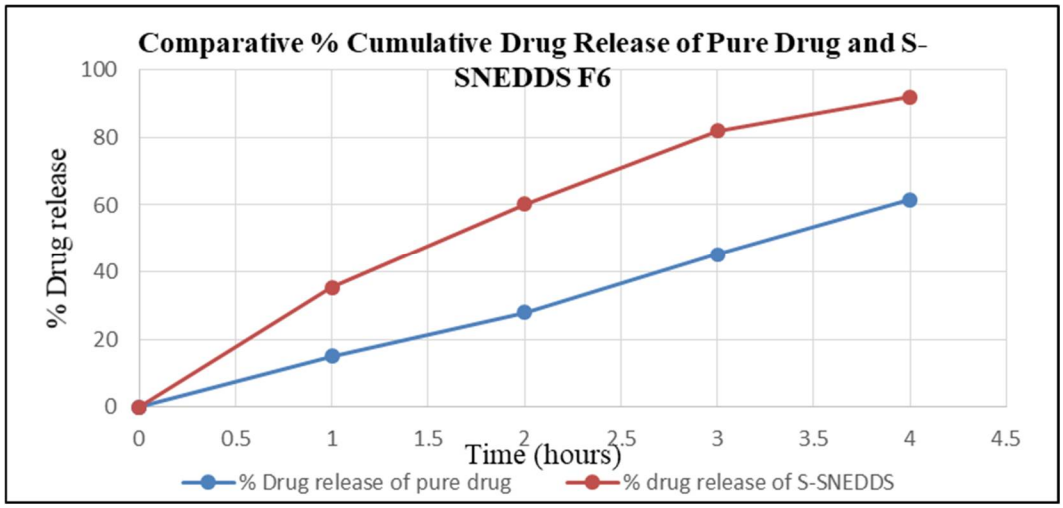



**Figure 11: Comparative % Cumulative Drug Release of Pure Drug and S-SNEDDS F6**

**Preparation of polymer plugs:**

The polymer plugs which were used for plugging the opening of capsule bodies were selected on the basis of swelling index and lag time.

**Swelling index**

Swelling index of different polymer is shown in figure 12. It was showed that Hydroxy Propyl Methyl Cellulose K100M shows highest swelling index and therefore selected for preparation of polymer plug.

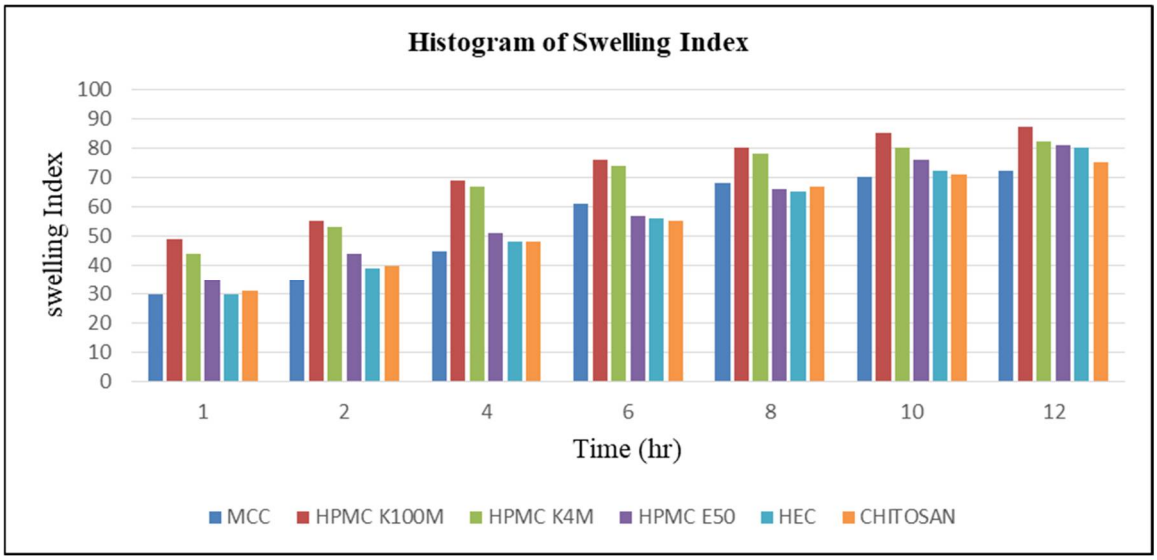



**Figure 12: Histogram of Swelling Index**

**Lag time**

From the result, it was showed that 70mg HPMC K100M polymer plug took 300min for optimum swelling and ejection from the capsule body. While 50mg HPMC K10M polymer plug took 280min. The optimum lag time (5hrs) was observed for 70 mg plug of HPMC K100M polymer and therefore it was selected for the preparation of polymer plug in pulsincap formulation.

***In vitro* study of pulsincap formulation**

During dissolution studies, it was observed that the enteric coating of the Eudragit S100 was intact for 2

hours in pH 1.2. The enteric coated cap of pulsincap was dissolved in phosphate buffer 7.4 and then the polymer plug absorbed the surrounding fluid, swelled and released a minor amount of drug through the swollen matrix. After complete swelling of a plug, it ejected out of the capsules body, released the drug into colon fluid of phosphate buffer 6.8. In accordance with the colon targeting and the site-specific treatment, the lag time criteria of 5 hrs. was satisfied by prepared enteric coated pulsincap formulation. After lag time the solid SNEDDS formulation showed better drug release profile. After 9 hrs, the cumulative percent drug release of optimized formulation was found to be 91.12% shown in figure 13.

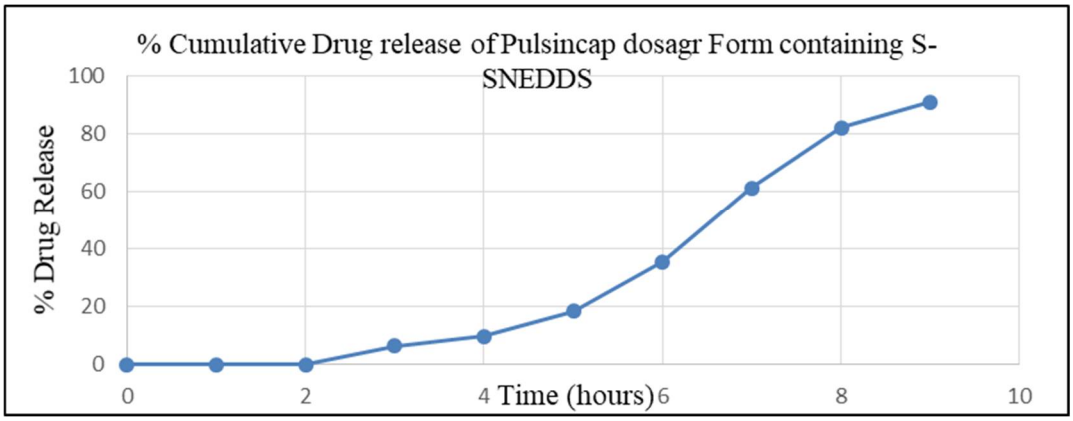



**Figure 13: Graph of % Cumulative Drug Release of Optimized Formulation F6**

**DISCUSSION**

The main aim of this study is to design and assess the Rifaximin SNEDDS for colon specific drug delivery for effective treatment of colonic bacterial infection in IBD patients. Where, SNEDDS will make Rifaximin available in soluble form and to enhance its solubility in region where it has very poor solubility or where it is mostly insoluble and enhance its antibacterial effect at lower doses. Pure Rifaximin was evaluated for its physical characteristics, analytical profiles and drug excipients compatibility study by using FTIR and DSC. From this study it was concluded that there was negligible difference occurred in between the drug and excipients used for the formulation of pulsincap of Rifaximin. From different oils Rifaximin shows maximum solubility in Clove oil, hence selected as an oil component. Among surfactants and co-surfactants tested Tween 80 and propylene glycol has shown maximum solubility. Therefore, Tween 80 as a surfactant and Propylene glycol as a cosurfactant were selected. From 1:1, 2:1 and 1:2 ratios of Surfactant and Co-surfactant respectively, 2:1 ratio gave good nano-emulsion area which was selected for further study. Based on the pseudo ternary phase diagrams, the formulation with the best self-nanoemulsifying properties, was formulated with varying amount of oil, surfactant and co-surfactant. All the formulation was tested for Visual assessment, Dispersibility test, Self-emulsification time, Robustness to dilution, Absolute drug content in L-SNEDDS as well as Globule size and Polydispersibility Index. The formulation F1, F2, F4, F6 and F8 emulsifies rapidly (in less than a minute) having a clear appearance. Formulation F3, F5, F7 and F9 emulsifies in less than 2 min having rapidly forming, slightly less clear emulsion. After diluting L-SNEDDS to 50, 100 and 1000 times with water, buffer pH 7.4 and pH 6.8 and storing for 12 h, it was observed that there was no sign of phase separation or drug precipitation in formulations.

From all formulations, F6 was selected as best formulation as it emulsifies rapidly (in less than a minute) having a clear appearance and there was no sign of phase separation or drug precipitation in formulations also formulation F6 show the highest drug content (98.37%) from all the other formulations as well as the globule size of the F6 was found to be 74.7 nm with polydispersibility index of 0.356. hence formulation F6 was selected as optimized formulation from all other formulation. For F6 Formulation S-SNEDDS was prepared by method adsorption on solid carriers using Aerosil 200. And further evaluated for micromeritics properties which shows good flow properties of S-SNEDDS of Rifaximin. FTIR and DSC showed there is no major differences occurred in between drug and formulation. The SEM image of liquid SNEDDS showed that particles are globular and well separated. However, the size was not uniform. The image of solid SNEDDS containing Rifaximin illustrates that particles have a narrow particle size distribution with an uneven surface area. The zeta potential value of the best formulation was found to be -38.2 mV. The negative zeta potential indicates the stable formulation. Also, the results of antibacterial activity showed that pure drug Rifaximin showed very negligible area (13mm) of zone of inhibition whereas S-SNEDDS of Rifaximin showed area of zone of inhibition 39mm which is greater than that of pure Rifaximin drug. The % drug release of optimized formulation was found to be 92.016% after 4 hours. Drug

release of Pure drug Rifaximin was found to be 61.534% after 4 hours. So, drug release of S-SNEDDS shows that % drug release in S-SNEDDS was significantly improved. Rifaximin in the form of SNEDDS which was releases the drug in pulsin manner. Capsule body was coated with ethyl cellulose and cap was coated with Eudragit S100 for colon targeted release. S-SNEDDS of Rifaximin was filled into treated capsule bodies and closed by plugging it with polymer HPMC K 100M. In dissolution studies, the enteric coating of the capsule was intact for 2 hours in pH 1.2 and dissolved in phosphate buffer 7.4 followed swelling of the plug releasing minor amount of drug with optimum lag time of 5 hours to eject the plug out of capsule body. After sufficient swelling of plug ejected out of the capsule body at the end and released drug into the colon fluid of phosphate buffer 6.8. The % cumulative drug release of S-SNEDDS formulation was found to be 91.124% after 9 hours. Pulse-in-cap device has been specially designed as a colon targeted drug delivery system which may maintain the antibacterial action of the drug.

## CONCLUSION

The current investigation demonstrates that pulincap system containing S-SNEDDS of Rifaximin have the ability to deliver the highest concentration of drug to the colon. The SNEDDS formulation was stable with a noticeably high zeta potential. The SNEDDS (F6) formulation showed lowest particle size about 74.7 nm with polydispersibility index of 0.356 were able to improve the antibacterial activity of Rifaximin as compared to the pure drug Rifaximin. Furthermore, these data support the overall improvement of all formulation related parameters suggests that pulsincap system containing SNEDDS of Rifaximin (F6) has potential for the colon specific delivery which enhanced the activity of Rifaximin and reduced the inflammatory condition in IBD by inhibiting the bacterial overgrowth.

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