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Formulation and Evaluation of Self-Emulsifying Drug Delivery System Of Methotrexate

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ABSTRACT

The objective of this study was to prepare the self-emulsifying drug delivery system of methotrexate for oral use. Preparation composed of soybean oil as oil phase, Span 80 as surfactants, isopropyl alcohol as co-surfactant, 0.1 N NaOH as the aqueous phase. Methotrexate is given orally in treatment of cancer and rheumatoid arthritis. The Anti-neoplastic drug MTX is having less aqueous solubility (50-60 %) and bioavailability of 60-70 %. Hence the present study is aimed to formulate and evaluate solid self-nano emulsifying drug delivery system with the aim of increasing the solubility and bioavailability which will decrease the dosing frequency in turn increase patient compliance. Liquid SNEDDS was prepared by adding drug to oil, surfactant and co-surfactant and heated up to at 60 ºC under continuous stirring until a clear solution is formed. All the formulations were optimized to get the best solubility results for MTX. Solid SNEDDS was prepared by mixing liquid SNEDDS with MCC in 1:1 proportion. The formulations were evaluated for angle of repose, bulk density, zeta potential, IR spectroscopy, *in vitro* dissolution, average particle size, size distribution and stability studies. The average particle size of the liquid SNEDDS was 60.4 nm and solid SNEDDS was 60.4nm. The surface charge was confirmed by the measurement of the zeta potential for the liquid SNEDDS and it was found to be -22.4 mV and that of the solid SNEDDS was -22.4 mV. The PDI value remained approximately around 0.565 indicated that all the nano particles were uniformly dispersed in the emulsion.

Keywords: Methotrexate, soyabean oil, span 80, solid self nano emulsifying drug delivery system, Antineoplastic, Pseudoternary phase

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INTRODUCTION

In recent years, the formulation of poorly soluble compounds presented interesting challenges for formulation scientists in the pharmaceutical industry. Up to 40% of new chemical entities discovered by the pharmaceutical industry are poorly soluble or lipophilic compounds, which leads to poor oral bioavailability, high intra and inter subject variability, and lack of dose proportionality.[1] Efforts are going on to enhance the oral bioavailability of lipophilic drugs in order to increase their clinical efficacy. There are several strategies to improve the bioavailability of poorly water soluble drugs include the solubilization and surfactants, the use of different polymorphic/ amorphic drug forms, the reduction of drug particle size, the complexation (e.g., cyclodextrins) and the formation of solid drug solutions/dispersions. For the therapeutic delivery of lipophilic active moieties (Class II drugs), lipid based formulations are inviting increasing attention.[2] There are several techniques that increase the rate & extent of drug absorption.

1. By increasing the rate or extent of dissolution.
2. By facilitating the absorption process. So to formulate a self-emulsifying formulation these approaches are generally used.

Self-emulsifying drug delivery systems (SEDDSs) have gained exposure for their ability to increase solubility and bioavailability of poorly soluble drugs.[1] Their solubilizing and absorption promoting effect is thought to lay in the reactivity of triglycerides and surfactants with the walls of the gastrointestinal tract (GIT). This bioavailability enhancing property has been associated with a number of *in vivo* properties of lipidic formulation including: the formation of fine dispersions and micellar suspensions, the ability of certain lipid compounds to initiate changes in the gastrointestinal fluid to favour improved drug absorption. Certain lipidic excipients are associated with selective drug uptake into the lymphatic transport system, thereby reducing the effect of first-pass drug metabolism in the liver.[3] Self-emulsification is influenced by the quality and nature of the concentration of surfactants, pair of oil/surfactant, and oil/surfactant ratio, and the physiological parameters in which it happens, including pH, and temperature. SEDDSs vary from conventional oral drug delivery systems in that digestion of enzymes significantly changes the excipients in the formulation· Self-emulsification is influenced by the quality and nature of the concentration of surfactants, pair of oil/surfactant, and oil/surfactant ratio, and the physiological parameters in which it happens, including pH, and temperature. SEDDSs vary from conventional oral drug delivery systems in that digestion of enzymes significantly changes the excipients in the formulation.[4] SEDDS or self-emulsifying oil formulations (SEOF) are defined as isotropic mixtures of natural or synthetic oils, solid or liquid surfactants or, alternatively, one or more

hydrophilic solvents and co-solvents/ surfactants.[3] Fine oil droplets would pass rapidly from the stomach and promote wide distribution of the drug throughout the GIT, thereby minimizing the irritation frequently encountered during extended contact between bulk drug substances and the gut wall.[5] The self-emulsification process is specific to the nature of the oil/surfactant pair, surfactant concentration, oil/surfactant ratio and temperature at which self-emulsification occurs. The ease of emulsification could be associated with the ease of water penetrating into the various liquids crystalline or gel phases formed on the surface of the droplet.[5]

When compared with emulsions, which are sensitive and meta-stable dispersed forms, SEDDS are physically stable formulations that are easy to manufacture. These systems advantageously present the drug in dissolved form and the small droplet size provides a large interfacial area for the drug absorption.[6] These characteristics result in faster drug release from emulsion in a reproducible manner, which can be designed further to make the release characteristics independent of the gastro intestinal physiology and the fed/fasted state of the patient. SEDDS formulations are physically stable because they are isotropic mixtures, clear and are resistant to small temperature changes. Particle size plays an important role as with the increase in particle size solubility of the product increases and the named itself indicates that it has the ability to emulsify when drug delivers at the site of action. This makes better as compared to conventional emulsion which does not emulsify when reaching the targeted site.[7]

MATERIALS AND METHOD

Pre-formulation study

Analytical Method Development

Determination of λ_{max}[8]:

The Standard drug solution of concentration 20 µg/ml was prepared using 0.1 N HCl and pH 6.8 phosphate buffer. The solution was scanned in UV visible spectrophotometer over wavelength 200 – 600 nm. From this scan, the peak of maximum absorbance was identified (λ_{max}) in each media and used for further analysis.

Calibration curve of MTX:

Preparation for standard stock solution:

Accurately weighed 50 mg of MTX was dissolved in 50 ml of the selected medium (HCl / pH 6.8 phosphate buffer) in a volumetric flask to get a final concentration of 1000 µg/ml.

Preparation of working standard solution:

2 ml of stock solution was diluted to 100 ml with the selected medium to produce a solution with a concentration of 20 µg/ml. Using this solution, 1, 2, 3, 4 and 5 ml were taken and diluted with 10 ml to produce final concentrations of 2, 4, 6, 8 and 10 µg/ml.

UV-spectroscopic determination:

The absorbance of the working standard solution was analyzed and a graph of concentrations of the solution was plotted against absorbance. The experiment was performed in triplicate and based on average absorbance and the equation for the best line was generated. Linear regression analysis was carried out in Microsoft Excel®.

Compatibility study by FTIR Spectroscopy.[9]

The compatibility between the drug and excipients is one of the important pre-formulation requirements which provide the information about the stability of the prepared formulation. The individual components, anhydrous mixtures and the formulations were studied by FTIR. S-SNEDDS was mixed with small quantity of IR grade Potassium bromide and scanned in the range of 4000-400 cm-1 (FTIR-4100, Jasco).

Formulation of Liquid Self-emulsifying system (SNEDDS)[10]

Determination of solubility of MTX in various oils, surfactants and co-surfactants:

To 1 ml of each of selected vehicles excess of the drug was added. Then, the mixture was kept on rotary shaker for 48 h to facilitate solubilization. The sample was centrifuged at 3000 rpm for 15 min to remove un-dissolved MTX. Then the supernatant was taken with micropipette and content of MTX was quantified spectrophotometrically after dilution with isopropyl alcohol.

Construction of Pseudo-ternary Phase Diagrams:[11]

Pseudo ternary phase diagram are representation of nano-emulsion system in two dimensions using three axes. Pseudo ternary phase diagram were constructed to examine the formation of o/w emulsion zone using oil, S_{mix} and distilled water. The phase diagram of oil, S_{mix} (surfactant: co-solvent) and water were developed using water titration method. Surfactant and co-solvent were mixed (S_{mix}) in different volume ratios (1:1, 2:1, 3:1, 1:2). These S_{mix} ratios were chosen to reflect increasing concentration of surfactant with respect to co-solvent for detailed study of the phase diagram in the nano emulsion formation. For each phase diagram, oil and specific S_{mix} ratio were mixed thoroughly in different volume ratios from 1:9 to 9:1 in different glass vials. Nine different combination of oil and S_{mix} 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 were made for study to delineate the boundaries of phases precisely formed in the phase diagram.

Pseudo ternary phase diagram were developed using ProSim Ternary Diagram software using the aqueous titration method. Slow titration with the aqueous phase was performed for each

combination of oil and S_{mix} separately. The calculation for the addition of aqueous phase was done by calculating the percentage of each component of the nano emulsion present at each water interval added. After each addition of the aqueous phase to the oil: S_{mix} mixture, visual observation was made. The results were recorded through various categories of visual observation.

Formulation selection:

Based on the pseudo ternary phase diagram studies, the formulations were selected which showed highest area of nano emulsion. Considering these criteria 12 formulations were selected as follows.

Table 1: Formulations for self-emulsifying drug delivery of MTX.

Ingredients	F1	F2	F3	F4	F5	F6
S_{mix}	1:1	1:1	1:1	1:1	1:1	1:1
Oil	1:9	2:8	3:7	4:6	5:5	6:4
Soyabean oil	10%	20%	30%	40%	50%	60%
Span 80	45%	40%	35%	30%	25%	20%
Isopropyl alcohol	45%	40%	35%	30%	25%	20%
MTX	2.5 mg	2.5 mg	2.5 mg	2.5 mg	2.5 mg	2.5 mg

Table 2: Various formulations for self-emulsifying drug delivery of MTX.

Ingredients	F7	F8	F9	F10	F11	F12
S_{mix}	2:1	2:1	2:1	2:1	2:1	2:1
Oil	1:9	2:8	3:7	4:6	5:5	6:4
Soyabean oil	10%	20%	30%	40%	50%	60%
Span 80	60%	53%	47%	40%	33%	27%
Isopropyl alcohol	30%	27%	23%	20%	17%	13%
MTX	2.5 mg	2.5 mg	2.5 mg	2.5 mg	2.5 mg	2.5 mg

Preparation of liquid SNEDDS formulations:[12]

Liquid SNEDDS was prepared by adding drug to oil, surfactant and co-surfactant mixture and heated up to at 60 ºC under continuous stirring. The obtained mixture was mixed by vortex until a clear solution formed.

Characterization of liquid SNEDDS

Drug content:

The percent drug content of MTX in Liquid SNEDDS was estimated by dissolving appropriate quantity of individual SNEDDS in 0.1N HCl. The samples were mixed thoroughly to dissolve the drug in 0.1N HCl. Proper dilution of the samples were made and sonicated using ultrasonicator for 15 min and analyzed using UV spectrophotometer at 243nm and absorbance was recorded using UV-visible spectrophotometer.

% Transmittance:

The % transmittance of the system after 100 times dilution was measured at 650 nm using UV visible double beam spectrophotometer keeping water as blank.

Dilution potential/Robustness on dilution:

The prepared formulation was diluted 100 times with distilled water. The emulsion was observed for any precipitation, to confirm stability of the emulsion.

Emulsification time and dispersibility:

For evaluation of self-emulsification properties of formulations, 0.1 ml of each formulation was added to 50 ml distilled water under continuous stirring (500 rpm) at 37 ºC and then spreadability and dispersibility tendency and emulsification progress was observed. Based on visual observation the following grades were assigned.

Centrifugation test:

To determine the stability of the emulsion under stress condition, centrifugation test was performed. Diluted formulations were centrifuged (Remi centrifuge) at 25 ºC at 3,500 rpm for 30 min and observed for any phase separation and precipitation of drug.

Viscosity:

The viscosity of the optimized formulation was evaluated by Brookfield viscometer at 25 ºC. Experiment was performed in triplicate for each sample and results were presented as average ± standard deviation.

Emulsification time:

The emulsification time is the time for a pre concentrate to form a homogenous mixture upon dilution. It was monitored by visually observing the disappearance of SNEDDS and the final appearance of the nano emulsion in triplicate. A USP dissolution apparatus was employed with 500 ml and with a paddle speed of 50 rpm at 37 ºC. The SNEDDS (1 ml) was added drop wise to the medium by a dropping pipette and the time required for the disappearance of SNEDDS was checked.

Thermodynamic stability: [13]

The stable liquid SNEDDS formulations after ternary phase studies were subjected to thermodynamic stability studies to evaluate effect of temperature variation and phase separation on SNEDDS formulations. During these studies the formulations were subjected to heating cooling cycles (4 and 45 ºC) and freeze thaw cycles (-21 and +25 ºC) with storage at each temperature for 2 days and observed visually for any phase separation. During centrifugation stress studies, SNEDDS formulations were subjected to centrifugation at 4,000 rpm for 15 min and formulations were visually observed for any phase separation.

***In vitro* dissolution test:** [14]

In vitro drug release from the formulations and dissolution of pure drug was carried out using USP type II dissolution apparatus (50 rpm; 37 ºC ± 0.5 ºC) in 0.1 N HCl. The selected SNEDDS formulations and pure drug were filled in transparent hard gelatin capsule size "00". At pre-determined time intervals, 5 ml was withdrawn and the drug concentration was determined by UV spectrophotometer. The volume removed was replaced each time with fresh dissolution medium. All experiments were carried out in triplicate and the results presented are the mean values of the three experiments.

Emulsion droplet size measurement, zeta potential and poly-dispersibility index: [15]

Average particle size and size distribution:

Particle size and polydispersibility index (PI) as a measure of the distribution of nano-emulsion was determined using dynamic light scattering using Malvern Zeta Sizer instrument. SNEDDS were diluted to 100 ml with distilled water. The droplet size distributions and polydispersibility index of the resultant nano emulsions were determined using Malvern Zetasizer.

Zeta potential:

The emulsion stability is directly related to the magnitude of the surface charge. The zeta potential of the diluted SNEDDS formulation was measured using a Malvern Zetasizer instrument. The SNEDDS was diluted with double distilled water (1:100) to get a uniform dispersion. The conductivity of the diluted sample was then measured at 25 °C.

Formulation of Solid SNEDDS (S-SNEDDS) by adsorption on a solid carrier:

S-SNEDDS was prepared by mixing liquid SNEDDS with MCC in 1:1 proportion. In brief liquid S-SNEDDS was added drop wise over MCC contained in broad porcelain dish. After each addition, mixture was homogenized using glass rod to ensure uniform distribution of formulation. Resultant damp mass was passed through sieve no. 120 and dried at ambient temperature and stored until further use.

Characterization and evaluation of S-SNEDDS:

Drug Content:

The percent drug content of MTX in S-SNEDDS was estimated by dissolving appropriate quantity of individual SNEDDS in isopropyl alcohol. The samples were mixed thoroughly to dissolve the drug in isopropyl alcohol. The sample was sonicated using ultrasonicator for 15 min and analyzed using UV spectrophotometer and absorbance was recorded using UV-visible spectrophotometer.

Reconstituted properties of S-SNEDDS:

Dilution study by visual observation

Dilution study was done to study the effect of dilution on S-SNEDDS, because dilution may better mimic the condition of stomach after oral administration. In this method, S-SNEDDS (100 mg) was introduced into 100 ml of double distilled water in a glass beaker that was maintained at 37 ºC and the contents mixed gently using a magnetic stirrer. The tendency to emulsify spontaneously and progress of emulsion droplets were observed with respect to time.

Angle of repose:

The angle of repose of S-SNEDDS was determined by funnel method. Accurately weighed sample were taken in a funnel. Height of the funnel was adjusted in such a way that the tip of the funnel just touches the apex of the heap of S-SNEDDS powder. The powders were allowed to flow through funnel freely onto the surface. The diameter of the powder cone was measured and angle of repose calculated using the following equation,

$$\text{Tan } \theta = h/r$$

Bulk Density:

Both loose bulk density (LBD) and tapped bulk density (TBD) were determined. A quantity of 2 g of S-SNEDDS was introduced into a 10 ml measuring cylinder. Initial volume was observed, the cylinder was allowed to fall under its own weight onto a hard surface from a height of 2.5 cm at 2 second intervals. The tapping was continued until no further change in volume was noted. LBD and TBD were calculated using the following formulae,

LBD=Weight of powder/ Volume of powder

TBD=Weight of powder/Tapped volume of powder

Compressibility Index (Carr's):

The compressibility of the granules was determined by Carr's Compressibility Index.

$$\text{Carr's compressibility index (\%)} = \text{TBD-LBD/TBD X100}$$

Hausner's ratio:

A similar index like compressibility index has been defined by Hausner's. Hausner's ratio can be calculated by formula:

$$\text{Hausner's ratio} = \text{TBD/LBD}$$

***In vitro* release:**

Apparatus type used was USP type II (paddle), dissolution medium was 900 ml of 0.1N Sodium hydroxide at 37 °C ± 0.5 °C, speed of rotation of paddle was 50 rpm, volume of sample withdrawn was 5 ml, sampling was carried for 180 min over entire duration of study. Required quantity of S-SNEDDS (equivalent to 2.5 mg) was filled into hard gelatin capsules. The capsules were placed into a dissolution medium and the dissolution test was carried out.

RESULTS AND DISCUSSION

Analytical Method Development:

Determination of λ_{max}

The UV absorption spectrum of MTX showed peaks at 307 nm and 243nm. The λ_{max} found at 243 nm was selected for the present study.

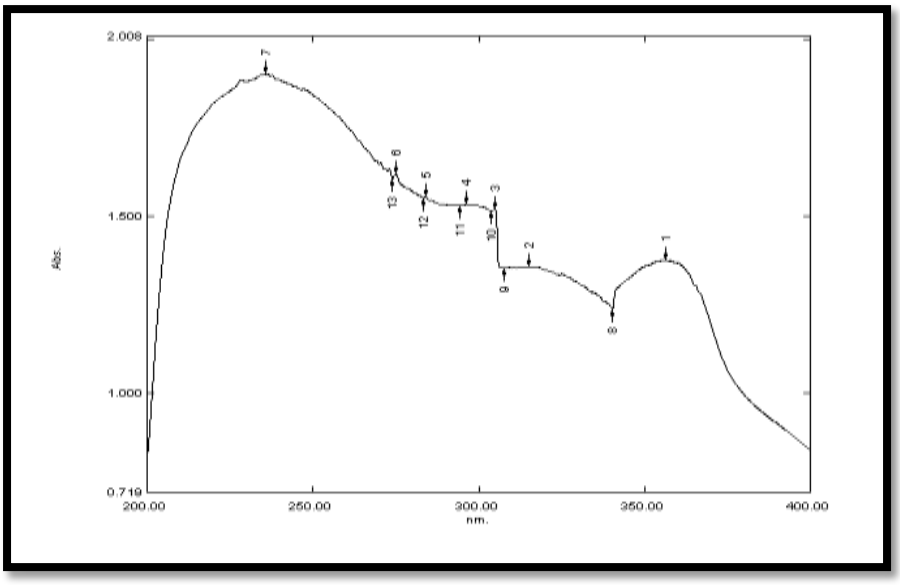

Figure 1: UV absorption spectrum of MTX

Development of calibration curve of MTX in 0.1 N HCl.

Calibration curve

Table 3: Calibration curve of MTX in 0.1 N HCl at 243 nm

Sr.no.	Concentration[µg/ml]	Absorbance*
1.	00	0.000
2.	2.0	0.182
3.	4.0	0253
4.	6.0	0.354
5.	8.0	0.456
6	10.0	0.579
7.	12.0	0.669
8.	14.0	0.752
9.	16.0	0.799
10.	18.0	0.862
11.	20.0	0.919

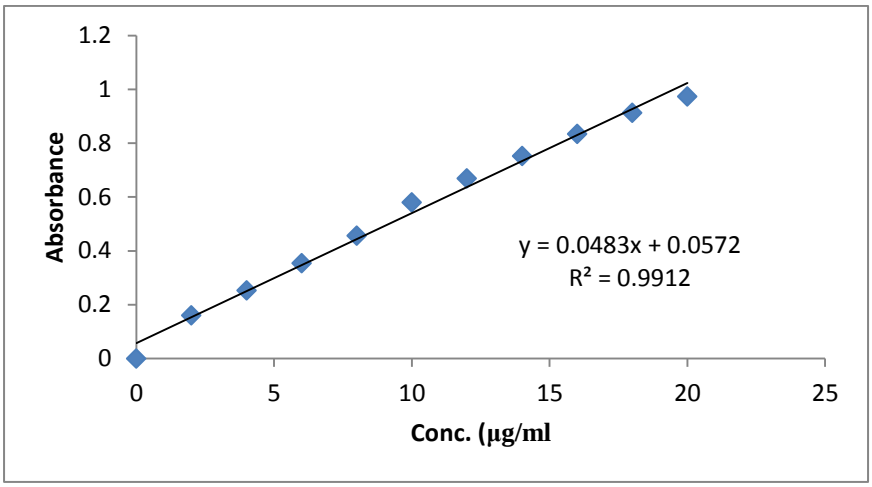

Figure 2: Calibration curve of MTX in 0.1 N HCl

A straight line equation (y = mx + c) was generated to facilitate the calculation of amount of drug. The equation is as follows: Y= m x + c

Compatibility studies by IR spectroscopy:

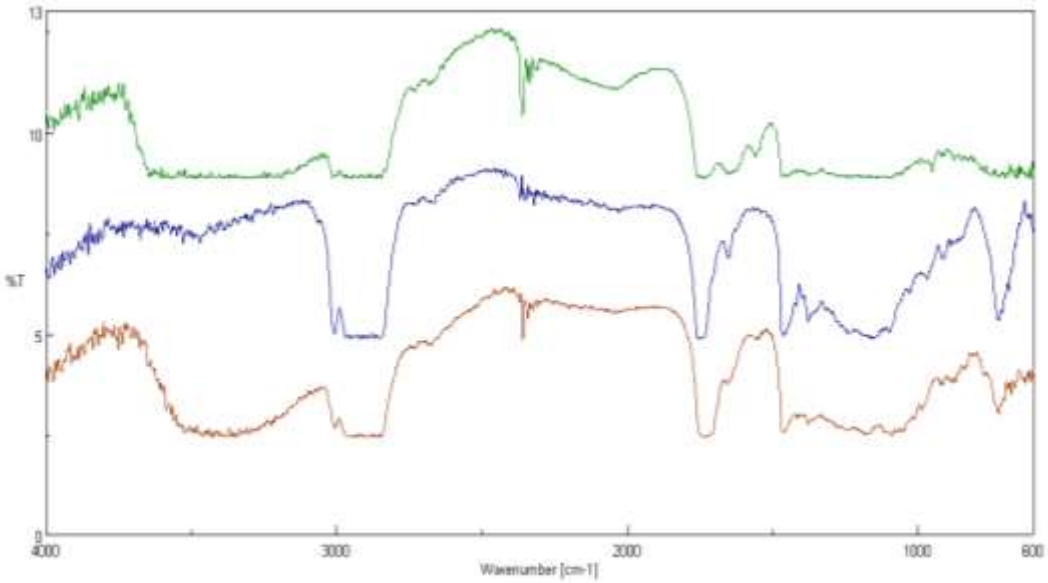

Figure 3: IR Spectra of formulation SF5 with excipients

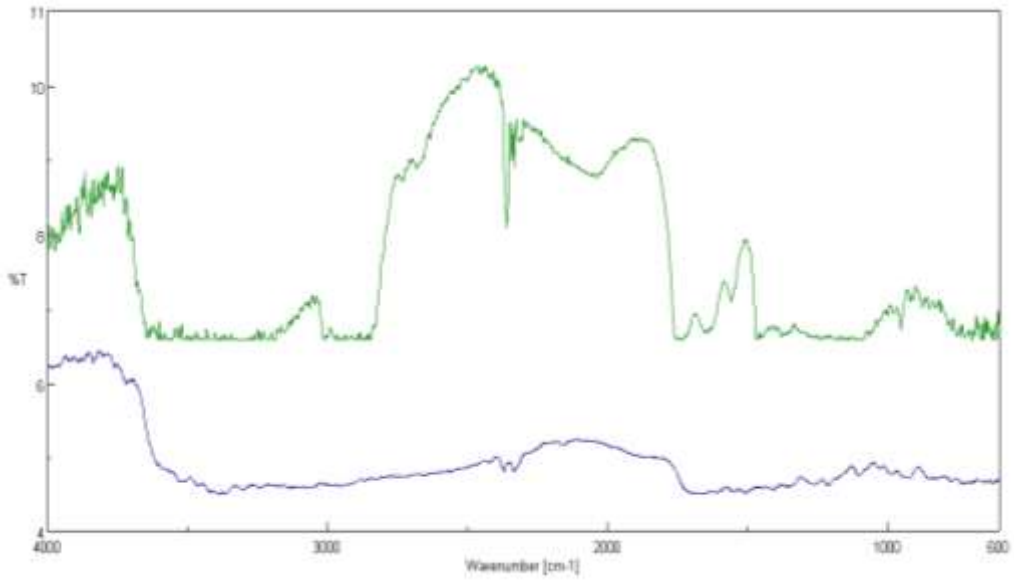

Figure 4: IR Spectra of pure drug with formulation SF5

FTIR spectrum of pure methotrexate – active substance (MTXAS) shows characteristic absorptions band as a broad signal at 3450 cm^{-1} (O–H stretching from carboxyl groups superposed with the O-H stretching from crystallization water), at 3080 cm-1 (primary amine N–H stretching), at 1670-1600 cm-1 assigned to C=O stretching (–C=O stretching from carboxylic group and C=O stretching from amidic group, so the C=O band is splited into a doublet in the MTXAS sample). The bands corresponding to N-H bending from amidic group appear in the 1550–1500 cm^{-1} spectral range, partly overlapping with the aromatic –C=C stretching. Another prominent bands, such as 1400-1200 cm^{-1} correspond to –C–O stretching from carboxylic group, 930 cm^{-1} O–H bending out of plane and 820 cm^{-1} to C–H adjacent hydrogens on an aromatic ring, para substitution. All the bands identified in the FTIR spectrum are in good agreement with the molecular structure of MTX and confirm its purity.

Formulation of Liquid SNEDDS:

Screening of oils

Based on solubility of drug:

Soyabean oil showed the highest solubility of MTX (35.58 mg/ml). Hence, soyabean oil was used as oil phase.

Table 4: Solubility of drug in various oils

Sr.no.	Oil	Solubility (mg/ml)
1.	Castor oil	21.60
2.	Soyabean oil	35.58
3.	Sunflower oil	26.35
4.	Sesame oil	22.55
5.	Olive oil	26.50

6.	Corn oil	28.39
7.	Peanut oil	19.30

Screening of surfactant:

Based on solubility of drug:

Span 80 showed the highest solubility of MTX (35.0 mg/ml) and highest % transmittance. Hence Span 80 was selected as surfactant.

Table 5: Solubility of MTX in surfactants

Sr.no.	Surfactant	Solubility (mg/ml)
1.	Span 20	25.40
2.	Span 80	35.00
3.	Tween 80	20.40

Based on ease of emulsification

Table 6: Number of flask inversions and % transmittance of oil and various surfactants

Sr.no.	Surfactant	No. of flask inversion	%Transmittance at 243 nm
1.	Tween 80	15	86.8
2.	Span 80	4	98.2
3.	Tween 20	7	96.2

Screening of co-solvent

Based on solubility of drug:

Isopropyl alcohol showed the highest solubility of MTX (95.0 mg/ml). Hence, Isopropyl alcohol was used as co-solvent.

Table 7: Solubility of drug in co-surfactant.

Sr.no	Co-solvent	Solubility
1.	Isopropyl alcohol	95.0
2.	PEG	65.70
3.	Glycerin	88.2

Construction of pseudo ternary phase diagram to select the best combination of oil/surfactant and co-solvent.

Preparation of S_{mix} (Surfactant + Co-solvent)

Stock solution of 60 ml was prepared in different ratios of surfactant and co-solvent, as follows,

1:1 = 30 ml surfactant + 30 ml co-solvent (S_{mix} A)

2:1 = 40 ml surfactant + 20 ml co-solvent (S_{mix} B)

3:1 = 45 ml surfactant + 15 ml co-solvent (S_{mix} C)

1:2 = 20 ml surfactant + 40 ml co-solvent (S_{mix} D)

Here span 80 was used as surfactant and isopropyl alcohol was used as co-solvent.

Water titration method:

Nine different combination of oil and S_{mix} 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 were made for study to delineate the boundaries of phases precisely formed in the phase diagram. Slow titration with the aqueous phase was performed for each combination of oil and S_{mix} separately. Pseudo ternary phase diagram were developed using ProSim Ternary Diagram software using the aqueous titration method.

Table 8: Water titration method for system A.

A	Ratio	Soyabean oil	S_{mix} A (1:1)	Water titration reading
A1	1:9	1 ml	9 ml	5.3 ml
A2	2:8	2 ml	8 ml	4.8 ml
A3	3:7	3 ml	7 ml	4.2 ml
A4	4:6	4 ml	6 ml	3.2 ml
A5	5:5	5 ml	5 ml	2.6 ml
A6	6:4	6 ml	4 ml	0.7 ml
A7	7:3	7 ml	3 ml	0.3 ml
A8	8:2	8 ml	2 ml	0.3 ml
A9	9:1	9 ml	1 ml	0.2 ml

Table 9: Water titration method for system B.

B	Ratio	Soyabean oil	S_{mix} B (2:1)	Water titration reading
B1	1:9	1 ml	9 ml	4.9 ml
B2	2:8	2 ml	8 ml	4.3 ml
B3	3:7	3 ml	7 ml	3.7 ml
B4	4:6	4 ml	6 ml	3.1 ml
B5	5:5	5 ml	5 ml	2.6 ml
B6	6:4	6 ml	4 ml	0.7 ml
B7	7:3	7 ml	3 ml	0.6 ml
B8	8:2	8 ml	2 ml	0.4 ml
B9	9:1	9 ml	1 ml	0.3 ml

Pseudo ternary phase diagram

Pseudo ternary phase diagram were developed using ProSim Ternary Diagram software using the aqueous titration method and the results are as follows,

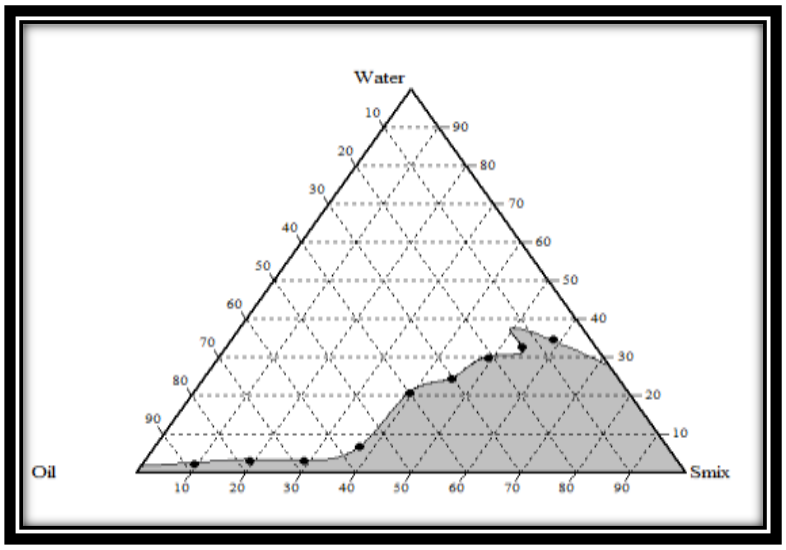

Figure 5: Ternary plots for formulation of system A.

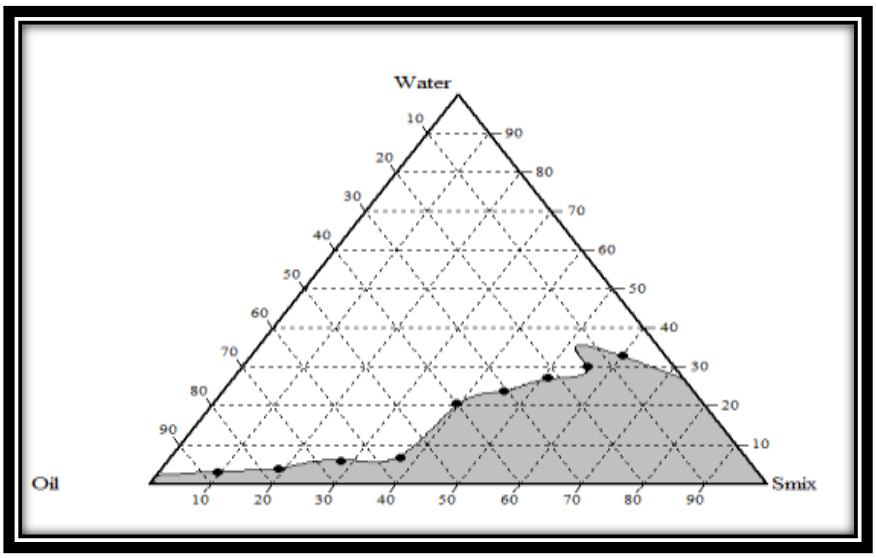

Figure 6: Ternary plots for formulation of system A.

Formulation of system A and system B showed wide area of nano emulsion when compared to system C and system D. Hence the system A and system B were selected for further studies. There was no notable change found in the area of the nano emulsion of system A and system B even after the addition of MTX drug.

Formulation Selection:

From the result of pseudo ternary phase diagram 12 different formulations were formulated.

Preparation of Liquid SNEDDS:

All the selected formulations of self-nano emulsifying drug delivery system were prepared by the formula given in the methodology. All the formulations were subjected to list of characterization studies.

Characterization and evaluation of liquid SNEDDS

Drug Content:

Table 10: % Drug content of various formulations.

Sr.no	Amount of MTX (mg)	% Drug Content
F1	2.23	89.2 %
F2	2.35	94.0 %
F3	2.22	88.8 %
F4	2.40	96.0 %
F5	2.43	97.2 %
F6	2.36	94.4 %
F7	2.39	95.6 %
F8	2.33	93.2 %
F9	2.38	95.2 %
F10	2.41	96.4 %
F11	2.34	93.6 %
F12	2.30	92.0 %

The percentage drug contents of the different formulations are shown in Table 10. The percentage drug content of all formulations was found to be in the range of ± 5 %.

% Transmittance:

Table 11: % Transmittance for various formulations

Formulation Code	% Transmittance
F1	96.1 %
F2	97.0 %
F3	77.3 %
F4	86.7 %
F5	99.1 %
F6	98.5 %
F7	89.3 %
F8	92.6 %
F9	93.4 %
F10	88.9 %
F11	96.5 %
F12	94.3 %

Many formulations like F1, F2, F5, F6, F8, F9, F11, F12 showed good result in % transmittance where as some formulations like F3, F4, F7, F10 failed to give good result in % transmittance.

Centrifugation test:

Table 12: Stability study on centrifugation

Formulation Code	Observation
F1	Stable, no precipitation & phase separation
F2	Stable, no precipitation & phase separation
F3	Stable, no precipitation & phase separation
F4	Stable, no precipitation & phase separation
F5	Stable, no precipitation & phase separation

F6	Stable, no precipitation & phase separation
F7	Stable, no precipitation & phase separation
F8	Stable, no precipitation & phase separation
F9	Stable, no precipitation & phase separation
F10	Stable, no precipitation & phase separation
F11	Stable, no precipitation & phase separation
F12	Stable, no precipitation & phase separation

All the formulations were subjected to stability on centrifugation. The results of which are shown in Table 12. From the results it was concluded that all the formulations were found to be stable and there was no evidence of precipitation and phase separation. Thus all the formulations passed the stability study on centrifugation and thus found to be stable.

Viscosity:

Table 13: Viscosity measurement for various formulations

Formulation Code	Viscosity (cps)
F1	27.58
F2	36.92
F3	33.67
F4	28.54
F5	42.36
F6	31.28
F7	34.55
F8	35.76
F9	30.77
F10	41.23
F11	33.88
F12	28.66

All the formulations were evaluated for viscosity by Brookfield viscometer at 25 ºC.

All the formulations were found to have viscosity in the range of 27 – 43 cps.

Emulsification time:

Table 14: Emulsification time for various formulations

Formulation Code	Emulsification time (sec)
F1	26
F2	32
F3	22
F4	38
F5	26
F6	33
F7	39
F8	25
F9	26
F10	22
F11	33
F12	35

The emulsification time is the time for a pre concentrate to form a homogenous mixture upon dilution. All the formulations were subjected to emulsification time, which was found out to be 22 – 39 sec. Thus emulsification times of all the formulations were found to be near to the average time.

Thermodynamic stability:

Table 15: Thermodynamic stability for various formulations

Formulation Code	Heating cooling cycles (4 ºC and 45 ºC	Freeze thaw cycles (-21 ºC and +25 ºC
F1	Stable, no precipitation	Stable, no precipitation
F2	Stable, no precipitation	Stable, no precipitation
F3	Stable, no precipitation	Stable, no precipitation
F4	Stable, no precipitation	Stable, no precipitation
F5	Stable, no precipitation	Stable, no precipitation
F6	Stable, no precipitation	Stable, no precipitation
F7	Stable, no precipitation	Stable, no precipitation
F8	Stable, no precipitation	Stable, no precipitation
F9	Stable, no precipitation	Stable, no precipitation
F10	Stable, no precipitation	Stable, no precipitation
F11	Stable, no precipitation	Stable, no precipitation
F12	Stable, no precipitation	Stable, no precipitation

All the formulations were subjected to thermodynamic stability (heating cooling cycle and freeze thawing cycle). The results of which are shown in Table 15**.** From the results it was concluded that all the formulations were found to be stable and there was no evidence of phase separation. Thus all the formulations passed thermodynamic stability study and thus found to be stable.

***In vitro* dissolution studies:**

Table 16: *In vitro* drug release study of formulations F1 – F6.

Time (min)	%CDR F1	%CDR F2	%CDR F3	%CDR F4	%CDR F5	%CDR F6
15	25.12	26.13	23.14	26.56	29.13	23.45
30	36.56	34.65	29.13	30.45	34.56	29.56
45	45.61	47.78	34.93	34.67	40.24	35.71
60	51.17	51.13	39.15	40.34	48.31	41.61
75	59.45	60.39	45.76	46.78	55.30	49.39
90	65.32	64.19	50.39	53.49	61.83	56.54
105	70.91	69.92	56.51	60.27	69.42	62.17
120	79.14	78.82	60.47	68.15	73.59	69.21
135	85.27	83.14	67.82	73.52	83.81	77.29
150	90.41	90.73	72.53	82.27	91.34	83.41
165	92.35	92.67	80.32	91.34	96.23	89.13
180	96.12	96.15	86.96	93.17	100	92.35
195	98.18	97.23	94.71	95.45		100
210	100	99.10	100	97.56		

Table 17: *In vitro* drug release study of formulations F7 – F12.

Time (min)	%CDR F7	%CDR F8	%CDR F9	%CDR F10	%CDR F11	%CDR F12
15	17.23	21.36	18.66	22.56	12.56	26.36
30	22.50	25.36	25.61	31.58	20.36	34.25
45	27.56	32.16	29.34	35.61	28.56	36.89
60	31.89	38.25	33.56	39.87	35.69	45.62
75	34.52	49.23	36.51	42.81	39.61	48.36
90	39.68	51.63	39.78	45.36	40.12	56.32
105	40.55	55.37	45.91	49.55	46.23	58.62
120	43.88	59.61	49.52	50.55	48.52	60.45
135	55.23	61.33	52.36	54.21	50.78	68
150	66.70	68.32	65.37	55.63	55.63	70.75
165	74.60	72.13	77.52	60.87	59.34	72.35
180	78.32	77.63	78.66	62.36	60.12	78.25
195	88.60	89.00	85.63	70.63	67.89	79.63
210	91.56	92.35	89.61	75.83	70.41	88.88

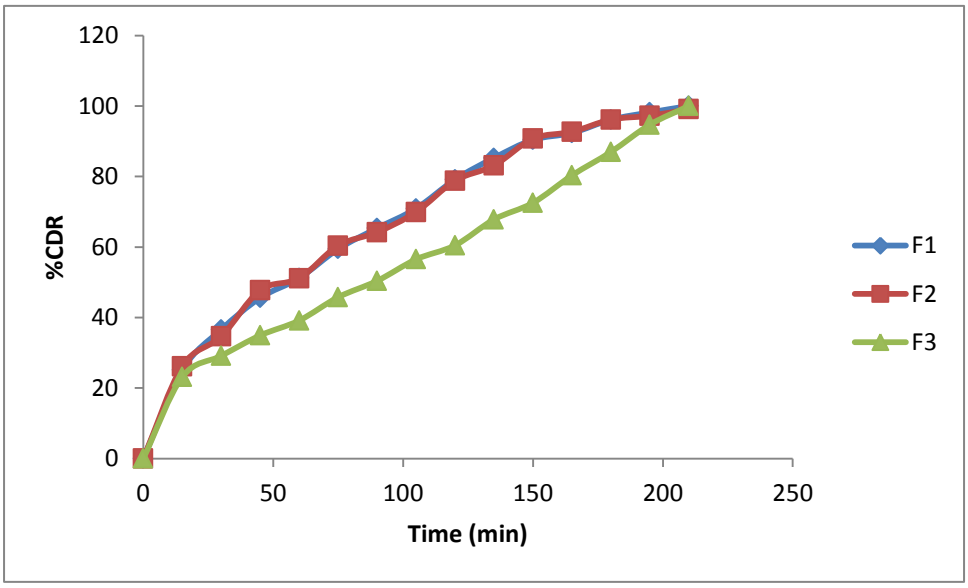

Figure 7: *In vitro* drug release study.

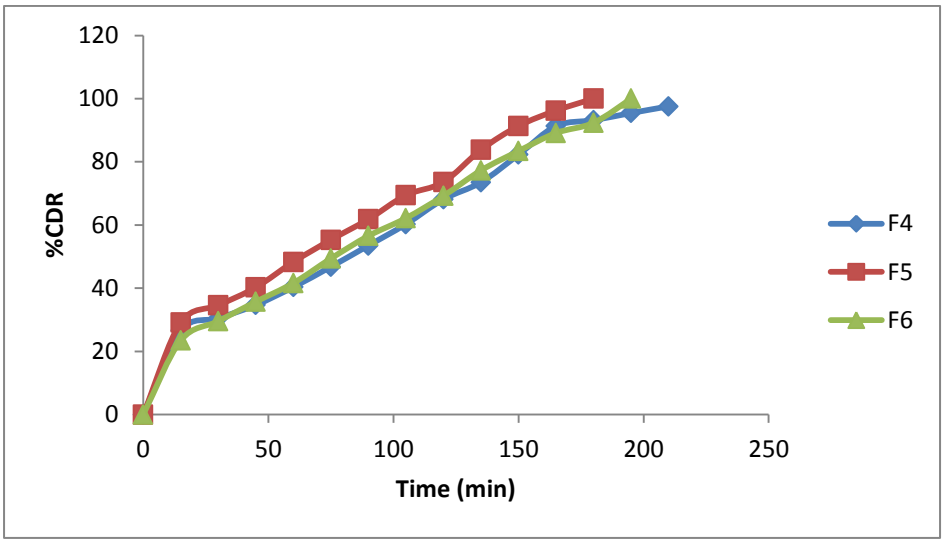

Figure 8: *In vitro* drug release study.

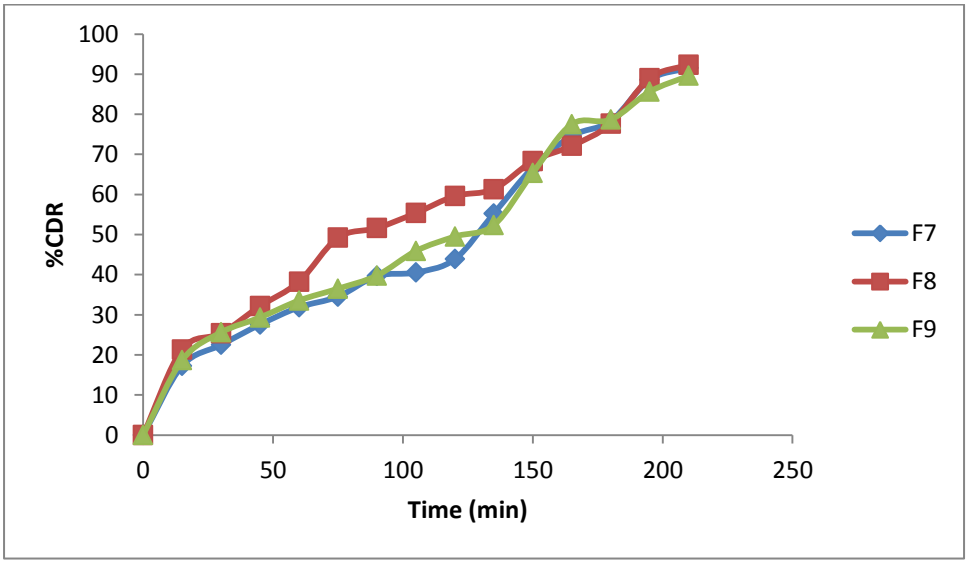

Figure 9: *In vitro* drug release study.

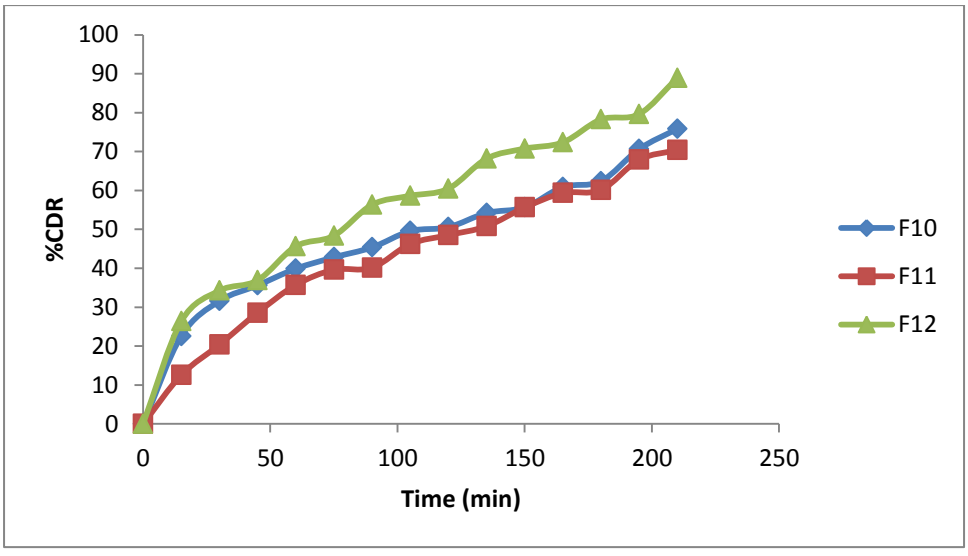

Figure 10: *In vitro* drug release study.

Among the 12 set of formulations, it was found that formulation F5 showed faster drug release when compared to other formulations. The formulation F5 showed a highest percentage drug release of 100 % in 180 min. Hence the formulation F5 was considered for further studies and was selected for the formulation of S-SNEDDS by adsorption process.

Average particle size and size distribution:

The mean particle size of the optimized formulation was found to be 60.4 nm and the PDI was found to be 0.565.

Zeta potential:

The zeta potentials of about -22.4 mV indicate good stability of formulation. This might be attributed to surfactant which decreases the electrostatic repulsion between the particles and statically stabilizes the nanoparticles by forming a coat around their surface.

Formulation of S-SNEDDS by adsorption on a solid carrier:

Formulation F5 was selected for conversion from liquid SNEDDS (F5) to S-SNEDDS (SF5). S-SNEDDS was prepared by mixing liquid SNEDDS with MCC in 1:1 proportion. Resultant damp mass was passed through sieve no. 120 and dried at ambient temperature and stored until further use.

Characterization and evaluation of solid SNEDDS:

Drug Content:

Table 18: Drug content of formulation SF5

Sr.no	Amount of MTX (mg)	% Drug Content
SF 5	2.5 mg	98.20±5

The percentage drug content of the formulation SF5 was found out to be 98.20 %. The percentage drug content of formulation SF5 was found to be in the range of ± 5 %.

Reconstituted properties of S-SNEDDS:

Dilution study by visual observation

Table 19: Dilution study by visual observation of formulation.

Formulation Code	Observations
SF5	Stable, no precipitation (25)

The formulation SF5 was subjected to stability on dilution study. The result of which is shown in Table 19. From the results it was concluded that the formulation was found to be stable and there was no evidence of precipitation found. Thus the formulation passed the stability study on dilution and found to be stable formulation. A clear nano emulsion was formed within 25 sec, hence it was judged to be qualitatively good.

Angle of repose:

Table 20: Angle of repose of formulation SF5.

Formulation	Angle of repose
SF5	27.82±0.07

The angle of repose of formulation SF5 was found out to be 27.82 which revealed excellent flow property of the formulation.

Bulk Density:

Table 21: Bulk density of formulation SF5.

Formulation	LBD	TBD
SF5	0.58	0.64

The bulk density of formulation SF5 was found out to be 0.58 (LBD) and 0.64 (TBD) which revealed good flow property of the formulation.

Compressibility Index (Carr's):

Table 22: Carr's index of formulation SF5.

Formulation	Carr's Index
SF5	18.28

The compressibility index of formulation SF5 was found out to be 18.28 which revealed good flow property of the formulation.

Hausner's ratio:

Table 23: Hausner's ratio of formulation SF5.

Formulation	Hausner's Ratio
SF5	1.05

The Hausner's ratio of formulation SF5 was found out to be 1.05 which revealed excellent flow property of the formulation.

***In vitro* release:**

Table 24: *In vitro* drug release of S-SNEDDS formulation SF5.

Time (min.)	%CDR (SF5)
15	23.56
30	34.38
45	45.21
60	50.62
75	58.46
90	60.25
105	65.55
120	68.23
135	70.88
150	75.63

165	77.34
180	89.22
195	96.20
210	100

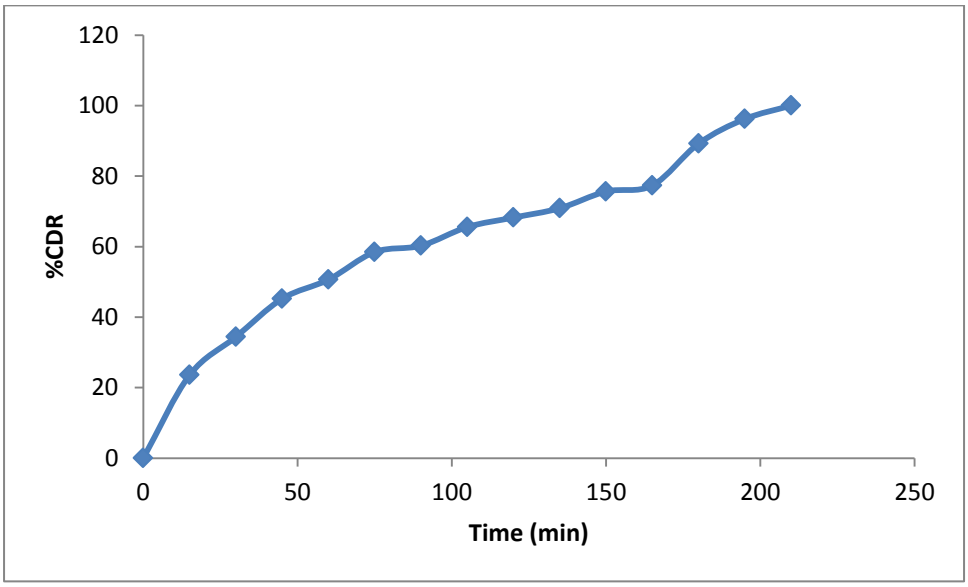

Figure 11: *In vitro* drug release study of SF5.

The formulation SF5 showed quick drug release of 70.88% only in 135 min. The formulation SF5 showed 100 % percentage drug release in 210 min. The formulation SF5 did not show any significant changes in *in vitro* drug release when compared with the liquid SNEDDS formulation. Hence there was a successful conversion of liquid SNEDDS formulation to S- SNEDDS formulation by adsorption process.

CONCLUSION

MTX is ingested orally in treating cancer and rheumatoid arthritis. However, the oral use of the marketed tablet is followed by various drawbacks including the low solubility, low bioavailability and increased dosing frequency. In the current work, an attempt was made to formulate and evaluate solid self nano emulsifying drug delivery system using soyabean oil, span 80 and isopropyl alcohol. The liquid SNEDDS was prepared by continuous stirring of oil phase, surfactant and co-solvent at 60ºC. The optimized formulation showed a size of 60.4 nm and a PDI value of 0.565 exhibiting smaller and a good uniformity in the size of the particles. The zeta potential was found to be -22.4 mV which revealed that the emulsion was stable. The S-SNEDDS was prepared by mixing liquid SNEDDS with MCC in 1:1 proportion. The FTIR analysis showed no change in the signal peaks thus clearly indicating the absence of any interactions between the ingredients. The *in vitro* drug release of S-SNEDDS showed drug release of 75% only in 180 min and 100 % drug release in 210 min which indicated increased drug release due to increase in its solubility. The

S-SNEDDS of MTX developed in this study has successfully increased the solubility and bioavailability when compared to marketed formulation. The present study showed lot of promise to scale up the method and to investigate further so that the said formulation can become commercially successful.

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