DEVELOPMENT AND EVALUATION OF A SELF-MICRO EMULSIFYING OMEGA-3 FATTY ACID DRUG DELIVERY SYSTEM USING FLAXSEED OIL

Abstract

Self micro-emulsifying drug delivery system is the mixture of oils, surfactants, and cosurfactants, which are emulsified in aqueous media under conditions of gentle agitation and digestive motility that would be encountered in the gastro-intestinal (GI) tract that leads improve the solubility, bioavailability and biocompatibility. Purpose of this work is to prepare self-microemulsifying drug delivery system (SMEDDS) to enhance the oral bioavailability and stability of linseed oil (Omega-3 fatty acid). Linseed contains very high amount of omega-3 fatty acid (55-60%). Solubility of linseed oil (Omega-3 fatty acid) was determined in various solvents. Based on screening for oil, surfactant and co-surfactant; Linseed oil, Cremophor RH-40 Surfactant, and Imwitor-988 Co-surfactant were selected. Pseudo-ternary phase diagrams were constructed to identify the efficient self-emulsification region and particle size distributions and zeta potential of the resultant microemulsions were evaluated. The release study of Omega-3-fatty acid from the SMEED formulation and marketed capsule formulation were compared, and shown to exhibit significantly higher release. Also GC chromatogram showed the better stability of omega-3-fatty acid in SMEED formation as compared to marketed capsule formulation. Our study demonstrated the potential and alternative formulation for efficient delivery of omega-3-fatty acid that contained in linseed
oil.

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I. INTRODUCTION

Although flaxseed oil is readily available and contains a high concentration of omega-3 fatty acids (55–60%), it is also highly susceptible to oxidation due to its high polyunsaturated fatty acid content (>75%) in the presence of oxygen, metal ions, and high temperatures. As a result, toxic hydro peroxides and off-flavoring compounds are produced during processing. Oleic acid (C18:1) is said to be 20 times more vulnerable to oxidation than - linolenic acid (ALA, C18:3, -3).

SEDDS, also known as self-emulsifying oil formulations (SEOF), are described as isotropic combinations of hydrophilic solvents and co-solvents, solid or liquid surfactants, and natural or synthetic oils. These systems can produce fine oil-in-water (o/w) emulsions, micro emulsions, or self-micro emulsifying drug delivery systems (SMEDDS) after light agitation and dilution in aqueous medium, such as gastrointestinal (GI) fluids. SMEDDS are defined as isotropic mixtures of hydrophilic solvents and co-solvents/surfactants that have the special ability to form fine oil-in-water (o/w) micro emulsions upon mild agitation followed by dilution in aqueous media, such as GI fluids.

The SMEDDS produce microemulsions with good thermodynamic stability and optical transparency. The size of the droplets makes a significant distinction between the aforementioned micro emulsions and conventional emulsions. In general, the size of the droplets of micro emulsions created by the SMEDDS ranges between 2 and 100 nm, while that of the droplets of ordinary emulsions is between 0.2 and 10 m. The bioavailability of the medicine is improved because the small particle size results in a much bigger total surface area for absorption and dispersion than that of a traditional emulsion. It can also easily traverse the gastrointestinal tract and be better absorbed.

Developing and evaluating the tests on a self-micro emulsifying drug delivery system (SMEDDS) to distribute omega-3 fatty acids from flaxseed oil more effectively and prevent oxidation was the aim of this effort. The purpose of the study was to produce a SMEED employing flaxseed oil to effectively deliver omega-3 fatty acids and to increase bioavailability. SMEED containing flaxseed was contrasted with flaxseed oil sold in soft gelatin capsules.

II. MATERIALS AND METHODS

1. **Materials:** Flaxseed oil was received as gift sample from RWNLF, Pune, Cremophore RH-40 procured from Himedia Ltd, Tween80 procured from S. D fine Chem. Ltd, Polyethylene Glycol (PEG400) procured from Suvidhinath Chemicals, Imwitor-988 received as gift sample from Olio-chemical, Jarman. All other reagents and chemical used of analytical grade.

2. **Methodology:**

   - **Screening of Surfactants:** 150 mg of the cosmophore RH-40 was added to 150 mg of the oily phase. The mixes were gradually warmed to 500C to homogenize the constituent parts. Then, distilled water was used to dilute each mixture, 100 mg, to 100

ml in a stoppered conical flask. The number of flask inversions necessary to produce a homogenous emulsion was used to determine how easy an emulsion was to produce. Emulsions were let to remain for two hours while a UV-Visible spectrophotometer measured their percent transmittance at 650 nm.

- **Screening of Co-Surfactants:** The several co-surfactants for the SMEEDS formulation were further screened using the chosen oil phase and surfactant. Similar to how it was done for the preliminary screening of surfactants, mixtures of 100 mg of co-surfactant, 200 mg of Cremophore RH-40 (surfactant), and 300 mg of linseed oil (Oil) were created and assessed.

- **Optimization of SMEDDS Formulations:** Surfactant and co-surfactant ratio were mixed at ratio 1:1, 1:2, 1:3, 1:4, 2:1, 3:1, 4:1. Take one by one ratio for further screening, surfactant-co-surfactant: oil ratio were mixed at ratio1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 respectively. Then add the distilled water up to 50-500 times and measure the % Transmittance by UV-Spectroscopy. After measuring the % Transmittance by observation take the selected batch for further preparation.

- **Pseudo Ternary Phase Diagram:** A valuable and significant tool for examining the size of the micro-emulsion zone and phase behavior is the pseudo ternary phase diagram. By adding distilled water drop by drop to a homogeneous liquid mixture of oil, surfactant, and co-surfactant at room temperature (the water titration method), the pseudo-ternary phase diagrams were created. A triangle with three coordinates can be used to represent a pseudo ternary phase diagram. Each coordinate stands for a specific part of the micro emulsion system. Oil phase, Surfactant-Co-surfactant phase (S mix), and Aqueous phase are the first three phases.

  To determine the necessary components and their concentration ranges that can lead to a large micro emulsion existence area, a pseudo-ternary phase diagram was created. S mix and oil were mixed at ratios of 0.1:0.9, 0.2:0.8, 0.3:0.7, 0.4:0.6, 0.5:0.5, 0.6:0.4, 0.7:0.3, 0.8:0.2, and 0.9:0.1 in pre-weighed vials at surfactant to co-surfactant ratios (1:1, 1:2, 2:1, and 3:1). The final combinations in the vials received drop-wise additions of distilled water. The moment at which the mixture turns from turbid to clear or slightly bluish emulsion was the end point of the water titration. Mixtures were now visually inspected for phase clarity and transmittance. Micro emulsion system was used to describe the final emulsion, which had a clear or slightly bluish appearance and had good stability and flow ability.

III. FORMULATION OF SMEDDS.

1. **Phase Titration Method (Water Titration Method):** Phase diagrams can be used to represent micro emulsions, which were created using the phase titration method. The creation of phase diagrams is a helpful method for studying the intricate web of interactions that might develop when several components are combined. Depending on the chemical makeup and concentration of each component, micro emulsions are created along with a variety of association structures (including emulsion, micelles, lamellar, hexagonal, cubic, and different gels and oily dispersion). The study's key components are the comprehension

of their phase equilibrium and the demarcation of the phase borders. Quaternary phase diagram (four component system) interpretation takes time and is challenging. To determine the various zones, including the micro-emulsion zone, pseudo ternary phase diagrams are frequently created. In these diagrams, each corner of the diagram represents 100% of the specific component. They can be divided into w/o or o/w micro emulsions by only taking the composition—whether it is oil- or water-rich—into account. Care should be taken when making observations to ensure that the meta-stable systems are excluded. In this approach, different mixtures of oil and Surfactant and Co-surfactant are generated at constant Surfactant and Co-surfactant ratios, and the water is added drop by drop. Each drop is added, and then the liquid is mixed and viewed with a polarizing filter or your unaided eye.

2. Evaluation of SMEDDS Formulation

- **Particle Size Analysis:** The particle size was determined using a Nano particle Analyzer (Horiba Scientific Nano Partica SZ-100, Japan). The average particle size was expressed in terms of nm. SMEDDS were diluted and put in the cuvette in analyser.

- **Zeta Potential Measurement:** Zeta potential distribution was determined by using a Zeta sizer (Horiba, SZ-100 Japan). One mg of freeze dried ITSLN were dispersedZP1in distilled water. To prevent the agglomeration, the dispersed solution was placed for 5minutes in ultra-sonication bath. Then the sample was taken in the glass cuvette and zeta potential was measured in range from -200 to + 200mv.

- **pH Determination:** Using a pH meter, the pH of each formulation was examined. Prior to each usage, the pH meter was calibrated using buffer solutions with a standard pH of 4 and 7. The pH was measured when the pH meter electrode was submerged in 10% aqueous liquid SMEDDS solution.

3. Thermodynamic Stability

- **Heating Cooling Cycle:** Six cycles between (4°C) and (45°C) in the refrigerator, with a minimum of 48 hours of storage at each temperature were examined. Centrifugation tests were performed on those formulations that remained stable at these temperatures.

- **Centrifugation:** Passed formulations were centrifuged at 5000 rpm for 30 min. Those formulations that did not show any phase separation were taken for the freeze thaw stress test.

- **Freeze thaw Cycle:** Three freeze thaw cycles between -10^0 C and 25^0 C with storage at each temperature for not less than 48 hrs was done for the formulations. Those formulations, which passed these thermodynamic stress tests, were further taken for the dispensability test for assessing the efficiency of self-emulsification.

IV. RESULT AND DISCUSSION

1. **Pre-Formulation Study:** The sample of linseed oil procured for study was identified and estimated for its purity. The sample was identified by organoleptic property, solubility study, UV spectrum and gas chromatography

Table 1: Organoleptic Properties

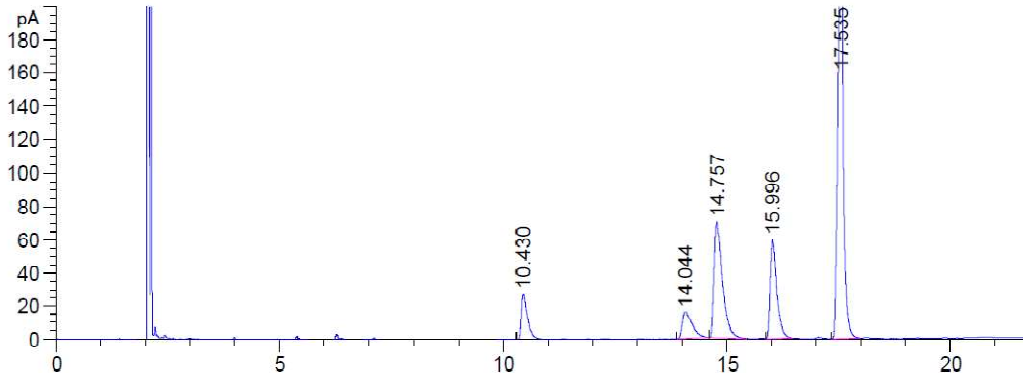
Sr. No	Parameter	Observation
1	Physical State	Liquid
2	Colour	Yellow to brown colour
3	Odour	Typical odor of flax seeds
4	Taste	Nutty flavor

2. **Solubility Study:** The solubility of linseed oil in various solvents was found to be:

Table 2: Solubility of Linseed Oil

Solvents	Solubility
Distilled water	Insoluble
Alcohol	Slightly Soluble
Light Petroleum	Soluble
n-Hexane	Soluble

3. **Gas Chromatographic Analysis of Flaxseed Oil:** GC study of drug sample showed a peak of ALA corresponding to its retention time and indicating its peak area and percent area



Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %	
1	10.430	BB	0.1168	272.43314	27.68369	6.25329	Palmitic acid
2	14.044	BB	0.1922	264.22073	16.13563	6.06478	Stearic acid
3	14.757	BB	0.1593	946.86005	69.88579	21.73373	Oleic acid
4	15.996	BB	0.1252	620.13000	59.68527	14.23414	Linoleic acid
5	17.535	BB	0.0988	2252.99463	271.47025	51.71406	ALA

Figure 1: Gas Chromatogram of Flaxseed Oil

4. **Standard Calibration Curve of Flaxseed Oil in Methanol:** Standard calibration curve of linseed oil was drawn by plotting absorbance Vs concentration. The λmax of linseed oil in methanol was found to be 313nm. The absorbance values are tabulated in Table 3. Standard calibration curve of linseed oil followed Beer's Lambert range between 2-20 ug/ml as shown in figure 2. The equation of line was found to be $y = 0.0179x + 0.0004$ with correlation coefficient $R^2 = 0.9995$.

Table 3: Absorbance Value of linseed Oil in Methanol

Sr. No.	Concentration (µg/ml)	Absorbance
1	2	0.034
2	4	0.069
3	6	0.111
4	8	0.146
5	10	0.179
6	12	0.217
7	14	0.253
8	16	0.291
9	18	0.324
10	20	0.353

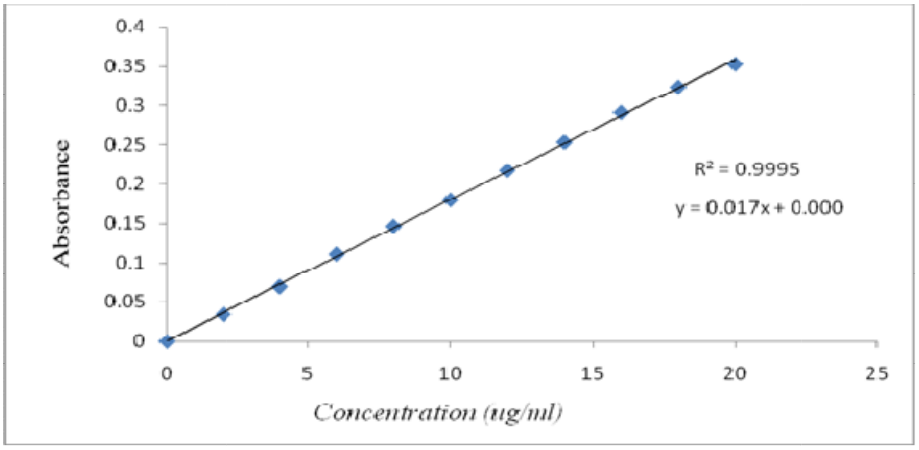


Figure 2: Standard Calibration Curve of Flaxseed Oil in Methanol

5. SMEDDS Formulation

- **Screening of Surfactants for SMEDDS Formulation:** In screening of surfactants 3 combinations were evaluated for ease of emulsification and percent transmittance (Table 4). After the screening for emulsification study; Cremophore RH-40 (surfactant) showed maximum transmittance. Hence selected for further screening

Table 4: Screening of Surfactants for SMEDDS Formulation

Sr. No.	Oil	Surfactant	No. of Flask Inversions		% Transmittance	
			Batch A	Batch B	Batch A	Batch B
1	Linseed oil	Cremophore RH-40	5	6	92.5	91.7
2		Solutol-HS-15	8	7	43.2	44.5
3		Tween-80	2	3	18.3	18.7

- **Screening of Co-Surfactants for SMEDDS Formulation:** Three combinations of co-surfactants were tested in an emulsification study for their ease of emulsification using the flask inversion method, and the percent transmittance was calculated. Linseed oil (oil), Cremophore-rh-40 (surfactant), and Imwitor-988 (co-surfactant) require the fewest number of flask inversions among the various combinations, and the resulting emulsion exhibits the highest transmittance (84.3%).

Table 5: Screening of co-surfactants for SMEDDS formulation

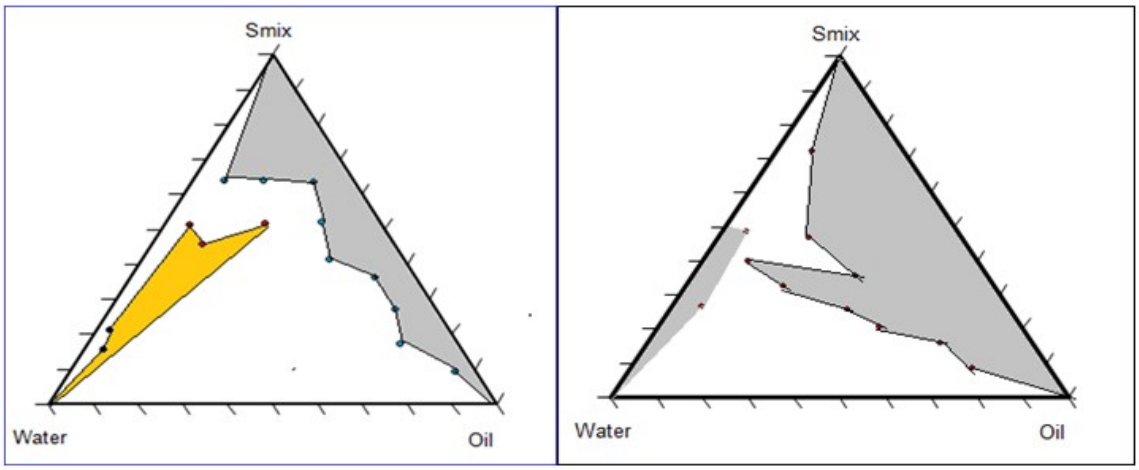
Sr. No.	**Oil**	**Surfactant**	**Co-surfactant**	**No. of Flask Inversions**		**% Transmittance**	
				Batch A	**Batch B**	**Batch A**	**Batch B**
1	Linseed oil	Cremophore RH-40	PEG 400	3	6	57.2	56.8
2			PEG 200	7	8	21.5	22.1
3			Imwitor-988	9	8	83.5	84.3

- **Optimization of SMEDDS Formulation:** Surfactant and co-surfactant ratio were mixed at ratio 1:1, 1:2, 1:3, 1:4, 2:1, 3:1, 4:1. Take one by one ratio for further screening, surfactant-co-surfactant: oil ratio were mixed at ratio1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 respectively. Then add the distilled water up to 50-500 times and measure the % Transmittance by UV-Spectroscopy.

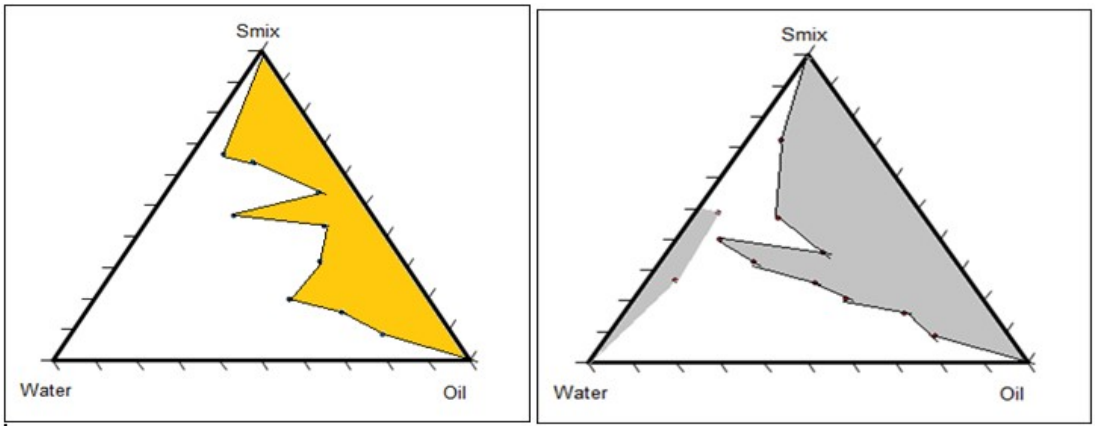
Table 6: Optimization of SMEEDS Formulation

Code no	S-cos: oil	S: cos ratio						
		01:01	**01:02**	**01:03**	**01:04**	**02:01**	**03:01**	**04:01**
F1	**1:9**	37.2	36.1	54.8	35.3	42.2	43.6	39.2
F2	**2:8**	2.1	3.4	1.4	3.2	6.1	6.9	4.1
F3	**3:7**	3.9	4.2	3.3	1.6	5.1	5.2	5.9
F4	**4:6**	4.9	5.1	4.3	1.9	7.1	6.9	6.1
F5	**5:5**	1.8	2.2	1.5	1.3	2.4	3.1	1.9
F6	**6:4**	2.9	3.2	2.4	11.1	5.3	4.2	4.2
F7	**7:3**	74.2	95.3	68.8	46.3	78.3	31.4	50.2
F8	**8:2**	87.2	94.4	92.2	73	99.4	92.6	96.4
F9	**9:1**	92.4	99.3	88.8	88.5	100.4	99.7	99.7

6. **Construction of Pseudo-Ternary Phase Diagrams:** A series of Micro-emulsion were prepared, and their micro-emulsifying properties were observed visually. The phase behaviours of Linseed oil and (Smix) showed (Figure 3-7). It was find out the best ratio 1:1 which gives more micro-emulsion region.



(A) (B)



(C) **(D)**

Figure 3: Ternary phase diagram of flaxseed oil: ratio of Cremophore RH-40: Imwitor-988 (S: cos) in the ratio 1:1, 2:1, 1:2 and 3:1 shown in fig (A), (B), (C), and (D) respectively.

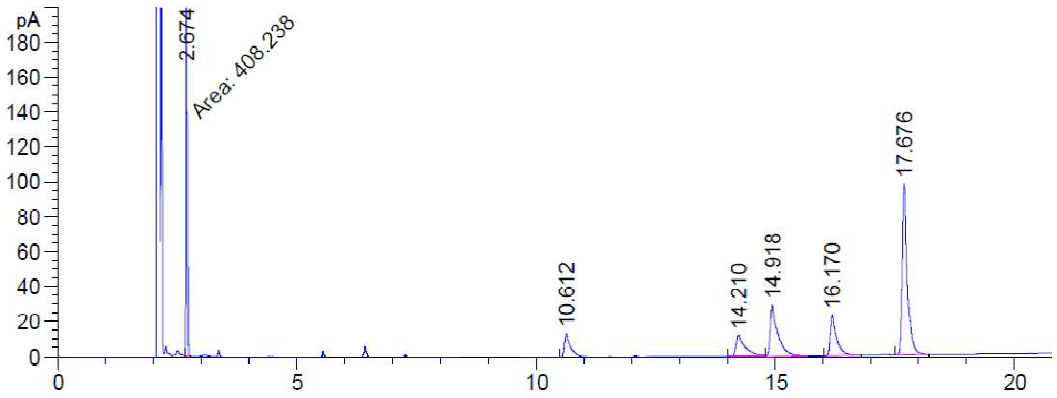
7. Evaluation of Self-Micro-Emulsion Drug Delivery System (SMEDDS)

- **Emulsification:** Transmittance study revealed that as the concentration of surfactant increases the transmittance of resulting emulsions increases the ease of emulsification or rate of emulsion formation was measured by UV-spectrophotometer.

Table 7: Ease of Emulsification, Phase Separation of Liquid SMEDDS

Formulation code	No. of Flask Inversions	% Transmittance	Phase separation
F_1	5	88.5	No
F_2	9	87.9	No
F_3	6	86.5	No
F_4	4	**90.2**	No
F_5	5	**91.9**	No
F_6	6	89.7	No
F_7	7	89.5	No
F_9	8	**90.5**	No

- **Gas Chromatography Study:** GC chromatogram shows the peak of ALA (Omega-3 fatty acid) at its corresponding retention time indicating its omega-3 fatty acid percent area (Figure 4). There was SMEDDS formulation shows the percentage of omega-3 fatty acid i.e. ALA. The fatty acid analysis of SMEDDS formulation revealed 36.70% ALA. F5 formulation was shown best result as compare other formulation so it was carried out for gas chromatography analysis.



Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %	
1	2.674	MM	0.0243	408.23837	280.55594	20.35208	Caprylic acid
2	10.612	BB	0.1212	119.94732	13.60708	5.97978	Palmitic acid
3	14.210	BV	0.1743	160.02948	12.10039	7.97802	Stearic acid
4	14.918	VB	0.1432	318.03986	29.34591	15.85537	Oleic acid
5	16.170	BB	0.1222	206.31609	23.55575	10.28556	Linoleic acid
6	17.676	BB	0.1064	736.28619	97.56111	36.70638	ALA
7	26.512	MM	0.1022	57.02350	9.30070	2.84282	

Figure 4: Gas Chromatogram of SMEDDS Formulation

- **Zeta Potential Measurements:** Zeta potential of optimized SMEDDS formulation was at -36.0 mv (Figure 5). Zeta potential in the range to -30 mv to + 30 mv is common for stabilized SMEDDS formulation, thus the zeta potential value indicated that the electrostatic repulsion between particles will prevent their aggregation and there by stabilize the nanoparticulate dispersion.

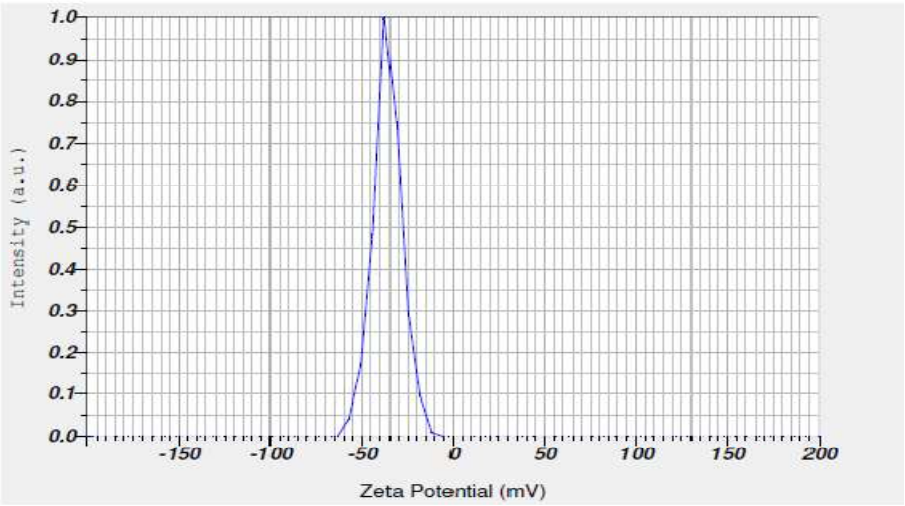


Figure 5: Zeta Potential of SMEDDS Formulation

- **Particle size Distribution:** The self-emulsification process is greatly influenced by the droplet size of the emulsion since it controls both the rate and degree of drug release as well as drug absorption. Additionally, it has been suggested that the emulsion droplets' lower particle size may promote the formulation's bioavailability and speed up absorption. The distribution of Omega-3 SMEDDS particle sizes after water diluting. Omega-3 SMEDDS have particles as small as 43.5 nm.

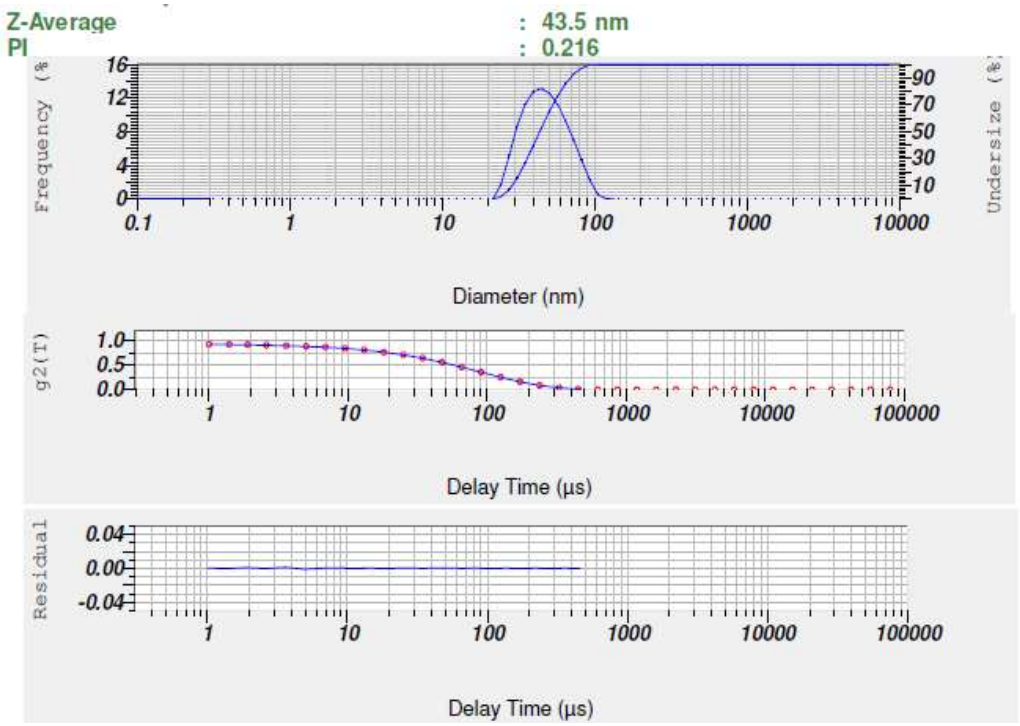


Figure 6: Particle Size of SMEDDS Formulation

- **pH Determination:** The pH optimized formulation of SMEDDS was found to be 2.9 & gastric pH of human is in the range of 1.5 to 3.5, so it was concluded that prepared formulation was compatible with human stomach

- **Thermodynamic Stability Study:** With no phase separation, creaming, or cracking, SMEDDS are thought to be thermodynamically stable systems that form at a concentration of oil, surfactant, and water. A number of stress stability tests, including centrifugation, freeze-thaw cycles, and heating-cooling cycles, were performed on a selected formulation from the phase diagram. Some formulations turned turbid and, in some phases, separated during physical stability testing. The Ostwald ripening, in which molecules move as a monomer and coalescence of small droplets occurs, may be one cause of this instability in micro emulsions. Diffusion processes driven by the gain in surface free energy result in the production of big droplets. The other explanation could be that instability of microbes results from temperature quench during stress stability research.

Table 8: Thermodynamic Stability Study Data

Sr. No	Formulation	Centrifugation	Heating Cooling Cycle	Freeze and Thaw Cycle
1	**F_1**	Pass	Pass	Fail
2	**F_2**	Pass	Fail	Fail
3	**F_3**	Fail	Fail	Fail
4	**F_4**	Pass	Fail	Fail
5	**F_5**	**Pass**	**Pass**	**Pass**
6	**F_6**	Pass	Fail	Pass
7	**F_7**	Pass	Fail	Pass
8	**F_8**	Fail	Fail	Fail
9	**F_9**	Pass	Pass	Fail

V. CONCLUSION

Measure focus of research work was to develop Self-Micro-Emulsifying Drug Delivery System (SMEDDS) by using flaxseed oil. This system is able to rapidly self-micro emulsifies in GI fluids and forms fine o/w micro-emulsion under the gentle agitation by GI tract movements. The drugs (linseed oil) in our work were identified and characterized by GC and UV spectroscopy. λmax of linseed oil was observed at 313 nm in methanol. Gas chromatography analysis revealed, ALA 51.7 %. Surfactant Cremophore RH-40, co-surfactant imwitor-988 showed maximum solubility. Cremophore RH-40 showed maximum %Transmittance 92.5%, co-surfactant imwitor-988 showed maximum % Transmittance 84.3%. The optimization of surfactant: co-surfactant (S/CoS) ratio was performed using pseudo ternary phase diagrams by water titration method. Pseudo ternary phase diagram showed 1:1 best ratio which gave more micro emulsions region. In SMEDDS formulation fatty acid analysis revealed 36.37% ALA Formed SMEDDS formulation having particle size less than 100nm. The particle size was observed in SMEDDS formulation 43.5 nm. Zeta potential of SMEDDS formulation -36mv was found. The range between zeta potential is -30mv to +30mv which shows the stable formulation. The SMEDDS formulation zeta potential was -36mv that indicate stability of SMEDDS formulation. Stability of SMEDDS formulation was found to be stable with respect to centrifugation, heating–cooling cycle and freeze thaw cycle indicating stability of formulation. Self-micro emulsifying drug delivery system for efficient delivery of omga-3-fatty acid is successfully developed.

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