**Development and Evaluation of a Self-Micro Emulsifying Omega-3 Fatty Acid Drug Delivery System using Flaxseed Oil**

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**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.

**Materials and Methods**

**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.

**Methodology:**

**Screening of surfactants**

Cremophore RH-40 (150 mg) was added to 150 mg of oily phase. The mixtures were gently heated at 500C for homogenization of the components. Each mixture, 100 mg, was then diluted with distilled water to 100 ml in a stoppered conical flask. Ease of emulsification was judged by the number of flask inversions required to yield homogenous emulsion. Emulsions were allowed to stand for 2hrs and their % transmittance was evaluated at 650 nm by UV-Visible spectrophotometer.

**Screening of co-surfactants**

The selected oil phase and surfactant were used for further screening of the different co-surfactants for the SMEEDS formulation. Mixtures of 100 mg of co-surfactant, 200 mg Cremophore RH-40 (surfactant), and 300 mg Linseed oil (Oil) were prepared and evaluated in a similar fashion as described in preliminary screening of surfactants.

**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:**

Pseudo ternary phase diagram is a useful and important tool to study the extent of micro emulsion region and phase behaviour. The pseudo-ternary phase diagrams were constructed by drop wise addition of distilled water to homogenous liquid mixture of oil, surfactant, and co-surfactant, at ambient temperature (water titration method). Pseudo ternary phase diagram can be represented in a triangular format (triangle) which has three coordinates. Each coordinate represents one component of micro emulsion system viz. (1) Oil phase (2) Surfactant-Co-surfactant phase (S mix) and (3) Aqueous phase

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.

**Formulation of SMEDDS.**

**Phase titration method (Water titration method)**

Micro emulsions were prepared by the phase titration method and can be depicted with the help of phase diagrams. Construction of phase diagram is a useful approach to study the complex series of interactions that can occur when different components are mixed. Micro emulsions are formed along with various association structures (including emulsion, micelles, lamellar, hexagonal, cubic, and various gels and oily dispersion) depending on the chemical composition and concentration of each component. The understanding of their phase equilibrium and Demarcation of the phase boundaries are essential aspects of the study. As quaternary phase diagram (four component system) is time consuming and difficult to interpret, Pseudo ternary phase diagram is often constructed to find the different zones including micro-emulsion zone, in which each corner of the diagram represents 100% of the particular component. They can be separated into w/o or o/w micro emulsion by simply considering the composition that is whether it is oil rich or water rich. Observations should be made carefully so that the meta-stable systems are not included. In this method, at a constant ratio of Surfactant and Co-surfactant, various combinations of oil and Surfactant and Co-surfactant are produced, and the water is added drop wise. After the addition of each drop, the mixture is stirred and examined through a polarized filter or by naked eye.

**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.

**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 -100 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.

**Result and Discussion**

**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 |

**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 |

**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





**Figure 1 Gas chromatogram of flaxseed oil**

**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 R² = 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 |

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Figure 2 Standard Calibration Curve of flaxseed oil in methanol

**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**

In emulsification study of co-surfactants 3 combinations were evaluated for ease of emulsification by flask inversion method and percent transmittance was measured. Among different combinations linseed oil (oil), Cremophore-rh-40 (surfactant) and Imwitor-988 (Co-surfactant) require only 4-5 flask inversion and resultant emulsion showed maximum 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 |

**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.



(B)

(A)

 ****

(D)

(C)

**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.**

**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** |
| **F1** | 5 | 88.5 | No |
| **F2** | 9 | 87.9 | No |
| **F3** | 6 | 86.5 | No |
| **F4** | 4 | **90.2** | No |
| **F5** | 5 | **91.9** | No |
| **F6** | 6 | 89.7 | No |
| **F7** | 7 | 89.5 | No |
| **F9** | 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.





**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 droplet size of the emulsion is a crucial factor in self-emulsification process because it determines the rate and extent of drug release as well as drug absorption. Also, it has been reported that the smaller particle size of the emulsion dro1plets may lead to more rapid absorption as well as enhance the bioavailability of the formulation. The particle size distribution of Omega-3 SMEDDS diluted with water. The particle size of omega-3 SMEDDS is 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**

SMEDDS are considered to be thermodynamically stable systems which are formed at a concentration of oil, surfactant, and water, with no phase separation, creaming or cracking. Selected formulation from phase diagram were subjected to different stress stability testing like heating cooling cycle, centrifugation and freeze thaw cycle. During physical stability testing some formulations became turbid and, in some phase, separation occurred. One reason of this instability in micro emulsions may be due to the Ostwald ripening in which molecules move as a monomer and coalescence of small droplets takes place, resulting in the formation of large droplets by diffusion processes driven by the gain in surface free energy. The other reason may be that when temperature quench occurs during stress stability study, instability of micro emulsion occurs due to separation of oil phase and droplet distribution of smaller size is favoured by the change in curvature free energy. Only **F5** formulation, which showed no phase separation, creaming, cracking, coalescence and phase inversion during stress stability tests,

**Table 8 Thermodynamic Stability study data**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sr. No** | **Formulation** | **Centrifugation** | **Heating Cooling Cycle** | **Freeze and Thaw Cycle** |
| 1 | **F1** | Pass | Pass | Fail |
| 2 | **F2** | Pass | Fail | Fail |
| 3 | **F3** | Fail | Fail | Fail |
| 4 | **F4** | Pass | Fail | Fail |
| **5** | **F5** | **Pass** | **Pass** | **Pass** |
| 6 | **F6** | Pass | Fail | Pass |
| 7 | **F7** | Pass | Fail | Pass |
| 8 | **F8** | Fail | Fail | Fail |
| 9 | **F9** | Pass | Pass | Fail |

**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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