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Impact of solidification on micromeritic properties and dissolution rate of self-nanoemulsifying delivery system loaded with docosahexaenoic acid

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Abstract

Development of self-nanoemulsifying drug delivery systems (SNEDDS) of docosahexaenoic acid (DHA) is reported with the aim to achieve enhanced dissolution rate. The optimized composition of liquid-SNEDDS (L-SNEDDS) formulation was Labrafil® M1944 CS, 47% v/v Tween 80, 27% v/v Transcutol P, and 0.1% v/v DHA. L-SNEDDS were solidified using Syloid XDP 3150 as solid porous carrier. The droplet size, polydispersity index, zeta potential, percentage drug loading and cloud point for L-SNEDDS were found to be 43.51 ± 1.36 nm, 0.186 ± 0.053, -19.20 ± 1.21mV, 93.23 ± 1.71 and 88.60 ± 2.54°C, respectively. Similarly, for solid-SNEDDS (S-SNEDDS) the above parameters were found to be 101.10 ± 1.87 nm, 0397 ± 0.043, -16.60 ± 2.18 mV, 91.23 ± 1.88 and 89.50 ± 1.18 °C, respectively. The formulations (L-SNEDDS, S-SNEDDS powder and S-SNEDDS tablet) showed significant ($p < 0.05$) improvement in dissolution rate of drug in 0.1N HCl (pH 1.2) and phosphate buffer (pH 6.8) as compared to unprocessed DHA. In both the dissolution media, the dissolution rate was found more that 85% in 90 minutes. Absence of drug precipitation, phase separation and turbidity during thermodynamic stability studies indicated that the developed SNEDDS were stable. Hence, it was concluded that SNEDDS have offered sufficient stability as well as dissolution rate of DHA.

Keywords: Solid-SNEDDS; Dissolution; Docosahexaenoic acid; Stability; TEM

1. Introduction

Docosahexaenoic acid (DHA) is a type of omega 3 fatty acid which is essential for the body. It is abundantly found in the human brain and plays a vital role in various stages of its growth and development including neural cell propagation, differentiation, migration, synaptogenesis etc (1). However, the brain is not able to synthesize DHA in sufficient amount and the uptake of DHA from circulating lipid pools is necessary to maintain homeostatic levels (2). DHA also finds its use in the treatment of various cardiovascular disorders and is helpful in decreasing triglycerides and cholesterol level (3). DHA and its metabolites are found to have a defensive potential in the treatment of human cancer by amelioration of symptomatic inflammation (4). Despite being such a potential candidate in the treatment of various disorders, use of DHA *i* is limited due to certain challenges such as poor aqueous solubility and first pass hepatic metabolism.

To overcome both the aforementioned challenges associated with DHA, self-nanoemulsifying drug delivery system (SNEDDS) of DHA have been formulated in the present research. SNEDDS constitute an isotropic mixture of oil, surfactant and co-surfactant which upon dilution form emulsion. Although there are numerous techniques for improving the poor aqueous solubility of drugs such as pro-drug formation, formulation of inclusion complexes, nanoparticles, solid dispersions and liquisolid compacts, their application is limited by certain physiological or pharmaceutical challenges. Physiological challenges include lack of protection of drug degradation due to acidic pH of stomach, GI enzymes or hepatic metabolism. Pharmaceutical challenges, on the other hand, include difficulty in scale up and stability of formulation. SNEDDS have proven their potential in enhancing the solubility as well as protecting the drugs from hepatic first pass metabolism (5). In SNEDDS systems, drug is present in dissolved form and their relatively smaller droplet size provides a large interfacial area. The large interfacial area enhances the activity of lipase (gastric as well as

pancreatic) to hydrolyze triglycerides, thereby promoting faster release of drug and formation of mixed micelles with bile salts. These micelles, in turn, form a cover over the drug to protect it against the enzymatic degradation (6, 7). In past, this technology has been reported toenhance the solubility as well as bioavailability of lipophilic drugs such as polypeptide k (8), curcumin and duloxetine (9), simvastatin (10), clopidogrel (11), rifampicin (12), andrographolide (13) and nabumetone (8). Despite having aforementioned advantages, SNEDDS suffer from certain limitations such as low stability (creaming, cracking, Ostwald ripening) during storage, difficulty in transportation, lack of dose accuracy, and interaction with the capsule shell.

These challenges associated with liquid SNEDDS (L-SNEDDS) can be overcome by their solidification. This is accomplished either by their direct adsorption onto the solid carriers such as silica and lactose, melt extrusion or by spray drying technique. It is important to note that liquid SNEDDS of DHA have been reported by Puri *et al*., 2015 (14) and solid SNEDDS by Singh et al., 2019 (15) using hydrophilic solid carriers. The reported formulations have shown excellent stability as well as therapeutic efficacy. In this manuscript, we have reported the formulation with hydrophobic adsorbants as well as screening of various oils to achieve optimised SNEDDS formulation. Impact of various hydrophilic and hydrophobic carriers on micromeritic properties and stability of developed formulation has also been discussed. This screening was considered to be significant due to liquid nature of DHA. Moreover, it is added in an isotropic mixture of oils and liquid surfactants. Hence, to solidify DHS-SNEDDS, more amount of hydrophilic carrier is required which, in turn, increases total weight of the formulation making it cumbersome to administer such high dose. Hydrophobic carriers such as aerosil or, other silica are known for excellent adsorption and flow properties due to very large surface area. This could offer solid DHA-SNEDDS using less amount of carrier with better flow properties. Hence, in the present study, an attempt has been made to

develop S-SNEDDS of DHA in order to improve its solubility, dissolution rate and physico-mechanical stability.

2. Material and methods

2.1. Material

DHA was purchased from Bliss Life Science, Indore, India. Mustard oil, sesame oil, olive oil, castor oil, eucalyptus oil, peanut oil, propylene glycol, poly ethylene glycol (PEG) 200, 400, 600, and 800, tween 20, 60, and 80, Aerosil 200 and sodium carboxy methyl cellulose (Na-CMC) were purchased from Central Drug House (CDH), New Delhi, India. Labrafac PG, Labrafil M1944 CS, Labrasol, and Transcutol P were received as gift sample from M/s Gattefosse, Mumbai, India. Capmule MCM was obtained from M/S Abitec Corporation, Mumbai, India. Microcrystalline cellulose PH102 (MCC PH102) was gifted by Colorcon Pvt. Ltd., Mumbai, India. Syloid XDP 3150 (SXDP) and Syloid 244FP (SFP) were gifted by Grace Materials Technologies, Discovery Science, Pune, India. Magnesium Stearate (MS) was purchased from S.D fine Chemical Ltd., Mumbai, India. In order to quantify the drug, UV–Visible double beam spectrophotometer (UV-1800, Shimadzu, Japan) was used. Magnetic Stirrer, REMI, India was used for mixing of solutions. Dissolution apparatus DS8000, Lab India, Mumbai, India was used to carry out release studies. All other chemicals and reagents used were of analytical grade.

2.2. Solubility studies

2.2.1. Solubility studies of unprocessed DHA in various oils, surfactant and co-surfactants

Solubility of unprocessed DHA was measured in various oils, surfactants and co-surfactants. Drug (100 mg, equivalent to 250 µl) was added in 1 mL of various oils (mustard oil, sesame oil, olive oil, castor oil, eucalyptus oil, peanut oil, Labrafac PG, Labrafil M1944CS, Labrasol, Capmul MCM), surfactants (propylene glycol, PEG 200, 400, 600, and 800, tween 20, 60,

and 80) and co-surfactants (Transcutol P). All the solutions were initially vortexed to ensure the uniform dispersion of drug. Mechanical shaking was carried out for all solutions for 48 h. The speed of shaker was maintained at 50 rpm and the temperature of bath was maintained at 37 ± 0.2°C. After shaking, all the samples were centrifuged at 5000 rpm for 10 min. (7, 9, 16-18). Supernatant was then collected and diluted with different solvents. All the surfactants were diluted using distilled water, whereas, oils were diluted using hexane. Transcutol P was diluted using ethanol. The diluted solutions were scanned under UV-Visible spectrophotometry at 275 nm. All experiments were carried out in triplicate and mean data was recorded.

2.3. Preparation of L-SNEDDS and Construction of pseudo-ternary phase diagram

From the results of solubility studies, it was observed that DHA had maximum solubility in Labrafil M1944 CS (oil), tween 80 (surfactant) and Transcutol P (co-surfactant). Hence, these were further screened for their optimum ratio to form SNEDDS. To find out the SNEDDS region in the phase diagram, twenty seven different batches of oil, surfactant, and co-surfactant was prepared by varying the ratio of oil and mixture of surfactant and co-surfactant (Smix) in 1:9 (Oil:Smix). The Smix (surfactant and co-surfactant) was varied in the ratio of 1:1, 1:2, and 2:1. The pseudo-ternary phase diagram was constructed using "Triplot"-software to find out the correct composition of the oil, surfactant, and co-surfactant which gives nano-emulsion. The prepared isotropic mixtures were diluted to 250 ml with distilled water and kept in a 500 ml glass beaker, maintained at 37 ± 02ºC on a magnetic stirrer at 50 rpm and time of emulsification was measured. The prepared emulsions were identified as nanoemulsion (transparent), microemulsion (translucent), and coarse emulsion (opaque) based on their visual observation. The prepared emulsion was further kept on storage for 48 h at room temperature for visual observation like phase separation, turbidity, creaming and cracking (7, 17, 18).

2.5. Preparation of S-SNEDDS

2.5.1. Oil adsorption capacity (OAC)

To increase the stability of the optimized L-SNEDDS formulations, they were converted to S-SNEDDS by physical adsorption technique with suitable carriers. Both the hydrophobic carrier Syloid® XDP 3150 (SXDP), Syloid® 244FP (SFP), Aerosil®-200 (A-200) Micro Crystalline Cellulose (MCC) PH102, Magnesium stearate (MS), lactose and hydrophilic carriers like Na-CMC were used in the formulation. To achieve better flow and compaction properties, it was important to know the OAC of porous carrier. OAC was determined by measuring the amount required for transforming the unit dose of liquid oily formulation into the solid free flowing powder by gravimetric method(9)

2.5.2. Preparation of S-SNEDDS using surface adsorption technique

The results showed the OAC of SXDP to be highest. Therefore, it was selected for solidification of L-SNEDDS. The optimized L-SNEDDS were converted into S-SNEDDS by Surface adsorption technique. Solid carrier was added into the optimized L-SNEDDS formulation and triturated until the formulation got converted into free-flowing powder. The obtained powder was passed through sieve no. 30 to break any possible lumps. Free flowing powder was then mixed with the excipients such as MCC PH102 (200 mg), PVK30 (200 mg), and starch powder (20 mg) and compressed into the tablets by direct compression method using 12 mm flat circular punch using rotary tablet compression machine with hydraulic pressure 8 kg/cm^2 (9).

2.6. Droplet size, zeta potential and polydispersity index (PDI)

Droplet size, PDI and zeta potential of formulation were determined using zeta sizer (nano ZS90, Malvern Instruments Ltd., UK). The readings were noted (in triplicate) using a laser beam (50 mV) at an angle of 90° in a disposable polystyrene cells maintained at 25°C. After

suitable dilution, samples were subjected for 12 sub-runs within 2 minutes to record the results

2.7. Drug Loading

Drug loading was calculated for the selected batch of L-SNEDDS using method reported by Garg *et al*., 2017. The L-SNEDDS as well the corresponding S-SNEDDS were diluted into 250 ml distilled water. Samples (5ml) were withdrawn, filtered and subjected to UV-Visible double spectrophotometer at 275 nm to check their absorbance (7, 9).

$$\% \text{ Drug Loading} = \frac{Amount\ of\ drug\ in\ known\ amount\ of\ L{-}SNEDDS}{Initial\ drug\ loading} \times 100$$

2.8. Evaluation of the optimized L-SNEDDS formulation for thermodynamic stability

Stability of the optimized L-SNEDDS formulation was evaluated using three parameters; temperature variation, centrifugation and cloud point. Samples were subjected to thermal stress by heating cooling cycles (4°C and 40°C), freeze thaw cycles (-21°C and +25°C), and storage stability at 40ºC for 48 h. Centrifugation stress was provided by centrifuging the diluted SNEDDS sample at 10000 rpm for 15 min. The diluted SNEDDS were prepared by addition of 1 mL of the formulation to 500 mL of distilled water. After centrifugation, the SNEDDS were visually observed for any instability (phase separation and drug precipitation). Cloud point was determined by heating 100 mL of diluted L-SNEDDS on a water bath as the temperature that was gradually increased from 25 to 100ºC. Heating was stopped upon appearance of cloudiness and temperature was recorded as its cloud point (7).

2.9. Robustness to dilution and pH change

The selected batch of SNEDDS was checked in various pH solutions such as distilled water, 0.1 M phosphate buffer (pH 6.8), 0.1 N HCL (pH 1.2) and phosphate buffer (pH 7.4). Different dilutions of these solutions were prepared (10,100, 250, 500 and 900 ml) and

isotropic mixture was added to each of these solutions, any change in droplet size and PDI was recorded (7, 18).

2.10. Viscosity measurement

The liquid SNEDDS formulations (F19) were subjected for viscosity measurement at 25±0.5℃as such, and after dilution by Brookfield Viscometer (Brookfield Engineering Labs, Middleboro, MA, USA) using spindle CC3-14 with shear rate at 100 rpm (19, 20). Solid SNEDDS were also subjected for viscosity measurement after dilution.

2.11. Characterization of developed S-SNEDDS formulation

2.11.1. Flow rate and Angle of repose

The angle of repose was determined using a fixed funnel and free-standing cone method by pouring the powder through the funnel till the apex of the cone and reaches the tip of the funnel. Then the average diameters of the base of the powder cones were measured (n=3) and the angle of repose was calculated using the equation 4.1 (9)

$$\tan \theta = 2h/D \qquad \text{Eq. (4.1)}$$

Here, h = Height of the heap of powder; D = Diameter of the base of the heap of powder.

2.11.2. Bulk density

The bulk density was determined using graduated measuring cylinder. Accurately weighed powder of S-SNEDDS was poured into a measuring cylinder and initial volume occupied by the powder was noted as the bulk volume and bulk density of the powder was calculated using the equation 4.2 (16).

$$\rho b = M/Vb \qquad \text{Eq. (4.2)}$$

whereas, ρb = bulk density, Vb= bulk volume, M= weight of powder

2.11.3. Tapped density

To determine its tapped density, the accurately weighed powder was taken into the measuring cylinder and the cylinder was tapped 100 times. The volume of tapped powder was noted and tapped density was calculated using the equation 4.3 (17).

$\rho t = M / Vt$ Eq. (4.3)

Where, Vt = Minimum volume occupied by the blend in the cylinder; M = Weight of the blend.

2.11.4. Carr's compressibility index

The Carr's compressibility index was calculated using equation 4.4.

CI = Tap density – Bulk density/Tap density × 100 Eq. (4.4)

2.12. *In vitro* dissolution studies

Dissolution study of L-SNEDDS, S-SNEDDS powder and S-SNEDDS Tablet were carried out and compared with that of unprocessed DHA and marketed formulation (OROMEGA-369, Pharvax, Bioscience, Chandigarh). The prepared L-SNEDDS and S-SNEDDS powder, were filled in '0' size hard gelatin capsule shells. The capsules of L-SNEDDS, S-SNEDDS powder and marketed formulation were kept in stainless sinker of dimension 21.3 x 9.4 mm capacity with 6 spirals and subjected for dissolution studies while, S-SNEDDS tablets were placed as such in the dissolution medium. The amount of DHA equivalent to 20 mg was present in all the formulations. USP type II dissolution apparatus and different dissolution media viz. 0.1 N HCL (pH 1.2), and 0.2 M phosphate buffer (pH 6.8) were used for the study. The temperature and stirring speed were maintained as 37 ± 0.5ºC and 50 rpm respectively. The study was carried out for 90 minutes and samples were withdrawn at predetermined intervals of 5, 10, 15, 30, 45, 60, 75 and 90 minutes. Sample (5ml) was withdrawn at

respective intervals and equal volume of medium was replaced to the vessels in order to maintain the sink condition. The collected samples were filtered and scanned using UV-Visible spectrometer for individual absorbance at 275 nm. The obtained results were recorded and percentage cumulative drug release was calculated (17).

2.13. Transmission Electron Microscopy (TEM)

To know the droplet morphology and any agglomeration for the optimized batches of S-SNEDDS, TEM was performed. The sample was diluted with distilled water. Formation of negative staining was done by putting a drop of emulsion or a carbon-coated copper grid for formation of a thin film. Excess amount of solution was removed by using filter paper. With the time lap of 10 minutes, phosphotungstic acid (PTA) was added dropwise on the copper grid and the excess amount was drained out. Then the grid was set for air drying and the required samples were analyzed through TEM (17).

2.14. Differential Scanning Calorimetry

The thermograms for DHA, Syloid XDP 3150, L-SNEDDS and S-SNEDDS formulation were recorded using DSC Q200 TA, Universal V 24.4 software, Bangalore, India, as per the procedure discussed Rajesh et al. (21). Samples (3 mg each) were crimped separately in an aluminium pan and heated from 0 to 300°C at a heating rate of 10°C/min. Nitrogen gas was purged at a flow rate of 50 mL/min during the scanning. An empty aluminium pan was used as reference. The melting points (Tm) were determined using TA-Universal Analysis 2000 software (version 4.7A).

2.15. Statistical analysis

All the experimental data are expressed as mean ± standard deviation (SD). Statistical assessment of the acquired information was performed using analysis of variance using

GraphPad Prism version 7.0 (GraphPad Software Inc., CA, USA). The $P < 0.05$ (wherever applicable) value showed a substantial difference in the outcomes achieved.

3. Results and discussion

3.1. Solubility studies

The result of the solubility studies indicated that maximum solubility of DHA were found in Labrafil M1944 CS (17.25 ± 1.24 µg/ml) as oil, Tween 80 (213.61 ± 2.2 µg/ml) as surfactant and Transcutol P (19.14 ± 1.44 µg/ml) as co-surfactant (Table 1). These were, therefore, selected for formulation of SNEDDS.

3.2. Construction of ternary phase diagram

Total 27 formulations (Table 2) were prepared by varying the ratios of Labrafil M1944 CS, Tween 80 and Transcutol P. Rapid emulsification was observed in all the formulations within 30 sec of addition of water. Formulation F1, F10, and F19-F22 showed very good SNEDDS region (**Fig.1.**). The region marked with red star in the diagram reveals the SNEDDS region which formed clear transparent oil in water emulsion upon gentle agitation. It was observed that at higher ratio of Transcutol P, spontaneity of the self-emulsification process got increased. Transcutol P has been also previously reported to provide excellent emulsification properties with improved drug loading (7, 17, 22, 23). The results also indicated that at higher concentration of surfactant mixture (S_{mix}) (i.e. Tween 80/ Transcutol P; >70%) or lower concentration of oil (Labrafil M1944 CS; < 30%), formation of clear transparent emulsions with nanosized droplets were observed. This could be due to higher HLB value of Tween 80 and better solubilization of DHA in Transcutol P. The combination of Tween 80 and Transcutol P has been reported in previous studies to prepare SNEDDS (18). It has been also reported that "right mixture of surfactants favourably adsorbed at interface and produces thermodynamically stable nanoemulsion by reducing the interfacial energy as well as providing a mechanical barrier to coalescence" (24). "In addition, co-surfactants increase

interfacial fluidity by penetrating into the surfactant film, creating void space between surfactant molecules" (9, 25-27). Due to these phenomena, the thermodynamic stability of formulations F1, F10, F19-F22 was found to be better as compared to that of other formulations. The results are reported in Table 3. It was observed that among the selected L-SNEDDS prototypes, formulation F19 has shown better stability against both thermodynamic stress and kinetic stress (centrifugation).

3.3. Formulation of S-SNEDDS

3.3.1. Oil adsorption capacity

In order to determine the oil adsorption capacity, various carriers were used to solidify the L-SNEDDS to S-SNEDDS. Among the different carriers, SXDP has shown highest OAC whereas, least OAC was observed for magnesium stearate. The decreasing orders of different carriers are given below:

Syloid XDP 3150 (300 mg) > Aerosil 200 (410 mg) > Syloid 244 FP (460 mg)> Microcrystalline cellulose PH 102 (490 mg) > Sodium carboxymethyl cellulose (510 mg) > lactose (620 mg) > Magnesium stearate (1600 mg)

The values (in parenthesis) of all the carriers indicate the individual amount required for adsorption of unit dosage form in the optimized SNEDDS formulation.

3.3.2. Micromeritic characterization of SNEDDS

The viscosity of SNEDDS is critical during its dispersion in the aqueous phase. Higher viscosities tend to slow down the emulsification rate which may affect *in vivo* drug release and bioavailability profiles. From viscosity determination results, it was observed that viscosity of L-SNEDDS (F19) prior to dilution was found to be 60.15 ± 2.14 cps. After dilution with 100 times distilled water the viscosity got decreased to 13.14±2.48 cps. The viscosity of solid SNEDDS using different carriers ranged between 28.22±2.87 cps and

56.88±4.21 cps after dilution. The results are shown in Table 4., formulation with SXDP has shown least viscosity and MS has shown highest viscosity. This could be attributed to the fact that the amount of SXDP required to solidify liquid SNEDDS was less as compared to that of other solidifying agents while the amount of MS required was highest. The presence of varying amounts of these carriers would have caused the increase in viscosity.

The S-SNEDDS powders prepared using different carriers were further characterized for flow rate, angle of repose, bulk density, tap density and Carr's index. Similar to the results of oil adsorption capacity, S-SNEDDS prepared using SXDP showed better flow and compression properties while MS showed poor flow and compression properties (Table 5). It is important to note that the values of Carr's index indicate that lactose and Syloid 244FP have better compressibility as compared to SXDP. However, based on overall performances such as amount of carrier required, angle of repose, viscosity etc., SXDP was found to be the best among all solid carriers. Based on these observations, S-SNEDDS prepared using SXDP were taken for further characterization.

3.4. Droplet size, PDI, zeta potential and Percentage drug loading of selected batch

Mean droplet size, PDI, zeta potential, percentage drug loading of the optimized L-SNEDDS were found to be and 43.51 ± 1.36 nm, 0.186 ± 0.053, -19.2 ± 1.21 mV and 93.23 ± 1.71 respectively. Similarly, values of these parameters were found to be 57.32 ± 1.87, 0.261 ± 0.043, -16.6 ± 2.18 mV and 91.23 ± 1.88 respectively for S-SNEDDS (Table 6).

3.5. Robustness to dilution and pH change

Since SNEDDS are the pre-concentrates that form o/w nanoemulsion only upon dilution, , chances of phase separation exist when the formulation undergoes infinite dilution in the GI fluids. This, in turn, could result in the precipitation of the drug owing to its poor aqueous solubility. During the passage of formulation through the GIT, there are wide variations in pH of GI tract from acidic environment in stomach to alkaline pH in intestine. These variations in

pH may cause the precipitation of drugs that show pH dependent solubility (28). To avoid such a situation, dilution study of DHA S-SNEDDS powder was carried out in 0.1N HCl, and phosphate buffer pH 6.8 with dilutions of 10, 100, 250, 500, and 900 times by volume. No phase separation was observed in any of the tested formulations. Interestingly, with the change in pH as well as upon increasing dilution factor from 10 to 500 times, a decrease in droplet size was observed (Table 7). Upon increasing the dilution factor to a certain level, better dispersion of droplets took place due to presence of surfactants at oil-water interface that resulted in formation of uniform, unaggregated and smaller droplets (29, 30). Upon further increasing the dilution, the size was found to increase. Increase in droplet size at 900 times dilution was found to be relatively low as compared to that in 10 times diluted samples. However, these changes were not significant ($p>0.05$) and clearly indicated the integrity of SNEDDS in nanoform upon change in the volume and pH of gastrointestinal tract (GIT).

3.6. *In vitro* dissolution studies

The *in vitro* dissolution profile of selected batch of L-SNEDDS, S-SNEDDS, marketed formulation and unprocessed drug revealed that the drug release profile of the formulated L-SNEDDS is better than that of S-SNEDDS, marketed drug and unprocessed drug in both the dissolution media (**Fig. 2 and 3**). In 0.1N HCl (pH 1.2), % drug release from L-SNEDDS, S-SNEDDS powder, S-SNEDDS tablet, marketed formulation and unprocessed drug were observed 102.92 ± 5.78%, 95.84 ± 4.45%, 93.69 ± 4.34%, 42.14 ± 3.15% and 14.21 ± 2.47%, respectively within 90 min. Similarly, In phosphate buffer (pH 6.8) at the end of 90 minutes, L-SNEDDS showed 98.11 ± 3.78 %, S-SNEDDS powder showed 91.25 ± 4.09%, S-SNEDDS tablet showed 88.35 ± 3.87% release of DHA while the unprocessed drug and marketed formulation showed only 10.15 ± 1.12% and 38.73 ± 2.19% drug release respectively, at 90 min..

The S-SNEDDS tablets showed slightly slow release of DHA from the formulation as compared to their L-SNEDDS counterpart. This can be attributed to S- the disintegration and de-aggregation process encountered during the dissolution process of the tablet. The physical interaction of SNEDDS with the hydrophobic surface of silica particles of SXDP may also be responsible for reduction in the dissolution rate of DHA at the initial stage. It is important to note that there was no significant difference ($p>0.05$) between the dissolution profiles of L-SNEDDS, S-SNEDDS powder and S-SNEDDS tablets at both pH values, indicating that the dissolution profile of DHA remains unaffected with change in GIT pH upon oral administration.

3.7.TEM analysis

The TEM analysis of DHA S-SNEDDS has shown uniform spherical droplets of the formulation at a 100 nm scale (**Fig.4**). The droplets looked intact and un-agglomerated indicating their uniform distribution. It is important to note that the droplet size of S-SNEDDS indicated by dynamic light scattering was 57.32 ± 1.87 nm which further endorsed the size indicated by TEM image which is in the same range.

3.8. DSC analysis

DHA oil showed endothermic peak at 18.5°C in DSC thermograms. However broader peaks in thermogram of L-SNEDDS and S-SNEDDS with no representative peak for DHA depicted the molecularly dispersion of DHA oil in both of the formulations. The results are shown in **Fig.5**.

4. Conclusion

SNEDDS were developed as a dosage form of DHA to enhance the dissolution profile of DHA. The goal of achieving a formulation of SNEDDS which is free from creaming, cracking, Ostwald ripening during storage could be achieved by preparing liquid as well as solid SNEDDS. The thermodynamic stability, pH-based dilution and dissolution studies have successfully demonstrated development of SNEDDS to overcome the problems of low aqueous solubility, gastric instability and dissolution rate of DHA. Upon conversion into solid SNEDDS other aspects such as stability and ease of handling of formulation has also been achieved. Syloid XDP 3150 has provided optimum flow properties to solid SNEDDS. The developed formulation can be taken further to scale-up and pre-clinical studies for safety and efficacy evaluation.

Conflict of interest

Declared None

Ethics statement:

This research article does not involve use of human or animal.

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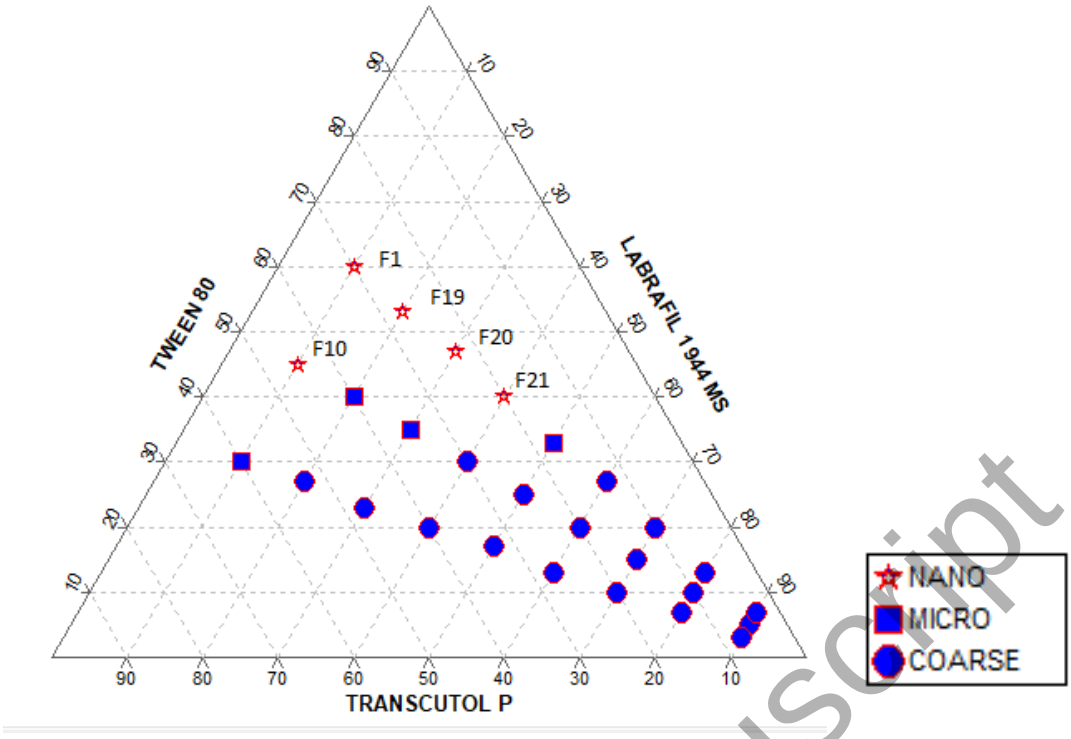

Fig. 1. Pseudo-ternary phase diagram.

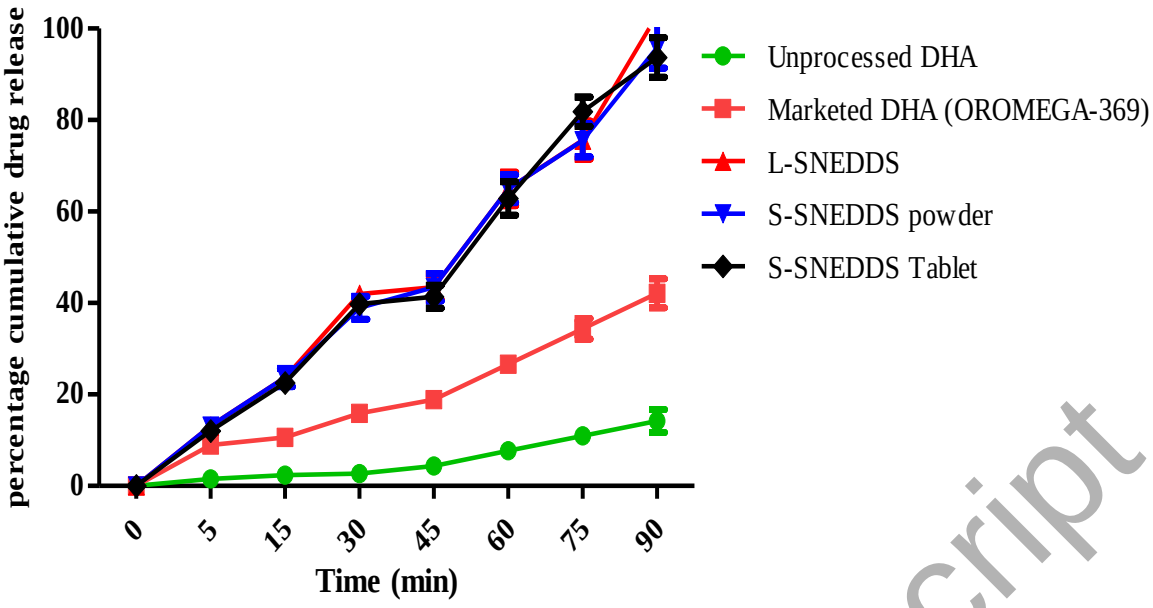

Fig.2. *In-vitro* dissolution studies of unprocessed DHA, marketed DHA, L-SNEDDS, S-SNEDDS powder and S-SNEDDS tablet in 0.1N HCl (pH 1.2).

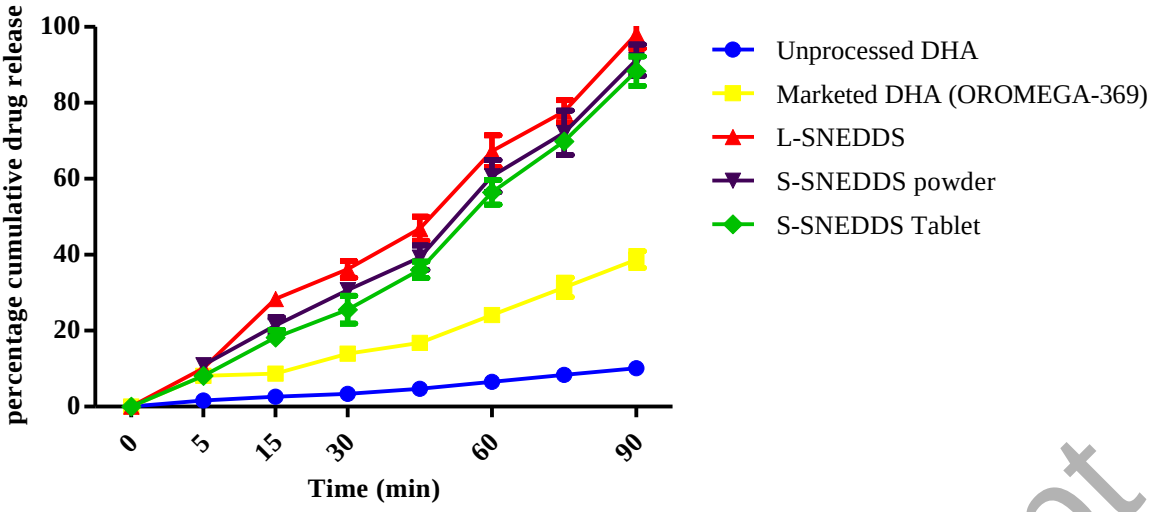

Fig.3. *In-vitro* dissolution studies of unprocessed DHA, marketed DHA, L-SNEDDS, S-SNEDDS powder and S-SNEDDS tablet in 0.2M phosphate buffer (pH 6.8).

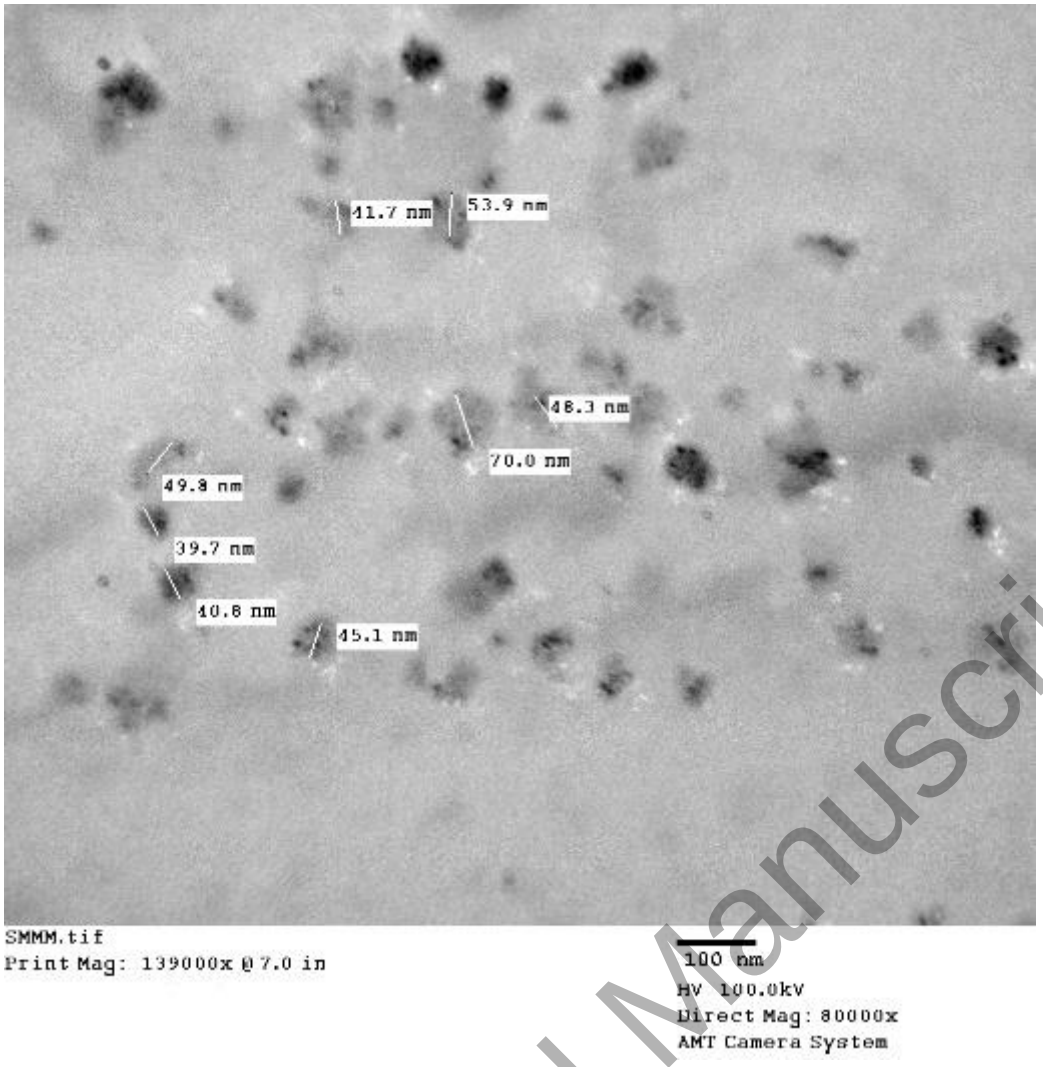

Fig. 4. TEM image of an optimized batch of S-SNEDDS.

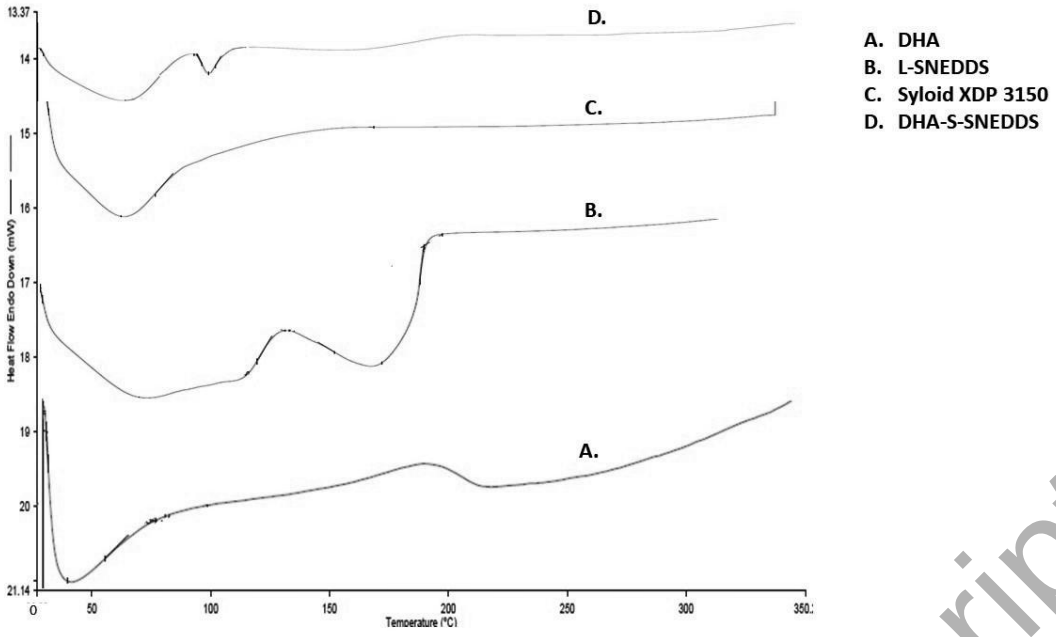

Fig.5. Overlay of DSC thermograms of DHA, L-SNEDDS, Syloid XDP 3150 and DHA-S-SNEDDS.

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Table 1.
The solubility of DHA in various vehicles (the mean representing each value + SD, N=3).

Vehicle	**The solubility of unprocessed DHA (µg/ml)**
Olive oil	12.06±2.32
Labrafil M®1944CS	**17.25±1.24**
Labrafac17.25 PG	2.71±0.17
Castor oil	13.08±0.51
Sesame oil	7.56±0.45
Peanut oil	6.24±0.32
Eucalyptus oil	4.65±2.14
Mustard Oil	10.67±0.54
Capmul® MCM	4.23±1.25
Labrasol®	3.23±0.25
Tween 20	1.2±0.29
Tween 60	2.28±1.25
Tween80	**213.61±2.2**
PEG 200	1.2±0.2
PEG 400	09.12±.21
PEG 600	2.1±017
Transcutol P	**19.14±1.44**

Table 2.
Composition of L-SNEDDS.

Oil (Labrafil M1944 CS)	Smix (Tween 80 : Transcutol P)		
	1:1	1:2	2:1
100	450:450 (F1)	300:600 (F10)	600:300 (F19)
200	400:400 (F2)	270:530 (F11)	530:270 (F20)
300	350:350 (F3)	230:470 (F12)	470:230 (F21)
400	300:300 (F4)	200:400 (F13)	400:200 (F22)
500	250:250 (F5)	170:330 (F14)	330:170 (F23)
600	200:200 (F6)	130:270 (F15)	270:130 (F24)
700	150:150 (F7)	100:200 (F16)	200:100 (F25)
800	100:100 (F8)	70:130 (F17)	130:70 (F26)
900	50:50 (F9)	70:30 (F18)	30:70 (F27)

Table 3.
Thermodynamic stability and cloud point determination.

Formulations	Parameters									
	Turbidity				Phase separation				Centrifugation (10000 RPM)	Cloud point (ºC)
	-21ºC	**4ºC**	**25ºC**	**40ºC**	**-21ºC**	**4ºC**	**25ºC**	**40ºC**		
F1	YES	NO	NO	NO	NO	NO	NO	NO	NO phase separation	65.2
F10	YES	NO	NO	NO	NO	NO	NO	NO	NO phase separation	78.5
F19	NO	NO	NO	NO	NO	NO	NO	NO	NO phase separation	88.6
F20	NO	NO	NO	NO	YES	NO	NO	NO	NO phase separation	81.7
F21	NO	NO	NO	NO	NO	NO	NO	YES	Phase separation	84.3
F22	NO	NO	NO	NO	NO	NO	NO	NO	Phase separation	82.2

Table 4.
Viscosity determination of formulations.

Component	**Viscosity (cps) (Mean ± SD)**
	Prior to dilution
Liquid SNEDDS	60.15 ± 2.14
	After dilution
Liquid SNEDDS	13.14±2.48
SXDP	28.22±2.87
Aerosil® 200	36.87±2.16
Syloid® 244FP	40.15±3.54
MCC PH 102	46.61±5.89
Na-CMC	51.52±3.98
Lactose	55.08±3.42
MS	56.88±4.21

Table 5.
Micromeritics properties of S-SNEDDS.

Component	Flow rate (g/s)	Angle of repose (θ)	Bulk Density (g/cm^3)	Tap Density (g/cm^3)	Carr's index
SXDP	4.74±0.21	24.22±0.32	0.210±0.14	0.275±0.32	23.63±2.12
Aerosil® 200	2.68±0.15	33.42±1.24.	0.291±0.08	0.408±0.35	28.67±1.32
Syloid® 244FP	2.42±0.25	33.42±1.52	0.262±0.12	0.320±0.15	18.12±1.12
Lactose	2.68±0.14	31.09±0.94	0.265±0.34	0.320±0.11	17.18±1.34
MS	1.01±0.81	45.11±1.89	0.291±0.02	0.456±0.02	36.18±3.90
MCC PH 102	0.53±0.34	43.53±2.12	0.301±0.09	0.423±0.02	28.84±0.68
Na CMC	1.21±0.03	34.89±1.54	0.294±0.87	0.406±0.02	27.58±1.42

Table 6.
Evaluation parameters of optimized batches of DHA L-SNEDDS & S-SNEDDS (F-19).

Formulation code	Mean droplet size (nm)	PDI	Drug loading (%)	Zeta potential (mV)	Cloud point (ºC)	Appearance	Phase separation after 48h and centrifugation
L-SNEDDS	43.51 ± 1.36	0.186 ± 0.053	93.23 ± 1.71	-19.2 ± 1.21	88.6 ± 2.54	TP	No
S-SNEDDS	57.32 ± 1.87	0.261 ± 0.043	91.23 ± 1.88	-16.6 ± 2.18	89.5 ± 1.18	TP	No

TP = Transparent

Table 7.
Robustness to dilution and pH change.

Dilution (mL)	Mean droplet size (nm)	PDI	Phase separation	Appearance	Zeta potential
pH 1.2					-10.1±1.23
10	215.90±1.23	0.460±0.018			
100	185.10±4.18	0.431±0.021	NO Phase	Transparent	-19.0±1.21
250	101.10±3.24	0.397±0.016	separation		-19.2±1.88
500	105.11±4.56	0.384±0.033			-18.8±2.23
900	115.45±1.34	0.389±0.045			-11.6±3.22
pH 6.8					
10	224.20±5.67	0.474±0.054			-8.68±1.43
100	188.40±3.18	0.398±0.032			-5.70±0.23
250	104.40±1.99	0.378±0.098	NO Phase	Transparent	-10.5±1.16
500	104.23±4.55	0.371±0.065	separation		-8.68±0.98
900	117.44±6.77	0.386±0.032			-19.0±0.88
Distilled water					
10	237.70±2.22	0.397±0.062			-2.46±1.87
100	185.50±4.98	0.397±0.043			-5.62±0.95
250	109.30±3.78	0.392±0.022	NO Phase	Transparent	-10.5±1.01
500	102.45±8.12	0.381±0.018	separation		-16.6±1.27
900	119.52±7.16	0.385±0.023			.-19.2±1.33