

Self-Nanoemulsifying Drug Delivery Systems of Poorly Soluble Drug Dutasteride: Formulation and In-Vitro characterization

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ABSTRACT

The present study aims to prepare and evaluate the self-nanoemulsifying drug delivery system (SNEDDS) to enhance the dissolution rate of a poorly soluble drug dutasteride. The formulation was prepared using capryol PGMC, Cremophor EL, and polyethylene glycol (PEG) 400 as oil, surfactant and co-surfactant, respectively. The pseudo-ternary phase diagrams with presence and absence of drug were plotted to find out the nanoemulsification range and also to evaluate the effect of dutasteride on the emulsification behavior of the phases. Prepared SNEDDS formulations were evaluated for its particle size distribution, nanoemulsifying properties, robustness to dilution, self-emulsification time, turbidity measurement, drug content and *in-vitro* dissolution. Furthermore, the optimized formulations were evaluated for heating cooling cycle, centrifugation studies, freeze thaw cycling, particle size distribution and zeta potential to confirm the stability of the formed SNEDDS formulations. The particle size, zeta potential and polydispersity index of the optimized formulation was found to be 35.45 nm, -15.45 and 0.19, respectively. The *in vitro* results revealed that the prepared formulation enhanced the dissolution rate of dutasteride significantly compared to pure drug. In 60 minutes, the formulation showed 83.15% and 82.04 % drug release for pH 6.8 and pH 1.2 respectively. Based on the results, it was concluded that the dutasteride-loaded SNEDDS revealed better dissolution compared to the raw drug suspension and commercial drug.

INTRODUCTION

Benign prostate hyperplasia (BPH) is the most common benign neoplasm which approximately 80 % of the elderly men (above 60 years old) develops the microscopic evidence of BPH (Dolder, 2006). Among them, half of the patients develop enlarged prostate gland, followed by symptoms such as nocturia, increase in urinary urgency and frequency, and reduced force of stream. For patients with enlarged prostate, the treatment would be invasive surgery and prostatectomy. Without surgery, the treatment would be to stop the static factor of prostate gland enlargement, which is dihydrotestosterone (DHT). DHT is a potent androgen, which is converted from testosterone to androstenedione. Inhibition of DHT formation can be a treatment strategy to reduce prostate size. Dutasteride inhibits 5-alpha

reductase (5AR), which is responsible for the conversion of testosterone to a more potent dihydrotestosterone (Dolder, 2006). Dutasteride is a drug of choice in the group of inhibitors. Compared to other 5AR inhibitor such as finasteride, dutasteride is superior as it binds to both types of alpha-reductases: type I 5AR and type II 5AR. Although type I 5AR is mainly active in skin and liver, and its inhibition, further, reduces the total circulating DHT (Kim, 2013). Dutasteride is 45 times more potent than finasteride to inhibit type I 5AR, and 2.5 times more potent to inhibit type 2 5AR, thus have more significant effect in reducing DHT count (Keam and Scott, 2008). In a study carried out by Dolder (2006), dutasteride decreases DHT by ~95%, as compared to 70% reduction in patient receiving finasteride based on a four-year observation. It is a recommended regimen for benign prostate hyperplasia treatment to improve symptoms. However, being classified as Biopharmaceutical Classification System (BCS) class II or IV, it has poor aqueous solubility that is only 0.038 ng/mL in water (Lee *et al.*, 2015) as well as a poor dissolution characteristic (USFDA, 2015).

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This property appears to affect the bioavailability of dutasteride, which slows the dissolution and the problems with absorption are limiting factors. With the marketed oral formulation (Avodart), dutasteride is dissolved in mono-di-glycerides of caprylic acid and capric acid, and then encapsulated in soft-gelatin capsule. However, the achieved bioavailability is still below satisfaction, with only between 40 to 60 per cent (Lee *et al.*, 2015). To achieve optimal therapeutic effects of dutasteride with minimal side effect, dissolution and absorption of dutasteride must be improved. Nowadays, several approaches are available to improve the bioavailability of dutasteride, such as solid dispersion (Kim *et al.*, 2015), polymeric nanoparticle (Choo *et al.*, 2013), Hydroxypropyl- β - cyclodextrin (HP- β -CD) nanostructures (Kim, 2013), and self-emulsifying drug delivery system (SEDDS). SEDDS is a promising method as it is anhydrous isotropic mixture of oil, surfactant, co-surfactant, and drug, which spontaneously form oil-in-water emulsion following the dilution in aqueous medium upon agitation (Abdalla *et al.*, 2008; Balakrishnan *et al.*, 2009; Gursoy and Benita., 2004). Lee *et al.*, (2015), designed supersaturable Self –microemulsifying drug delivery system (S-SMEDDS) of dutasteride for oral administration. Ali *et al.*, (2014) designed nanoemulsions of dutasteride as transdermal patch. SEDDS formulation is mainly useful for oral formulation where dutasteride can form oil in water (O/W) emulsion in gastrointestinal tract using the GI motility to agitate the mixture (Ammar *et al.*, 2014; Gursoy and Benita., 2004; Tang *et al.*, 2007). Recently, there are many studies has been conducted to improve the bioavailability of the poorly soluble dutasteride. Among them, oral self nanoemulsifying drug delivery system (SNEDDS) of dutasteride has not been researched yet. SNEDDS is selected for formulating because it is simple and cost effective in manufacturing process by omitting high energy emulsification process. SNEDDS is physically a stable lipid solution. It shows a better dissolution rate and expected bioavailability. Hence, the drug dose can be reduced, and brings the adverse effects (Gupta *et al.*, 2011). Khan *et al.*, 2012 developed atorvastatin as SNEDDS and showed 90% of increased dissolution rate. Zakia *et al.*, 2012 developed SNEDDS of atorvastatin which showed a faster rate (97.24%) of drug release and 1.87-fold increase in bioavailability. Rajinikanth *et al.*, (2012) investigated on the SNEDDS of valsartan which showed a 6-fold increase in the bioavailability. Balakumar *et al.*, (2013) formulated rosuvastatin as SNEDDS revealed a 2.45-fold increase in bioavailability. The above studies revealed a significant increase in the bioavailability. The nano sized oil droplet dispersed in gastrointestinal fluid increases the interfacial area; hence it increases its dissolution rate and increases the bioavailability (Amrutkar *et al.*, 2014).

Based on the above findings, the objectives of the study are to develop, to characterize the dutasteride loaded SNEDDS formulation, and to compare the pure drug suspension to the marketed dutasteride.

The strength of marketed dutasteride (Avodart) is 0.5 mg soft gelatin capsule. In this study, the self-nano-emulsifying drug delivery system (SNEDDS) is employed with the strength of 0.5

mg to enhance the dissolution rate that might increase the bioavailability of dutasteride.

MATERIALS AND METHODS

Materials

Dutasteride was kindly offered by RA Chem Pharma (Hyderabad, India), Capryol PGMC, Labrasol, Labrafilm 1966CS, Capryol 90, Cremophor EL, Cremophor 40 RH, Propylene Glycol, Transcutol HP, Solutol HS were obtained as gift samples from Gattefosse (France), sesame oil, and olive oil were obtained from Sigma (USA), Tween 80 was purchased from Merck (Germany) Canola oil was purchased from Lam soon group (Malaysia). PEG 400 and methanol were purchased from SIME Scientific, and hydrochloric acid was purchased from R&M Chemicals (UK). Methanol was purchased from Merck scientific (Germany), and other solvents used in this study were of analytical grade.

Solubility studies

The criteria for screening the excipients are the solubility of drug in oils, surfactants and co-surfactants. This screening is performed to determine the solubility of various components and the highest soluble component will be chosen to formulate the SNEDDS. Shake flask method was used to determine equilibrium solubility of dutasteride in various excipients. The solubility of drug in various excipients was determined by adding 2 mL of each of the selected components to each vial containing an excess of drug. After sealing, the mixture was heated at 40° C in a water bath to facilitate the solubilization. Mixing of the systems was performed using a vortex mixer. Formed suspensions were then shaken with a thermodynamically controlled shaker at 30° C for 72 hours followed by equilibrium for 24 hours. After reaching equilibrium, each vial was centrifuged at 3000 rpm for 15 minutes, and excess insoluble drug was discarded. Then the supernatant was taken and filtered through the 0.45 μ m membrane filter. Later, supernatant 10 μ L oil was taken and suitably diluted with methanol. The concentration of drug was then quantified through RP-HPLC validated method. Methanol 80% and phosphate buffer pH 6.8 20% were used as mobile phase at the wavelength of 230 nm with the flow rate of 1 mL/min.

Construction of ternary phase diagrams

Pseudoternary phase diagrams of oil, surfactant/ co-surfactant (S/CoS), and water was developed using the water titration method. The mixture of chosen oil and S/CoS at certain weight ratio was diluted with water in a drop-wise manner. For each phase diagram at a specific ratio of S/CoS (i.e., 1:1,1:2,1:3, 2:1, 3:1, and 2:3 volume/volume), a transparent and homogenous mixture of oil and S/CoS (Smix) was formed by vortexing for 5 minutes. For each phase diagram a specific Smix ratio was mixed thoroughly in various volume ratios from 0:10 to 10:0. Nine different combinations of oil and Smix (0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, 10:0) were blended. Each mixture was then titrated with water and visually observed for phase clarity and

flowability. The concentration of water at which turbidity-to-transparency and transparency-to-turbidity transitions occurred was derived. To determine the effect of drug addition on the nanoemulsion boundary, phase diagrams were also constructed in the presence of drug using drug-enriched oil as the hydrophobic component. The physical state of nanoemulsion was marked on a pseudo ternary phase diagram with one axis representing the aqueous phase, second oil and third a mixture of surfactant and co-surfactant at a fixed ratio.

Preparation of SNEDDS

A series of nanoemulsion formulations was prepared after selecting few of the formulations from the nanoemulsion region of the pseudoternary phase diagram. Accurately weighed drug was placed in a glass vial, oil was added and vortexed until it dissolves, then, surfactant, and finally co-surfactant was added. Then, the components were mixed by gentle stirring, vortex mixing and heating at 40° C on a magnetic stirrer, until the drug completely dissolves. The mixture was then stored at room temperature for further study.

Determination of droplet size, polydispersity index and zeta-potential

One hundred microliters of each formulation was diluted to 10 mL in a beaker and gently mixed using a magnetic stirrer. Immediately, the average droplet size and zeta potential were determined by dynamic light scattering equipment (Malvern, UK). All studies were done in triplicates.

Determination of Self-emulsification time

Evaluation of the self-emulsifying properties of formulations were performed by visual assessment. Visual assessment was performed by addition 1 mL of the pre-concentrate into 500 mL of distilled water in dissolution apparatus with a paddle speed of 50 rpm. Precipitation was evaluated by visual inspection of the resultant emulsion after 24 hours. The formulations were then categorized as clear (transparent or transparent with bluish tinge), unclear (turbid), stable (no precipitation at the end of 24 hours), or unstable (showing precipitation within 24 hours). The assessment for the efficiency of the emulsion system is also made according to the following grading system

Grade A: Rapidly forming emulsion having a clear or bluish appearance

Grade B: Rapidly forming, translucent bluish appearance

Grade C: Fine milky emulsion forming within 30 seconds

Grade D: Slow forming, slightly oily appearance

Robustness to dilution

The prepared formulations were diluted infinitely (i.e. 500 times) with distilled water, Phosphate buffer pH 6.80 and acid

buffer pH 1.2 in three separate glass vials. The diluted formulations were shaken and then visually inspected after 24 hours for any form of instability.

Determination of viscosity and refractive index

The viscosity of the SNEDDS formulation was measured by Brookfield viscometer (DV 2T) using at 10 rpm. Each reading was taken after equilibrium of the sample at the end of two minutes. The samples were repeated three times. The refractive index of the system was measured using Refractometer (Atago Automatic Digital Refractometer RX-5000, USA) by placing one drop of the formulation on the slide in triplicate at 25°C.

Thermodynamic stability testing

Heating cooling cycle

Six cycles of cooling and heating between refrigerator temperature 4°C and elevated temperature 45° C with exposure to each temperature for not less than 48 hours was carried out. The stable formulations were subjected to centrifugation test.

Centrifugation study

The formulations that pass the heating cooling cycle were centrifuged at 3500 rpm for a period of 30 minutes. The formulations which did not have any phase separation, were considered for the freeze thaw stress test.

Freeze Thawing

Freeze thawing was employed to evaluate the stability of formulations. The formulations were subjected to 3 to 4 freeze-thaw cycles, which included freezing at -4°C for 24 hours followed by thawing at 40°C for 24 hours. The formulations were observed for phase separation. The stable formulations should not show any phase separation. Those stable formulations which didn't show any phase separation was used for further study.

Attenuated Total Reflectance-Fourier –Transform infrared spectroscopy (ATR- FT-IR) study

FT –IR spectrum of pure drug, additives and formulation was obtained by FT-IR spectrophotometer. The spectrum obtained from the formulation was compared to the pure drug and marketed product.

Differential Scanning Calorimetry (DSC) study

DSC curves of the pure drug and the formulation was obtained by DSC endothermic peaks and exothermic peaks. The curves of the formulation were compared to the pure drug and marketed product.

In vitro dissolution study

Dissolution studies were performed in 900 mL of buffer pH 1.2 and 6.8 using US Pharmacopeia XXIV dissolution apparatus 2. The formulation was instilled into the apparatus containing 900 mL of buffer pH 1.2 and 6.8 in a separate run. The temperature was maintained at 37° C with the paddle speed of 50

rpm. Samples (5 mL) were withdrawn at 5, 15, 30, 45, and 60 minutes with replacement of an equal volume of temperature-equilibrated media. The SNEDDS formulations were used for drug release studies and the results were compared with those of plain drug. The content of the drug in the samples were analyzed (n=3) by using RP-HPLC (Perkin-Elmer).

Accelerated stability testing

The selected batches of the formulations (F4 and F6) were stored in an incubator at 40° C with 75% of relative humidity for 8 weeks. Visual assessment, droplet size and zeta potential analyses were conducted for selected formulations at the end of the study.

RESULTS AND DISCUSSION

Solubility studies

The solubility of dutasteride in various vehicles at room temperature was presented in Fig.1. Components were selected based on two criteria; first, on the solubility profile and second on HLB values. Capryol PGMC is propylene glycol monocaprylate, a water insoluble surfactant but is soluble in many organic solvents, containing more than 90 % of monoester of C8 fatty acid, caprylic acid. It also exerts inhibition to CYP3A4 enzyme, which is useful for drugs which are metabolized by it (Khan *et al.*, 2012). Capryol PGMC has shown highest solubility (17.35 mg/mL) among the selected oils, thus chosen as oil for preparation of SNEDDS formulation. Cremophor EL (solubility=7.82 mg/mL) and PEG 400 as surfactants and co-surfactant (solubility= 6.85 mg/mL) respectively.

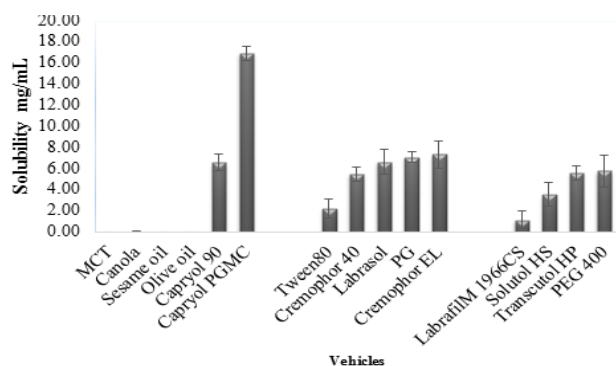


Fig. 1: Saturation solubility of dutasteride in various oils, surfactants and co-surfactants

Cremophor EL, a hydrophilic surfactant with a HLB value of 12-14, is an ideal surfactant to be used in SNEDDS formulation. According to Weerapol *et al.*, (2014), Cremophor EL has structural significance on the final emulsion droplet size. In their study, they concluded that a branch alkyl structure of Cremophor EL had an effect on the penetration of oil onto the curved film interface, thus resulting in the self-formation of nanoemulsion. Cremophor EL showed highest solubility of drug (7.82 mg/mL) as compared to other surfactants, so this has been chosen as surfactant for SNEDDS formulation. PEG 400 is a

hydrophilic surfactant that is miscible in water, consists of alcohol and ether.

It shows the highest solubility of dutasteride among the co-surfactants and thus it is chosen as co-surfactant. Selection of oil, surfactant and co-surfactant not only based on the solubility, but also on the HLB values. The HLB values for the blend of components was determined by William Griffin's method. Based on William Griffin (1949), the desired HLB value for oil in water emulsion should be in-between 8-18. So based on the HLB values, suitable component was chosen for an O/W emulsion.

Construction of pseudo ternary phase diagram

Based on the results obtained, one of the formulations from the phase diagram was chosen. Oil, surfactant and co-surfactant were grouped into 6 different combinations to study the phase diagrams (Fig.2). Water titration method was used to construct the phase diagram. Pseudo ternary phase diagram was constructed with 5% to 95% increment of addition of water. It was also found that by increasing the oil content, the system showed the appearance of coarse emulsion. When co-surfactant is increased in the Smix system, it was observed that there is a decreasing property of spontaneous nanoemulsions. From the observation it is clear that the surfactant is playing an important role in the spontaneous formation of nanoemulsions at appropriate range.

Pseudo ternary phase diagrams were constructed. The samples which were clear or bluish transparent in appearance were chosen as nanoemulsions. From the six different phase diagrams, it was observed that, when Smix ratios increased, nanoemulsions region also increased. The Smix 2:3 resulted in the largest self-nanoemulsifying region than that of other Smix ratios. There was no significant difference in the emulsification region in the 2:3 ratios of the oil and surfactant/ co-surfactant with (0.5 mg) and without drug Fig.2.

The droplet size is based on the transparency of the formulation. The more turbid the formulation, droplet size will be bigger and vice-versa. So it is determined that the transparency of the nano-emulsion is inversely proportional to the size of the droplets. At a point, gel was formed with the addition of water, and this will be useful for the gel formulations which can be used as emulsion for external use.

Preparation of Self-nanoemulsifying drug delivery systems (SNEDDS)

After identifying the emulsification region, the components were selected for further optimization. Capryol PGMC, Cremophor EL and PEG 400 were weighed accurately in the ratio of 2:3 and mixed well. Appropriately weighed dutasteride 0.5 mg was mixed with capryol PGMC until the drug dissolved. Then, the surfactant and co-surfactant were added and mixed thoroughly. Nine points (Table.1) were chosen from the self-nanoemulsifying region and formulation was prepared and was subjected to further studies.

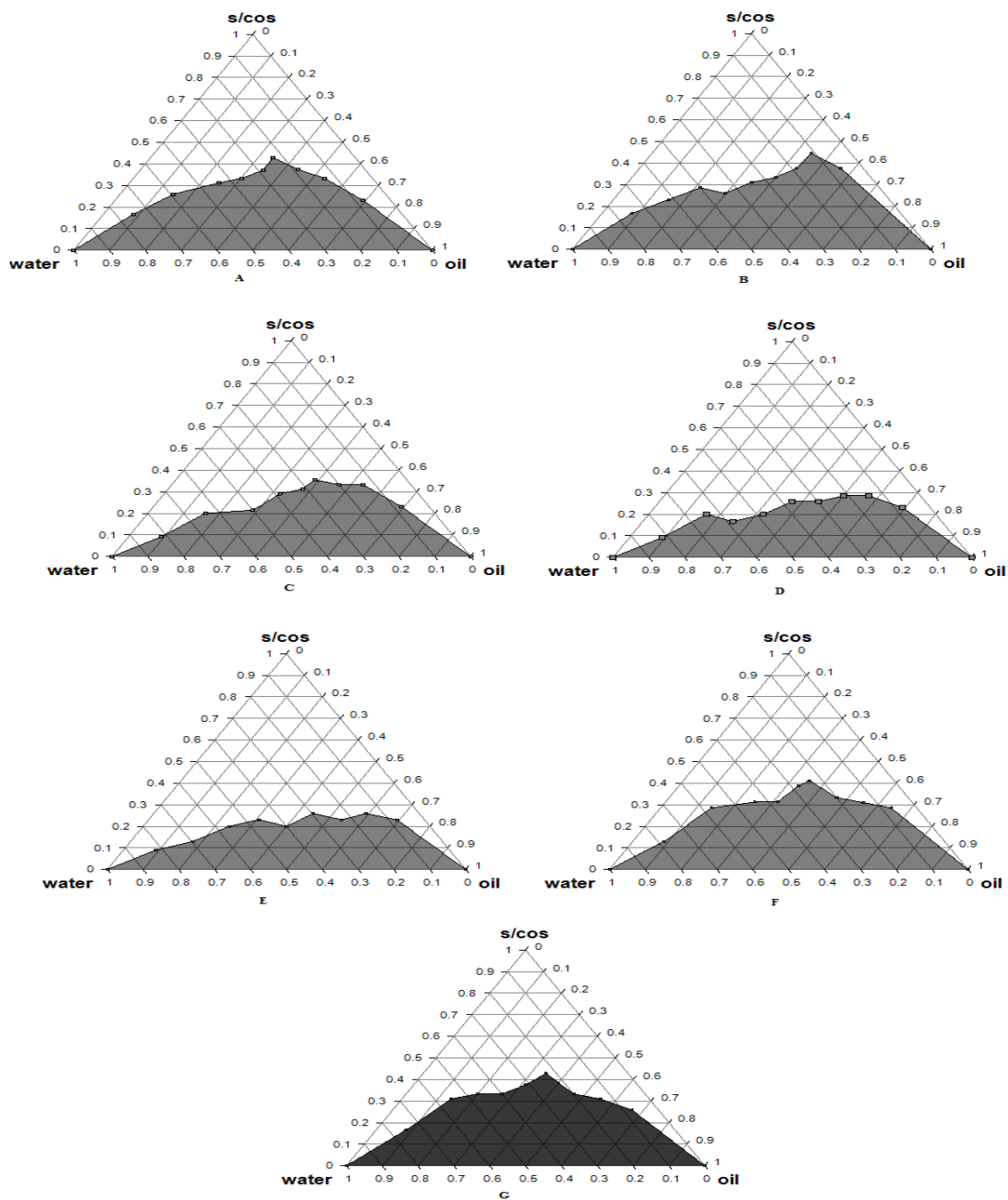


Fig. 2: Pseudoternary phase diagrams of oil (Capryol PGMC), Smix (Cremophor EL and PEG 400) and water with the Smix ratio of A) Smix 1:1; B) Smix 1:2; C) Smix 3:1; D) Smix 1:3; E) Smix 2:1; F) Smix 2:3; G) Smix 2:3 with 0.5mg of dutasteride.

Table 1: Composition each excipient for optimized SNEDDS formulation .

Formulation	Capryol PGMC (% w/v)	Cremophor EL (% w/v)	PEG 400 (% w/v)	Dutasteride (mg)
F1	20	32	48	0.5
F2	30	28	42	0.5
F3	40	42	18	0.5
F4	40	24	36	0.5
F5	40	35	25	0.5
F6	35	35	30	0.5
F7	30	35	35	0.5
F8	33	35	32	0.5
F9	30	45	25	0.5

Determination of droplet size, polydispersity index and zeta-potential

Determining the particle size is of great importance in formulations as nanoemulsions. Small particle size contributes to greater interfacial area, which then provided better drug partitioning and absorption at GIT. However, there is no consensus in the literature on the exact size range of a nanoformulations. The average particle size and zeta potential of optimized SNEDDS formulation were determined by Photon correlation spectroscopy (PCS) analysis using zeta sizer. The average particle size and zeta potential of nanoemulsions were found to be 45.59 and 11.23 mV, respectively (Table 5). PDI measures the size distribution of the nanoparticles in a sample. It has been reported that the mono dispersed samples with uniform particle size distribution has a PDI range from 0.0 to 0.3, whereas the sample with a broad distribution has a PDI value > 0.4. In our study, PDI value of all the batches of SNEDDS formulation was found to be between 0.178 to 0.488. Formulation F1, F2, F4, and F9 indicated uniformity of droplet

size suggesting that they were monodispersed with uniform particle size distribution (less than 0.3) as shown in Table 5 and Fig.4. Whereas PDI values for F3, F5, F6, F7, F8 showed non uniformity (more than 0.4) of the particles in nanoemulsions formulations. However, ideally accepted, desirable value for PDI is < 0.5 (Rajinikanth *et al.*, 2012; Sunitha *et al.*, 2015). Comparison of particle size with the oils, surfactants, and co-surfactant were presented in the Fig.3. The zeta potential represents the stability of the nanodispersion. It has been reported that nanodispersion with a minimum zeta potential of < -60 mV has excellent stability, and minimum zeta potential of < -30 mV has physical stability (Gursoy *et al.*, 2004). All the developed SNEDDS formulation in this study showed zeta potential value, between -11.23 mV to -16.86 mV as shown in Table 5. The small particle size as well as uniform size distribution with optimum zeta potential of developed nanoemulsion was suitable for the development of formulation with enhanced bioavailability (García-Díaz *et al.*, 2012; Yoo *et al.*, 2010).

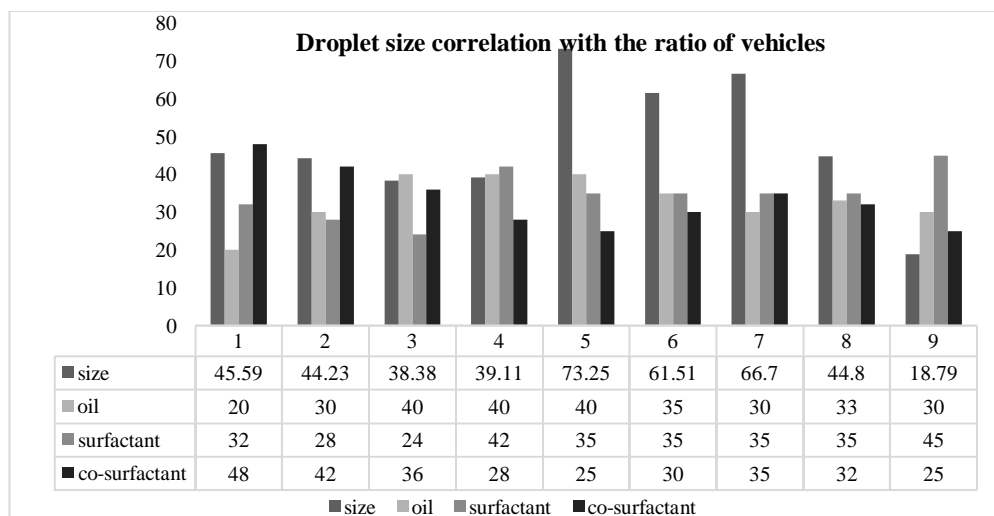


Fig. 3: Particle size (nm) correlation with comparison excipients

Results

Z-Average (d.nm): 39.11	Peak 1: 42.64	% Intensity: 96.7	St Dev (d.n...) 16.28
Pdl: 0.194	Peak 2: 4299	3.3	965.8
Intercept: 0.932	Peak 3: 0.000	0.0	0.000

Result quality **Good**

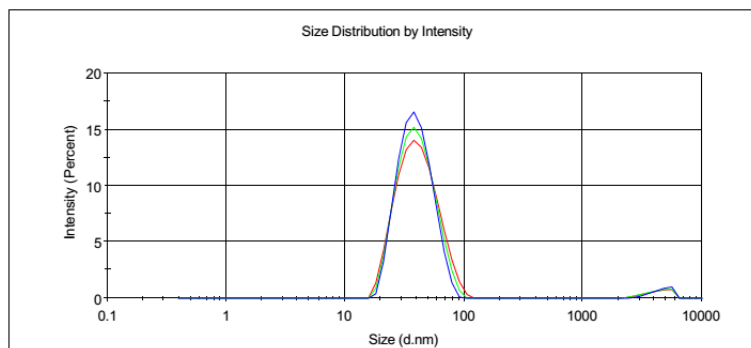


Fig.4: Particle size distribution graph of SNEDDS formulation obtained from Zeta sizer.

Determination self-emulsification time, viscosity and refractive index

Most of the prepared SNEDDS formulations formed the nanoemulsion in less than 1 minute with grade A and B as shown in Table 3. The formulation F4 and F6 were formed the nanoemulsion within 20 seconds with grade A system in PBS and acid buffer. The results are indicating that the prepared SNEDDS formulations form a good and stable nanoemulsion in different dissolution with short time duration (<1 minute). The viscosity and refractive index were found to be within the range as shown in Table 5.

Thermodynamic stability testing

It was found (Table.2) that there was no sign of precipitation as well as phase separation for all the formulations except F1 and F8 in heating and cooling cycle, freeze thaw cycle and centrifugation. Hence almost all the SNEDDS formulations passed the thermodynamic stability testing. This reveals that the formulations were robust against the storage of extreme temperatures.

Table 2: Thermodynamic stability testing and self-emulsification parameters of optimized SNEDDS formulation

Formulation	Heating and cooling cycle	Centrifuge	Freeze thaw	Self-emulsification (seconds)	Assessment of self-emulsification
F1	F	P	P	25.00 ± 2.00	GRADE A
F2	P	P	P	23.56 ± 1.52	GRADE A
F3	P	P	P	26.00 ± 2.00	GRADE A
F4	P	P	P	18.24 ± 2.45	GRADE A
F5	P	P	P	21.34 ± 2.00	GRADE A
F6	P	P	P	17.20 ± 3.24	GRADE A
F7	P	P	P	25.10 ± 3.00	GRADE B
F8	P	F	P	23.45 ± 2.76	GRADE B
F9	P	P	P	22.00 ± 1.45	GRADE B

P = Passed; F = Failed

Robustness to dilution and precipitation

The dilution capability of the formulations was tested to determine the capability of the formulation to withstand possibly infinite dilutions with water. This was because, upon ingestion, the gastrointestinal fluids are responsible for the dilution, and it is impossible to accurately identify the amount of water present to form emulsion with the formulation. Robustness to dilution was performed diluted with excess of water (500-900 mL) and was stored for 12 hours, and the result indicated that there was no precipitation or phase separation as shown in Table 3.

The ability of SNEDDS formulation to withstand aqueous dilution was found to be fascinating. The phenomenon was attributed to the high solubilising properties of the excipients, and also the capability to form a relatively stable emulsion with small droplet sizes. This implied that these formulations were stable at infinite water dilution.

Table 3: The average droplet size of selected SNEDDS formulations at various dilution factors with water.

Formulation	Dilution factor	Droplet size (nm)	PDI
F4	100	51.32 ± 0.46	0.352 ± 0.007
F4	500	44.34 ± 0.32	0.202 ± 0.003
F4	900	38.76 ± 0.92	0.197 ± 0.004
F6	100	105.22 ± 0.41	0.472 ± 0.002
F6	500	82.49 ± 0.30	0.429 ± 0.004
F6	900	62.22 ± 0.76	0.414 ± 0.005

Effect of droplet size and zeta potential in different dispersant media

Due to considerable pH variations along gastrointestinal tracts, it is rational to observe the consequence of different media on the SNEDDS. The optimized SNEDDS formulation was studied in order to find out if there are any changes of particle size and PDI and zeta potential when it is mixed with different dispersion medium such as water, phosphate buffer pH 6.8 and acid buffer pH 1.2. The results from Table 4 clearly indicated that there were no significant changes in particle size distribution and zeta potential which confirms the stability of the formulation under different pH conditions.

Table 4: Mean droplet size, PDI and Zeta potential at 500-time dilution with different dispersion medium (n=3)

Formulation	Dispersion Media	Droplet size (nm)	PDI	Zeta potential (mV)
F4	Water	39.11 ± 2.32	0.194 ± 0.005	11.45 ± 1.42
F4	Acid buffer pH1.2	41.10 ± 1.02	0.212 ± 0.007	8.10 ± 0.67
F4	PBS pH16.8	40.61 ± 0.29	0.201 ± 0.001	7.95 ± 0.54
F6	Water	61.51 ± 0.64	0.410 ± 0.010	11.67 ± 0.13
F6	Acid buffer pH1.2	64.11 ± 1.10	0.419 ± 0.002	6.23 ± 0.87
F6	PBS pH16.8	63.32 ± 0.69	0.414 ± 0.005	7.98 ± 1.04

ATR-FT-IR studies

FT-IR (Spectrum 100, PerkinElmer Life and analytical sciences, USA) was studied for SNEDDS and pure drug to identify any chemical interaction. Compared to the pure drug (Fig.5), it was found that the spectrum of SNEDDS (Fig.6 has same the functional group of dutasteride as follows. Carboxamide, RCONR₂. To prove the functional group of amide, there are presence of peaks at N-H stretch (in pure drug 3192.30 cm⁻¹, SNEDDS 3486.01 cm⁻¹ and 3423 cm⁻¹), N-H bend (1592.86 cm⁻¹ in pure drug and 1640.23 cm⁻¹ in SNEDDS) and C=O stretch at 1670.95 cm⁻¹ in pure drug, whereas 1736.60 cm⁻¹ in SNEDDS. C=C, symmetric stretch at the peak of 1670.95 cm⁻¹ was observed in pure drug; and 1640.23 cm⁻¹ in SNEEDS demonstrates the presence of the aromatic ring. As well as the presence of C=C-H, asymmetric stretch at 3391.60 cm⁻¹ in pure drug; 3486.01 cm⁻¹ in SNEDDS reveals the presence of aromatic ring. Presence of C-X is observed by the peak at 1142.68 cm⁻¹ in the pure drug and 1105.93 cm⁻¹ in SNEDDS. In conclusion, ATR FT-IR spectrum of pure drug and SNEDDS were almost similar because of the presence of the same functional groups.

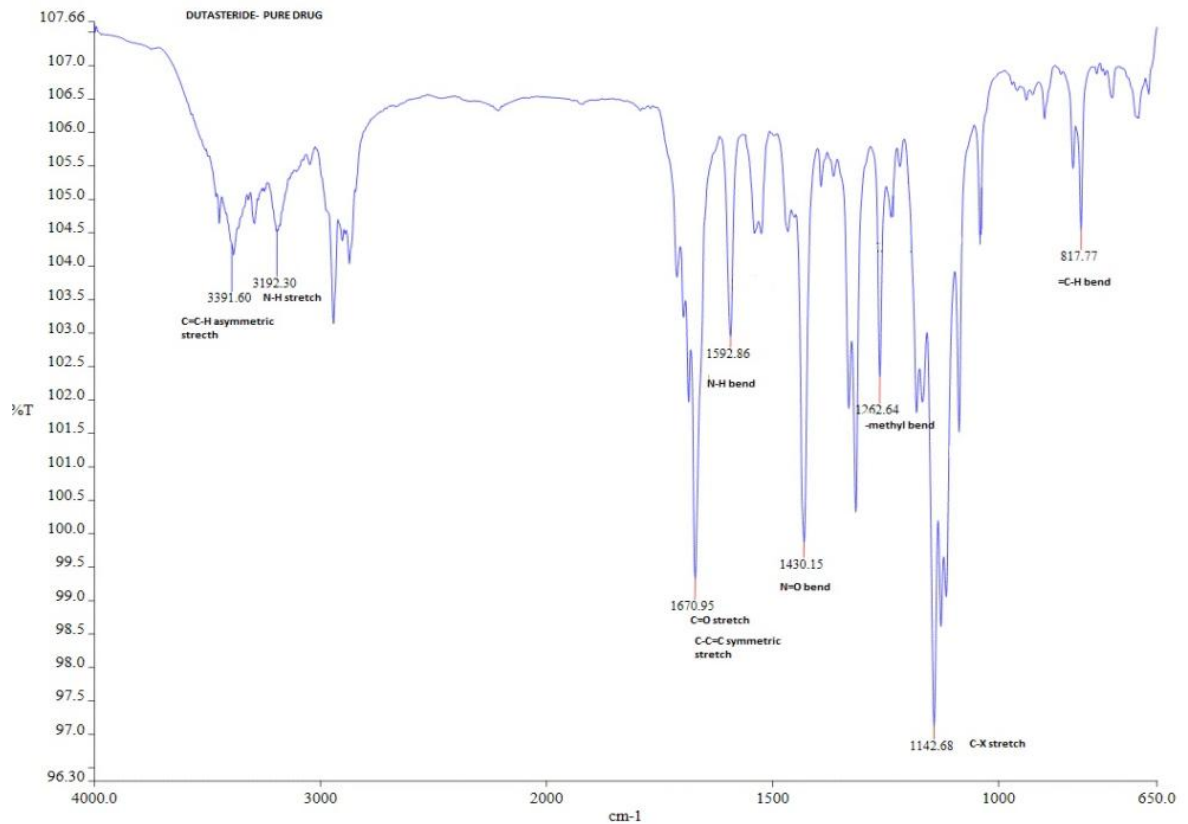


Fig.5: A Chromatogram of FTIR for standard dutasteride.

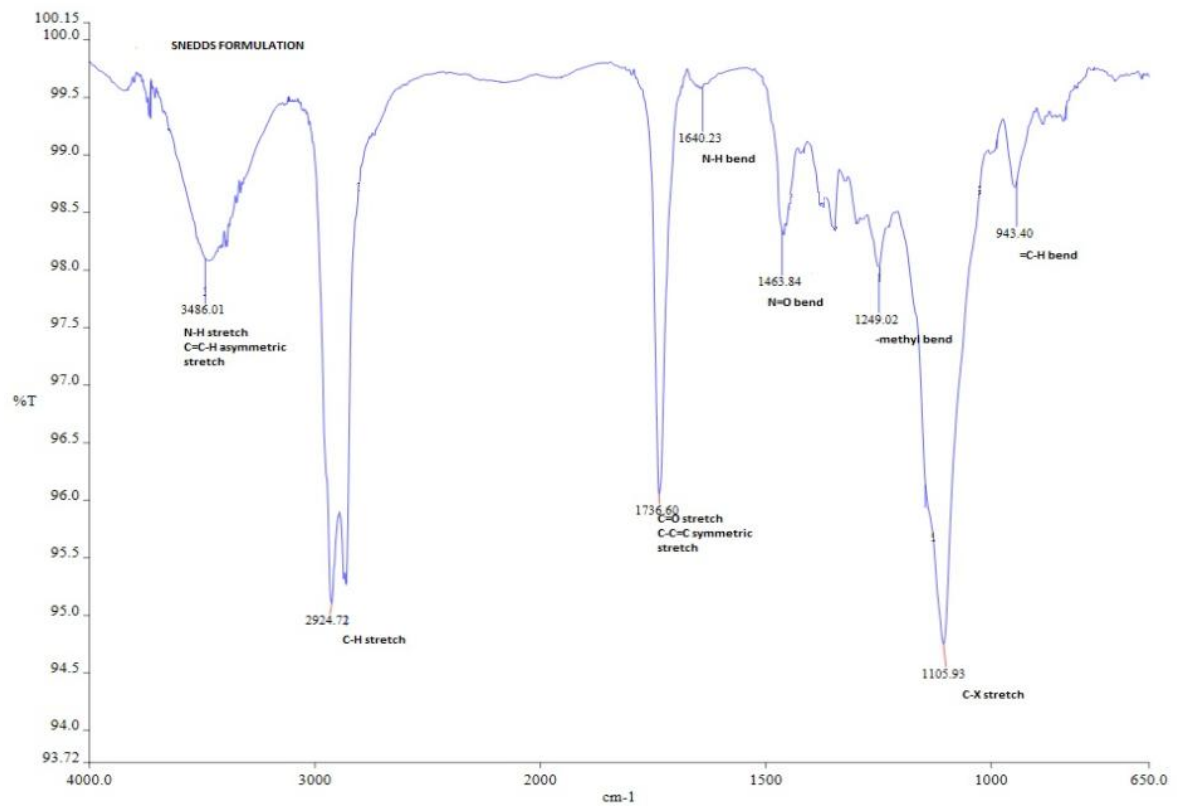


Fig.6: A representative Chromatogram of FTIR for SNEDDS formulation.

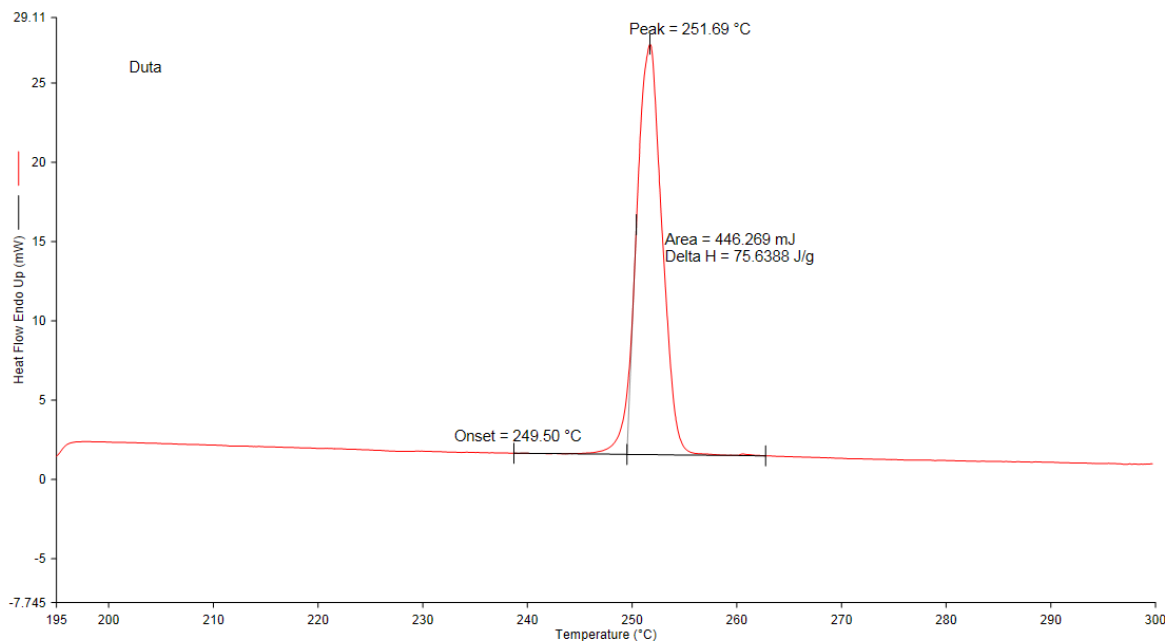


Fig.7: A representative Chromatogram of DSC for standard dutasteride.

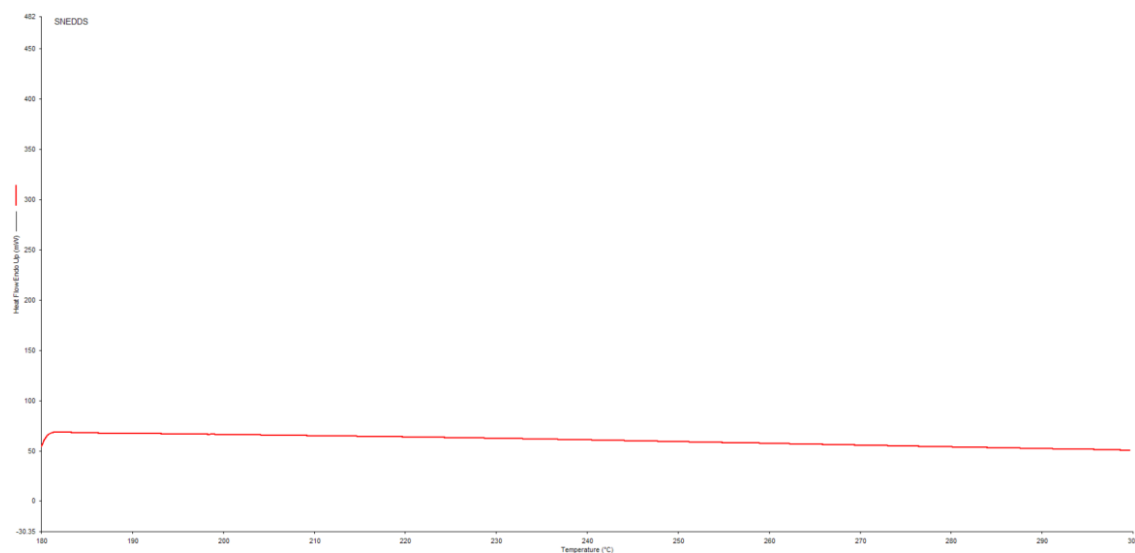


Fig.8: A representative Chromatogram of DSC for SNEDDS formulation.

Differential Scanning Calorimetry (DSC) studies

DSC (Spectrum 100, PerkinElmer Life and analytical sciences, USA) was used to determine the enthalpy changes by which the transmission of crystalline form of drug to amorphous form was determined. The melting point of dutasteride is between 242 to 250 °C. The analysis temperature range was chosen between 180-300°C. Obtained chromatogram is presented in the Fig.7 (pure drug) and Fig. 8 (SNEDDS formulation). It is observed that, there is no curve obtained in the SNEDDS formulation. This could be due to exothermic and endothermic reaction of the drug in the formulation with the excipients. The peak of drug has disappeared in the formulation due to chemical reactions with the other ingredients contained in the formulation. This shows the change of dutasteride. When prepared as SNEDDS formulation, it

changed from crystalline to amorphous form. Vadher *et al.* (2009) studied on the SMEDDS of Neusilin and Ibuprofen also noticed that there was no significant line for the formulation in the chromatogram of DSC studies. They concluded that the drug in the SMEDDS formulation was either present in the amorphous form or in a disordered crystalline form.

In vitro dissolution study

The *in vitro* dissolution study of the optimized SNEDDS formulations, raw dutasteride and marketed dutasteride (Avodart) were performed in both acid buffer pH 1.2 and phosphate buffer pH 6.8 and the results were shown in Fig.9 and Fig 10.

Among all the formulations, in pH1.2 (Fig.9) as well as pH6.8 (Fig.10) F4 formulation showed the highest dissolution rate

in SNEDDS. Based on the Fig.9, there is a significant difference at the pH1.2, between the raw drug and the SNEDDS formulations. SNEDDS showed better dissolution profile compared to the pure form of the drug at the pH 6.8 (Fig.10). From the observation (Fig.9 and Fig.10), it is understood that the drug release was better in the pH6.8 than pH 1.2. Comparison of the formulations and raw drug indicated that the dissolution profile of the raw drug is worse. This could be due to the poor aqueous solubility. There is no significant ($p \geq 0.05$) difference between the pH 1.2 and pH 6.8, which resembles that the drug is not affected by the pH. F4 showed better drug release at the end of 60minutes than other formulations, commercial product and the raw drug. SNEDDS formulation resulted in better drug release than the raw drug due to relatively low particle size. Rajinikanth *et al.* (2012) in SNEDDS formulation of valsartan, suggested that the small particle size

permitted a faster rate of drug release than the marketed and powder drug. The proper blend of the components, might have played an important role in the globule size reduction, that could have improved the dissolution of the SNEDDS. Balakumar *et al.* (2013), advised that “the maximum drug release was observed in the proper combination of the components; and also suggested that higher amount of oil, could be the reason for reduced drug release”. A better dissolution rate and more predictable bioavailability of SNEDDS indicates that the reduction in drug dose and, perhaps, eliminate the dose-related side effects (Gupta *et al.*, 2011). Therefore, the amount of ingested drug dutasteride could be reduced. Khan *et al.*, (2012) developed self-emulsifying drug delivery system with poorly water-soluble atorvastatin and the results revealed a 90 % of increased dissolution.

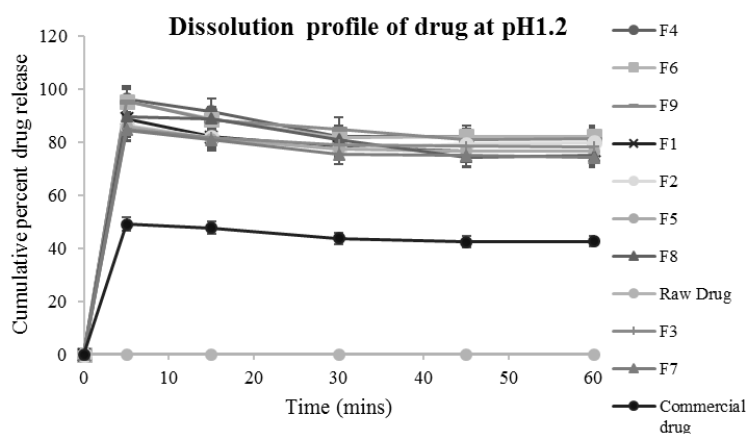


Fig. 9: *In vitro* dissolution profile of SNEDDS formulation as compared with raw dutasteride using acid buffer pH 1.2.

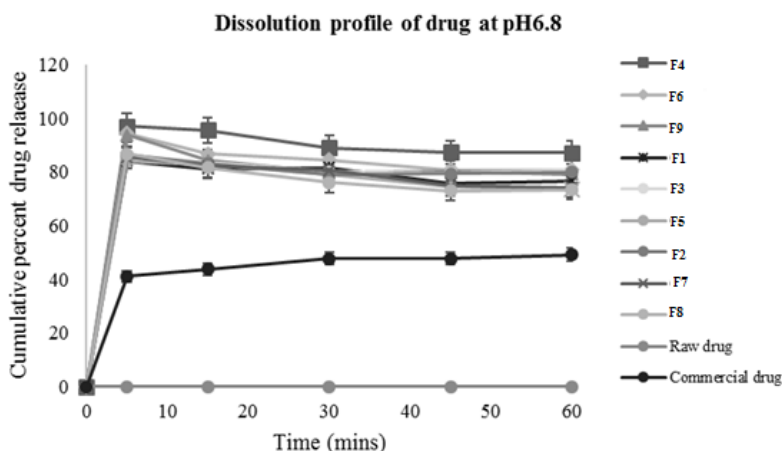


Fig.10: *In vitro* dissolution profile of SNEDDS formulation as compared with raw dutasteride using PBS buffer pH

Table 5: Droplet size, polydispersity index and zeta-potential.

Formulation	Droplet size (nm)	PDI	Zeta potential (mV)	Viscosity	Refractive Index	Drug content (%)
F1	45.59 ± 0.11	0.185 ± 0.01	11.23 ± 0.19	47.4 ± 1.62	1.443±0.01	90.45
F2	44.23 ± 0.27	0.178± 0.03	-13.73 ± 0.03	40.8 ± 1.64	1.444±0.02	89.87
F3	38.38 ± 0.36	0.436 ± 0.03	-14.03 ± 0.09	44.6 ± 1.45	1.440±0.01	91.46
F4	39.11 ± 2.32	0.194 ± 0.05	15.30 ± 0.12	40.7 ± 1.37	1.449±0.02	96.84
F5	73.15 ± 0.33	0.402 ± 0.01	15.51 ± 0.34	40.8 ± 1.42	1.443±0.05	93.22
F6	61.51 ± 0.64	0.410 ± 0.01	-11.67 ± 0.13	45.4 ± 1.02	1.440±0.01	91.21
F7	66.70 ± 1.04	0.415 ± 0.02	-14.29 ± 0.21	46.2 ± 1.38	1.443±0.02	92.14
F8	44.80 ± 0.16	0.488 ± 0.04	-16.86 ± 0.09	40.2 ± 1.54	1.440±0.02	91.74
F9	48.79 ± 0.19	0.317 ± 0.02	-12.71 ± 0.15	46.8 ± 1.87	1.441±0.02	93.74

Table 6: The droplet size, PDI and zeta potential of formulation F4 and F6 before and after accelerated stability study (n=3).

Formulation	Accelerated stability study (Before)			Accelerated stability study (After)		
	Droplet size(nm)	PDI	Zeta potential (mV)	Droplet size(nm)	PDI	Zeta potential (mV)
F4	39.11 ± 2.32	0.194 ± 0.005	10.30 ± 0.12	42.41 ± 1.24	0.192 ± 0.07	10.98 ± 0.10
F6	61.51 ± 0.64	0.410 ± 0.010	11.67 ± 0.13	66.45 ± 1.43	0.394 ± 0.02	11.01 ± 0.16

Accelerated Stability studies

The accelerated stability studies were performed at 45°C for 3 months. It was observed that there was no phase separation, drug precipitation in the formulations F4 and F6 at the end of 3 months of the accelerated temperature. From the Table 6, the results revealed that there was no significant changes in the particle size, PDI and zeta potential before and after the accelerated study.

CONCLUSION

The dutasteride loaded-SNEDDS has been successfully developed using Capryol PGMC, Cremophor EL and PEG 400. The SNEDDS formulations have good droplet size below 100 nm and pass thermodynamic stability study and robustness to dilution study, confirming the stability of the formulations. The formulations also have the ability to form nanoemulsion less than 1 minute, forming grade A and grade B nanoemulsion. The optimized SNEDDS formulations (F2) shows the highest percentage drug release, which is >85 % at 15 minutes while other formulations show 80-82 % at 15 minutes. This result draws a big contrast compared to raw drug, whereby negligible amount (< 2 %) dutasteride was detected in the *in-vitro* dissolution study due to the fact that it is practically insoluble in water. From the study, it is concluded that the SNEDDS formulation is a potentially effective formulation that can be applied to improve the bioavailability of the drug, thereby improve their therapeutic efficacy and minimize the toxicities.

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