Self-Emulsifying Drug Delivery System (SEDDS) for Enhancement of Solubility and Photostability of Amlodipine Besilate

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ABSTRACT

Amlodipine besilate (AMB) with IUPAC name of 3-ethyl-5- methyl-(4RS)-2-((2-aminoethoxy) methyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate monobenzenesulfonate is the most widely used drug for the treatment of hypertension and ischemia by blocking dihydropyridine calcium-channel. Although AMB has excellent performance against the treatment of hypertension, but its low water solubility (2.93 g/L (0.0052 mol/L) in water at 32 °C) is one of the limitations of using AMB in pharmaceutical industry. AMB is in class IV of the biopharmaceutical classification system with slight solubility in water and sparingly soluble in ethanol. To increase the therapeutic efficacy of AMB, the solubility of AMB should be increased in aqueous systems because drugs with low aqueous solubility have poor absorption and low bioavailability. Amlodipine is also known as photosensitive since light catalyzes oxidation of amlodipine to pyridine derivatives that are therapeutically ineffective. To overcome the problem of solubility and photosensitivity, Amlodipine was formulated in the form of Self-Emulsifying Drug Delivery System (SEDDS). Liquid SEDDS was prepared by dissolving amlodipine in various S_{mix} which were further evaluated and F1 and F2 were found to be optimized. F1 and F2 were solidified using spray drying method. After evaluation of Solid SEDDS F1 and F2 batch showed 91.041±2.96 % and 93.059±1.53% Drug release and increase in Photostability was observed.

KEYWORDS - Amlodipine, Solubility, Absorption, Bioavailability, Photostability, Self-Emulsifying Drug Delivery System (SEDDS)

1. INTRODUCTION

Amlodipine besilate (AMB) with IUPAC name of 3-ethyl-5- methyl-(4RS)-2-((2-aminoethoxy) methyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate monobenzenesulfonate (Figure 1.1) is the most widely used drug for the treatment of hypertension and ischemia by blocking dihydropyridine calcium-channel. Although AMB has excellent performance against the treatment of hypertension, but its low water solubility (2.93 g/L (0.0052 mol/L) in water at 32 °C) is one of the limitations of using AMB in pharmaceutical industry. AMB is in class IV of the biopharmaceutical classification system with slight solubility

in water and sparingly soluble in ethanol. To increase the therapeutic efficacy of AMB, the solubility of AMB should be increased in aqueous systems because drugs with low aqueous solubility have poor absorption and low bioavailability (1). Amlodipine is also known as photosensitive since light catalyzes oxidation of amlodipine to pyridine derivatives that are therapeutically ineffective (2).

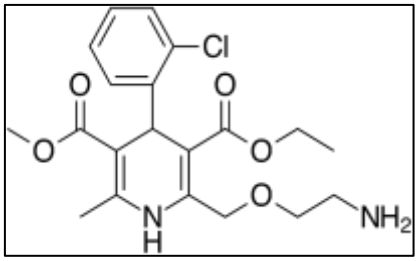


Figure 1.1 – Structure of Amlodipine

Sufficient aqueous solubility is one of the essential requirements for oral administration of a drug. Limited solubility can lead to insufficient dissolution and further reduce the bioavailability of a drug (3, 4) As a result, a variety of effort has been made to enhance the oral bioavailability of poorly water soluble drugs through lipid based emulsion systems, specifically the self-emulsifying drug delivery system (SEDDS) (5-8). SEDDSs are defined as homogeneous mixtures of natural or synthetic oils, surfactants, and co-surfactants that easily form emulsion upon mild agitation and generate a high surface area of interactions between the SEDDS formulation and the gastrointestinal (GI) fluid (9). Moreover, SEDDS has been identified as a prominent technology for drug delivery, because the formulations have great solubilization capacity and a tiny droplet size, which could improve permeation across the GI membrane.

In this present study attempt has been taken to prepare liquid as well as solid self-emulsifying drug delivery system of poorly water soluble and highly photosensitive drug amlodipine. The objective of preparing liquid as well as self-emulsifying drug delivery system is to enhance the solubility of the selected drug which may also increase the dissolution rate and bioavailability of the drug.

2. MATERIAL AND METHOD

2.1. Material

Amlodipine besilate BP, a dihydropyridine calcium-channel blockers was obtained as gift sample from Zydus Cadila, Goa. Capmul PG-8, Capmul MCM, Captex 500 were obtained as Gift sample from Abitec Corporation, US. Labrafil M 1944 CS/ 2125 CS both were gifted by Gattefosse India Pvt Ltd, Mumbai. Oils such as Oleic acid, Isopropyl myristate, Tween 40/60/80, Span 20/80, PEG 200/400/600, Carbitol were purchased from Research lab, Mumbai. Other Solvent used during the Research were of HPLC Grade.

2.2. Screening of Excipients

2.2.1. Solubility study

The solubility of amlodipine in various oils, surfactants, and co-surfactants was measured, respectively. An excess amount of amlodipine was added into 2 ml of each of the selected oils, surfactants, co-surfactants and distilled water in 5-ml stoppered vials separately, and mixed by vortexing. The mixture vials were then kept at 25 ± 1.0°C in an isothermal shaker for 72 h to reach equilibrium. The equilibrated samples were removed from shaker and centrifuged at 3000 rpm for 15 min. The supernatant was taken and filtered through a 0.45 µm membrane filter. The concentration of amlodipine was determined in oils, surfactants, co-surfactants and water using UV- spectrophotometer at 360 nm (9, 10).

2.2.2. Preliminary screening of surfactants

Emulsification ability of various surfactants was screened. Briefly, 300 mg of surfactant was added to 300 mg of the selected oily phase. The mixture was gently heated at 45–60^{0}C for homogenizing the components. The isotropic mixture, 50 mg, was accurately weighed and diluted with double distilled water to 50 ml to yield fine emulsion. The ease of formation of emulsions was monitored by noting the number of volumetric flask inversions required to give uniform emulsion. The resulting emulsions were observed visually for the relative turbidity. The emulsions were allowed to stand for 2 h and their transmittance was assessed at 360 nm by UV-spectrophotometer (UV V-630, Jasco) using double distilled water as blank (11).

2.2.3. Preliminary screening of co-surfactants

The turbidimetric method was used to assess relative efficacy of the co-surfactant to improve the Nano emulsification ability of the surfactants and also to select best co-surfactant from the large pool of co-surfactants available for peroral delivery. Surfactant, 0.2 gm. was mixed with 0.1 gm. of co-surfactant. Labrafil M 1944 CS, 0.3 gm., was added to this mixture and the mixture was homogenized with the aid of the gentle heat (45–60^{0}C). The isotropic mixture, 50 mg, was accurately weighed and diluted to 50 ml with double distilled water to yield fine emulsion. The ease of formation of emulsions was noted by noting the number of flask inversions required to give uniform emulsion. The resulting emulsions were observed visually for the relative turbidity. The emulsions were allowed to stand for 2 h and their transmittance was measured at 360 nm by UV-spectrophotometer (UV V-630, Jasco) using double distilled water as blank. As the ratio of co-surfactants to surfactant/s is the same, the turbidity of resulting Nano emulsions will help in assessing the relative efficacy of the co-surfactants to improve the nanoemulsification ability of surfactants (12).

2.3. Drug – Excipients Compatibility Study

The Drug – Excipients Compatibility Studies were performed in order to confirm the drug- excipients compatibility. This study mainly include DSC given below, The DSC study was carried out for pure amlodipine, Tween 20, PEG 400, Labrafil M 1944 CS & physical mixtures of all excipients that were expected to be used in the development of formulation like oil phase, emulsifier, surfactant and co-surfactant etc. The DSC patterns were recorded on a METTLER TOLIDO DSC1 STAR SYSTEM. Each sample (2-4mg) was heated in crimped aluminium pans at a scanning rate of 10^{0}C/min in an atmosphere of nitrogen using the range of 30^0-400^{0}C. The temperature calibrations were performed periodically using indium as a standard and thermograms obtained were observed for any interaction and reported in the section 3.2. (Table 3.2) (13).

2.4. Construction of Pseudo-ternary phase diagram

A pseudo-ternary phase diagram was constructed by titration of four component mixtures of oil, surfactant and co-surfactant with water at room temperature. After equilibrium, the mixture was visually observed. The generated sample which was clear or slightly bluish in appearance was determined as microemulsion. On the basis of the solubility studies of drug, select the oil phase, surfactants and co-surfactants. Water was used as an aqueous phase for the construction of phase diagrams. Surfactant : co-surfactant (S_{mix}) are mixed in different weight ratios 1:0, 0.5:1(1:2), 1:1, 1:0.5 (2:1), 3:1. These S_{mix} ratios were chosen in increasing concentration of surfactant with respect to co-surfactant and increasing concentration of co-surfactant with respect to surfactant for detailed study of the phase diagrams. For each phase diagram, oil and specific S_{mix} ratio was mixed thoroughly in different weight ratios from 1:9 to 9:1 in different glass vials. Sixteen different combinations of oil and Smix, 1:9, 1:8, 1:7, 1:6, 1:5, 2:8(1:4), 1:3.5, 1:3, 3:7(1:2.3), 1:2, 4:6(1:1.5), 5:5(1:1), 6:4(1:0.7), 7:3 (1:0.43), 8:2(1:0.25), 9:1(1:0.1), were made so that maximum ratios were covered for the study to delineate the boundaries of phases precisely formed in the phase diagrams. Pseudo ternary phase diagrams were developed using aqueous titration method. Slow titration with aqueous phase was done to each weight ratio of oil and Smix and visual observation was carried out for transparent and easily flowable o/w microemulsions. The mixture was visually examined for transparency. After equilibrium was reached, the mixtures were further titrated with aliquots of distilled water until they showed the turbidity. Clear and isotropic samples were deemed to be within the microemulsion region. No attempts were made to completely identify the other regions of the phase diagrams. Based on the results, appropriate percentage of oil, surfactant and co-surfactant was selected, correlated in the phase diagram and were used for preparation of SEDDS containing amlodipine. All studies were repeated thrice, with similar observations being made between repeats and results of phase diagram were reported in section 3.3. (14, 15)

2.5. Selection of Formulation from Pseudo ternary Phase Diagram

From each phase diagram, constructed, different formulations were selected from micro-emulsion region it is reported in section 3.4, so that drug could be incorporated into the oil phase on the following bases.

- The oil concentration should be such that it solubilizes the drug (single dose) completely depending on the solubility of the drug in the oil. 5 mg of amlodipine will dissolve easily in 1 mL of oil.
- To check if there was any effect of drug on the phase behaviour and microemulsion area of the phase diagram.
- The minimum concentration of the S_{mix} used for that amount of oil was taken.

- For convenience purposes, 1mL was selected as the microemulsion formulation, so that it can be increased or decreased as per the requirement in the proportions. (14)

Selected formulations were subjected to diff erent thermodynamic stability and Dispersibility tests.

2.5.1. Thermodynamic stability studies

1. Heating cooling cycle

Six cycles between refrigerator temperature (4^0C) and 45^0C with storage at each temperature of not less than 48h was studied. Those formulations, which were stable at these temperatures, were subjected to centrifugation test (16, 17).

2. Centrifugation

Passed formulations were centrifuged at 3000 rpm for 30 min. Those formulations that did not show any phase separation were taken for the freeze thaw stress test (16, 18).

3. Freeze thaw cycle

Three freeze thaw cycles between -21^0C and +25^0C with storage at each temperature for not less than 48 h was done for the formulations (16, 19).

Those formulations, which passed these thermodynamic stress tests, were further taken for the Dispersibility test for assessing the efficiency of self-emulsification.

2.5.2. Dispersibility test

The efficiency of self-emulsification of oral microemulsion was assessed using a standard USP XXII dissolution apparatus 2 (Disso TDT 08L, Electrolab). One millilitre of each formulation was added to 500 mL of water at 37±0.5^0C. A standard stainless steel dissolution paddle rotating at 50 rpm provided gentle agitation. The in-vitro performance of the formulations was visually assessed using the following grading system:

Grade A: Rapidly forming (within1min) Nano emulsion, having a clear or bluish appearance.

Grade B: Rapidly forming, slightly less clear emulsion, having a bluish white appearance.

Grade C: Fine milky emulsion that formed within 2 min.

Grade D: Dull, greyish white emulsion having slightly oily appearance that is slow to emulsify (longer than 2min).

Grade E: Formulation, exhibiting either poor or minimal emulsification with large oil globules present on the surface.

Those formulations that passed the thermodynamic stability and also Dispersibility test in Grade A, Grade B and Grade C was selected for further studies. The results were reported in section 3.4 (Table 3.6 and 3.7)

2.6. Preparation of Liquid SEDDS Formulations

The formulations were prepared by dissolving the formulation amount of amlodipine (5 mg/mL) in the mixture of surfactant, oil and co-surfactant (Table 2.1). Tween 20, Labrafil M 1944 CS, Polyethylene glycol 400 (PEG 400), and amlodipine were accurately weighed and transferred into a borosilicate glass vial. Using magnetic stirrer, the ingredients were mixed for 10 min at 60–65^0C until a yellowish transparent formulation was attained. Amlodipine SEDDS formulations were then allowed to cool to room temperature before they were used in subsequent studies (21).

Table 2.1- Data for Preparation of Liquid SEDDS Formulations

Ingredients	Group I (S_{mix} 2:1)			Group II (S_{mix} 3:1)		
	A	B	C	D	E	F
Amlodipine (gm.)	0.005	0.005	0.005	0.005	0.005	0.005
Labrafil M 1944 CS (% w/w)	20	25	30	20	25	30
S_{mix} (% w/w)	80	75	70	80	75	70

Where S_{mix} is Tween 20 and PEG 400

2.7. Evaluation of Liquid SEDDS Formulations

1. Determination of emulsification time

The emulsification time of SEDDS was determined according to United State Pharmacopeia (USP) XXIII, dissolution apparatus II (Disso TDT 08L, Electrolab). In brief, 0.5 mL of each formulation (Table 2.1) was added drop wise to 500mL of purified water at 37^0C. Gentle agitation was provided by a standard stainless steel dissolution paddle rotating at 50 rpm (21). The emulsification time was assessed visually as reported by Bachynsky et al. (22) and it was reported in section 3.6. (Table 3.8).

2. Turbidimetric evaluation

Self-emulsifying system (0.2 mL) was added to 0.1 mol L^{-1} hydrochloric acid (150 mL) under continuous stirring (50 rpm) on a magnetic plate (Remi 5-MLH DX) at ambient temperature, and the increase in turbidity was measured until equilibrium was achieved using a turbidimeter (Digital Nephlo-Turbidity Meter 132,Systronics,India) and it was reported in section 3.6.

3. Drug Content

Amlodipine from pre-weighed SEDDS was extracted by dissolving in 25 mL methanol. Amlodipine content in the methanolic extract was analyzed by UV-spectrophotometer (UV V-630, Jasco) at 360 nm, against the standard methanolic solution of amlodipine and it was reported in section 3.6.(23, 24)

4. Globule size analysis

Droplet size distribution of SEDDS diluted with water was determined using a photon correlation spectrometer (Zetasizer 3000 HAS, Malvern Ltd., UK) based on the laser light scattering phenomenon. Samples were diluted 200 times with purified water. Diluted samples were directly placed into the module and measurements were made in triplicate after 2-min stirring. Droplet size was calculated from the volume size distribution and it is reported in section 3.6. (25, 26)

5. Drug release studies

Drug release studies from SEDDS were performed using USP XXIII, dissolution apparatus II (Disso TDT 08L, Electrolab) with 500 mL of 0.1N HCl as medium at 37 ± 0.5^0C. The speed of the paddle was adjusted to 100 rpm. 1 mL of the formulation was (5 mg of drug) and marketed tablet (Amlocard®, Cipla) directly introduced into the medium and an aliquot (2 mL) of sample was collected at designated times and analyzed for the content of amlodipine by UV-spectrophotometer at 360 nm. An equivalent volume (2 mL) of fresh dissolution medium was added to compensate for the loss due to sampling and results of drug release study were reported in section 3.5.(23, 24)

2.8. Preparation of solid SEDDS

Maltodextrin was dissolved in 100 ml distilled water by Magnetic stirring. The liquid SEDDS was then added with constant stirring, and the solution was kept at 50^0C for 10 min to obtain a good o/w emulsion. The emulsion was spray dried with a Labultima spray dryer (LU 222 ADVANACED) apparatus. Conditions and parameter for spray drier are shown in Table 2.2. (27)

Table 2.2 - Data for Spray Drying Parameters

Sr. No.	Parameters	Condition at which the formulations were prepared
1	Inlet temperature	120^0C
2	Outlet temperature	100^0C
3	Feed pump	2.5 mL/min
4	Aspirator Speed	40mmWC
5	Vacuum	25 PSI
6	Cycle time	45 min

Table 2.3 - Data for Preparation of Solid SEDDS Formulations

Ingredients	Group I (S_{mix} 2:1)	Group II (S_{mix} 3:1)
	F_1	F_2
Maltodextrin (g)	10	10
Liquid SEDDS (g)	10	10
Water (mL)	100	100

2.9. Evaluation of Solid SEDDS Formulations

1. Reconstitution properties of solid SEDDS

A. Reconstitution

Solid SEDDS (100mg) prepared was dispersed with 10 ml distilled water, respectively, by vortex mixing (30s), and then incubated for 30 min at 25^0C and the results of reconstitution was reported in section 3.8.

B. Droplet size of reconstituted emulsions

The average droplet size, size distribution emulsions from solid SEDDS were assessed by photon correlation spectrometer (Zetasizer 3000 HAS, Malvern Ltd., UK) and results of droplet size was reported in section 3.8. (25)(26)

2. Drug Content

Amlodipine from pre-weighed solid SEDDS was extracted by dissolving in 25 mL methanol. Amlodipine content in the methanolic extract was analyzed UV-spectrophotometrically (UV V-630, Jasco) at 360 nm, against the standard methanolic solution of amlodipine and results of drug content was reported in section 3.8.(23)(24)

3. Drug release study

Drug release studies from solid SEDDS were performed using USP XXIII, dissolution apparatus II (Disso TDT 08L, Electrolab) with 500 ml of 0.1N HCl pH 1.2 as a medium at 37 ± 0.5^0C. The speed of the paddle was adjusted to 100 rpm. Amlodipine-loaded solid SEDDS (equivalent to 5 mg of amlodipine) were placed in a dissolution tester. At predetermined time intervals an aliquot (2 ml) of the sample was collected, filtered and analyzed for the content of amlodipine by UV-spectrophotometer (UV V-630, Jasco) as mentioned above. An equivalent volume (2 ml) of fresh dissolution medium was added to compensate for the loss due to sampling and results of drug release study was reported in section 3.8. (23)(24)

4. Morphological analysis of solid SEDDS

The outer macroscopic structure of the solid SEDDS was investigated by Scanning Electron Microscope (SEM) with a Scanning Electron Microscope (JEOL JSM- 6360, Japan), operating at 10 kV and results of SEM was reported in section 3.8. (28)

5. Solid state characterization of solid SEDDS

A. DSC

The physical state of amlodipine in solid SEDDS was characterized by the differential scanning calorimetry thermogram analysis. The DSC patterns were recorded on a METTLER TOLIDO DSC1 STAR SYSTEM. Each sample (2-4mg) was heated in crimped aluminum pans at a scanning rate of 10^0C/min in an atmosphere of nitrogen using the range of 30-400^0C. The temperature calibrations were performed periodically using indium as a standard. The DSC curves are shown in Figure 3.10**.** (29)

2.10. Photostability study

A. Preparation of sample for irradiation test

All samples were passed through a sieve no. 40 to obtain fine powders with uniform particle sizes before irradiation tests.

B. Irradiation by fluorescent lamp

The irradiation test was employed utilizing a fluorescent lamp (FL-15 Watt, vacuum tube). Each sample of pure amlodipine powder, solid SEDDS of amlodipine was placed and spread uniformly as a thin film

on an aluminium foil. The fine powders on the aluminium foil were discrete enough to allow for uniform irradiation. Irradiation was conducted inside a light cabinet (Photostability chamber TP00000906, Thermolab) to protect samples from extraneous light. The accelerated irradiation test using this lamp was carried out at ambient temperature. Samples were assayed for their content of amlodipine prior to exposure and at 4, 8, 12, 24, 36, and 60 h of continuous exposure using HPLC assay method (30). The obtained chromatograms at different times were shown in Figure 3.11 and 3.12.

3. RESULT AND DISCUSSIONS

3.1. Screening of Excipients

3.1.1. Solubility study

The self-emulsifying formulations consisted of oil, surfactants, co-surfactants and drug should be clear and monophasic liquids at ambient temperature when introduced to aqueous phase and should have good solvent properties to allow presentation of the drug in solution. Solubility studies were aimed at identifying suitable oily phase and surfactant/s for the development of amlodipine SEDDS. Identifying the suitable oil, surfactant/co-surfactant having maximal solubilizing potential for drug under investigation is very important to achieve optimum drug loading (31, 32). The solubility of amlodipine in various oily phases, surfactants and co-surfactant is reported in Table 3.1, 3.2, 3.2 respectively and it is represented graphically in Figure 3.1

According to Solubility tested in this study, Tween 20, a medium-length alkyl chain with HLB 16.7 was selected as appropriate surfactant because non-ionic surfactants are less toxic than ionic surfactants, has good biological acceptance; is powerful permeation enhancer, less affected by pH and ionic strength, and highest solubility of Amlodipine was obtained. Furthermore, Transcutol-P (Diethylene glycol monoethyl ether), Polyethyleneglycol 400 (PEG 400) were selected as a co-surfactants because of their potential to solubilize the drug (33).

Table 3.1 - Data for Solubility study of Amlodipine in Various Oils

Sr No	Oil	*Solubility of Amlodipine (mg/ml) at 25°C
1	Labrafil M 1944 CS	10.24 ±6.23
2	Isopropyl Myristate	11.83 ±4.40
3	Labrafil M 2125 CS	17 ±5.68
4	Capmul PG 8	9.2 ±5.23
5	oleic acid	6.16 ±7.24

* Represents mean ± S.D. (n = 3)

Table 3.2 - Data for Solubility study of Amlodipine in Various Surfactants

Sr No	Surfactant	*Solubility of Amlodipine (mg/ml) at 25°C
1	Tween 20	90.12 ±6.74
2	Span 20	115.93 ±29.42
3	Tween 60	120.68 ±7.25
4	Span 80	74.5 ±22.33

* Represents mean ± S.D. (n = 3)

Table 3.3 - Data for Solubility study of Amlodipine in Various Co-Surfactants

Sr No	Co-Surfactant	*Solubility of Amlodipine (mg/ml) at 25°C
1	Ethanol	160.95 ±5.04

2	PEG 200	204.82 ±5.75
3	PEG 400	228.95 ±5.39
4	PEG 600	154.16 ±4.49
5	Capmul MCM	64.46 ±47.66
6	Captex 500	11.66 ± 1.53
7	Carbitol	330.91 ±8.6

* Represents mean ± S.D. (n = 3)

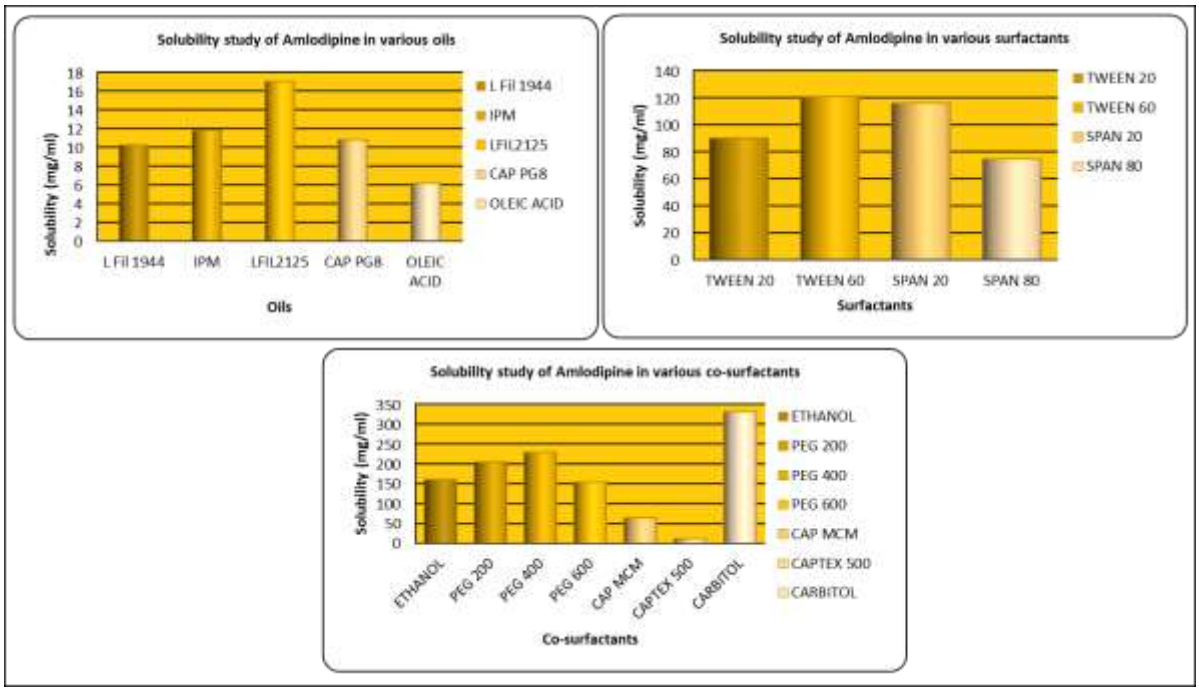


Figure 3.1 – Solubility of Amlodipine in various Oils, Surfactants and Co-surfactants

3.1.2. Preliminary screening of surfactants

Non-ionic surfactants are generally considered less toxic than ionic surfactants. They are usually accepted for oral ingestion. The surfactants were compared for their emulsification efficiencies using different oily phases. It has been reported that well formulated SEDDS is dispersed within seconds under gentle stirring conditions. Transmittance values of different mixtures are demonstrated in Table 3.4. From results it was inferred that the oily phase Labrafil M 1944 CS exhibited the highest emulsification efficiency with Tween 20, requiring only 5 flask inversions for homogenous emulsion formation. On the other hand, Labrafil M 2125 CS showed poor emulsification properties with Tween 20, requiring a minimum of 40 flask inversions (34).

The aforementioned results suggested the use of Labrafil M 1944 CS as an oily phase with Tween 20 as a surfactant for further study.

Table 3.4 - Data for Emulsification efficiency of surfactant

Sr. No.	Oils	% Transmittance
		Tween 20
1.	Labrafil M 1944 CS	98
2.	Labrafil M 2125 CS	68

3.1.3. Preliminary screening of co-surfactants

Addition of a co-surfactant to the surfactant-containing formulation was reported to improve dispersibility and drug absorption from the formulation. In view of current investigation, two co-surfactants,

polyethyleneglycol 400, Transcutol-P, were compared for ease of emulsification (34). As reported Table 3.5, the Labrafil M 1944 CS exhibited good emulsification with both co-surfactants, i.e. PEG 400 showing maximum transmittance (98.6%) followed by Transcutol-P (89%).

Table 3.5 - Data for Emulsification efficiency of Co-surfactant

Sr. No.	Co-surfactants	% Transmittance
		Labrafil M 1944 CS
1.	Polyethyleneglycol 400	98.6
2.	Transcutol-P	89

Based on the results of preliminary screening, one distinct system was selected which was:
Labrafil M 1944 CS as oily phase, Tween 20 as surfactant, polyethyleneglycol 400 as co-surfactant for further studies.

3.2. Drug – Excipients Compatibility Study

Compatibility of drug and excipients can be determined by diff erential scanning calorimetry. Endothermic peaks of Amlodipine at 208^{o} C disappeared in the curves of Labrafil M 1944 CS + Amlodipine, Tween 20+ Amlodipine, PEG 400 + Amlodipine and combination drug & all these excipients. It might be explained as excipients inhibited the crystallization of Amlodipine, because oil, surfactant and co-surfactant produces the molecular dispersion of Amlodipine. According to DSC graph drug and excipients are compatible to each other (35).

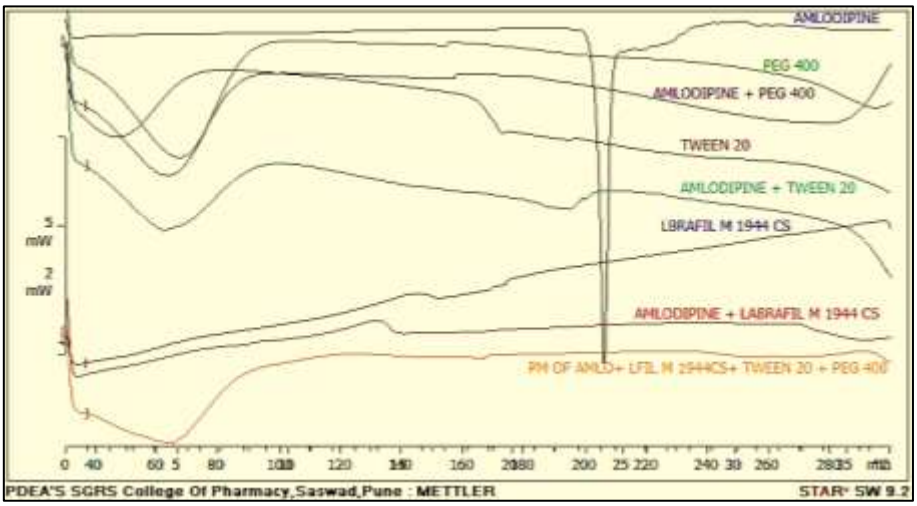


Figure 3.2 - DSC Spectra of Amlodipine and Excipients

3.3. Construction of Pseudo ternary phase diagram

The consideration for screening formulation of SEDDS usually involves: the formulation composition should be simple, safe, and compatible; it should possess good solubility; a large efficient self-emulsification region which should be found in the pseudo-ternary phase diagram, and have efficient droplet size after forming microemulsion. Thus, pseudo-ternary phase diagrams were constructed to identify the self-emulsifying regions with maximum drug loading and to optimize the concentration of oil, surfactant and co-surfactant in the SEDDS formulations and to obtain transparent and stable O/W micro-emulsions.The shaded areas in the pseudo-ternary phase-diagrams shown in Figure 3.3 represented the existence field of stable, clear and transparent O/W micro-emulsions containing Labrafil M1944 as oil and with the Tween 20: PEG 400 fixed mixing ratio, respectively. For any selected composition of surfactant and co-surfactant ratio from self-emulsifying region of ternary phase diagram (shaded) the addition of great volumes of continuous phase allowed the clear system.

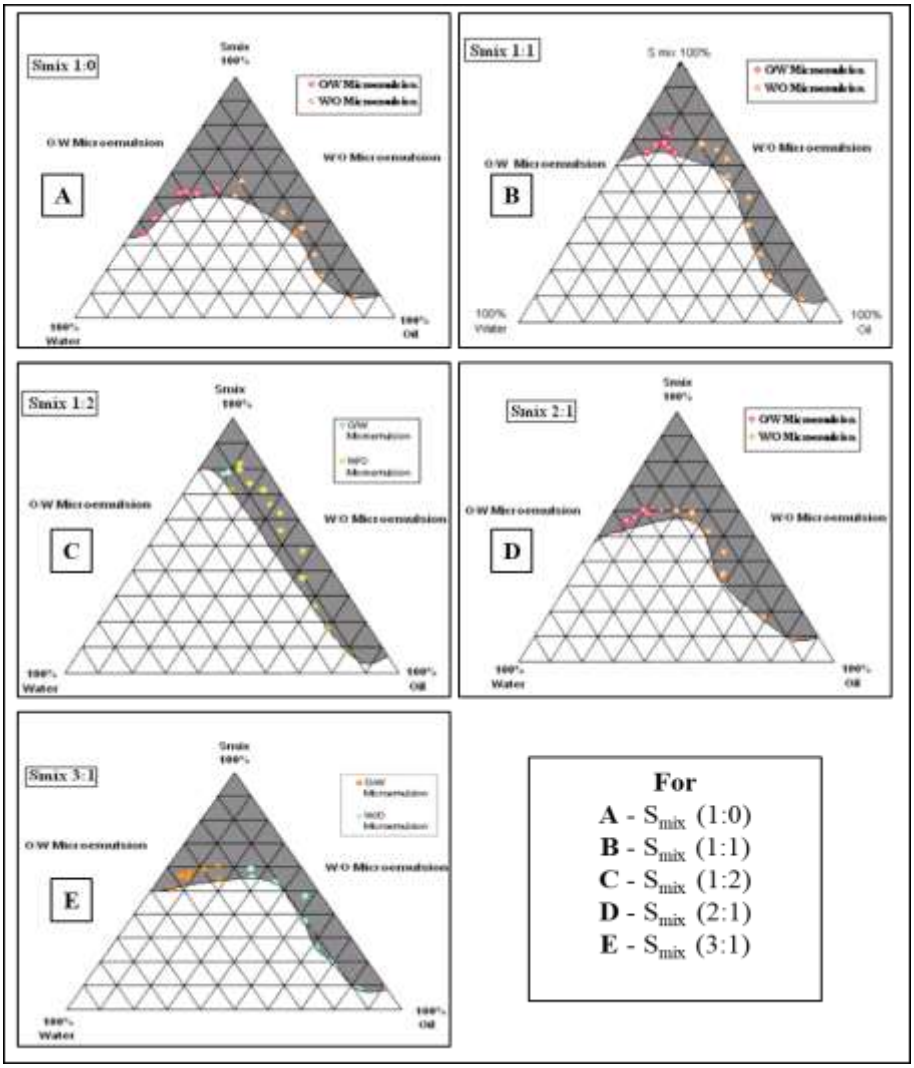


Figure 3.3 - Phase diagram of Labrafil M 1944 CS (oil)-S_{mix} (Tween 20 and Polyethylene glycol 400) were water system having different S_{mix} ratio.

It can be also seen that microemulsion region exists at S_{mix} ratio 1:0 (i.e. without co-surfactant). However, equal mixture of surfactant and co-surfactant decreases the microemulsion region (Figure 3.3 B). Increasing the concentration of surfactant (2:1) resulted in even larger area of microemulsion region (Figure 3.3 D). Further increasing surfactant concentration from 2:1 to 3:1 resulted in no influence on microemulsion region (Figure 3.3 E). The influence of concentration of co-surfactant on the microemulsion region was also seen by constructing the phase diagram in ratio of 1:2. It was seen that the region of microemulsion was decreased with increase in concentration of co-surfactant (Figure 3.3 C).The existence of large or small microemulsion region depends on the capability of a particular surfactant or surfactant mixture to solubilize the oil phase. The extent of solubilization resulted in a greater area with clearer and homogenous solution. It was seen that when the surfactant (Tween 20) was used alone, the oil phase was solubilized to a lesser extent at higher concentration of surfactant implying that surfactant alone was not able to reduce the interfacial tension of oil droplet to a sufficiently low level and thus was not able to reduce the free energy of the system to an ultra-low level desired to produce microemulsions. When a co-surfactant was added, the interfacial tension was reduced to a very low level and very small free energy was achieved which helps in larger microemulsion region. With further increase in surfactant from 1:1 to 2:1 and 3:1 further drop in interfacial tension and free energy was achieved resulting in maximum region of microemulsion/ self-emulsifying formation. Thus, pseudo-ternary phase diagram for S_{mix} 2:1 and 3:1 were selected for the formation of drug loaded self-emulsifying drug delivery system.

3.4. Selection of Formulation from Pseudo ternary Phase Diagram

It is well known that large amounts of surfactants cause GI irritation; therefore, it is important to determine the surfactant concentration properly and use minimum concentration in the formulation. S. Shafiq et al. reported the basis of selecting different nanoemulsion or microemulsion formulations from the phase diagram, as hundreds of formulations can be prepared from nanoemulsion region of the diagram. From the data shown in different pseudo-ternary phase diagrams (D and E), it was understood that oil could be solubilized up to the extent of 50% w/w. Therefore, from phase diagram (D and E) different concentrations of oil, which formed nanoemulsions, were selected at a difference of 5% (20, 25, 30, 35, 40, 45 and 50%) so that maximum formulations could be prepared covering the nanoemulsion/ self-emulsification area of the phase diagram (Tables 3.6 and 3.7). For each percentage of oil selected, only those formulations were taken from the phase diagram, which needed minimum concentration of S_{mix}. There was no sign of change in the phase behavior and nanoemulsion area of phase diagrams when Amlodipine (5 mg) was incorporated in the formulations, which was indicated as the formation and stability of Nano- and microemulsions consisting of nonionic components is not affected by the pH and or ionic strength (36-41).

3.4.1. Thermodynamic stability studies

Nanoemulsions are thermodynamically stable systems and are formed at a particular concentration of oil, surfactant and water, with no phase separation, creaming or cracking. It is the thermostability which differentiates Nano- or microemulsion from emulsions that have kinetic stability and will eventually phase separate (36). Thus, the selected formulations were subjected to different thermodynamic stability testing by using heating cooling cycle, centrifugation and freeze thaw cycle stress tests. Those formulations, which passed thermodynamic stability tests, were taken for dispersibility test (Table 3.6 and 3.7).

Thus it was concluded that the efficiency of surfactant and co-surfactant mixture was unaffected after exposing to extreme conditions.

3.4.2. Dispersibility test

When infinite dilution is done to nanoemulsion formulation, there is every possibility of phase separation, leading to precipitation of a poorly soluble drug as nanoemulsions are formed at a particular concentration of oil, surfactant and water. For oral nanoemulsions the process of dilution by the GI fluids will result in the gradual desorption of surfactant located at the globule interface. The process is thermodynamically driven by the requirement of the surfactant to maintain an aqueous phase concentration equivalent to its CMC (36).

In the present study, we used distilled water as a dispersion medium because it is well reported that there is no significant difference in the nanoemulsions prepared using nonionic surfactants, dispersed in either water or simulated gastric or intestinal fluid (36, 40). Formulations in Group I (Table 3.6) and Group II (Table 3.7) that passed dispersibility test in Grade A, B and C were taken for further study, as Grade A and B formulations will remain as nanoemulsions when dispersed in GIT. Formulation falling in Grade C could be recommended for self-emulsifying drug delivery formulation.

So from the study, total six formulations were selected for further study three from each group i.e. F_1, F_2, F_3 from Group I and F_1, F_2, F_3 from Group II.

Table 3.6 - Data for Thermodynamic stability test of different formulations selected from Group I (Figs. 10.10 D) at a difference of 5% w/w of oil.

Group I (Fig. 10.10 D) S_{mix} ratio (S:CoS) 2:1	**Percentage w/w of different components in formulation**		**Observations based on the preparation, thermodynamic stability studies and dispersibility tests**				**Inference**
Formulation	**Oil**	**S_{mix}**	**H/C**	**Cent.**	**Freez. Tha.**	**Disperse.**	
F_1	20	80	√	√	√	Grade A	Selected
F_2	25	75	√	√	√	Grade B	Selected
F_3	30	70	√	√	√	Grade C	Selected

F_4	35	65	√	√	√	Grade D	Rejected
F_5	40	60	√	√	√	Grade D	Rejected
F_6	45	55	√	√	√	Grade E	Rejected
F_7	50	50	√	√	√	Grade E	Rejected

Where, Heating cooling cycle (H/C), Freeze-thaw cycle (Freez. Tha.), Centrifugation (Cent.), Dispersibility test (Disperse.)

Table 3.7 - Data for Thermodynamic stability test of different formulations selected from Group II (Figs. 10.10 E) at a difference of 5% w/w of oil

Group II (Fig. 10.10 E) S_{mix} ratio (S:CoS) 3:1	**Percentage w/w of different components in formulation**		**Observations based on the preparation, thermodynamic stability studies and dispersibility tests**				**Inference**
Formulations	**Oil**	**S_{mix}**	**H/C**	**Cent.**	**Freez. Tha.**	**Disperse.**	
F_1	20	80	√	√	√	Grade A	Selected
F_2	25	75	√	√	√	Grade B	Selected
F_3	30	70	√	√	√	Grade C	Selected
F_4	35	65	√	√	√	Grade D	Rejected
F_5	40	60	√	√	√	Grade D	Rejected
F_6	45	55	√	√	√	Grade E	Rejected
F_7	50	50	√	√	√	Grade E	Rejected

Where, Heating cooling cycle (H/C), Freeze-thaw cycle (Freez. Tha.), Centrifugation (Cent.), Dispersibility test (Disperse.)

3.5. Preparation of Liquid SEDDS Formulations

Formulations selected in section 2.6 were prepared as per the composition reported in Table 2.1 and found to be thermodynamically stable even after addition of a drug.

3.6. Evaluation of Liquid SEDDS Formulations

1. Determination of emulsification time

In SEDDS, the primary means of self-emulsification process is visual evaluation (41). The efficiency of self-emulsification could be estimated by determining the rate of emulsification. The rate of emulsification is an important index for the assessment of the efficiency of emulsification (31) that is the SEDDS should disperse completely and quickly when subjected to aqueous dilution under mild agitation. The emulsification time of liquid SEDDS are presented in Table 3.8. Emulsification time study showed that all the formulations emulsified within 20 s. Among the tested formulations, formulations A and D showed shortest emulsification time than others.

2. Turbidimetric evaluation

The results of turbidimetric evaluation of liquid SEDDS are presented in Table 3.8. Formulations A and D showed low turbidity values (23.1 NTU and 31 NTU, respectively) owing to the presence of adequate amounts of surfactant (Tween 20), which primarily governs the resultant droplet size and its distribution. Formulation C and F, with moderate quality of emulsion formation because of high concentration of oil and showed very high and variable turbidity (94.2±15.8 NTU and 82.1±12.8, mean ± SD, n = 3) and coarser droplets. Formulation B and E showed moderate turbidity values (41.1 NTU and 31.7 NTU, respectively).Thus the droplet size distribution is strongly dependent on concentration of surfactant/co-surfactant.

3. Drug Content

The drug content of all formulations ranged between 5.79 and 7.95 mg/mL (Table 3.8) and passed uniformity of content.

4. Globule size analysis

Droplet size of SMEDDS is a critical parameter in the adapted strategy of enhancing drug bioavailability (42). Droplet size analysis revealed the effect of varying amounts of Tween 20 and PEG 400 in the formulated SEDDS. Changes in Tween 20 to PEG 400 ratios are most likely to alter the resultant HLB of the system and the properties of liquid crystal (LC) interfaces. This in turn governs the size of droplets formed (43). Thus it is the appropriate choice of surfactant and co-surfactant together with their proper concentrations, which provides an optimum self-emulsifying formulation. The mean droplet sizes of the reconstituted microemulsions are reported in Table 3.8. As shown in the table, the average droplet sizes of all microemulsions were less than 700 nm. The data for size distribution for various emulsions containing amlodipine measured by photon correlation spectroscopy are shown in Figure 3.4.

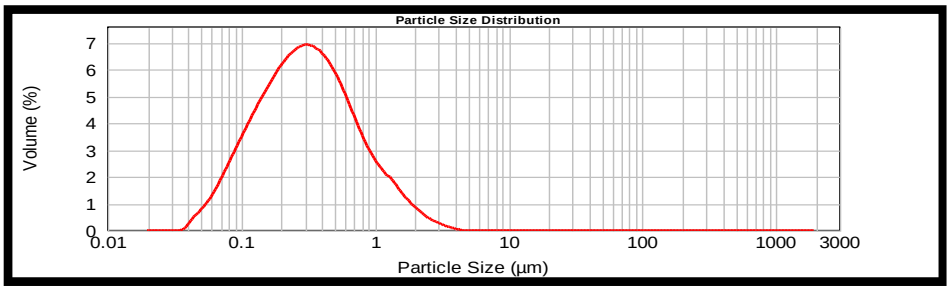
Table 3.8 - Data for Evaluation of Liquid SEDDS formulations

Evaluation Parameters	Group I (S_{mix} 2:1)			Group II (S_{mix} 3:1)		
	A	B	C	D	E	F
Emulsification Time (S)[a]	12±2	17±3	19±4	14±3	16±2	18±1
Turbidity (NTU)[a]	23.1±2.28	41.1±3.41	94.2±15.8	31±4.76	31.7±2.7	82.1±12.8
Drug Content (mg/ml)[a]	5.79±0.05	7.95±0.043	7.11±0.067	6.38±0.9	6.29±0.02	7.87±0.11
Mean Droplet Size (µm)	0.306	0.518	0.690	0.315	0.348	0.366

[a]Mean ± SD, n = 3.

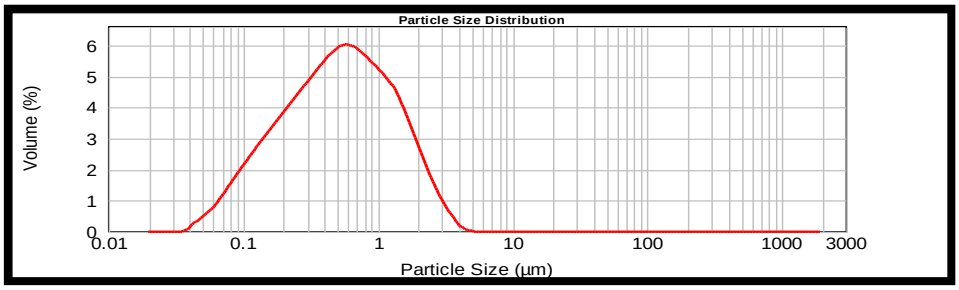
Formulation A

D (0.1) = 0.102µm, d (0.5) = 0.306 µm, d (0.9) =0.967 µm, Uniformity = 0.907, Span =2.753



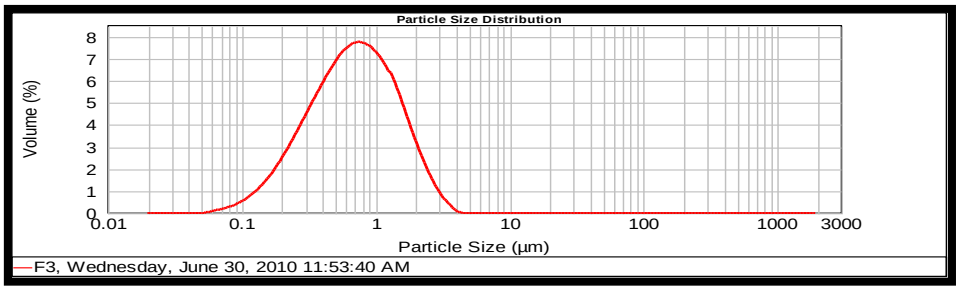
Formulation B

D (0.1) = 0.129 µm, d (0.5) = 0.518 µm, d (0.9) =1.653 µm Uniformity = 0.914, Span =2.943



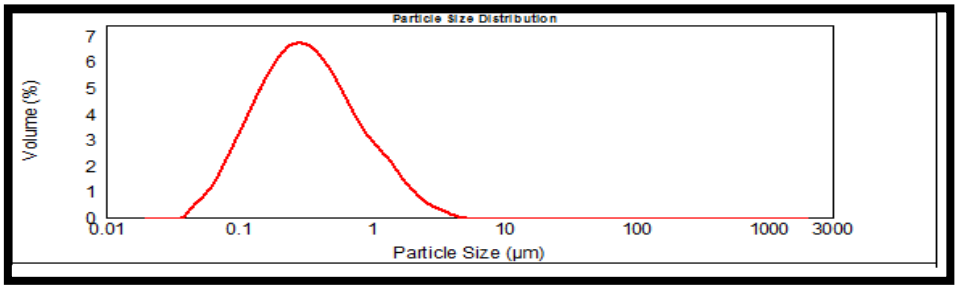
Formulation C

D (0.1) = 0.238 µm, d (0.5) = 0.690 µm, d (0.9) =1.708 µm Uniformity = 0.665, Span =2.130



Formulation D

D(0.1)=0.106 µm, d(0.5)=0.315 µm, d(0.9)=1.100 µm, Uniformity: 0.995



Formulation E

D(0.1)=0.110 µm, d(0.5)=0.348 µm, d(0.9)=1.216 µm, Uniformity: 1

Figure 3.4 - Results of Droplet size distributions of Liquid SEDDS

5. Drug release studies

The in- vitro drug release study of liquid SEDDS were performed in 0.1N HCl. The percent drug release for different formulations is shown in Table 3.9. In the self-emulsifying systems, the free energy required to form an emulsion was very low, thereby allowing spontaneous formation of an interface between the oil droplets and water. It is suggested that the oil/surfactant/co-surfactant and water phases effectively swell and eventually there was increase the release rate. It was clear from the Figure 3.5 and 3.6. The maximum percentage of the drug released within 5min because of fast emulsification. The marketed formulation (Amlocard®) showed only 61% drug release at the end of 60 min. This clearly demonstrated the superior performance of the developed Liquid SEDDS as compared to Amlocard®. The SEDDS represented Amlodipine in solubilized form in gastric fluids after ingestion and hence provided large interfacial area for Amlodipine absorption. Therefore, the optimized formulations (C and F), had higher drug release than marketed preparation, optimum globule size, and stability of emulsion and drug and above all, lower surfactant concentration was selected for the further study (28).

Table 3.9 - Dissolution data for Liquid SEDDS formulations in 0.1N HCl

Time (in min)	*Percent drug dissolved						
	A	B	C	D	E	F	MT
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000
05	96.86±1.33	96.09±0.65	94.66±1.25	97.58±1.25	96.59±1.78	94.54±1.12	59.8±1.23
15	97.48±1.4	96.27±0.59	90.92±1.57	96.64±1.45	96.95±1.05	94.47±1.36	60.91±1.54
30	97.99±2.76	96.32±1.01	91.45±2.45	96.48±1.54	96.48±1.19	94.59±1.55	61.68±0.98
60	98.45±2.06	96.32±1.3	91.97±2.68	96.12±1.68	96.89±1.45	94.63±1.48	61.61±0.85

*Represents mean ± S.D. (n = 3), MT: Marketed Tablet of Amlodipine

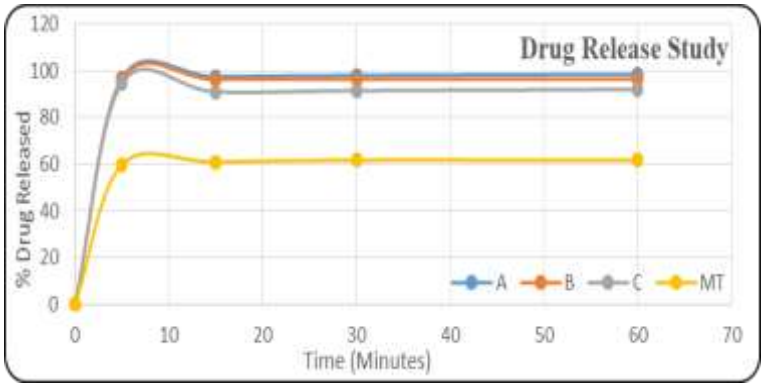


Figure 3.5 - In- vitro drug release profile of Liquid SEDDS Formulations A, B, C and MT in 0.1N HCl

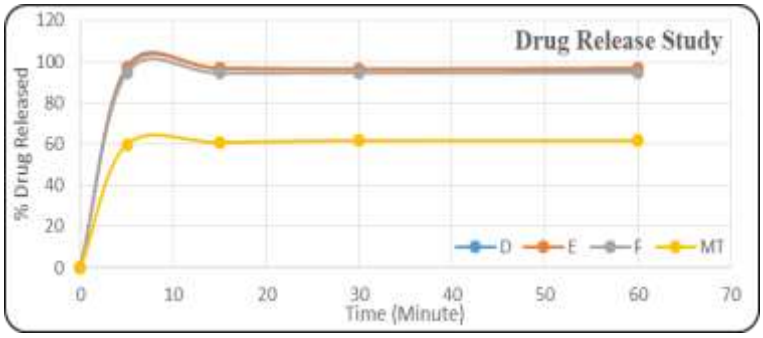


Figure 3.6 - In- vitro drug release profile of Liquid SEDDS Formulations D, E, F and MT in 0.1N HCl

3.7. Preparation of Solid SEDDS

Solid SEDDS were prepared as per the composition reported in Table 2.3.

3.8. Evaluation of Solid SEDDS Formulations

1. Reconstitution properties of solid SEDDS

The mean droplet sizes of the solid SEDDS is presented in Table 3.10. As shown in the table, the z-average droplet sizes of both systems were less than 1µm. The emulsion droplet size distribution and solid SEDDS (Figure 3.7) further confirmed the self-emulsification nature of the solid SEDDS. The droplet size of the emulsion from the solid SEDDS was slightly increased, compared to the liquid SMEDDS. At the same time, a broader size distribution was observed.

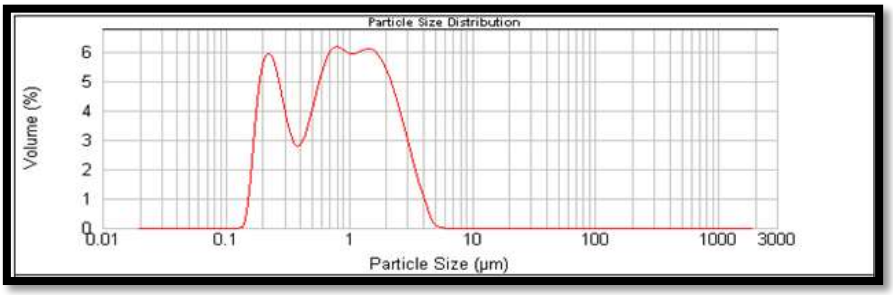
The solid SEDDS preserved the self-emulsification performance of the liquid SEDDS.

Table 3.10 - Data for Evaluation of Solid SEDDS formulations

Evaluation Parameters	Group I (S_{mix} 2:1)	Group II (S_{mix} 3:1)
	F_1	F_2
Emulsification Time (S)[a]	20±2	15±3
Drug Content (% w/w)[a]	2.59±0.85	2.52±0.48
Mean Droplet Size (µm)	0.839	0.623

*Represents mean ± S.D. (n = 3)

Formulation A (Solid SEDDS S_{mix} 2:1)
d(0.1) = 0.220µm, d (0.5) = 0.839µm, d (0.9) = 2.406µm



Formulation B (Solid SEDDS S_{mix} 3:1)

d (0.1) =0.229µm d (0.5) =0.623µm d (0.9) =1.605µm

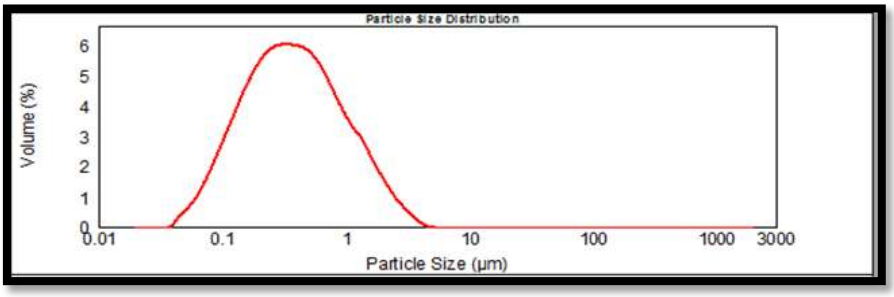


Figure 3.7 - Results of Droplet size distributions of Solid SEDDS

2. Drug Content

The drug content of both formulations ranged between 2.50 and 2.60 % w/w (Table 3.10).

3. Drug Release Study

The in-vitro drug release studies were performed in order to ensure the quick release of the drug in the dissolution medium. In-vitro dissolution studies also give an idea about the self-emulsification efficiency of the developed system. The in-vitro drug release profile of F_1 and F_2 was evaluated in 0.1N HCl (n = 3). It was observed that both the solid SEDDS formulations F_1 and F_2 released more than 90% of Amlodipine within 60

min. Both the formulations dispersed almost instantaneously indicating the high self-emulsion efficiency of the developed formulations.

The graphs of the drug release profile are shown in Figure 3.11. Amlodipine from the solid SEDDS was completely and rapidly dissolved in medium without affecting the dissolution pattern also.

Table 3.11 - Dissolution data for formulations in 0.1N HCl

Time (Minute)	*Percent drug dissolved	
	F_1	F_2
0	00.000	00.000
05	88.419±1.06	91.338±1.80
15	89.201±2.60	91.898±3.95
30	89.906±1.10	92.497±1.71
60	91.041±2.96	93.059±1.53

Represents mean ± S.D. (n = 3)

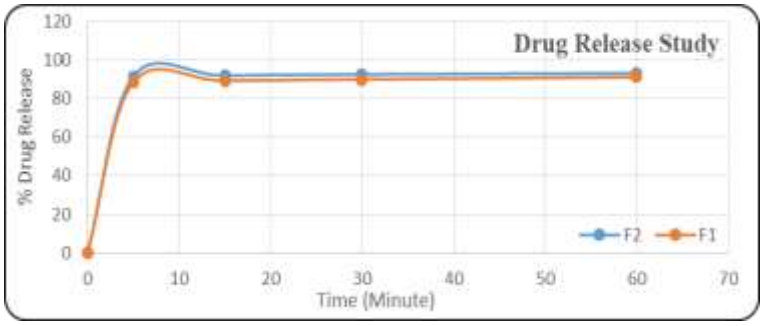


Figure 3.8 - In- vitro drug release profile of Solid SEDDS Formulations F_1 and F_2

4. Morphology of the Solid SEDDS

The outer macroscopic morphology of the Solid SEDDS revealed well separated spherical particle with smooth surface seen in SEM images of the Solid SEDDS. Figure 3.9 shows the scanning electron micrographs of the Maltodextrin powder and Solid SEDDS formulation. Maltodextrin (Figure 3.9 A and 3.9 B) appeared with a rough surface with porous particles. However, the solid SEDDS (Figure 3.9 C and 3.9 D) appeared as smooth-surfaced Maltodextrin particles, indicating that the liquid SEDDS is adsorbed or embedding inside the pores of Maltodextrin. Following spray-drying, maltodextrin is known to produce deep and abundant surface dents and the limited agglomeration of particles was probably due to maltodextrin ability to diminish the degree of particle agglomeration (44, 45) and to the storage of products in closed vials protected from humidity; hence preferred as carrier in the study.

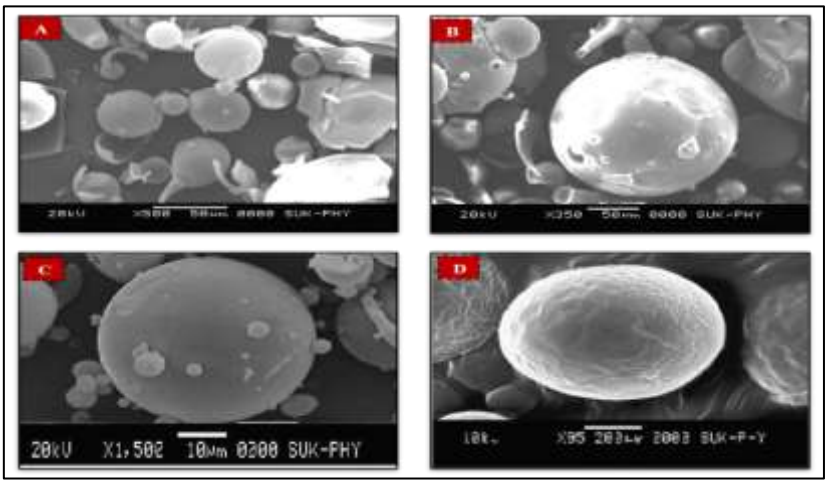


Figure 3.9 - Scanning electron micrographs: (A & B) Maltodextrin; (C & D) Solid SEDDS

5. Solid state characterization of solid SEDDS

1. DSC

The physical state of amlodipine in the solid SEDDS was investigated since it would have an important influence on the in-vitro and in-vivo release characteristics. DSC curves of pure amlodipine, and the solid SEDDS of amlodipine are shown in Figure 3.10. Pure amlodipine showed three sharp endothermic peaks at temperatures between 205^0 and 210^0C. No obvious peaks for amlodipine and oil were found in the solid SEDDS of amlodipine. It might be explained that the melting behavior of the oil was changed by maltodextrin and the crystallization of amlodipine was inhibited by maltodextrin and surfactants.

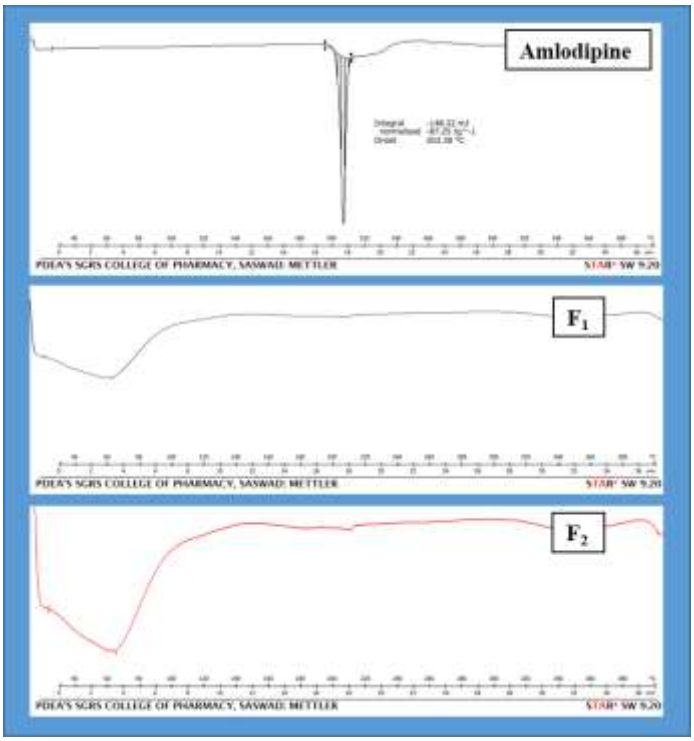


Figure 3.10 - DSC Spectra of pure Amlodipine and Solid SEDDS.

3.9. Photostability study

The Photostability studies of pure amlodipine and Solid SEDDS were done by exposing these samples to the fluorescent light using Photostability chamber (TP 00000906, Thermolab). The Samples were assayed for their content of amlodipine prior to exposure and at 4, 8, 12, and 24 h of continuous exposure using HPLC assay method. The decomposition of pure amlodipine was found to be remarkable upon exposure to fluorescent lamp or sunlight (which is the main source of light during manufacturing, storage and handling). The retention time for amlodipine and its degradation product was found to be 3.3 ± 0.18 and 2.9 ± 0.14 respectively.

In this study, Solid SEDDS was prepared by spray drying the Liquid SEDDS with relatively excess amount of maltodextrin compared to amlodipine. The outer macroscopic morphology of the Solid SEDDS observed by SEM (Figure 3.9 C & 3.9 D) suggests that most of the amlodipine was encapsulated in the maltodextrin matrix. Therefore the improved photostability of Solid SEDDS might be due to the compact physical barrier composed of maltodextrin as observed as the smooth surface of the Solid SEDDS powder (Figure 3.9 C & 3.9 D).

This study indicated that the rate of photo degradation is very slow in Solid SEDDS as compared to pure amlodipine powder; thus Solid SEDDS conferred photostability to drug.

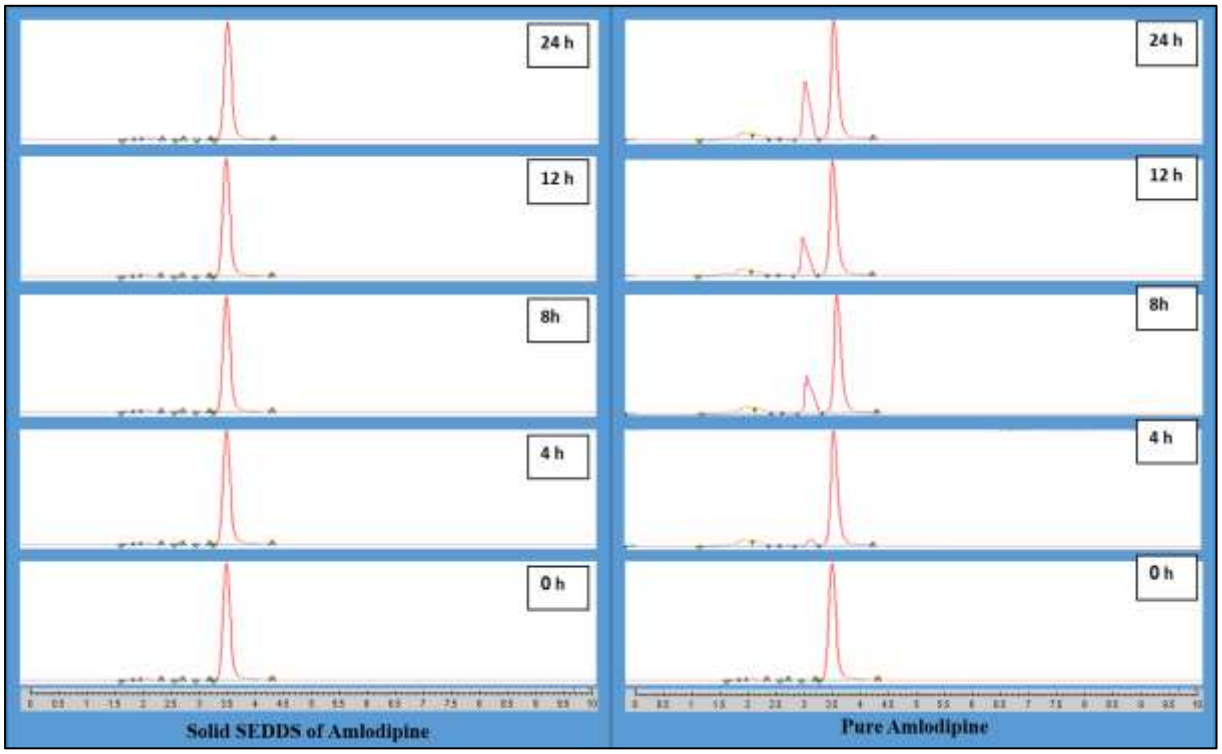


Figure 3.11 - Chromatograms of Solid SEDDS of Amlodipine and Pure Amlodipine at different time interval.

CONCLUSION

In the present study, Amlodipine was formulated in the form of Self-Emulsifying Drug Delivery System (SEDDS) to increase its solubility which will result in enhancement in Dissolution Rate and Bioavailability and also to increase its Photostability. Firstly, Liquid SEDDS was prepared by dissolving Amlodipine in various S_{mix} which were further evaluated and F1 and F2 were found to be optimized. F1 and F2 were solidified using spray drying method and Preparation of Solid SEDDS took place. The in-vitro drug release profile of F_1 and F_2 was evaluated in 0.1N HCl. It was observed that both the solid SEDDS formulations F_1 and F_2 released more than 90% of Amlodipine within 60 min. Further Photostability studies of pure amlodipine and Solid SEDDS (F1 and F2) were done by exposing these samples to the fluorescent light using Photostability chamber. The Result of photostability study indicated that the rate of photo degradation is very slow in Solid SEDDS (F1 and F2) as compared to pure amlodipine powder; thus we can say that Photostability of amlodipine increase when it is formulated in the form of SEDDS.

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CONFLICT OF INTEREST

All authors declared no conflicts of interest.

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