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DEVELOPMENT AND EVALUATION OF POMALIDOMIDE SUPERSATURABLE SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEM FOR IMPROVED SOLUBILITY AND DISSOLUTION RATE

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Keywords:

Pomalidomide, S-SNEDDS, solubility, Pseudo ternary phase diagram, Anti-neoplastic agent.

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ABSTRACT: Background: Pomalidomide belongs to BCS class IV drug with low solubility and undergoes first-pass metabolism leads to reduced bioavailability of 15%. **Objective:** The current study aimed to develop pomalidomide Super saturable-SNEDDS to enhance solubility. **Methods:** Preliminary solubility studies were performed to identify oil, surfactant, and co-surfactant ratios. Pseudo tertiary phase diagram constructed to select the areas of nanoemulsion and based on monophasic region. Nineteen ratios were selected for drug loading study having oil: Smix ratio in 3:1, from which 15 formulations of pomalidomide SNEDDS were prepared with 4mg drug loading and screened for visual observation and turbidity measurement studies. These SNEDDS are further characterized for robustness, content of drug, entrapment efficiency, and *in-vitro* dissolution analysis. The optimized SNEDDS formulation was selected for screening of precipitation inhibitor by adding 2% precipitation inhibitors, and concentration-time profiles were studied under non-sink conditions from which best PI was selected and then *in-vitro* dissolution studies conducted for the super saturable SNEDDS (S-SNEDDS). The optimized final formulation S-SNEDDS with the highest drug release was analyzed for FTIR, particle size, Z average, zeta potential, SEM analysis, and stability studies. **Results:** Based on pseudo tertiary phase diagram, Akomed E oil - Caprol PGE 860 - and PEG 600 as oil, surfactant, and co-surfactant selected, respectively. All the formulations were stable with no phase separation and maximum % transmittance. The formulation F11 was selected as optimized one based on maximum drug release of 99.04% within 60 min. By adding PVP K17 as a precipitation inhibitor to conventional SNEDDS, a super saturable system was prepared. Firstly, the prepared SNEDDS played an important role in increasing the aqueous solubility and hence oral absorption due to nano-range size. Secondly, the S-SNEDDS found to be advantageous over SNEDDS for having a higher drug load and inhibition of dilution precipitation of pomalidomide. Formulated S-SNEDDS (F11) showed drug release of 99.98%. The particle size, PDI and zeta potential of the optimized formulation F11 S-SNEDDS were 49.0 nm, 0.318, and -24.4 mV, respectively. The FTIR and SEM studies did not indicate any drug excipient interaction and confirmed nanosized which is stable. **Conclusion:** Pomalidomide-loaded S-SNEDDS could potentially be exploited as a delivery system for improving solubility and increase drug release..

INTRODUCTION: Pomalidomide, an analog structurally similar to thalidomide, is an immune-modulatory agent with antineoplastic activity. In *in-vitro* cellular assays, pomalidomide inhibited proliferation and induced the apoptosis of hematopoietic tumor cells and showed immune-modulatory activity.

It is anti-angiogenic and also acts as an immune modulator in the treatment of multiple myeloma. Pomalidomide is marketed by Celgene under the brand names Imnovid® and Pomalyst®. Imnovid® and Pomalyst® are supplied for oral administration as immediate-release hard gelatin capsules in four different strengths: 1, 2, 3, and 4 mg. Pomalidomide is a BCS class IV product, having

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low permeability and low solubility. The drug substance is practically insoluble in water [1, 2]. More than 60% of the discovered drugs have the problem of low aqueous solubility, which leads to their poor dissolution and reduced bioavailability. There are many techniques to overcome this problem: cyclodextrin complexation, salt formation, particle size reduction, solid dispersion, lipid-based formulations, *etc.* Self-nano emulsifying drug delivery system (SNEDDS) is one of the techniques which is gaining more attention for improving the solubility of the lipophilic drug. SNEDDS is an isotropic mixture of oil, surfactant, and co-surfactant, which forms oil in water (o/w) nanoemulsion with slight agitation. Oil is selected based on its solubility capacity, and both surfactant and co-surfactant are selected based on their emulsifying ability.

To prevent the precipitation of the drug and to reduce the dosing frequency, suitable precipitation inhibitors can be used (maintains supersaturation state and blocks the formation and growth of the crystals). By introducing precipitation inhibitors into the formulation, the surfactant concentration can be minimized (reduce GI side effects). Hence, Super saturable SNEDDS (S-SNEDDS) is an effective method for the oral delivery of poorly water-soluble drugs to improve its bioavailability [3, 4]. The present work described an innovative approach by designing a supersaturated self-emulsifying formulation (S-SNEDDS) to improve the solubility and dissolution of a poorly soluble drug, pomalidomide.

MATERIALS AND METHODS

Materials: Pomalidomideis gifted by Hetero Labs Limited, Hyderabad. Akomed E, Castor oil, Corn oil, Caprylic acid, Capryol 90, Imwitor, Miglyol 810, Neobee M5, Oleic acid, Triacetin, Brij35, tween20, Caprol PGE 860, Cremophor EL, CremophorHS15, Labrasol. Cremophor RH 40, Tween 85, D-alpha Tocopheryl Polyethylene Glycol, PEG-400, PEG-600, Propylene glycol, lauro glycol FCC, HPMCK4M, PVPK30, EudragitL100, and Poloxamer 407 purchased from Gattefosse, Mumbai.

Solubility of Pomalidomide in Vehicles: Naturally occurring different vegetable oils, various surfactants and co-surfactants were studied for pomalidomide solubility in order to identify the components for construction of ternary phase diagrams. An excess amount of pomalidomide was placed in screw-capped glass vials containing 1 g of vehicle (*i.e.,* oil or surfactant or co-surfactant). Glass vials were sealed with caps and vortexed for 10 min using a cyclomixer in order to facilitate proper mixing of pomalidomide with the vehicles. Then vials were shaken reciprocally using a mechanical rotary shaker for 48 h at 25 °C and allowed for another 24 h to attain equilibrium conditions without shaking at the same temperature. The vials were centrifuged at 3000 rpm for 10 min using a centrifuge to obtain a clear supernatant liquid. Supernatant (100 mg) was collected extracted for pomalidomide and filtered through a millipore membrane filter (0.45 µm) and diluted suitably with methanol and analyzed for pomalidomide using UV spectrophotometer at 251 nm. The amount of pomalidomide dissolved in various vehicles was calculated [5, 6].

Construction of Pseudo-Ternary Phase Diagrams: From the solubility study, oil, surfactant and co-surfactant were chosen based on the maximum solubility of the drug in it; the chosen vehicles were mixed in various ratios ranging from 1:9 to 9:1 (oil: Smix). Smix is the mixture of surfactant and co-surfactant prepared in defined ratios of 1:1, 2:1, and 3:1. Ternary phase diagrams comprising surfactant, co-surfactant, and oil were plotted, each of them representing an apex of the triangle. Akomed E oil as oil phase Caprol PGE 860 as Surfactant and PEG 600 as co-surfactant were selected (based on the solubility studies). Varying ratios of oil: Smix were filled in 2 ml Eppendorf tubes shaken, and kept aside. These mixtures were gently mixed with 100 ml of water taken in a beaker and checked for phase separation and turbidity. The ratios with no phase separation and clear appearance with no turbidity were separated and checked for transmittance using UV spectrophotometer [7]. The transmittance value of more than 90 indicated nano-size droplets formation; hence these ratios were noted and used for plotting pseudo-ternary phase diagram [8]. A pseudo ternary phase diagram is constructed using CHEMIX software

Effect of Pomalidomide Loading: The drug loading has considerable influence on the

spontaneously emulsifying systems' globule size and phase behaviour. In view of this, the effect of pomalidomide loading on the transmittance, phase behaviour and area of nanoemulsion formation was studied on Akomed *E. oil* - Caprol PGE 860 - and PEG 600 compositions with Smix in 3:1 ratio, which gave more area of nano emulsification region among the other ratios. Nineteen compositions of varying ratios of Akomed E oil - Caprol PGE 860 - and PEG 600 were taken, and in 1ml composition of each ratio were incorporated with 2 mg, 4 mg, and 8 mg of pomalidomide (i.e. 19*3=57 formulations). A required amount of pomalidomide was added to the screw-capped glass vials containing the required amount of surfactant and co-surfactant. The drug was solubilized using a vortex mixer or by heating at 40 °C in a water-bath wherever necessary. Finally required amount of oil was added to the vials and vortex mixed for 2 min for proper mixing. The transmittance of the resulting dispersions up on diluting 25 mg of the formulations with 50 mL distilled water was measured using UV spectrophotometer at 600 nm. The area of nano emulsification region was identified as described above by constructing pseudo-ternary phase diagrams [9].

Preparation of Pomalidomide SNEDDS: A series of SNEDDS (F1- F15, the composition was shown in Table 1) which showed transmittance values more than 90) were selected from 4 mg loaded pomalidomide system and prepared as described above 8. About 1ml of the formulation (equivalent to 4 mg of the pomalidomide) was filled in size '00' hard gelatin capsules, sealed and stored at ambient temperature (25 °C) until used. These SNEDDS were evaluated for visual observations, turbidity, the effect of pH of the dispersion media on globule size and zeta potential, robustness to dilution and *in-vitro* dissolution study, and optimized [10].

TABLE 1: COMPOSITION OF POMALIDOMIDE SNEDDS

S. no.	Formulation code	Pomalidomide drug (mg)	Ratios of Oil: Smix	Oil	Smix 3:1	
				(Akomed E oil)	Surfactant (Caprol PGE 860)	Co-surfactant (PEG 600)
1	F1	4	01:01	50	37.5	12.5
2	F2	4	01:02	33	49.5	16.5
3	F3	4	03:01	75	18.75	6.25
4	F4	4	02:01	66	24.75	8.25
5	F5	4	02:03	40	45	15
6	F6	4	09:02	81.8	13.6	4.6
7	F7	4	07:02	77.7	16.7	5.5
8	F8	4	05:02	71	21.3	7.1
9	F9	4	03:02	60	30	10
10	F10	4	03:04	42.6	42.6	14.8
11	F11	4	03:07	30	52.5	17.5
12	F12	4	08:03	72.7	20.25	6.75
13	F13	4	07:03	70	22.5	7.5
14	F14	4	05:03	62.5	28.12	9.3
15	F15	4	04:03	57.1	31.95	10.65

TABLE 2: GRADING SYSTEM FOR VISUAL OBSERVATIONS OF SELF-EMULSIFYING FORMULATIONS

Grade	Dispersibility	Appearance	Self-Emulsification Time
A	Rapid and spontaneous emulsification	Clear or slightly bluish	<1 min
B	Rapid emulsification	Slightly less clear and bluish-white	<2 min
C	Slow emulsification	Bright white emulsion	<3 min
D	Slow emulsification	Dull, grayish-white emulsion slightly oily	>3 min
E	Poor or minimal emulsification	Large oil droplets present on the surface	>3 min

Visual Observations: To assess the self-emulsification properties, pomalidomide SNEDDS (4 mg) was introduced into 50 mL of distilled water in a glass Erlenmeyer flask at 37 °C, and the contents were gently stirred manually [11]. After equilibrium, time of self-emulsification, dispersibility, and appearance were observed and rated according to grading system **Table 2** [12].

Turbidity Measurement: Turbidity of the prepared dispersions was measured using Nephelo Turbidity Meter using 30 mL of the dispersion [13].

Robustness to Dilution: Robustness of Pomalidomide SNEDDS to dilution was studied by diluting 25 mg of SNEDDS with 50, 100, and 1000 mL of distilled water, 0.1N HCl, pH 4.5 acetate buffer, and pH 6.8 phosphate buffer. The diluted nanoemulsions were stored for 24 h and observed for any signs of phase separation or drug precipitation [13].

Percentage Drug Content: The percentage drug content was estimated according to the reported method [14].

Entrapment Efficiency: The entrapment efficiency was estimated based on reported method [15].

***In-vitro* Dissolution Studies:** *In-vitro* dissolution studies were performed on developed pomalidomide SNEDDS, Pomalidomide pure drug-using USP dissolution Apparatus II (Lab India DS 8000, Mumbai, India). Hard gelatin capsules, size "1" filled with pomalidomide SNEDDS formulation were introduced into 900 mL of freshly prepared aqueous hydrochloric acid 0.1 N, maintained at 37 ± 0.5 °C and the speed of the paddle was set at 50-100 rpm. Capsules were held to the bottom of the vessel using copper sinkers. 5 mL of samples were withdrawn at pre-determined time intervals using a syringe and immediately replaced with 5 mL of fresh medium maintained at 37 ± 0.5 °C (FDA recommended dissolution data was followed-accessdata.gov.in) [16]. The pomalidomide content in each dissolution sample was quantified spectrophotometrically at the wavelength of 251 nm as reported in the literature and compared with *in-vitro* dissolution profiles of pomalidomide pure drug. All measurements were done in triplicate [17].

Screening for A Precipitation Inhibitor: *In-vitro* precipitation experiments were used to estimate the apparent drug concentration-time profile and the duration of the super-saturated state. Polymers such as HPMCK4M, PVPK30, EudragitL 100, and Poloxamer 407 were employed tostabilize the supersaturated pomalidomide solution. A 100 mL aliquot of simulated gastric fluid (SGF) was maintained at 37 °C with the stirring speed held at 100 rpm. One gram of optimized pomalidomide SNEDDS formulation with various polymers was added into the medium. One milliliter samples of the solution were taken without volume replacement at 5, 15, 30, 45, 60, 90, 120, 180, and 240 min, and the aliquots were centrifuged at 3000 rpm for 3 min. The supernatant was diluted with methanol, and the concentration of pomalidomide was assayed by UV analysis at 251 nm 6.

Characterization of Optimised Pomalidomide SNEDDS Formulation:

Fourier Transform-Infrared Spectroscopy (FTIR): Excipient compatibility studies are conducted mainly to predict the potential incompatibility of the drug (API) in the final dosage form. These studies provide justification for the selection of excipients and concentrations in the formulation as required in regulatory filings. These studies are important in the drug development process, as the knowledge gained from excipients compatibility studies is used to select dosage form components, delineate the stability profile of the drug, and identify degradation products. Pomalidomide and excipients were analyzed by FT-IR spectrophotometer with data acquisition system OPUS [18].

Globule Size and Zeta Potential: The globule Size and zeta potential of optimized formulation was determined by a Zetasizer Nano ZS90 dynamic light scattering particle size analyzer (Malvern Instruments, Malvern, Worcestershire, UK) at a wavelength of 251 nm, a scattering angle of 90 °C and at 25 °C.

Surface Morphology (SEM Studies): Scanning electron microscopy studies (JEOL JEM 2100 F, USA) were carried out for optimized formulation by diluting the same with distilled water to 1000 times and then plunging on a 2% uranyl acetate solution stained carbon grid [19].

Forced Degradation and Accelerated Stability Studies: Pomalidomide is very susceptible to decomposition when it is exposed to oxygen, light, temperature, humidity, carbon dioxide, and acidic pharmaceutical excipients. Particularly amorphous pomalidomide is not only very unstable toward acid excipients but also very susceptible to

oxidative degradation. Pomalidomide decomposes very rapidly under an acidic environment to form atorvastatin lactone. Therefore it is an object of the present invention to study the pomalidomide stability of SNEDDS in the gastrointestinal (GI) environment and other environmental factors such as temperature, humidity, and light. Hence forced degradation studies and accelerated stability studies for optimized SNEDDS were conducted

Forced Degradation Studies: In order to know the stability of the prepared SNEDDS in the GI environment, forced degradation studies (gastric, intestinal, and neutral degradation) were conducted on optimized pomalidomide SNEDDS, pure drug. Pomalidomide is insoluble in aqueous solutions of pH below 4.0. In addition, pomalidomide is very slightly soluble in distilled water and pH 6.8 phosphate buffer and is slightly soluble in ethanol. As pomalidomide is freely soluble and stable in methanol, it was used as a co-solvent in all forced degradation studies [20]. All solutions for use in forced degradation studies were prepared by dissolving optimized SNEDDS and pure drug in small volume of methanol and diluted with the respective forced degradation medium *i.e.* with methanol or distilled water for neutral degradation, with simulated gastric fluid (aqueous 0.1 N HCl was adjusted to pH 1.2 with NaCl) without pepsin for gastric degradation and with simulated intestinal fluid (aqueous pH 6.8 phosphate buffer) without pancreatin for intestinal degradation to achieve a concentration of 100 g/mL of each solution. All solutions were stored at room temperature. At different time intervals (0, 4, 6, 12, and 24 h), aliquots of these solutions were diluted suitably to yield 10 g/mL concentrations and analyzed for the drug-using UV method. The percentage degradation of pomalidomide was calculated (ICH Harmonized Tripartite guideline on "Stability Testing of NewDrug Substances and Products Q1A (R2)" 6 February 2003) [21].

Accelerated Stability Studies: Optimised formulation was filled in hard gelatin capsules packed in HDPE screw-capped bottles and kept in humidity chambers maintained at 40 ± 2 °C/ 75 ± 5% RH per ICH guidelines for Zone III and stored for 6 months. Samples were evaluated for entrapment efficiency, drug content, and drug release (ICH Harmonized Tripartite guideline on "Stability Testing of New Drug Substances and Products Q1A (R2)", 6 February 2003).

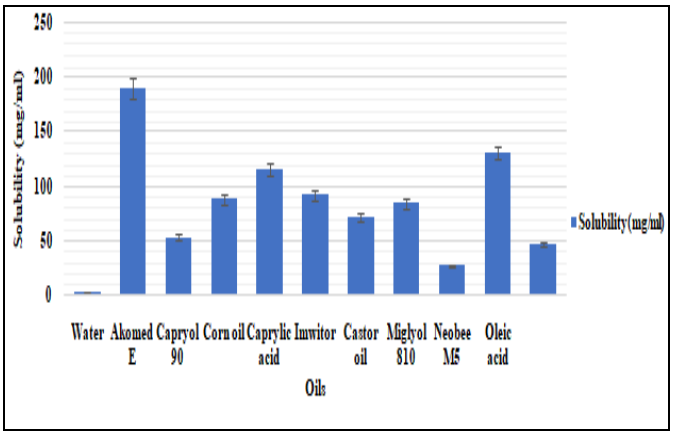

FIG. 1: SOLUBILITY OF POMALIDOMIDE IN VARIOUS OILS

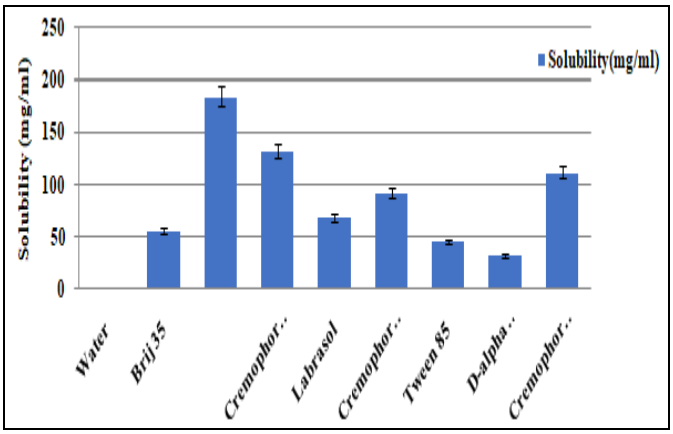

FIG. 2: SOLUBILITY OF POMALIDOMIDE IN VARIOUS SURFACTANTS

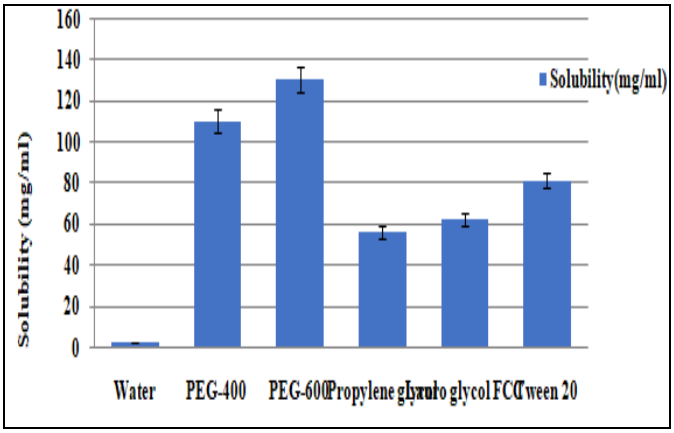

FIG. 3: SOLUBILITY OF POMALIDOMIDE IN VARIOUS CO-SURFACTANTS

RESULTS AND DISCUSSION:

Determination of Pomalidomide Solubility In Various Excipients: Akomed E oil was selected as oil phase due to its higher solubilization (190.54 ± 0.94 mg/ml) of pomalidomide compared to other oils **Fig. 1.** Surfactant Caprol PGE 860 and co-

surfactant PEG 600 were selected for further studies due to their higher solubilizing capacity towards Pomalidomide **Fig. 2, 3.**

Construction of Ternary Phase Diagrams: The region of nano emulsification was indicated as a shadow area encircled by a solid line and the points indicate the compositions of the system explored. Akomed E oil - Caprol PGE 860 - and PEG 600 system with Smix ratio in 3:1 exhibited larger nano emulsification region as compared to 1:1 and 2:1 Smix ratio **Fig. 4.**

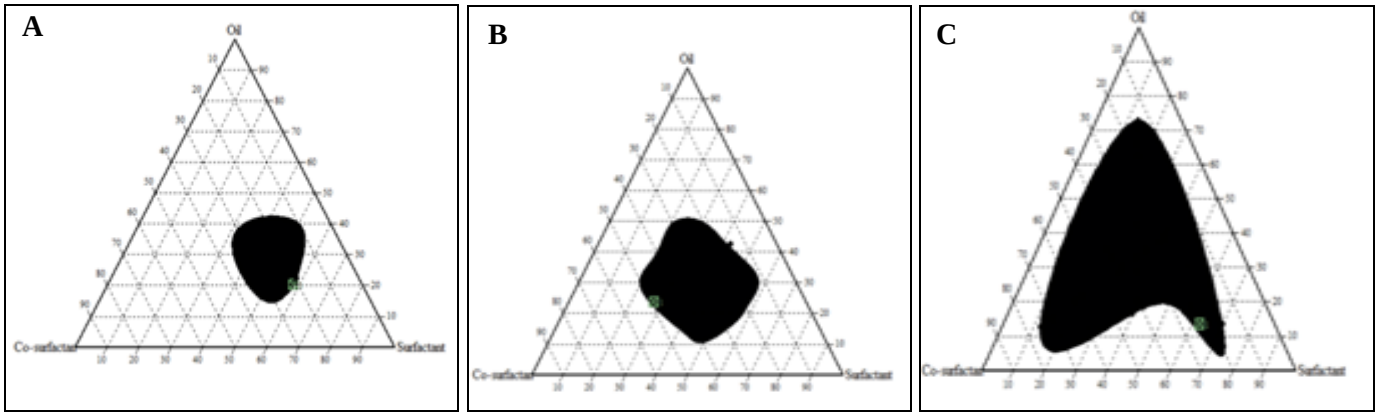

FIG. 4: TERNARY PHASE DIAGRAM FOR AKOMED E OIL - CAPROL PGE 860 - AND PEG 600 WITH SMIX IN 1:1 RATIO(A); SMIX IN 2:1 RATIO; SMIX IN 3:1 RATIO (KEY: THE FILLED REGION WITHIN THE TERNARY PHASE DIAGRAM INDICATES NANO EMULSIFICATION AREA WHERE THE TRANSMITTANCE IS GREATER THAN 90)

The mean globule size was decreased with an increase in surfactant concentration. Hence the systems containing Akomed E oil - Caprol PGE 860 - and PEG 600 with 3:1 Smix ratio were selected for further studies due to their larger nanoemulsifying area, greater capacity for incorporation of oily phase with uniformity of dispersion and high transmittance values.

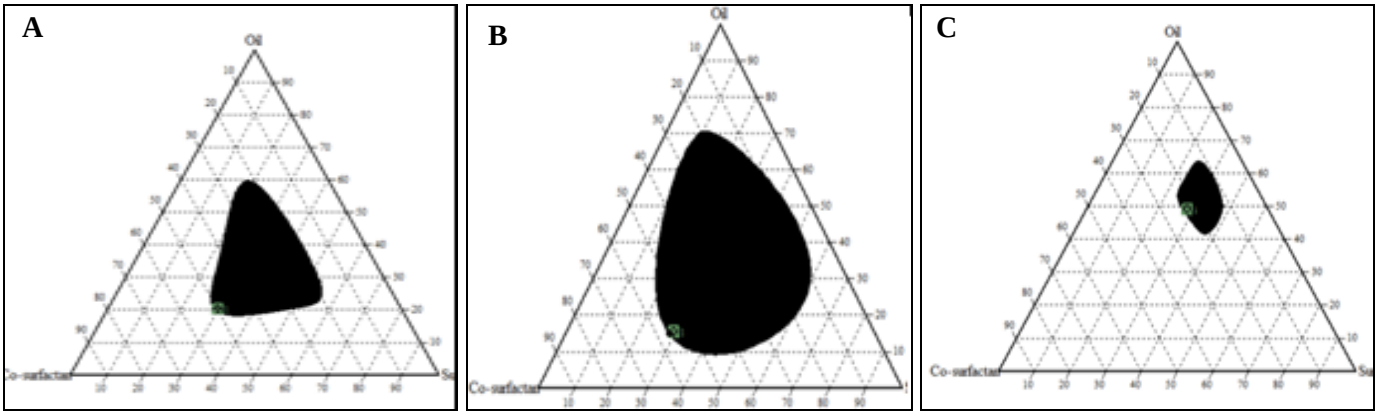

FIG. 5: TERNARY PHASE DIAGRAM FOR (A)2 MG OF POMALIDOMIDE;(B) 4 MG OF POMALIDOMIDE (C) 8MG OF POMALIDOMIDE LOADED AKOMED E OIL - CAPROL PGE 860 - AND PEG 600 SYSTEM WITH SMIX IN 3:1 RATIO (KEY: THE FILLED REGION WITHIN THE TERNARY PHASE DIAGRAM INDICATES NANOEMULSIFICATION AREA WHERE THE TRANSMITTANCE IS GREATER THAN 90)

Effect of Pomalidomide Loading: Incorporation of Pomalidomide (2 mg, 4 mg, and 8 mg) led to a considerable increase in transmittance values for 2 mg and 4 mg **Figure 8, 9** and **10**. But there was a decrease in the values for 8mg drug loading; this behaviour could be thought that undissolved drug in the compositions affected the clarity and thereby transmittance value to decrease with increased pomalidomide amount. Oil globules were observed on the surface after dispersion on standing for the majority of the compositions containing high pomalidomide. The area of nano emulsification was considerably reduced with an increase in pomalidomide loading from 4 to 8mg into the Akomed E oil -Caprol PGE 860 - and PEG 600system with 3:1 Smix ratio.Hence for the

stability reasons of the SNEDDS, a system containing 4 mg of pomalidomide was chosen **Fig. 5** for the formulation of pomalidomide SNEDDS and further studies.

Preparation and Evaluation of Pomalidomide SNEDDS: From the above results, it was found that Akomed *E. oil* concentration in the range of 30-82% w/w, Caprol PGE 860 in the range of 13-53% w/w, and PEG 600 in the range of 4-18% w/w in 3:1 of oil: Smix ratio with 4mg of loaded Pomalidomide drug produced the SNEDDS having the transmittance greater than 90, with good stability. A series of SNEDDS was prepared in the above-mentioned ranges of oil- surfactant-co-surfactant ratios and were evaluated for visual observations, turbidity measurements, robustness to dilution and *in-vitro* dissolution study.

Visual Observations: Visual observations indicated that at higher surfactant levels, the spontaneity of the self-emulsification process was increased. This may be due to excess penetration of water into the bulk oil causing massive interfacial disruption and ejection of droplets into the bulk of the aqueous phase. When a co-surfactant, PEG 600, was added to the system, it further lowered the interfacial tension between the o/w interfaces and also influenced the interfacial film curvature.

Turbidity Measurement: Turbidity values (NTU) have been reported to be of use in SNEDDS characterization.

TABLE 3: VISUAL OBSERVATION AND TURBIDITY MEASUREMENT VALUES

Formulation code	Visual Observation	Turbidity (NTU)
F1	A	17.65
F3	B	21.24
F4	A	18.32
F5	A	17.05
F6	B	21.85
F7	B	21.56
F8	B	20.02
F9	A	17.95
F10	A	17.42
F11	A	15.24
F12	B	20.64
F13	A	19.03
F14	A	18.08
F15	A	17.77

From these results, it can be generalized that the formulations that have low turbidity (<20) gave a transmittance value of more than 90, indicating rapid and spontaneous emulsification within 1min; hence it gives a good correlation between transmittance and turbidity values **Table 3.**

Robustness to Dilution: Nanoemulsions resulting from the dispersion of pomalidomide SNEDDS (F1-F15) with distilled water, 0.1N HCl, pH 4.5 acetate buffer, and pH 6.8 phosphate buffer were found to be robust to all dilutions, and no separation or drug precipitation was observed even after 24 h of storage.

Percentage Drug Content and Entrapment Efficiency: The drug content of all formulations ranged between 96.09 ± 1.37 to 99.52 ± 1.38 %, with maximum value exhibited by F11 **Table 4.** The entrapment efficiency of all formulations varies between 96.19 ± 1.48 to 99.85 ± 1.63 %, with the maximum value displayed by F [11].

***In-vitro* Dissolution Studies:** Comparative dissolution profiles of Pomalidomide pure drug and pomalidomide SNEDDS formulations (F1-F15) are shown in **Fig. 11.** Faster release rates were observed for pomalidomide SNEDDS than the pure drug. All Pomalidomide SNEDDS formulations (F1-F15) completed dissolution within 60 min, and all formulations released more than 95% of the drug, whereas pure drugs released 31.99 ± 0.72 %. Formulation F11 exhibited the highest drug release of 99.84 ± 0.69 % within 60 min. The release of the drug from SNEDDS formulation was increased proportionally with the increase in surfactant concentration and hence F11 exhibited high drug release from these results; it was evident that there was a significant improvement in the release of drug from the pomalidomide SNEDDS (01-15) compared to pure drug. This greater availability of dissolved pomalidomide from the SNEDDS could be due to their nano range globule size and the presence of surfactant/co-surfactants. This availability of the drug in the dissolution media may lead to higher absorption and oral bioavailability. Pomalidomide SNEDDS formulation F11 was selected as an optimized formulation due to the lower turbidity values, faster drug release values among the other SNEDDS.

***In-vitro* Evaluation of Precipitation:** In this study, the degree of supersaturation of the S-SNEDDS

was determined using HPMC E5LV, PVP K17, Maltodextrin, and Soluplusas precipitation inhibitors under non-sink conditions. The total volume of the selected medium was 100 mL, equivalent to the total volume of residual stomach fluid based on physiological considerations.

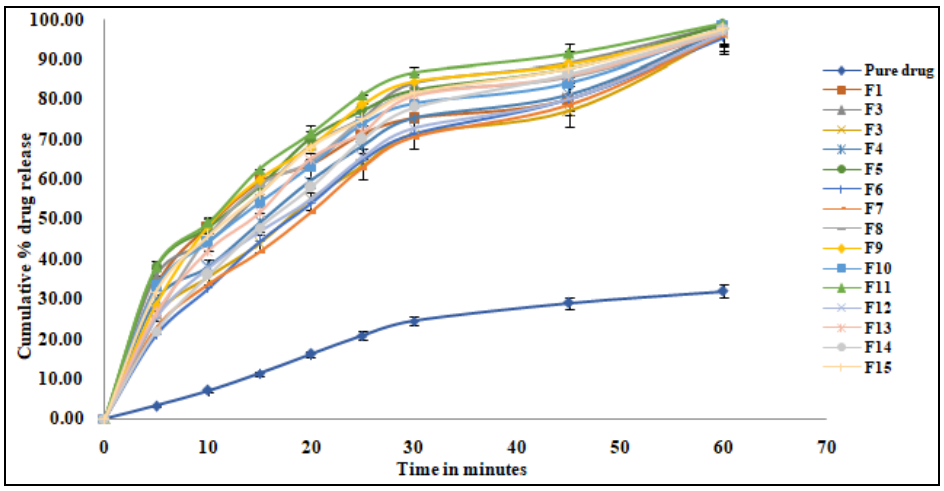

FIG. 6: COMPARATIVE DISSOLUTION PROFILE OF POMALIDOMIDE PURE DRUG AND POMALIDOMIDE SNEDDS FORMULATION (F1-F15)

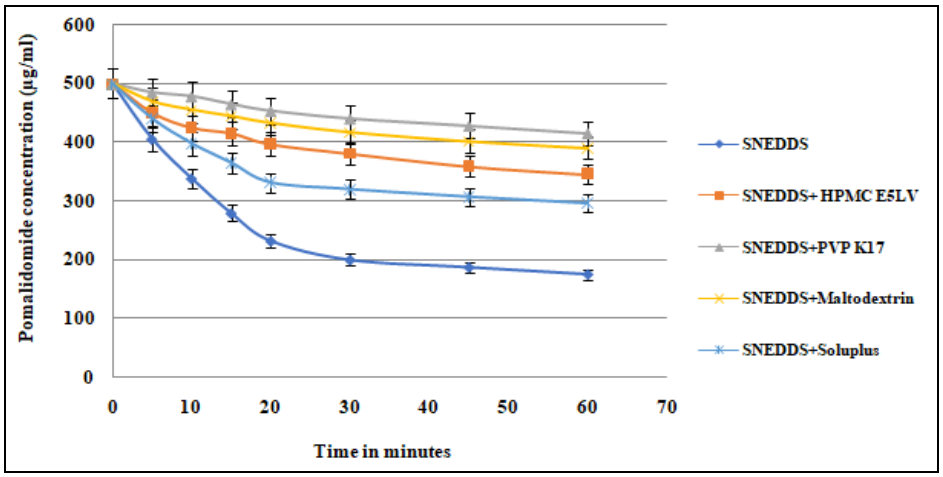

FIG. 7: *IN-VITRO* CONCENTRATION RELEASE PROFILES OF POMALIDOMIDE FROM SNEDDS FORMULATION WITHOUT PRECIPITATION INHIBITORS AND S-SNEDDS FORMULATION CONTAINING DIFFERENT PRECIPITATION INHIBITORS

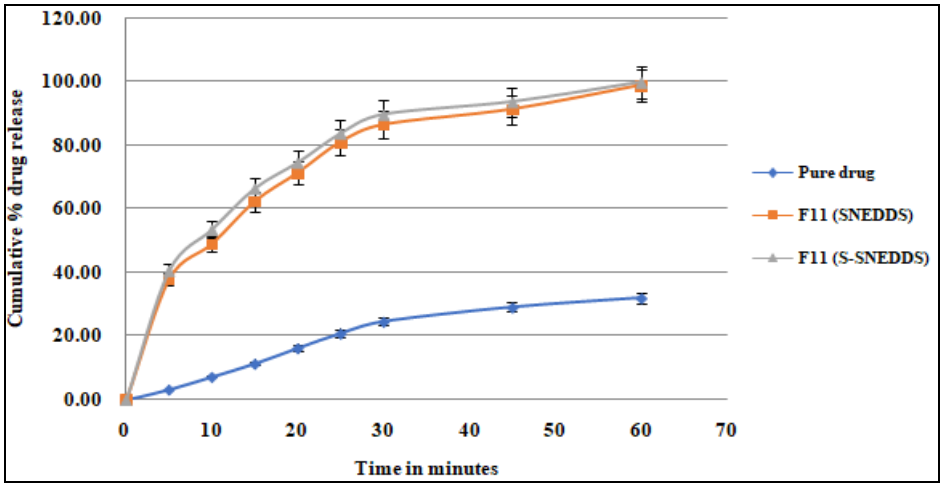

FIG. 8: COMPARATIVE DISSOLUTION PROFILES OF POMALIDOMIDE PURE DRUG, POMALIDOMIDE SNEDDS AND POMALIDOMIDE S-SNEDDS

In the medium, pomalidomide exists in one of three states, namely, as (1) free drug molecules (2) solubilized molecules partitioned into the nanoemulsion, and (3) precipitated solid particles. The distribution of pomalidomide among these states is dynamic and changed rapidly over time. The apparent concentration-time profiles of pomalidomide are described in. The amount of precipitation inhibitor in each formulation was 2% relative to the SNEDDS vehicle. As shown in **Fig. 8**, the precipitation profiles showed that the S-SNEDDS had better inhibition of pomalidomide precipitation than the SNEDDS (the same composition but without precipitation inhibitor) during the 60 min of the study. Upon mixing with the SGF, the SNEDDS formulation initially appeared as a nanoemulsion with a bluish reflection. After 30 min, solid precipitates of pomalidomide were observed, which suggested that the medium was in a supersaturated state.

For the SNEDDS formulation, at t = 20 min, the concentration of pomalidomide declined to about 232.28 µg/mL, and decreased rapidly to about 175.69 µg/mL after 60 min due to the precipitation. In contrast, the S-SNEDDS formulation showed consistently higher apparent pomalidomide concentration-time profile as compared to the SNEDDS formulation. The pomalidomide concentration in the S-SNEDDS formulation decreased rapidly when soluplus were applied as the precipitation inhibitors. The concentration declined to < 350 µg/mL within 30 min (320.45 µg/mL), indicating that soluplus was unable to sustain the apparent pomalidomide concentration. Although HPMC E5LV, PVP K17, Maltodextrin, and Soluplus could all effectively inhibit pomalidomide precipitation, PVP K17 performed better than HPMC E5LV and maltodextrin. Because the highest concentration of pomalidomide 415.52 µg/mL after 60 min was observed with PVP K17; for comparison, the concentrations achieved with HPMC E5LV and maltodextrin were 345.82 and 390.94 µg/mL, respectively.

In-vitro dissolution studies for S-SNEDDS of formulation F12 with 2% PVP K17 as precipitation inhibitor were studies. Comparative dissolution profiles of pomalidomide pure drug, pomalidomide SNEDDS, and pomalidomide S-SNEDDS is shown in **Fig. 9.** which indicates the release of drug from pomalidomide S-SNEDDS was highest with 99.98% at the end of 60 min.

Globule Size and Zeta Potential: The particle size for the optimized formulation of S-SNEDDS (F11) was found to be 49.0 nm with PDI 0.318. This might be due to the addition of precipitation inhibitor PVP K17 (in S-SNEDDS) which formed a physical barrier around oil droplets and prevented aggregation from giving a smaller sized nanoemulsion. S-SNEDDS was found to have comparably higher zeta potential than plain SNEDDS and thus was more stable. The negative value of zeta potential of -24.4 mV might be due to anionic groups of free fatty acids and glycol present in the oil, surfactant, and co-surfactant. This, in turn, implies having sufficient repulsion among emulsion droplets to give a kinetically stable emulsion system. The particle size and zeta potential were shown in **Fig. 9, 10.**

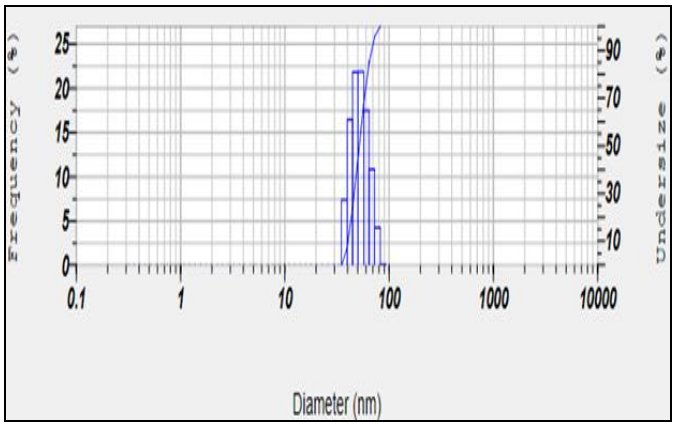

FIG. 9: PARTICLE SIZE OF OPTIMISED SNEDDS SNEDDS FORMULATION

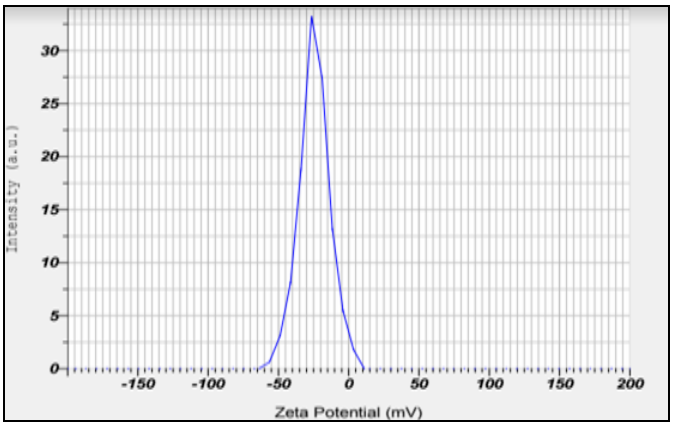

FIG. 10: ZETA POTENTIAL OF OPTIMISED OF POMALIDOMIDE FORMULATION OF POMALIDOMIDE (F11) FTIR STUDIES

The FTIR spectrum of pomalidomide displayed all characteristic peaks at 844.85, 956.72, and 1107cm$^{-1,}$ and the spectrum contained stretching vibrations of Pomalidomide C=O stretching vibration (1242.2 cm^{-1}), hydrocarbon stretching vibration of long fatty chain (2924.18 and 2924.18 cm^{-1}), and P–O stretching vibration (1107.18 cm^{-1}) one stretching vibration at 3392.9 cm^{-1} (Chen Z, Zhai J *et al.*). The presence of prominent characteristic peaks confirms pomalidomide's purity as per the established standards **Fig. 11, 12.**

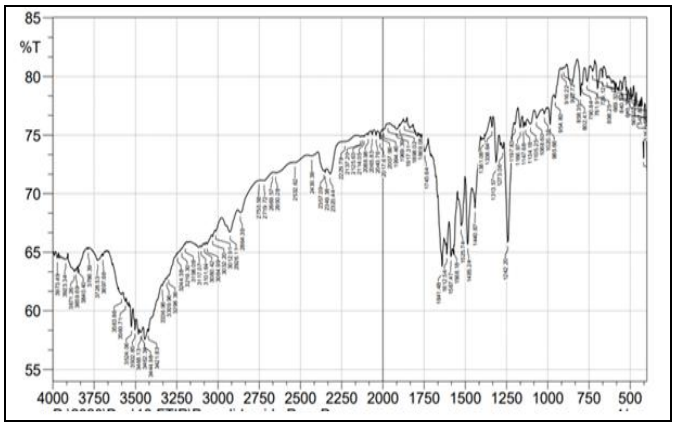

FIG. 11: FTIR SPECTRUM OF PURE DRUG POMALIDOMIDE

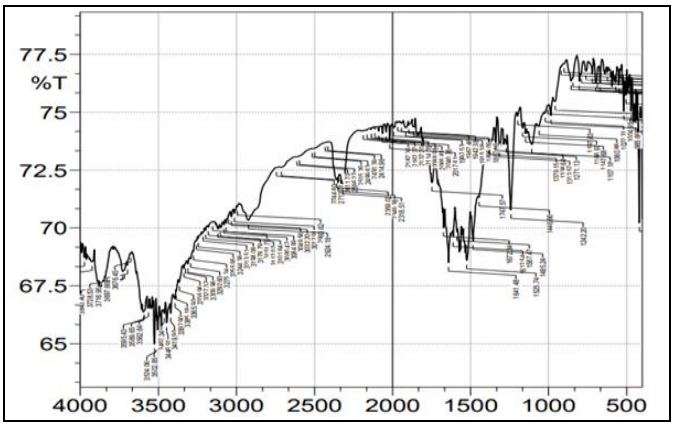

FIG. 12: FTIR OF OPTIMISED POMALIDOMIDES-SNEDDS (F14)

SEM Studies: Morphological and structural examination of the optimized batch F11 of Pomalidomide loaded SNEDDS was carried out using a transmission electron microscope. These results were in accordance to that of globule size analysis, and it was observed that the size of all droplets of SNEDDS F11 was less than 200 nm as furnished in **Fig. 13 A, B, C**. However, the shape of droplets was found to be spherical.

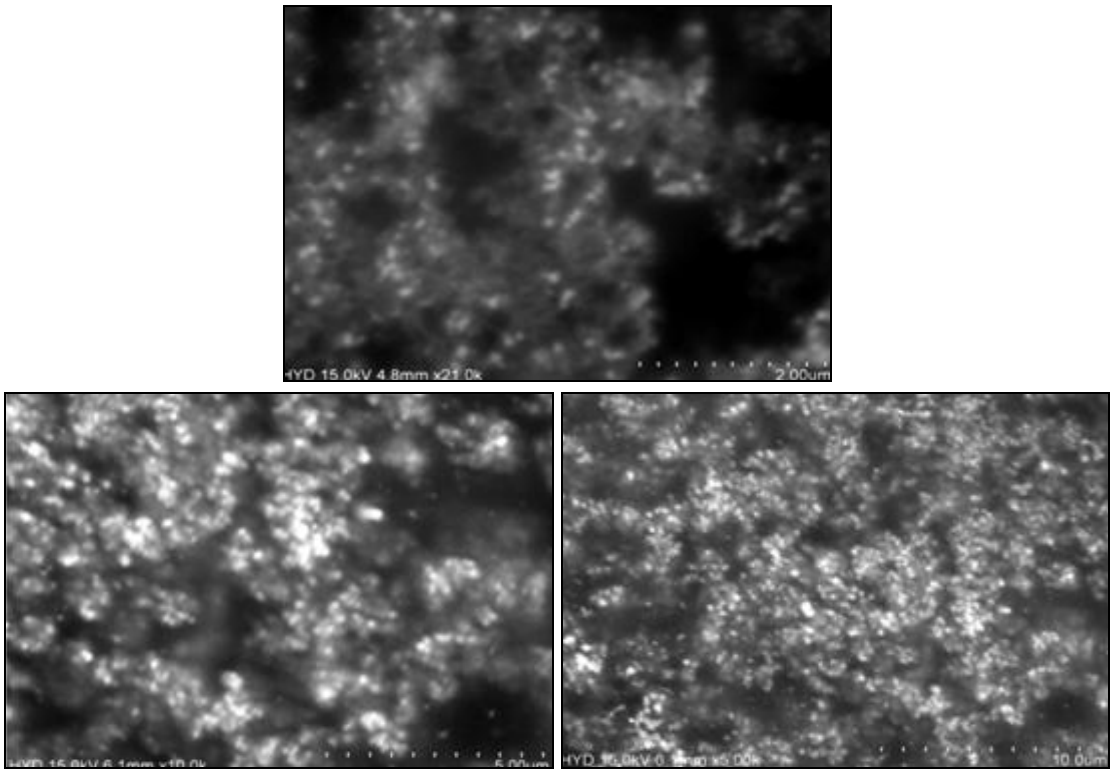

FIG. 13: SEM IMAGES OF OPTIMISED FORMULATION OF POMALIDOMIDE SNEDDS F11 (A, B& C)

Forced Degradation and Accelerated Stability Studies of Optimized SNEDDS: Forced degradation and accelerated stability studies were conducted in order to know the stability of

pomalidomide in the gastric environment and in SNEDDS formulation.

Forced Degradation Studies: Stability of pomalidomide from pomalidomide SNEDDS in the gastrointestinal environment was assessed by conducting forced degradation studies (acid, alkali, and neutral degradation) and compared with pure drug **Table 4.** Optimized pomalidomide SNEDDS, the pure drug showed no degradation even after 24 h storage in methanol, distilled water, and pH 6.8 phosphate buffer. Pure drug present in 0.1N HCl solution showed 26.48% degradation within 4 h, and the degradation was increased with time (70.05% degradation was found at 24thh). Pomalidomide showed very less decomposition (<1% degradation) for up to 4 hs and then decomposed with the time in 0.1N HCl solution. Pomalidomide showed 0.04, 14.64, 26.75, and 34.49% degradation in 4^{th}, 6^{th}, $12^{th,}$ and 24^{th} h, respectively.

The degradation of pomalidomide from optimized pomalidomide SNEDDS formulation was significantly less when compared to the pure drug degradation.

TABLE 4: PERCENT DEGRADATION OF POMALIDOMIDE FROM PURE DRUG AND OPTIMIZED POMALIDOMIDE SNEDDS IN FORCED DEGRADATION STUDY

Formulation code	Time (hr) / Diluting Solvent	%Drug Degraded (%, mean ± SD, n=3)				
		0 H	4^{th}H	6^{th}H	12^{th}H	24^{th}H
Pure drug	Methanol	0.01 ± 0.46	0.02 ± 1.06	0.01 ± 0.58	0.11 ± 0.37	0.1 ± 1.38
	Water	0.01 ± 1.86	0.01 ± 2.47	0.02 ± 0.84	0.02 ± 1.83	0.03 ± 1.38
	0.1 N HCl	0.08 ± 1.48	26.48 ± 1.64	28.67 ± 0.63	44.91 ± 1.74	70.05 ± 1.65
	pH 6.8 phosphate Buffer	0.03 ± 0.53	0.03 ± 1.48	0.04 ± 1.84	0.05 ± 1.37	0.06 ± 1.79
F11 S-SNEDDS	Methanol	0.03 ± 1.97	0.14± 0.74	0.17 ± 1.48	0.02± 1.59	0.01 ± 1.36
	Water	0.02 ± 2.49	0.25 ± 1.85	0.03 ± 2.03	0.03 ± 1.48	0.08 ± 1.38
	0.1 N HCl	0.02 ± 1.74	0.04 ± 1.48	14.64 ±2.38	26.75 ± 2.83	34.49 ± 1.72
	pH 6.8 phosphate Buffer	0.02 ± 1.37	0.05 ± 0.29	0.06 ± 1.27	0.09 ± 1.02	

Above parameters are communicated as Average ± Standard Deviation; (n=3)

From these results it can be concluded that the pH dependant degradation of pomalidomide could be minimized to some extent by formulating the drug into SNEDDS. Moreover, under normal physiological conditions, gastric emptying occurs from the stomach within 4 h. Hence, SNEDDS may be considered a suitable formulation approach for improving the therapeutic efficiency of pomalidomide.

Accelerated Stability Studies: Stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light and enables recommended storage conditions. No visible physical changes were observed in the F11 super saturable SNEDDS formulation (F11 S-SNEDDS) withdrawn from the humidity chambers. The samples were assayed for %entrapment efficiency, % drug content, and in-vitro drug release, and the results are shown in **Table 5.** No significant difference was observed after storage at accelerated conditions at 40 ± 2 °C / 75 ± 5% RH for a period of six months.

TABLE 5: STORAGE AT 40 ±2 °C / 75 ± 5% RH FOR 6 MONTHS

Retest time for optimized formulation F4	%Drug content	% Entrapment efficiency	*In-vitro* drug release (%)
0 days	99.52±1.38	99.85±1.63	99.98±0.72
30 days	99.25±0.53	99.64±0.68	99.67±0.37
60 days	98.93±0.46	99.42±0.38	99.43±0.28
90 days	98.82±0.74	99.18±0.94	98.95±0.64
180 days	98.69±0.65	98.78±0.94	98.45±0.14

Above parameters are communicated as Average ± Standard Deviation; (n=3)

CONCLUSION: The pomalidomide SNEDDS formulation was prepared using Akomed E oil - Caprol PGE 860 - and PEG 600 aided by monophasic zone in the pseudo tertiary phase diagram. The 15 developed formulations were subjected to thermodynamical physical stability

studies, drug content, percentage entrapment efficiency, and drug dissolution analysis. The formulation F11 was chosen optimal with maximum drug content of 99.52 % with no phase separation during the thermodynamic stability study. The in vitro drug dissolution of 99.04% is indicative of reduced particle size and enhanced solubilizing capacity of F11 than pure drugs. By adding PVP K17 as a precipitation inhibitor to conventional SNEDDS, a super saturable system was prepared.

Firstly, the prepared SNEDDS played an important role in increasing the aqueous solubility and hence oral absorption due to nano-range size. Secondly, the S-SNEDDS was found to be advantageous over SNEDDS for having a higher drug load and inhibition of dilution precipitation of pomalidomide. Formulated S-SNEDDS (F11) showed drug release of 99.98%. The FT-IR data assured the retention of the major peaks of pomalidomide in the F11, forecasting no interaction.

The particle size, PDI, and zeta potential of the optimized irinotecan formulation F11 S-SNEDDS were 49.0 nm, 0.318, and -24.4 mV. The SEM studied of F11 indicated the narrow uniform distribution of the drug in the formulation.

The stability studies carried out for 6 months indicate no significant variation in the optimized formulation drug release and drug content. Hence, these investigations advocated the suitability of super saturable-SNEDDS loaded pomalidomide in the enhancement of solubility and drug release.

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CONFLICT OF INTEREST: NIL

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