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Research Article

Solubility Enhancement of Poorly Water-Soluble Drug Aprepitant for Oral Delivery by Self-Micro Emulsifying Drug Delivery System

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Abstract

The objective of the present study was to develop self microemulsifying drug delivery system (SMEDDS) for improving the delivery of a BCS class IV antiemetic drug, aprepitant (APT). The *in-vitro* self-emulsification properties, droplet size analysis, drug content, polydispersity index, zeta potential etc. of these formulations upon their addition to water under mild agitation conditions were studied. The solubility of APT was found to be high in Capryol90 (12.53 ± 0.35); Labrasol (13.30 ± 0.23); Transcutol HP (49.15 ± 0.28) mg/ml. The range of mean droplet size was 13.97 ± 0.72 nm to 124.90 ± 0.20 nm while the polydispersibility values were in the range of 0.169 to 0.604 with a zeta potential of -41.1 mV. More than 90% of the drug released within 30 minutes when compared with pure drug release of 13% in 30 minutes. The SMEDDS formulations followed first order kinetics. The APT formulations were proved to be robust, stable to pH, and thermodynamically stable and formed clear transparent micro emulsions in few seconds. Thus, the study confirmed that the Aprepitant SMEDDS formulation can be used as a possible alternative to traditional oral formulations to improve its bioavailability.

Keywords: Aprepitant; SMEDDS; Ternary Phase Diagram; Micro Emulsion; Solubility

Abbreviations

API: Active Pharmaceutical Ingredients; APT: Aprepitant; BCS: Biopharmaceutic Classification System; LOD: Limit of Detection; LOQ: Limit of Quantification; SEDDS: Self-Emulsifying Drug Delivery Systems; SGF: Simulated Gastric Fluid; SIF: Simulated Intestinal Fluid; SMEDDS: Self Microemulsifying Drug Delivery System; o/w: Oil-in-Water; PDI: Polydispersibility Index; TEM: Transmission Electron Microscope; ZP: Zeta Potential

Introduction

The oral delivery is considered as the major route of drug administration for numerous diseases. However, various potent lipophilic drugs exhibit low oral bioavailability due to their poor aqueous solubility. Approximately 40% of active substances emerging from drug delivery candidates are poorly water soluble, presenting the pharmaceutical scientist with several problems when developing formulations for such active pharmaceutical ingredients (API) [1,2]. The formulation skill plays an important role in overcoming this shortcoming of poorly water-soluble drugs. In this regard, various formulation strategies were exploited, including the use of surfactants, lipids, permeation enhancers, micronization, salt formation, cyclodextrins, nanoparticles and solid dispersions [2-6]. In recent years, much attention has been paid to lipid based formulations with particular emphasis on self-emulsifying drug delivery systems (SEDDS) to improve the oral bioavailability of lipophilic drugs [7,8].

Aprepitant (APT) is an antiemetic drug that blocks the neurokinin 1 receptor. It is used in preventing highly emetogenic chemotherapy induced nausea and emesis [9-11]. It is a white to off-white crystalline solid which is a non-polar substance. It is relatively lipophilic (log P at pH 7 is 4.8). According to Biopharmaceutic Classification System (BCS), aprepitant can be categorized into BCS class IV drug being neither "highly soluble" nor "highly permeable" [12]. It has very limited solubility in water but has reasonably high solubility in non-polar liquids such as oils. It is sparingly soluble in ethanol and isopropyl acetate and is slightly soluble in acetonitrile.

Aprepitant has been reported to have an aqueous solubility of 3-7 µg/ml [13]. The compound exhibits moderate permeability in the Caco-2 model (7.85 I x I10^{-6} cm/s) [13]. Based on these values it was concluded that low oral bioavailability of aprepitant can be attributed to its poor dissolution [14]. The delivery of aprepitant is fraught with inter-patient variability when delivered as a tablet formulation. The poor solubility of aprepitant in aqueous media and poor permeability characteristics pose a tremendous challenge to the pharmaceutical formulation scientists in its delivery in adequate concentrations into the systemic circulation [15]. The oral bioavailability of aprepitant is limited by poor dissolution of the compound in the gastrointestinal tract which is more prominent in the fasted state. There is a significant positive food effect on the solubility of aprepitant. Even though the oral bioavailability of this drug is around 65%, there is a high inter subject variability

in the plasma profile when the drug is administered orally. Product development of aprepitant has been focused on self-micro emulsifying drug delivery systems as it known that SMEDDS act as super solvents to drugs and enhance their solubility [16].

Objectives of the Study

The objective of the present study was to develop and evaluate self microemulsifying drug delivery system (SMEDDS) for improving the solubility of poorly water soluble antiemetic drug, aprepitant (APT) for oral administration. Self-emulsifying drug delivery systems (SEDDS) are isotropic mixtures of natural or synthetic oils, solid or liquid surfactants, or alternatively, one or more hydrophilic solvents and co-solvents/surfactants (Gursoy and Benita, 2004). Upon mild agitation followed by dilution in aqueous media, such as GI fluids, these systems can form fine oil-in-water (o/w) emulsions or micro emulsions (SMEDDS). SEDDS typically produce emulsions with a droplet size between 100 and 300 nm, while SMEDDS form transparent micro emulsions with a droplet size of less than 50 nm [3]. Many researchers have reported using SMEDDS as a drug delivery system for improving the drug solubility, enhancing the dissolution rate and improving the bio-availability of poorly water soluble drugs like ritonavir [14], saquinavir [14]; cyclosporine A [17]; celecoxib [18]; carvedilol [19]; atorvastatin [20]; fenofibrate [21]; griseofulvin [22]; oridonin [23]; exemestane [24]; itraconazole and bicalutamide [24]. They have shown that SMEDDS proved to be an efficient and good delivery system for improving the bioavailability of drugs.

Materials and Methods

Materials

Aprepitant, was purchased from HaiHang Industry, China. Propylene Glycol Monocaprylate (Capryol®90), Caprylocaproylmacrogol-8 glycerides (Labrasol®), Diethyleneglycol monoethylether (Transcutol®HP), Oleoylmacrogol- 6 glycerides (Labrafil® M1944 CS), Propylene glycol dicaprylocaprate (Labrafac® PG), Glycerolmono-oleate 40 (PeceolTM) were purchased from Gattefosse (USA). All other chemicals and reagents used were of analytical grade.

Analytical Method

The HPLC system comprised of an Agilent pump (Model G1312A, Agilent technologies, (1200 series) fitted with 20 µl sample loop, a UV-visible detector (Agilent technologies). A reverse phase Merck C_{18} (5µm, 150 x 4.6 mm ID) column (Merck, Germany) fitted with a LICHROCART PUROSPHER STAR guard column packed with replaceable RP-18 E 4-4 guard cartridge (5 µm) Merck, Germany was used. Data acquisition was undertaken using Agilent 1200 series software. The mobile phase consisted of phosphate buffer and acetonitrile (40:60% v/v) for aprepitant. The mobile phase was isocratically pumped at a flow rate of 1.6 ml/min, detected at a wavelength of 210 nm with an injection volume of 20 µl. The quantities were determined using peak height measurements.

Solubility studies of aprepitant in various vehicles

The solubility of aprepitant, was studied in various oils, surfactants, and co-surfactants i.e. Capryol®90, Labrafil®M1944CS, Labrasol®, PEG-300, PEG - 600, Tween 20, Transcutol®HP, Cremophor®EL, Tween20 and Tween80 to find out the solubilizing capacity for the drug. An excess amount of aprepitant was added to 1 ml of each selected oil, surfactant and co-surfactant mixture in 2 ml Eppendorf tubes. The mixture was vortexed for two minutes using Stuart vortex mixer SA8, and then shaken in an oscillating mechanical shaker bath (Certomat®H Model S11, Sartorius stedim) at 200 oscillations/minute maintained at 25°C ± 1°C for 72 hours respectively. The Eppendorf tubes were examined periodically for the drug solubility. If any additional drug was needed it was added and recorded. The supernatant solution was poured into separate Eppendorf tubes and centrifuged at 4,000 rpm for 15 minutes using Mini Spin plus Centrifuge, (Eppendorf mini spin plus). The supernatant was filtered and the concentration was quantified using HPLC after dilution with methanol (n = 3).

Construction of pseudo-ternary phase diagrams

The pseudo-ternary phase diagram was constructed using a water titration method [25]. Mixtures comprising 1 ml of oil-capryol 90, surfactant - cremophor EL and co-surfactant - transcutol HP were prepared for aprepitant. The surfactants and co-surfactants were mixed in different ratios of 1:1, 2:1 and 3:1 but constant ratio of 2:1 was maintained between the co-surfactants.

The above surfactant mixtures (Surfactant + Co-surfactant) were mixed at three ratios 1:1, 2:1 and 3:1% v/v. For each ratio, the oil (capryol 90) was mixed with surfactant mixtures at ratios of 9:1, 8.5:1.5, 8:2, 7.5:2.5, 7:3, 6.5:3.5, 6:4, 5.5:4.5, 5:5, 4.5:5.5, 4:6, 3.5:6.5, 3:7, 2.5:7.5, 2:8, 1.5:8.5, 1:9, 0.5:9.5, and 0:10, in 10 ml glass test tubes. Water was added in small increments at 5% v/v (0.5 ml) of the total mixture in each glass tube. The water addition was continued until the clear mixture in each glass tube became turbid and the amount of water added was noted, as this was the beginning of phase inversion area. On further addition of water, the turbid mixture turned clear (starting of o/w micro-emulsion area), and finally turbid once again with the continuous further addition of water (end of micro-emulsion area) [26]. For each addition of water, the mixture in glass tube was vortexed for 2 minutes, and placed in a vortex mixer (Stuart model SA8) maintained at 25°C for two hours at 100 oscillations/minute. The resultant mixture was evaluated visually for phase clarity. The micro-emulsion area was calculated from the graph (n = 3).

Determination of drug solubility in the compositions of microemulsions

The eleven different compositions of microemulsions were selected from the micro-emulsion area of capryol 90: cremophor EL and transcutol HP (3:1) for the drug aprepitant where their compositions are shown in tables 1a. All the self-micro emulsifying drug delivery system (SMEDDS) were prepared by initially dispersing the drug in capryol 90, cremophor EL, and transcutol HP. The final mixture was vortexed using a vortex mixer (Stuart model SA8) until a clear solution was formed. These mixtures were observed for signs of turbidity or phase separation for a period of 48 hours.

Formulation Code	Oil Capryol 90 (% v/v)	Surfactant Cremophor EL (% v/v)	Co-surfactant Transcutol HP (% v/v)	Amount of pure drug aprepitant solubilized (mg/ml)
APT1	11.00	66.75	22.25	16.71
APT2	8.00	69.00	23.00	26.38
APT3	7.00	69.75	23.25	27.98
APT4	15.00	63.75	21.25	24.25
APT5	25.00	56.25	18.75	27.88
APT6	29.00	53.25	17.75	29.92
APT7	17.00	62.25	20.75	30.65
APT8	26.00	55.50	18.50	25.58
APT9	33.00	50.25	16.75	31.31
APT10	42.00	43.50	14.50	31.07
APT11	20.00	60.00	20.00	35.62

Table 1a: Composition details of SMEDDS formulations containing aprepitant.

In-vitro characterization of SMEDDS formulations containing drug

Drug content

Each batch of the prepared SMEDDS was tested for drug content. One mL of formulated self-micro emulsifying drug delivery system was taken and dispersed in ten mL of methanol. This was vortex mixed for 30 minutes. The vortexed sample was taken and diluted further with the mobile phase (buffer:acetonitrile :: 40:60% v/v). This solution was filtered through a membrane filter (Microporous Nylon 66) of pore size 0.45 µm and diameter of 47 mm. The drug content was determined using HPLC at 210 nm. The amount of drug present in the formulation was determined using the prepared standard calibration curve of the plain drug in methanol.

Droplet diameter measurement

Samples were loaded into a cuvette in a thermostatic chamber (n = 3). The formulations prepared with each drug were studied for the droplet size measurement [27,28]. The droplet diameter was measured at zero time and at the end of 24 hours. Monitoring the droplet size changes over a period of 24 hours was considered as adequate, as the drug usually stays in the gastrointestinal tract for no longer than 24 hours.

Transmission Electron Microscope (TEM)

From the results of thermodynamic stability studies the formulation APT7 was selected for the morphological characterization using transmission electron microscope (TEM). TEM of Hitachi 7500, Japan was used as a visualizing aid. Samples of SMEDDS APT7 (5-10 µl) were dropped onto Formvar-coated copper grids. After complete drying, the samples were stained using 2% w/v phosphotungstic acid. Digital Micrograph and soft Imaging Viewer software was used to perform the image capture and analysis, including particles sizing [2,29-32].

Effect of dilution

The study was conducted to evaluate the stability of SMEDDS formulations upon dilution. The volume of the stomach in the fasted stated varies from 10 - 100 ml and 500 - 1000 ml in the fed state. The formulations were diluted 10, 100, and 1000 times with distilled water, to mimic the process of dilution in the gastrointestinal tract after oral administration and the droplet diameter was determined.

Effect of pH in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) media

Study in SGF

The SMEDDS formulations were diluted 100 times with SGF (without enzymes), according to the method suggested by United States Pharmacopoeia. SGF was prepared by dissolving 2 gm of sodium chloride in 7 ml of 1N HCL and was diluted to 1000 ml with distilled water) [33]. From each of the eleven formulations, 0.1 ml of the solution was drawn and diluted to 10 ml with SGF. The physical appearance of the mixtures was recorded and the droplet size was measured using Malvern zetasizer (Model: Nano-ZS, Malvern, UK).

Study in SIF

The SMEDDS formulations were diluted 100 times with SIF (without enzymes), according to the method suggested by United States Pharmacopoeia [33]. SIF was prepared by dissolving 6.8 gm of monobasic potassium phosphate in 250 ml of water, mixed and finally pH was adjusted to 7.5 with 0.2 N sodium hydroxide [33]. From each of the eleven formulations 0.1 ml of the solution was drawn and was diluted to 10 ml with SIF. The physical appearance of the mixtures for each of the test sample was recorded and the droplet size was measured using Malvern zetasizer.

Thermodynamic stability studies

The formulations were subjected to different thermodynamic stability studies by using heating-cooling cycle and centrifugation stress tests. Those formulations which passed the freeze thaw cycle were subjected to centrifugation by rotating the samples at a speed of 4200 rpm for 30 minutes. The formulations were observed for phase separation in the mixtures.

Evaluation of emulsification time

The efficiency of self-emulsification of oral SMEDDS was assessed using a standard USP type II dissolution apparatus. A dissolution apparatus (Electrolab, TDT08 plus dissolution test apparatus, USA) was employed with 900 ml of distilled water, with a paddle speed of 50 rpm and the dissolution medium was maintained at 37ºC 0.5°C. The SMEDDS formulation of 1 ml was delivered via syringe at 1 cm below the surface of dissolution medium. The emulsification time was monitored by visual inspection and the experiment was carried out in triplicate.

In-vitro dissolution studies

The *in-vitro* drug dissolution study was performed by using dissolution test apparatus-paddle II USP (Electrolab, TDT08 plus dissolution test apparatus, USA) [19,24,28] at 37 ± 0.5ºC using 900 ml of various dissolution media viz., water, SGF and SIF with stirring speed of 50 rpm. At predetermined time intervals of 5, 10, 15, 20 and 30 minutes, a test solution of 5 ml was withdrawn and substituted with the same volume of dissolution medium. The solutions were filtered through 0.45 µm cellulose membrane filter (Whatman, USA) and suitably diluted with respective dissolution medium. The samples were taken in triplicate and were analyzed employing HPLC after suitable dilution with methanol at 210 nm. Release behavior of micro emulsions was compared with that of plain aprepitant suspension.

Stability studies

Optimized SMEDDS formulation APT7 was subjected to 40°C/75% RH for 30, 90 and 180 days of storage. The formulations were kept in a desiccator containing saturated calcium chloride at 75% RH and the desiccator was placed in an oven (Memmert, Germany) maintained at 40°C. The samples were taken at preset time intervals over a period of 6 months and the drug concentration in the sample was analyzed using the HPLC.

Stability studies after 24 hr in water

A sample of 0.1 ml was withdrawn from each of the formulations APT1 to APT11 and diluted to 10 ml with pure water. These samples were kept at room temperature for about 24 hours. After 24 hours of stay at room temperature, samples were analyzed for droplet size, zeta potential and PDI by using Malvern zetasizer instrument.

Stability studies after 2hr and 8 hrs in SGF and SIF

This test was performed after 2hr and 8hr in SGF to see the nature of droplet size during the transit time in the stomach and intestine, respectively. A sample of 0.1 ml of the formulation was withdrawn from each of the formulations APT1 to APT11. The samples were diluted to 10 ml with SGF and were thoroughly mixed. These samples were centrifuged at 14,000 rpm for 20 minutes. A volume of 1 ml was collected from the supernatant solution and its droplet size, zeta potential and PDI was determined.

Accelerated stability studies

Accelerated stability studies were carried out for optimized formulations. The optimized SMEDDS formulation APT7 was kept at a temperature of 40°C ± 1°C and 70% RH and studied for six months. The droplet size and dissolution profiles were studied at time points of one month, three months and six months.

Kinetic studies

Kinetic studies of correlation coefficient and first order release kinetics were carried out for the optimized formulations of APT1, APT7, APT8, APT11 and pure drug.

Results and Discussion

Aprepitant is a relatively lipophilic drug which has a log P value of 4.8 at pH 7.0 and possesses very low aqueous solubility of 3 - 7 µg/ml in the pH range of 2 - 10 [13,16,34]. The compound aprepitant, when administered unformulated has limited oral bioavailability in the fasted state and exhibits marked positive food effect [13]. The delivery of aprepitant is fraught with inter-patient variability when delivered as a tablet formulation. A nano-particulate capsule-based composition may serve to overcome this problem. The poor solubility of aprepitant in aqueous media and poor permeability characteristics pose a tremendous challenge to the pharmaceutical formulation scientists in its delivery in adequate concentrations into the systemic circulation [15]. The oral bioavailability of aprepitant is limited by poor dissolution of the compound in the gastrointestinal tract which is more prominent in the fasted state. There is a significant positive food effect on the solubility of aprepitant. Product development of aprepitant has been focused on self-micro emulsifying drug delivery systems as it known that SMEDDS act as super solvents to drugs and enhance their solubility [16]. The aprepitant formulations were subjected to various *in-vitro* formulation studies to see the significance of SMEDDS for efficient oral drug administration.

Analytical method

The HPLC method employed for the estimation of aprepitant was validated by determining its linearity, accuracy, precision, specificity, limit of detection (LOD), and limit of quantification (LOQ). The method was found to have a high selectivity for the analyte; since no interfering peaks from the compounds were observed at the retention time for the drug in any of the six independent blank samples. The regression equation between aprepitant concentration (µg/ml) and its peak height was found to be y = 4.025x- 0.2091. The figure 1a and 1b shows the chromatogram of the drug aprepitant with a retention time of 3.539 minutes with a regression value of R^2 = 0.9992. Hence, this HPLC method is highly reproducible, precise and highly accurate. It is also having selectivity, specificity and linearity.

Solubility studies of aprepitant in various vehicles

The drug aprepitant has very low solubility in oils, surfactants and in cosurfactants, though it has a high low P value of 4.8, so aprepitant may be considered as a drug which is poorly soluble in both lipid and water. However, as the dose of the drug is small, its formulation into SMEDDSs would be a good approach. Among the various oils, screened, Capryol 90 showed the highest solubility potential for aprepitant (12.53 ± 0.35 µg/ml) compared to Labrafil M1944 (4.00 ± 0.09 µg/ml), Labrafac (2.34 ± 0.21 µg/ml) and Olive oil (5.13 ± 0.02 µg/ml). The solubility in Capryol 90 was significantly higher ($p < 0.02$) when compared to solubility in other oils. Among the various surfactants screened, such as Cremophor EL, Tween 20, Tween 40, Tween 80, Span 20 and Labrasol, highest solubility was seen in Cremophor EL. The solubility in Cremophor EL was significantly higher than the solubility in Tween 40 and Span 20 ($p < 0.01$).

The drug aprepitant showed maximum solubility in Transcutol HP (49.15 ± 0.28 µg/ml). This value was significantly higher ($p < 0.05$) than the values of solubility in other cosurfactants. The solubility in various vehicles is shown in figure 1c.

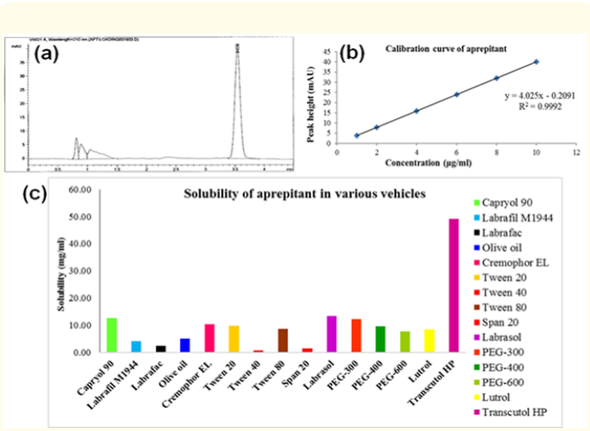

Figure 1: (a and b) Standard graph of Aprepitant; (c) solubility of aprepitant in various components.

Construction of pseudo-ternary phase diagrams

SMEDDS form a fine oil-water emulsion with only gentle agitation, upon their introduction into the aqueous media. The surfactants and cosurfactants used in the formulations initially adsorbed at the interface, reducing the interfacial energy and providing a mechanical barrier to coalescence. Initially ternary phase diagrams were constructed by using various vehicles in which the solubility of the drug aprepitant was highest. Then the mixtures were observed for stability. Lack of stability resulted in phase separation. In this regard, various combinations of vehicles like oil - Capryol 90, Smix (Labrasol and Transcutol HP) were prepared in different ratios of 1:1% v/v, 2:1% v/v and 3:1% v/v. These mixtures resulted in good micro emulsion regions, but after observation for 24hours, they formed phase separation and found to be unstable. The phase separation might have resulted due to low emulsification of the surfactant, Labrasol, so a highly viscous surfactant like Cremophor EL was chosen in place of Labrasol. The solubility of aprepitant was found to be next highest in this surfactant. Trials were then run with 1:1%v/v composition containing oil - Capryol 90, Smix (Cremophor EL + Transcutol HP), (surfactant and cosurfactants mixture) was observed for 24 hours for homogeneity and stability. Phase separation was observed as a sign of instability.

Various combinations like [Oil (0.7% v/v) + Smix (0.15% v/v)]; [Oil (0.55% v/v) + Smix (0.225% v/v)]; [Oil (0.1% v/v) + Smix (0.45% v/v)]; [Oil (0.9% v/v) + Smix (0.05% v/v)] were run to check for micro emulsion regions. Similarly, the above compositions were repeated with a change in Smix containing (PEG 300 + Transcutol HP) keeping the oil proportion same. The Smix containing (PEG 300 + Transcutol HP) formed a hazy and turbid mixture but on observation for 24 hours, it resulted in phase separation. However, Smix containing Cremophor EL + Transcutol HP, formed a light pale yellow colored homogeneous mixture and there was no phase separation when observed for 24 hours. This combination was found to be fruitful and further studies were carried out for water titration with all the combinations of 1:1, 2:1, 3:1% v/v. This Smix combination containing Cremophor EL and Transcutol HP was found to be giving formulations that were homogeneous and stable without any phase separation even after 24 hr. This stable nature might be due to the viscous nature of Cremophor EL which gave good consistency to the mixture.

The use of Cremophor EL as a surfactant with Capryol 90 as an oil produced a gel structure that required a long time for emulsification although they had good ability to emulsify Capryol 90. Hence, Transcutol HP was used in combination with the surfactant. Inclusion of higher oil content led to a lower requirement for surfactants and cosurfactants. Further, it was considered important to formulate SMEDDS with the least concentration of surfactant because it was reported [35] that high concentration of surfactants could cause irritation of the gastrointestinal tract. Hence, among the three systems studied, System C [Capryol 90: Cremophor EL/Transcutol HP (3:1); Water] was chosen for formulating SMEDDS. This system showed no distinct conversion from water-in-oil (w/o) to oil-in-water (o/w) microemulsion. The translucent and low viscosity microemulsion area was represented in the phase diagram and was marked as ME (microemulsion area). It may be observed from figure 2, that the drug effect on phase diagram showed no significant difference in self-emulsifying performance, when compared with the placebo formulation.

Determination of drug solubility in the compositions of microemulsions

Capryol 90 was taken as the oil and Cremophor EL and Transcutol HP were taken for Smix. The compositions were arrived at by considering the micro-emulsion region in the figure 2c. The eleven compositions were chosen from the microemulsion region of figure 2c and shown in figure 2d. The solubility of aprepitant in these compositions was determined. The amount of aprepitant dissolved ranged from 16.71 mg/ml in APT1 to 35.62 mg/ml in APT11. More drug was solubilised by formulations having higher oil content or by formulations having surfactant and cosurfactants in the range of 14 - 20%v/v.

Among the various oils, screened, Capryol 90 showed the highest solubility potential for aprepitant (12.53 ± 0.35 µg/ml) compared to Labrafil M1944 (4.00 ± 0.09 µg/ml), Labrafac (2.34 ± 0.21 µg/ml) and Olive oil (5.13 ± 0.02 µg/ml). The solubility in Capryol 90 was significantly higher ($p < 0.02$) when compared to solubility in other oils. Among the various surfactants screened, such as Cremophor EL, Tween 20, Tween 40, Tween 80, Span 20 and Labrasol, highest solubility was seen in Cremophor EL. The solubility in Cremophor EL was significantly higher than the solubility in Tween 40 and Span 20 ($p < 0.01$). Among the co-surfactants studied, aprepitant showed maximum solubility in Transcutol HP (49.15 ± 0.28 µg/ml). This value was significantly higher ($p < 0.05$) than the values of solubility in other cosurfactants.

Based on the solubility values of aprepitant in various compositions, eleven formulations of SMEDDS were designed. The compositions of these eleven formulations were so chosen as to cover the total area of the micro emulsion forming region figure 2d. It may be understood from figure 2c, that, if a composition is prepared by taking oil and Smix in the quantities indicated by a point in the area of microemulsion, it would be having the properties of a SMEDDS. If a drug in the quantity that it can dissolve is incorporated in it, and is administered to a patient, it is expected that the SMEDDS would form a micro emulsion in the gastric fluid and in the intestinal fluid and would release the drug in a fast manner. This is expected to result in a much-enhanced dissolution rate and consequently bioavailability.

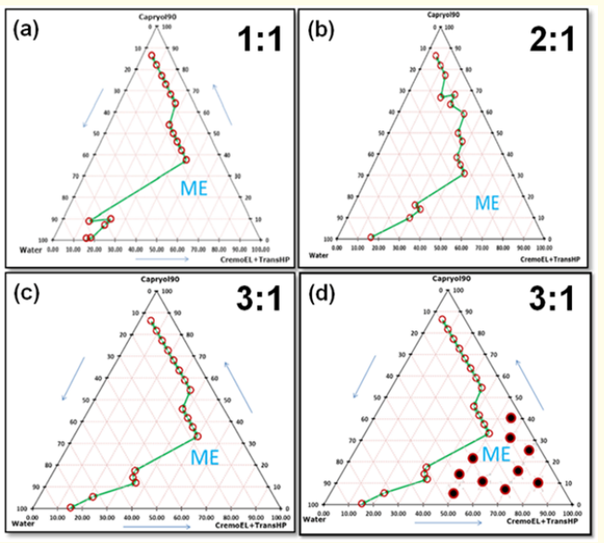

Figure 2: Pseudo ternary phase diagrams indicating micro-emulsion area constructed with Oil, Capryol90; Cremophor EL/ Transcutol HP (S/CoS) and Water. System (a) 1:1 (%v/v), System (b) 2:1 (%v/v), System (c) 3:1 (%v/v). ME: Micro-Emulsion Region; (2d) representation of aprepitant formulations selected from ternary phase diagram (3:1) constructed using Capryol90, Cremophor EL/Transcutol HP (S/CoS) and water.

In-vitro characterization of SMEDDS formulations containing drug

Drug Content

The drug content for each batch of SMEDDS formulated estimated was found to have a good consistency of about 98.5% in all the eleven formulations (Figure 3a). The formulations show that there is consistent uniformity when the drug is mixed with the other formulations. The results show that the drug is uniformly distributed and homogeneous.

Droplet diameter measurement

The immediately formulated formulations were suitably diluted with water and analyzed in a zetasizer for the measurement of droplet size, polydispersibility index (PDI) and zeta potential (ZP). The droplet diameter values, PDI and ZP of aprepitant SMEDDS formulations are shown in table 1b. The average droplet size ranged from min 13.98 ± 0.72 (APT 2) with a max droplet size of 124.90 ± 0.20 (APT9). However, the droplet size was below 125 nm which is highly suitable for a micro emulsion formulation [36]. The formulation of microemulsion could affect droplet size by the type of cosurfactant and surfactant used. The minimum PDI was observed to be 0.169 ± 0.011 (APT 3) and maximum 0.604 ± 0.089 (APT 7). All these values are low and the inference is that the prepared SMEDDS formulations were uniformly distributed. The ZP ranged from -9.1 ± 3.14 (APT 3) to -45.8 ± 1.71 (APT7). The low SD values for PDI as well as ZP indicate that all the formulations were uniform and the preparation procedure was consistent and reproducible. Higher zeta potential values as shown for formulations APT1 and APT7, this may be because they have relatively higher concentrations of surfactant than the other formulations. The SMEDDS containing high oil concentrations (42.0% w/v) showed low ZP values and this is irrespective of the surfactant concentrations. APT3 contained low oil concentration and high concentration of surfactant, but showed a low ZP value of -9.1 ± 3.14 mV. This indicates that the ZP value is not a simple function of certain factors but involves some complex interactive factors. All the formulations showed negative zeta values indicating the adsorption of negatively charged surfactants on the droplets.

Formulation Code		Average ± SD (n=3)	
	Droplet size (nm)	Polydispersity Index	Zeta potential (mV)
APT1	18.26 ± 2.07	0.406 ± 0.096	-41.1 ± 11.01
APT2	13.98 ± 0.72	0.172 ± 0.069	-30.9 ± 4.14
APT3	15.04 ± 0.77	0.169 ± 0.011	-9.1 ± 3.14
APT4	17.35 ± 0.34	0.240 ± 0.020	-11.5 ± 1.87
APT5	114.97 ± 1.27	0.322 ± 0.041	-9.5 ± 6.16
APT6	120.13 ± 0.57	0.290 ± 0.004	-12.4 ± 0.35
APT7	33.09 ± 0.76	0.604 ± 0.089	-45.8 ± 1.71
APT8	88.29 ± 0.67	0.316 ± 0.005	-14.4 ± 3.10
APT9	124.90 ± 0.20	0.278 ± 0.010	-13.0 ± 0.35
APT10	117.97 ± 1.07	0.238 ± 0.011	-12.5 ± 1.08
APT11	75.07 ± 1.53	0.359 ± 0.046	-11.1 ± 0.53

Table 1b: Average values of droplet size, polydispersity index and zeta potential of various aprepitant formulations studied after immediate formulation development.

It is well known that the addition of surfactants to the micro-emulsion system causes the interfacial film to stabilize and condense, while the addition of cosurfactants causes the film to expand; thus, the relative proportion of surfactant to cosurfactants has varied effects on the droplet size [36,37].

TEM

The TEM photograph of SMEDDS of APT7 was shown in figure 3b. The figure indicates that all the globules were spherical and of very low size (< 150 nm) i.e. in nanometers. Figure 3c indicates the

intensity of droplet size distribution was narrow and the percent intensity was high, indicating the uniformity in the preparation. The intensity of droplet size distribution of aprepitant SMEDDS formulation APT7 with found to have a mean particle size of 33.07 nm and the TEM picture shows that the droplets of microemulsion was almost of spherical shape with smooth surface and there was no aggregation among the droplets of microemulsion. The morphology of microemulsion by TEM analysis showed the spherical shape and uniform droplet size of microemulsion. It was found that the droplet size increased as the concentration of oil proportion increased.

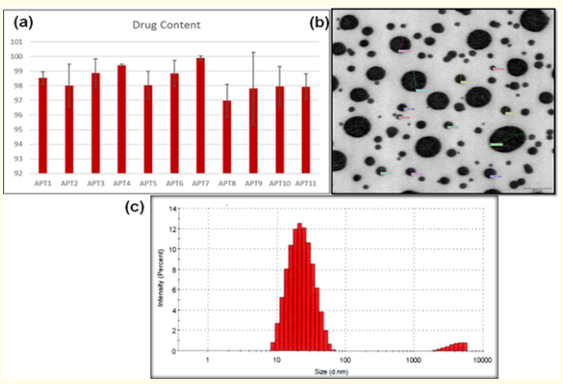

Figure 3: (a) Percent drug content values (Mean ± SD) of various self-micro emulsifying drug delivery systems of aprepitant formulations; (b) Transmission Electron Microscope image of aprepitant SMEDDS formulation APT7; (3c) Intensity of droplet size distribution of aprepitant SMEDDS formulation APT7 with a mean particle size of 33.07 nm.

Effect of dilution

The robustness of the prepared SMEDDS, APT1 to APT11, was studied by subjecting them to dilution studies with water, SGF and SIF. The objective of these studies was to understand the behavior of the SMEDDS when they go into the gastro intestinal tract. The robustness of the prepared SMEDDS of aprepitant was studied by studying the effect of dilution and the effect of change in pH on their properties, such as droplet diameter, PDI and ZP. The effect of dilution of the lipid based self-micro emulsifying compositions of aprepitant with various dilution levels was investigated by observing the potential change and were shown in figures 4a-4c if any for the globule size, PDI and ZP, respectively.

Dilution to 1:100 level had no effect on droplet size of the SMEDDS. But on further dilution to 1:500 and 1:1000, there was a variation in the droplet size. However, even at the highest dilution, all the formulations showed droplet sizes below 100 nm. So, it may be reasoned that these SMEDDS are suitable as oral dosage forms. APT1, APT3 and APT4 showed an increase in droplet size upon dilution, whereas remaining formulations showed a decrease in droplet size upon dilution. ZP values showed no variation when SMEDDS were diluted with water at 1:100 level but showed some variation when they were diluted to 1:500 and 1:1000 level. APT3, APT5, APT6, APT7, APT9, APT10 and APT11 showed an increase in ZP on dilutions with water. APT1, APT2, APT4 and APT8 showed a decrease in ZP dilution with water. However, at all dilution levels, the negative charge remained same. This indicates that the surfactant was firmly adsorbed onto the oil/water interface. Dilution with water had no effect on PDI values. SMEDDS could retain their homogeneous structure even on dilution with water.

Dilution with all the diluents did not cause any change in the visual clarity. Thus, the robustness studies reveal a small change in globule size, no change in PDI and drastic change in zeta potential.

Effect of pH in SGF and SIF media

The aprepitant was studied in various simulated fluids like SGF and SIF, as the drug passes thru the various phases of the GI tract. The effect of pH will reveal whether the formulation is stable in the GI fluids. When the formulation undergoes infinite dilution in gastrointestinal fluids, it is likely that the drug may precipitate owing to the poor aqueous solubility of the drug. Gastrointestinal stability studies are important to rule out the possibility of precipitation of drug *in-vivo* [38]. The values of droplet size, PDI and ZP with respect to pH in various media were shown in figures 4d, 4e, and 4f, respectively.

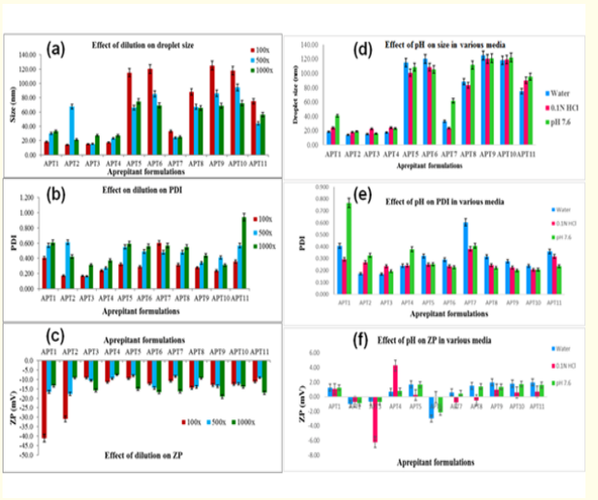

Figure 4: (a) Droplet size of various aprepitant formulations at different dilution levels with water; (b) Polydispersity index of various aprepitant formulations at different dilution levels with water; (c) Zeta potential of various aprepitant formulations at different dilution levels with water; (d) Droplet size of various aprepitant formulations after incubation with Water, SGF and SIF media; (e) Polydispersity Index of various aprepitant formulations after incubation with Water, SGF and SIF media; (f) Zeta potential of various aprepitant formulations after incubation with Water, SGF and SIF media.

Minimum droplet size of 13.98 ± 0.72 (APT2) and maximum of 124.90 ± 0.20 (APT9) was observed when studied in water. Min 17.68 ± 0.11 (APT2) and max 120.07 ± 1.07 (APT9) was observed in SGF medium. Min 15.83 ± 0.98 (APT3) and max of 122.10 ± 0.30 (APT10) was observed in SIF medium. The results show that all the formulations are below 150nm which ideally represents quality of a SMEDDS.

Minimum PDI in water, SGF and SIF observed to be as 0.169 ± 0.011 (APT3), 0.206 ± 0.006 (APT10) and 0.194 ± 0.021 (APT3),

respectively whereas maximum PDI values 0.604 ± 0.089 (APT7), 0.317 ± 0.047 (APT11) and 0.765 ± 0.076 (APT1) respectively. As the values are below 1.0 the formulations represent that they are uniformly distributed and prepared.

Minimum ZP in water, SGF and SIF observed to be -1.1 ± 0.53 (APT11), -0.07 ± 3.96 (APT6) and -0.9 ± 0.61 (APT2), respectively; whereas maximum ZP values -43.8 ± 1.71 (APT7), -30.81 ± 4.14 (APT7) and 36.45 ± 0.24 (APT7) respectively. Ideal ZP values must lie between -30 to -60 mv.

Thermodynamic stability studies

Thermodynamic stability studies were carried out on all the eleven formulations of SMEDDS of aprepitant. Thermodynamic stability study involved subjecting the SMEDDS to five (5) cycles of freezing at -80 °C for 24 hours and thawing at room temperature (25°C) for 24 hours. After the end of the thermodynamic stability study the SMEDDS were studied for droplet size, PDI and ZP and the results were shown in figures 5i-a, i-b and i-c, respectively.

The droplet size comparatively decreased after freeze thaw cycle. The decrease was not much significant but however, they are less than 150nm. The droplet size for APT7 was 61.7 ± 4.39 before 5 cycles and 57.97 ± 0.96 (APT7) after 5 cycles. Freezing and thawing caused a variable type of change in the eleven formulations with respect to size of droplets. In some formulations (APT2, APT3, APT6, APT7, APT8, APT10 and APT11) there as was only a small change in droplet size, whereas in others (APT1, APT4, APT5 and APT9) the change in droplet size was considerable. But none of the formulations showed more than 120 nm. There was no much change in the PDI or the ZP of the formulations, before and after the cycles of freezing and thawing. On the whole, it may be inferred that the freeze thaw cycles had a small effect on droplet size but no effect on polydispersity index and zeta potential.

Evaluation of emulsification time

It was found that all the SMEDDS formulated had an emulsification time of less than one minute. The optimized formulation APT7 formed a clear and slight bluish tint micro emulsion. The results of the study on emulsification time are shown in table 1c gives the time taken for emulsification and a grade given to each product of SMEDD, based on the type of micro emulsion that is obtained. All the eleven SMEDDS formulated gave micro emulsions in less than one minute and hence may be declared as of very good quality. However, APT6, APT9 and APT10 have taken comparatively longer time than others. APT2, APT3, APT4, APT9 and APT10 showed micro emulsions which are of Grade - B in terms of their color and appearance. Hence, it was considered appropriate to proceed further, for dissolution studies with formulations of SMEDDSs, APT1, APT7, APT8 and APT11. Figure 5b shows the emulsification for formulation APT 7.

Formulation No.	Time ± SD (Sec)	Turbidity	Grade
APT 1	26.32 ± 1.102	Clear Bluish tint	A
APT 2	26.05 ± 0.693	Bluish white	B
APT 3	23.80 ± 0.801	Bluish white	B
APT 4	31.14 ± 0.510	Bluish white	B
APT 5	42.81 ± 0.202	Clear Bluish tint	A
APT 6	51.82 ± 0.473	Clear Bluish tint	A
APT 7	30.73 ± 0.413	Clear Bluish tint	A
APT 8	47.81 ± 0.992	Clear Bluish tint	A
APT 9	54.75 ± 0.661	Bluish white	B
APT 10	58.45 ± 0.709	Bluish white	B
APT 11	40.60 ± 0.366	Clear Bluish tint	A

Table 1c: Emulsification time for aprepitant SMEDDS formulations (Mean ± SD, n = 3).

In-vitro dissolution studies

It was felt necessary to optimize among the prepared SMEDDS and select four formulations for the purpose of carrying out *in-vitro* dissolution studies. The selection process was based on the following considerations. The average drug content per ml of SMEDDS varied from a minimum of 16.7 mg/ml in APT1 to a maximum of 35.62 mg/ml in APT11. One consideration was to select formulations at different mid points in the range 16.71 - 35.62 mg/ml, and another consideration was to discard SMEDDS having globule size greater than 100 nm. On this basis formulations APT5, APT6, APT9 and APT10 were not taken for processing by in-vitro dissolution studies. In the evaluation of emulsification time, APT2, APT3 and APT4 were seen to be of Grade B i.e. they showed an opalescence which was not acceptable. Hence, formulations APT1, APT7, APT8 and APT11 were selected for *in-vitro* dissolution studies. These represent drug concentrations at different points in the range 16.71 - 35.62 mg/ml.

Formulations APT1, APT7, APT8 and APT11 were considered good candidates for further processing based on their attributes of good drug content solubilized, good amount of drug content per low amount of surfactant, optimum size of globule, lack of change of globule size on pH dilution, good emulsification time and clear emulsion formation. The dissolution profile of the formulations in water, SGF and SIF were shown in Figures 5iii-a, iii-b and iii-c, respectively. The dissolution profile in water for various formulations of APT1, 7, 8 and 11 were found to be more than 80% where as in pure drug it was found to be 13.36%. The dissolution profile in SGF for various formulations of APT1, 7, 8 and 11 were found to be more than 92.51% where as in pure drug it was found to be 42.77%. The dissolution profile in SIF for various formulations of APT1, 7, 8 and 11 were found to be more than 91.79% where as in pure drug it was found to be 19.51%.

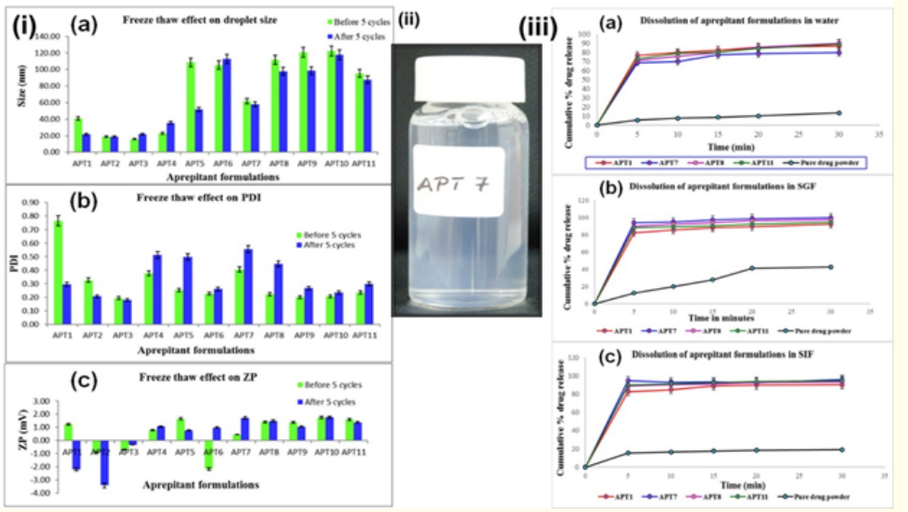

Figure 5(i): (i-a) Effect of freeze thaw on particle size of various aprepitant formulations; (i-b) Effect of freeze thaw on polydispersity index of various aprepitant formulations; (i-c) Effect of freeze thaw on zeta potential of various aprepitant formulations. Figure 5 (ii): Visual appearance of APT7 SMEDDS formulation during emulsification time. Figure 5 (iii): (a) Drug release profiles of aprepitant SMEDDS formulations in water; (b) Drug release profiles of aprepitant SMEDDS formulations in SGF; (c) Drug release profiles of aprepitant SMEDDS formulations in SIF.

Stability studies

Stability studies after 24 hr in water

Stability studies, carried out on prepared SMEDDS were shown in figure 6a that there was no significant change in the droplet size or PDI upon incubating in water for 24 hours, but there was change in zeta potential, which was significant in many cases (APT1, APT2, APT3, APT4, APT8, APT9).

Stability studies after 2hr and 8 hrs in SGF and SIF

There was no significant change in the droplet size or PDI or zeta potential of any SMEDD on incubating with SGF after 2 hr (Figure 6b), however significant changes after 8 hr for some formulations of SMEDDS (APT1, APT4) in droplet size. But no significant change in the PDI or zeta potential of any SMEDD on incubating with SIF for 8 hours and shown in figure 6c which shows that the formulations were consistent and stable in gastric fluids.

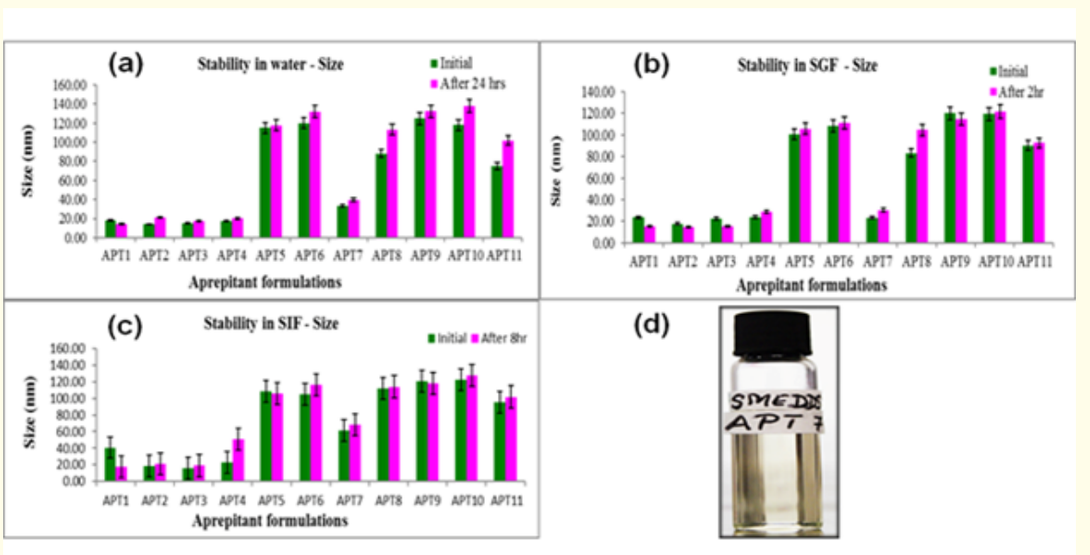

Figure 6: (a) Stability after 24hours on droplet size of aprepitant formulations in water; (b) Stability after 2hours on droplet size of aprepitant formulations in SGF; (c) Stability after 8hrs on droplet size of aprepitant formulations in SIF; (d) Finished product of aprepitant SMEDDS formulation APT7.

Accelerated stability studies

Accelerated stability studies carried out on formulation APT7 gave results which are shown in table 2a. They indicate that there was no significant change in the droplet diameter or drug content or dissolution profile of APT7 which it was placed in a stability chamber for 6 months at 40°C and 75% RH. This indicates the robustness of the optimized formulation.

Aprepitant SMEDDS - APT 7							
Droplet size in SGF (nm)				**Droplet size in SIF (nm)**			
Initial value	1 month	3 months	6 months	Initial value	1 month	3 months	6 months
23.38 ± 2.81	23.87 ± 1.97	23.64 ± 0.31	23.77 ± 1.54	61.70 ± 4.39	68.47 ±3.26	68.47 ± 2.03	69.79 ± 1.69
Dissolution in SGF (Cumulative % drug release)				**Dissolution in SIF (Cumulative % drug release)**			
Initial value	1 month	3 months	6 months	Initial value	1 month	3 months	6 months
97.25 ± 0.95	97.45 ± 0.82	97.52 ± 0.66	97.52 ± 0.86	93.56 ± 0.25	94.40 ±0.74	95.12 ± 0.46	96.03 ± 0.20
Drug Content (Assay)							
Initial		1 month		3 months		6 months	
99.89 ± 0.158		99.58 ± 0.167		99.69 ± 0.118		99.79 ± 0.128	

Table 2a: Accelerated stability data for aprepitant SMEDDS formulation APT7 at 40ºC+75 % RH (n = 3).

Kinetic studies

Results shown in table 2b indicate that the drug release profiles in all the three media studied of APT1, APT7, APT8 and APT11 follow first order kinetics. The correlation coefficient values in almost all cases (except pure drug in water and SIF and APT7 in SGF and in SIF) are more in value in the case of first order. Hence it may be inferred that the release profiles are following first order kinetics.

The first order rate constants are shown in table 2c. These are calculated for the second phase of the release profile. It may be inferred from these values also, as from the release profiles, that the release rate is much faster from SMEDDS than from pure drug. After the fast release in the first five minutes, the release is slow and steady during the remaining period of the dissolution study.

	Water		**0.1N HCl**		**pH 6.8 buffer**	
Formulation Code	**Zero order (r)**	**First order (r)**	**Zero order (r)**	**First order (r)**	**Zero order (r)**	**First order (r)**
APT1	0.9728	0.9855	0.9813	0.9981	0.9123	0.9493
APT7	0.9075	0.9254	0.9763	0.9617	0.9154	0.8959
APT8	0.9856	0.9951	0.9431	0.9783	0.9438	0.9512
APT11	0.9697	0.9902	0.9927	0.9921	0.9764	0.9934
Pure drug	0.9974	0.9864	0.9464	0.9447	0.9771	0.9011

Table 2b: Correlation coefficient (r) values in the release kinetics of aprepitant formulations.

Formulation Code	Water K_1 (hr^{-1})	SGF K_1 (hr^{-1})	SIF K_1 (hr^{-1})
APT1	0.028	0.035	0.030
APT7	0.021	0.131	0.016
APT8	0.044	0.058	0.023
APT11	0.037	0.032	0.029
Pure drug	0.005	0.002	0.002

Table 2c: First order release kinetics of aprepitant formulations.

Conclusions

The SMEDDS of aprepitant formulations could be successfully developed and the optimized aprepitant formulations could able to show dramatic improvement in the dissolution rate when compared with the pure drug aprepitant. In the current study, the solubility of aprepitant was increased significantly from 3 - 7 µg/ml to 30.65 mg/ml (APT7 SMEDDS), which is a 10216 folds increase (30650/3) and is to be considered as highly significant. The APT7 SMEDDS final formulation showed a good drug release of more than 90% than that of pure drug which released 13%. The SMEDDS drug delivery system was found to be a potential drug delivery system for enhancing the solubility of poorly water-soluble drugs.

The optimized SMEDDS of aprepitant formulation APT 7 compositions consisting of oil phase (Capryol 90 - 17%), surfactant (Cremophor EL - 62.25%) and co-surfactant (Transcutol HP - 20.75%) based on the result of solubility test, self-emulsifying grading test, droplet size analysis and ternary phase diagrams test. The optimized SMEDDS formulation of aprepitant was successfully prepared and evaluated for its drug delivery potential. The solubility of aprepitant in the developed SMEDDS formulation

was increased significantly and the drug loading was enough for making this drug clinically applicable. The APT7 SMEDDS final formulation as shown in figure 6d, showed a good drug release of more than 90% than that of pure drug which released 13%. The SMEDDS drug delivery system was found to be a potential drug delivery system for enhancing the solubility of poorly water-soluble drugs.

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Conflict of Interest

There is no conflict of interest and disclosures associated with the manuscript.

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