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Optimization and Evaluation of Acyclovir Loaded Liquid and Solid Self Nanoemulsifying Drug Delivery System

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ABSTRACT

Acyclovir has low bioavailability mainly due to low solubility. In this study, solid self-nanoemulsifying drug delivery systems (S-SNEDDS) of acyclovir were developed with the objective of improving its solubility. Initial screening was carried out to select the excipients. Ternary phase diagrams were constructed to detect the nanoemulsification region. The nanoemulsion systems selected from the phase diagram were characterized for their robustness to dilution and cloud point temperature. Box- Behnken design was then applied for further optimization. The formulations obtained were further characterized for their droplet size and entrapment efficiency. The best formulation was converted to S-SNEDDS by simple adsorption technique. The liquid SNEDDS (L-SNEDDS) was adsorbed onto microcrystalline cellulose in 1:1 ratio. Zeta potential, differential scanning calorimetry, scanning electron microscopy was then carried out. In vitro drug release was studied by comparing the S-SNEDDS with pure drug. The L-SNEDDS formulation which was converted to S-SNEDDS showed particle size of 147 nm. The formulation was found to be robust to dilution and showed cloud point at 86 ◦C. Negative zeta potential meant, there was no coalescence of globules. SEM studies of nanoemulsion demonstrated that the globules in L-SNEDDS were indeed adsorbed on the MCC. Results of DSC confirmed that the drug was incorporated in the S-SNEDDS that was formulated. The *in vitro* drug release from acyclovir S-SNEDDS was found to be considerably higher in comparison to that of the pure drug. Therefore, it can be concluded that acyclovir loaded S-SNEDDS improved the solubility and release characteristics of the drug.

Keywords: Acyclovir, Self nanoemulsifying drug delivery systems (SNEDDS), Box-Behnken design, Powder SNEDDS

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INTRODUCTION

The formulation of hydrophobic drugs present interesting challenges in the pharmaceutical industry. Up to 40% of new chemical entities discovered are poorly water soluble or hydrophobic compounds, which lead to poor oral bioavailability, high intra and inter subject variability and lack of dose proportionality[1]. New techniques are being developed to improve the oral bioavailability of hydrophobic drugs in order to enhance their clinical efficacy[2]. Self emulsifying drug delivery systems have been shown to be successful in increasing the oral bioavailability of hydrophobic drugs[3].

Self emulsifying drug delivery system (SEDDS) also called as self emulsifying oil formulation is a combination of oil and surfactant(s), which are ideally isotropic, and sometimes contain co-solvents, which emulsify spontaneously to produce fine oil in water emulsion, when introduced into aqueous phase under gentle agitation[4,5]. Solubility enhancements caused by co-solvent addition generally occurs because of changes in the bulk properties of the isotropic solution. Mechanisms include: (1) creation of a single phase condition, (2) decrease in the interfacial tension, and (3) swelling by solubilization of the cosolvents within the phase[6].

Self-Nanoemulsifying (SNEDDS), Self-Microemulsifying (SMEDDS) and Self-Emulsifying Drug Delivery Systems (SEDDS) are the techniques utilised to enhance the oral bioavailability of poorly water-soluble drugs[7-9].

Self nanoemulsifying drug delivery systems possess the following qualities[10]:

1. The oil droplet size is less than 100nm
2. The appearance is optically clear
3. The required Hydrophilic Lipophilic Balance (HLB) value is greater than 12

Acyclovir [9-(2-hydroxyethoxylmethyl) guanine], a synthetic purine nucleoside analog derived from guanine, is the most widely used antiviral agent. It is effective in the treatment of herpes simplex virus (HSV), mainly HSV-1 and HSV-2, and varicella-zoster virus[11]. The pharmacokinetic parameters of acyclovir following oral administration generally are highly variable. Peak plasma values have been shown to be 0.46 to 0.83 or 0.63 to 1.21 µg/L after a single oral dose of 200 or 400 mg, respectively[12] and have been generally obtained 1.5 to 2.5 hrs after oral administration[12, 13]. Acyclovir absorption in the gastro intestinal tract is slow, variable and incomplete. The bioavailability of acyclovir after oral administration ranges from 10%- 30%[13]. Approximately 80% of the oral dose is never absorbed and is excreted through faeces. The main excretory organ is kidney. The plasma half life of acyclovir on average in adults is 3 hrs with normal renal function[14].

MATERIALS AND METHOD

Acyclovir was obtained as a gift sample from FDC limited (Goa, India). Cremophor RH40 was a gift sample from Tyco healthcare, Canada. Capmul PG- 12 was gifted by Abitech Corp., USA where as capryol 90 by Gattefosse, France. Olive oil, methanol, polyethylene glycol 200 (PEG 200), PEG 400, Tween 20, Tween 80 and Span 80 were purchased from Loba Chemie Pvt. Ltd., Mumbai. Ethanol was purchased Greenfield chemicals, Canada. The materials used were recorded as "generally recognized as safe"(GRAS), endorsed safe for use in oral drug delivery by the World Health Organization, and were within their tolerable limits. All other chemicals used in this study including those stated were of analytical reagent (A.R.) grade.

Equlibrium solubility studies of acyclovir

Components for the development of SNEDDS were selected on the basis of equilibrium solubility studies so as to incorporate the desired dose of acyclovir. Solubility of acyclovir was determined in various oils (capmul PG- 12, capryol 90, oleic acid, olive oil), surfactants (Tween 20, Tween 80, Triton X 100, Cremophor RH 40), co-surfactants (ethanol, PEG 200, PEG 400, Span 80). An excess amount of acyclovir was mixed with the solvents (n = 3) and kept in a mechanical shaker for a period of 48 hours. The solvent- drug mixture were then taken and centrifuged at 3000 rpm for 10 minutes. The supernatant was filtered through Whatman filter paper and then diluted with glacial acetic acid. The amount of acyclovir solubilized was quantified using ultraviolet spectophotometer (SHIMADZU UV-1800) at 255 nm [15].

Self emulsifying grading test

The efficiency of self-emulsification of oral nanoemulsion is assessed using a standard USP XXII dissolution apparatus II. One milliliter of each formulation is added to 500 ml of water at 37 ± 0.5°C. A standard stainless steel dissolution paddle rotating at 50 rpm provides the gentle agitation required. The *in vitro* performance of the formulations is visually assessed using the following grading system.

Grade A: Rapidly forming (within 1 min). Nanoemulsion, have a clear or bluish appearance.

Grade B: Rapidly forming. Slightly less clear emulsion and have a bluish white appearance.

Grade C: Fine milky emulsion that forms within 2 minutes

Grade D: Dull, greyish white emulsion that have a slight oily appearance and is slow to emulsify (takes longer than 2 min).

Grade E: Formulation exhibits either poor or minimal emulsification. Large oil globules are present on the surface[16].

CONSTRUCTION OF PSEUDO-TERNARY PHASE DIAGRAMS

For the development of acyclovir-loaded SNEDDS, pseudoternary phase diagrams were constructed to recognize the zone of nanoemulsion formation. Phase diagram was constructed using Cremophor RH 40 and PEG 400 as Smix (surfactant + co-surfactant) in the ratio of 1:1, 2:1, 1:2. For the construction of phase diagram, oil (PG-12) and specific Smix ratios were mixed carefully in diverse weight ratios from 9:1 to 1:9 (% w/w). Additionally, each weight ratio of oil and Smix mixture was titrated slowly with distilled water with gentle stirring to allow equilibration[17]. % transmittance at 638.2 nm was checked for each of the formulation.

Preparation of liquid self-nanoemulsifying mixtures

Self-nanoemulsifying (SE) properties of SNEDDS strongly depend upon the selected oils, surfactants, co-surfactants and their relative amounts. The utilization of lipid and surfactant(s) mixtures gives the possibility to optimize the SNEDDS for a particular drug. The Smix were prepared by mixing cremophor RH 40 and PEG 400 in the mentioned ratios which were then added to the lipidic drug solution (drug and PG-12), while stirring at high speed using a magnetic stirrer. 27 such mixtures were prepared[18]. The prepared solutions were then examined for clarity (% transmittance)[19] and also characterized using self emulsifying grading test[20].

Robustness to dilution and Cloud point measurement

After identifying the best formulations on the basis of clarity and self emulsifying grading test, the selected formulations were further characterized by subjecting them to robustness to dilution and cloud point temperature measurement. Robustness to dilution was checked by diluting the SNEDDS 50, 100, 250 and 1000 times with distilled water, pH 1.2 buffer and pH 6.8 buffer and then evaluated for changes in transmittance whereas cloud point temperature was identified by diluting the SNEDDS with distilled water, pH 1.2 buffer and pH 6.8 buffer in the ratio of 1:250, by placing them in a water bath and temperature is increased gradually. Cloud point was measured as the temperature at which there was a sudden appearance of cloudiness[21].

EXPERIMENTAL DESIGN

Box- Behnken design was applied to the best formulation which was identified on evaluating the robustness to dilution and cloud point temperature measurement. Design Expert version 9 software (Stat-Ease, Minneapolis, Minnesota) was used. Cremophor RH 40 as surfactant (X_1), PEG 400 as co-surfactant (X_2) and PG-12 as oil (X_3) were selected as the three factors for further optimization of formulation. overall, a set of 13 formulations were studied as per the experimental design matrix. The response considered were droplet (particle) size(Y_1) and entrapment efficiency(Y_2).

Response surface plot and contour plot was conducted to predict the possible solutions.

Droplet size determination

The average droplet size and PDI of reconstituted nanoemulsions were determined by means of photon correlation spectroscopy. Measurements were made using NANOPHOX- NX0088 (Sinhgad Institute of Pharmacy, Pune). Light scattering was monitored at 25°C at a 90-degree angle. Reading was recorded thrice[22].

Entrapment efficiency

Drug from pre-weighed SNEDDS is extracted by dissolving in a suitable solvent. Drug content in the solvent extract is analyzed by suitable analytical method against the standard solvent solution of drug[23,24]. In this case, acyclovir from pre-weighed SNEDDS was extracted by dissolving in glacial acetic acid and entrapment efficiency was analyzed using ultraviolet spectrophotometer against blank glacial acetic acid.

FORMULATION OF SOLID SELF NANO-EMULSIFYING DRUG DELIVERY SYSTEM

Micro crystalline cellulose (MCC) was used as solid adsorbent carrier to formulate solid acyclovir SNEDDS in the ratio of (adsorbent: acyclovir L-SNEDDS) 0.25:1, 0.5:1, 1:1. Briefly the fixed aliquot of acyclovir SNEDDS was added and mixed vigorously with the adsorbent in the mortar. The granular mass obtained was passed through 250 μm mesh to obtain uniformity in particle size. The powder samples were then stored in a desiccator until further evaluation[25].

Zeta Potential (ζ potential) and Scanning Electron Microscopy

The zeta potential of the nanoemulsion was determined by a Malven zetasizer Nano-ZS (Malven Instruments, Malven, UK). SEM studies were also carried out to determine morphology of S-SNEDDS.

Differential Scanning Calorimetry (DSC)

DSC of pure acyclovir and acyclovir S-SNEDDS was performed in order to characterize and identify potential interaction between acyclovir and the adsorbent. Accurately weighed samples (2–5 mg) were placed in perforated aluminium pans and scanned through a temperature range of 20–270 ◦C at a heating rate of 10 ◦C/min, under a nitrogen purge gas flow of 50 ml/min. The instrument was calibrated for temperature and energy using indium standards[26]. DSC was performed on the instrument, METTLER STAR SW 9.01.

***In vitro* drug release studies**

Dissolution profile of S- SNEDDS as well as pure acyclovir, was determined using rotating basket apparatus (Electrolab, Mumbai, India). S-SNEDDS was filled in capsules. Dissolution conditions

were: 0.1 M hydrochloric acid, 37 ± 0.5 ◦C, 900 ml, 100 rpm and pH 6.8 buffer, 37 ± 0.5 ◦C, 900 ml, 100 rpm . At fixed time intervals (5, 10, 15, 20, 30, 45, 60), samples of 4 ml were withdrawn from the medium. Sink conditions were maintained at all times. All samples were filtered using Whatmann filter paper and assayed for acyclovir[26]. Acyclovir concentration was determined spectrophotometrically at 259 nm for 0.1M HCl and 256 nm for pH 6.8 buffer (Shimadzu UV-1800, Japan). The amount of acyclovir that was removed during sampling was taken in consideration while calculating the percentage of released drug.

Accelerated stability studies

The optimized S-SNEDDS formulation was subjected to accelerated stability studies, carried out at 40 ± 2 ◦C/75% ± 5% RH, as per the ICH guidelines, for the climatic zone IV, at time points of 0, 1, 3 and 6 months. The S-SNEDDS powder formulation was introduced in capsule of size 0 and was assayed for % transmittance[27] and drug content[28].

RESULTS AND DISCUSSION

Acyclovir solubility and permeability, restrict its oral bioavailability. SNEDDS proved their potential to improve oral bioavailability of similar lipophilic drug facing metabolic deterrents, such as atorvastatin[29] and amphotericin B[30]. SNEDDS spontaneously form nanoemulsions when exposed to GIT fluids. The spontaneous formation of nanoemulsions presents the drug in a dissolved form. The resultant small droplet size provides a large interfacial surface area for drug release and absorption. In addition, the specific components of the system promote the intestinal lymphatic transport of drugs. Main mechanisms include increasing membrane fluidity to facilitate transcellular absorption, opening tight junction to allow paracellular transport, inhibiting P-gp and/or CYP450 to increase intracellular concentration and residence time by surfactants, and stimulating lipoprotein/chylomicron production by lipid. In order to prepare an efficient SNEDD system of acyclovir, the formulation should be designed for such a drug. Proper type and ratio of oily phase, surfactant mixture and proper globule size should be selected. Furthermore, optimal formulation should posses a cloud point higher than 37 ◦C (i.e. above the body temperature) and a promising release profile, as detailed in the following sections.

Equilibrium solubility studies of acyclovir

Oils

Solubilizing capacity of an oily phase is the outlook of consideration regarding oil selection [31]. The solubility of the drug was tested in four different oily phases (capmul PG- 12, capryol 90, oleic acid, olive oil) that are commonly utilized in SEDDS and SNEDDS formulation. Results of

solubility studies in oily phases are depicted in Figure 1. The figure demonstrates that solubility of acyclovir was found to be highest in the PG- 12.

Surfactants

Solubility of the drug was then checked in surfactants. Cremophor RH 40, Triton X- 100, TWEEN- 20 and TWEEN- 80 were the surfactants utilized for the study. Figure. 2 depicts the solubility of acyclovir in surfactants. Solubility was found to be highest in cremophor RH 40 followed by Triton X- 100

Co-surfactants

Solubility of the drug was also checked in co-surfactants. PEG 200, PEG 400, SPAN 80, ethanol utilized as co-surfactants in the study. Figure. 3 depicts the solubility of acyclovir in co-surfactants. Solubility was found to be maximum in PEG 400 followed by PEG 200.

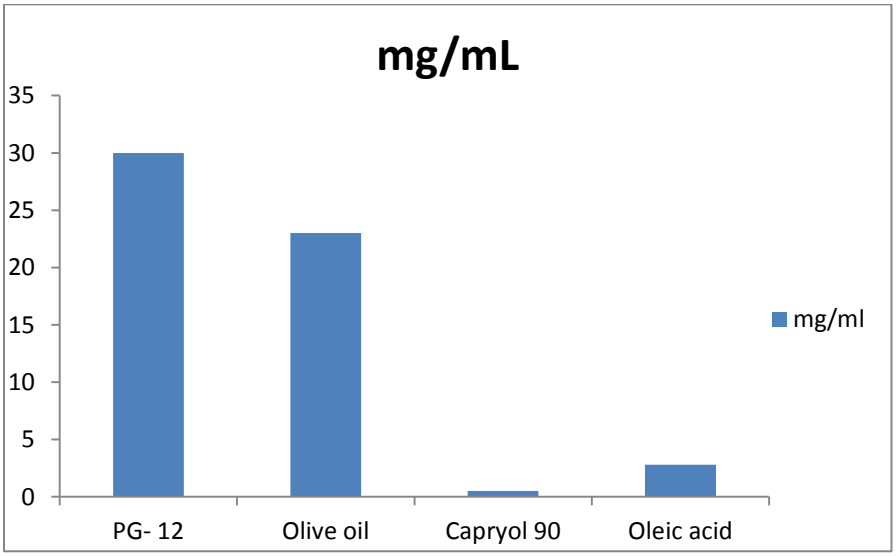

Figure. 1: Solubility of acyclovir in oils.

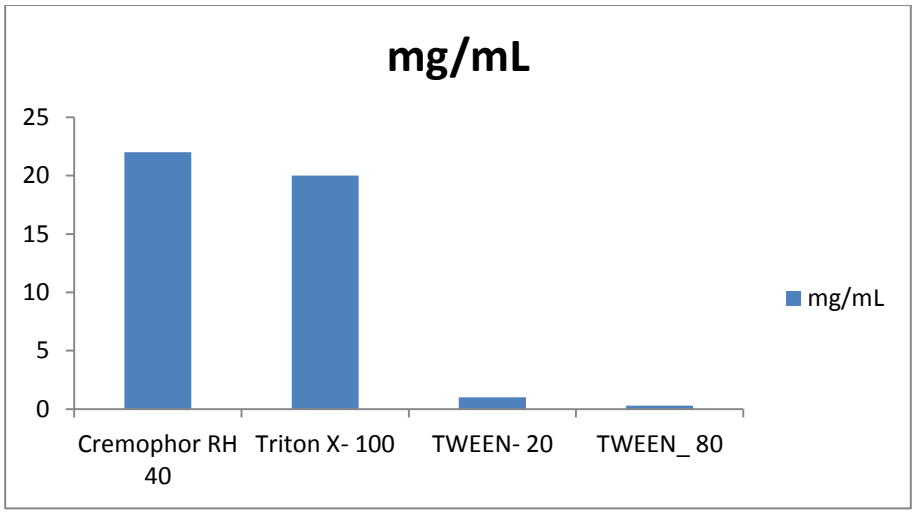

Figure. 2: Solubility of acyclovir in surfactants.

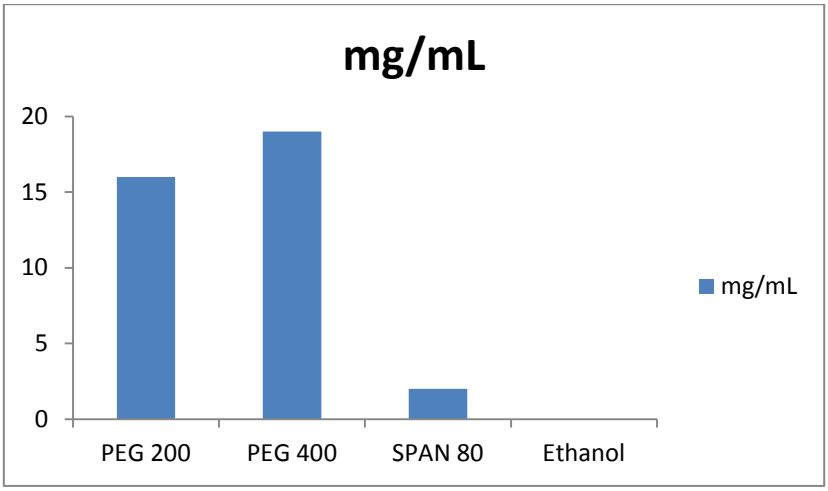

Figure. 3: Solubility of acyclovir in co- surfactants.

Screening of Surfactants and co- surfactants

It has been reported that well formulated SNEDDS is dispersed within seconds under gentle stirring conditions[31]. The surfactants were compared for their emulsification efficiencies using PG-12 as oil phase. Addition of a co-surfactant to the surfactant-containing formulation was reported to improve dispersibility and drug absorption from the formulation[32](Table 1). The system comprising of PG- 12 and Cremophor RH 40 showed grade A when emulsification grade testing was carried out following the method as mentioned. This meant that there was rapid dispersion and the emulsion formed was transparent (clear). Co- surfactants were added to this system and emulsification grade was determined(Table 2).

Table 1: Emulsification efficiency of various surfactants using PG-12 as oil.

Oil+ surfactants	Emulsification grade
PG- 12+ Cremophor RH 40	A
PG- 12+ Triton X- 100	B
PG- 12+ TWEEN 20	D
PG- 12+ TWEEN 80	D

Table 2: Emulsification efficiency of various co-surfactants using PG-12 as oil and Cremophor RH 40 as surfactant.

Oil+ surfactant= co-surfactants	Emulsification grade
PG-12+ Cremophor RH 40+ PEG 200	B
PG-12+ Cremophor RH 40+ PEG 400	A
PG- 12+ Cremophor RH 40+ SPAN 80	C
PG- 12+ Cremophor RH 40+ Ethanol	D

From the results obtained, system comprising of PG- 12 as oil phase, Cremophor RH 40 as surfactant and PEG 400 as co- surfactant was finalised by considering the emulsification grade test. Grade A implies rapid formation (dispersion) and transparent emulsion.

Construction of ternary phase diagram

Based on the results of the preliminary screening of the excipients, a ternary phase diagram was constructed to determine the nanoemulsification region by using the method described above. All the components were converted to weight/weight percent (w/w%) before constructing the phase diagrams (Figure. 4). The highlighted area enclosed in the triangle represents the region of self-emulsification. Within this area the SNEDDS form superior oil in water emulsion with only gentle agitation. Surfactant and co-surfactant get preferentially adsorbed at the interface, reducing the interfacial energy as well as providing a mechanical barrier to coalescence then improves the thermodynamic stability of the nanoemulsion formulation[33]. Furthermore, co-surfactants increase interfacial fluidity by penetrating into the surfactant film creating void space among surfactant molecules[34].

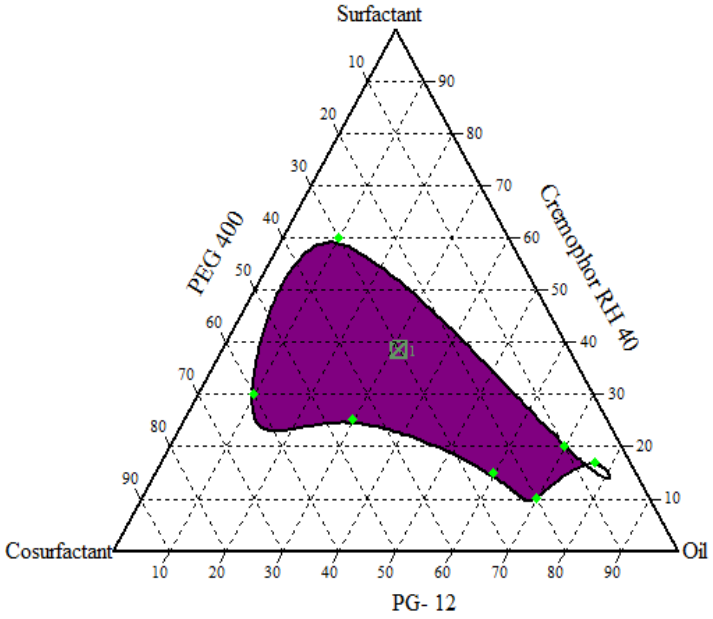

Figure. 4: Ternary phase diagram of the system containing PG-12 as the oil phase, Cremophor RH 40 as surfactant and PEG 400 as co-surfactant. The red region depicts the nanoemulsifying region.

The highlighted region in the ternary phase diagram is the nanoemulsification region. As mentioned already, within this area the SNEDDS form superior oil in water emulsion with only gentle agitation. Table 3 depicts the details about concentration of oil and S_{mix} in % w/w and the 27 formulations that were prepared by varying % of oil phase, as described in the method above. The table also depicts the % surfactant and % co-surfactant in the $S_{mix.}$ % transmittance along with self emulsifying grading was checked for each of the formulations. The details are depicted in

Table 4. Out of the 27 formulations, F-2, F-3, F-8, F-17, F-19, F-20, F-21 show % transmittance >85% and form transparent nanoemulsions rapidly.

Table 3: Concentration of oil and S_{mix} in % w/w and the surfactant: co-surfactant ratio in the $S_{mix.}$ in each of the 27 formulations.

Formulations	% Oil Phase (% w/w)	% Smix (% w/w)	% Surfactant in S_{MIX}	% Co-surfactant in S_{MIX}
F-1	10	90	45	45
F-2	10	90	60	30
F-3	10	90	30	60
F-4	20	80	40	40
F-5	20	80	53.3	26.6
F-6	20	80	26.6	53.3
F-7	30	70	35	35
F-8	30	70	46.6	23.3
F-9	30	70	23.3	46.6
F-10	40	60	30	30
F-11	40	60	40	20
F-12	40	60	20	40
F-13	50	50	25	25
F-14	50	50	33.3	16.6
F-15	50	50	16.6	33.3
F-16	60	40	20	20
F-17	60	40	26.6	13.3
F-18	60	40	13.3	26.6
F-19	70	30	15	15
F-20	70	30	20	10
F-21	70	30	10	20
F-22	80	20	10	10
F-23	80	20	13.3	6.6
F-24	80	20	6.6	13.3
F-25	90	10	5	5
F-26	90	10	6.6	3.3
F-27	90	10	3.3	6.6

Table 4: % transmittance along with self emulsifying grading test of the 27 formulations.

Formulations	% Transmittance (S.D.= ±0.05)	Grade
F-1	79.10	B
F-2	86.10	A
F-3	91.84	A
F-4	81.50	B
F-5	76.72	B
F-6	79.93	B
F-7	75.21	B
F-8	90.21	A
F-9	76.52	B
F-10	64.24	B

F-11	71.08	B
F-12	63.05	B
F-13	74.94	B
F-14	58.88	C
F-15	67.06	B
F-16	67.21	B
F-17	87.97	A
F-18	69.71	B
F-19	86.21	A
F-20	88.16	A
F-21	86.63	A
F-22	58.39	C
F-23	24.71	D
F-24	22.01	D
F-25	10.05	D
F-26	6.04	D
F-27	6.02	D

Robustness to dilution and cloud point temperature measurement

It is important to ensure that uniform emulsions are formed from self-emulsification of SEDDS/SNEDDS at different dilutions. Also, dilution may influence drug release profile and the drug may get precipitated at higher dilutions 'in vivo', which may significantly hinder its absorption[35]. Table 5 depicts the % transmittance of the selected formulations at various dilutions in distilled water, pH 1.2 buffer and pH 6.8 buffer.(Table 5, 6, 7)

The cloud point is the temperature above which a clear formulation turns cloudy(turbid). At temperatures higher than the cloud point, an irreversible phase separation occurs due to dehydration of its ingredients, which may affect drug absorption[15]. Hence, to avoid this phenomenon, the cloud point for SNEDDS should be above body temperature (37 ◦C). From the table (Table 8), it is clear that formulation F-3 is robust to dilution even when diluted by 1000 times. Neither precipitation of the drug nor any phase separation was observed when the SNEDDS was diluted up to 1000 times, showing the stability of the reconstituted emulsion.

Table 5: Robustness to dilution using distilled water as medium

Formulations	50X (%)	100X (%)	250X (%)	1000X (%)
F-2	86.08	86.91	87.01	87.53
F-3	91.24	91.84	92.76	93.58
F-8	92.20	92.46	92.78	93.40
F-17	88.22	88.48	88.96	89.91
F-19	86.74	87.08	87.24	88.09
F-20	88.28	88.63	89.02	90.14
F-21	86.99	87.03	87.58	88.12

Table 6: Robustness to dilution using pH 1.2 buffer as medium

Formulations	50X (%)	100X (%)	250X (%)	1000X (%)
F-2	86.45	86.93	87.04	87.56
F-3	91.18	91.78	92.63	93.41
F-8	92.08	92.32	92.77	93.31
F-17	88.20	88.46	88.82	89.93
F-19	86.70	87.01	87.15	88.09
F-20	88.30	88.61	88.97	89.99
F-21	86.89	86.99	87.44	88.07

Table 7: Robustness to dilution using pH 6.8 as medium

Formulations	50X (%)	100X (%)	250X (%)	1000X (%)
F-2	86.21	86.83	87.06	87.74
F-3	91.22	91.80	92.66	93.40
F-8	92.14	92.37	92.86	93.34
F-17	88.21	88.49	88.86	89.90
F-19	86.73	87.08	87.18	88.11
F-20	88.31	88.66	88.94	89.92
F-21	86.94	87.03	87.48	88.11

Table 8: Cloud point temperature of the formulations

Formulations	cloud point temperature (°C)
F-2	71
F-3	86
F-8	81
F-17	74
F-19	73
F-20	76
F-21	72

Also, the cloud point temperature of the reconstituted SNEDDS formulation F-3 was 86◦C well above body temperature and higher than other formulations that were selected. Hence, formulation F-3 was considered.

Box- Behnken design

The design was applied to formulation F-3. The formulation was further optimised using the design. 13 formulations were obtained (X_1- X_{13}). Details are shown in Table 9,10. The aim of the optimization of pharmaceutical formulations is generally to determine the levels of the variables from which a superior product with high quality characteristics may be produced[36]. The approximation of responses were fitted to linear mathematical model. Coefficients with R^2 closest to 1 and p-value less than 0.05 had a significant effect on the prediction efficacy of the model for the measured response. The approximation of response values of Y1 and Y2 based of the linear model was the most suitable. The values of the coefficients X1, X2 and X3 are related to the effect of these variables on the response. A positive sign of coefficient indicates a synergistic effect while

a negative term indicates an antagonistic effect upon the response obtained[37] (Table 11). The larger coefficient means the independent variable a strong influence on the response. To identify the significance of the effects and interactions between them, analysis of variance (ANOVA) was performed for each parameter.

Table 9: Variables in Box- Behnken design

Factors	Independent variables	Levels (%)		
		LOW (-1)	MID (0)	HIGH (+1)
X_1	Cremophor RH 40	25	30	35
X_2	PEG 400	55	60	65
X_3	PG-12	05	10	15

Dependent Variables

Y_1: Droplet (particle) size, Y_2: Entrapment efficiency

Table 10: Observed responses for the 13 formulations of Box–Behnken design. Y1: droplet (particle) size; Y2: Entrapment efficiency.

Formulations	Y_1 (nm)	Y_2 (%)
X-1	153.24	77.98
X-2	237.11	73.18
X-3	239.54	72.96
X-4	255.64	71.31
X-5	269.54	71.02
X-6	228.32	75.11
X-7	208.32	76.02
X-8	247.71	72.65
X-9	278.63	72.03
X-10	215.86	75.31
X-11	150.75	78.50
X-12	147.12	79.61
X-13	239.54	75.97

From Table 11, it is clear that the effect of the surfactant on globule size is negative i.e. it produces antagonistic effect on the response which means that there is an inverse proportionality between the surfactant and globule size. This implies that on increasing

Table 11: Mathematical relationship in the form of factors coefficients and its corresponding P-values for the measured responses.

Model	Coefficient	Y_1	Y_2
Linear	X1	-15.47	+1.42
	X2	+16.68	-1.13
	X3	+30.01	-1.99
	S.D.	30.81	1.96
	R2	0.5209	0.5775
	p- Value	0.0380	0,0198

surfactant, tendency to produce smaller SNEDDS increases. In case of co- surfactant, the effect is positive on the response. This means that there is direct proportionality between co-surfactant and globule size. This implies that on increasing co-surfactant, a higher tendency to produce larger SNEDDS is observed. Addition of surfactants to the nanoemulsion systems causes the interfacial film to stabilize and condense, while the addition of co-surfactant causes the film to expand[38-40].

It is also clear that the effect of the co-surfactant on entrapment efficiency is negative i.e. it produces antagonistic effect on the response which means that there is an inverse proportionality between co-surfactant and entrapment efficiency. This implies that on increasing co-surfactant, a higher tendency to produce SNEDDS with low entrapment efficiency is observed. In case of surfactant, the effect is positive on the response. This means that there is direct proportionality between surfactant and entrapment efficiency. This implies that on increasing surfactant, a higher tendency to produce SNEDDS with high entrapment efficiency is observed. This means that the addition of surfactant to the formulation helps in increased entrapment efficiency due to the drug getting completely solubilized in the formulation.

Response surface plots based on ANOVA results were constructed to elucidate the statistically significant relationship between the dependent and independent variables previously reported. From Figure 5 (for Y_1 response), we can observe that as the co-surfactant (PEG 400) is increased from lower level to higher level, there is an increase in globule size, but as the surfactant (Cremophor RH 40) is increased from lower to higher, there is a decrease in globule size.

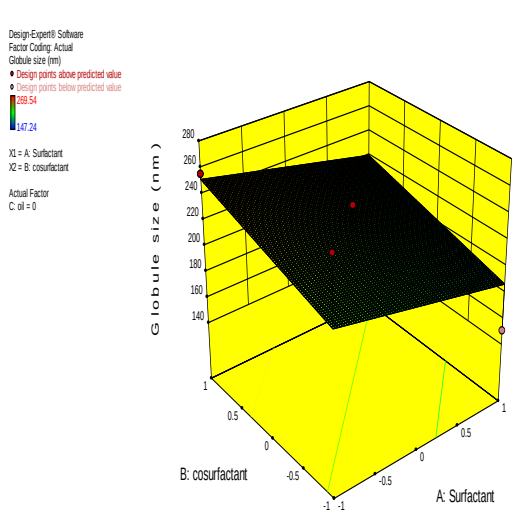

Figure. 5: Response curve when globule size [Y_1(dependent variable)] is considered as a response. Concentration of oil, surfactant and co-surfactant were considered as independent variables.

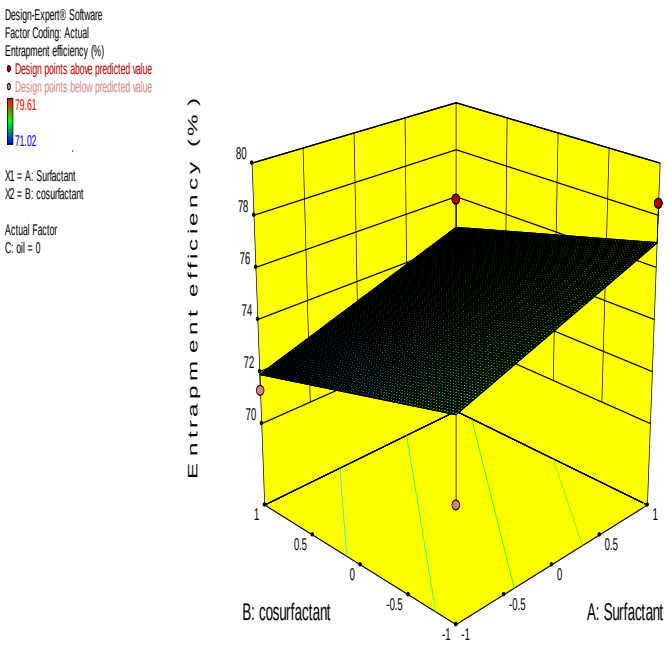

Figure.6: Response curve when entrapment efficiency [Y_2 (dependent variable)] is considered as a response. Concentration of oil, surfactant and co-surfactant were considered as independent variables.

From Figure 6, (for Y_2 response), we can observe that as the surfactant (Cremophor RH 40) is increased from lower level to higher level, there is a sharp increase in entrapment efficiency, but as the co-surfactant (PEG 400) is increased from lower to higher, there is a decrease in the entrapment efficiency.

From the observed responses in Table 10, we can conclude that the formulation X-12 has the lowest globule size of 147.12 nm, highest entrapment efficiency of 79.61% and is transparent. Formulation composes of Cremophor RH 40 (30%), PEG 400 (55%), PG- 12 (05%). At higher concentrations of the oily phase, proportion of the surfactant mix that facilitates water penetration decreases and the mixture becomes more lipophilic with increasing difficulty of emulsification[41]. The concentration of oily phase in this formulation is at the lowest level.

Formulation of S-SNEDDS

Self emulsifying powder was prepared to overcome the disadvantages associated with L-SNEDDS. Hence, to increase the stability and patient compliance, optimized formulation X-12 was adsorbed onto micro crystalline cellulose (MCC) at various carrier loads. The amount of MCC adsorbed to produce the free flowing powder was 1 g MCC/g of SNEDDS formulation (Figure. 7).

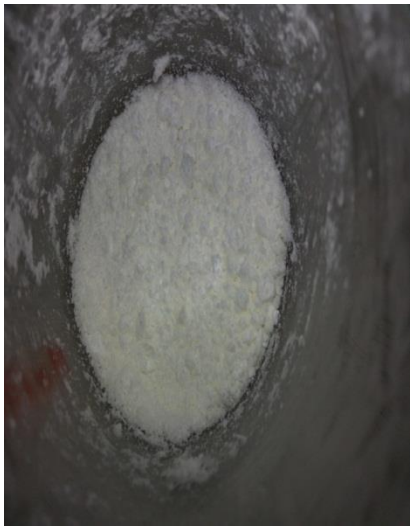

Figure. 7: S-SNEDDS by adsorbing L-SNEDDS on MCC.

Zeta Potential (ζ potential) and Scanning Electron Microscopy (SEM)

The zeta potential of the formulation X-12 was found to be -8.79 mV (Figure. 8). Negative value of zeta potential interprets an increase in electrostatic repulsive forces, thus ruling out the possibility of coalescence. As such, the result also clearly shows that phase separation did not occur, which indicate the formation of stable SNEDDS[27]. SEM studies were carried out to determine the morphology of formulated S-SNEDDS (Figure 9A and 9B). Fig 9A shows the spherical powder particle (MCC) whereas Figure 9B shows small spherical globules of liquid SNEDDDS adsorbed on the MCC.

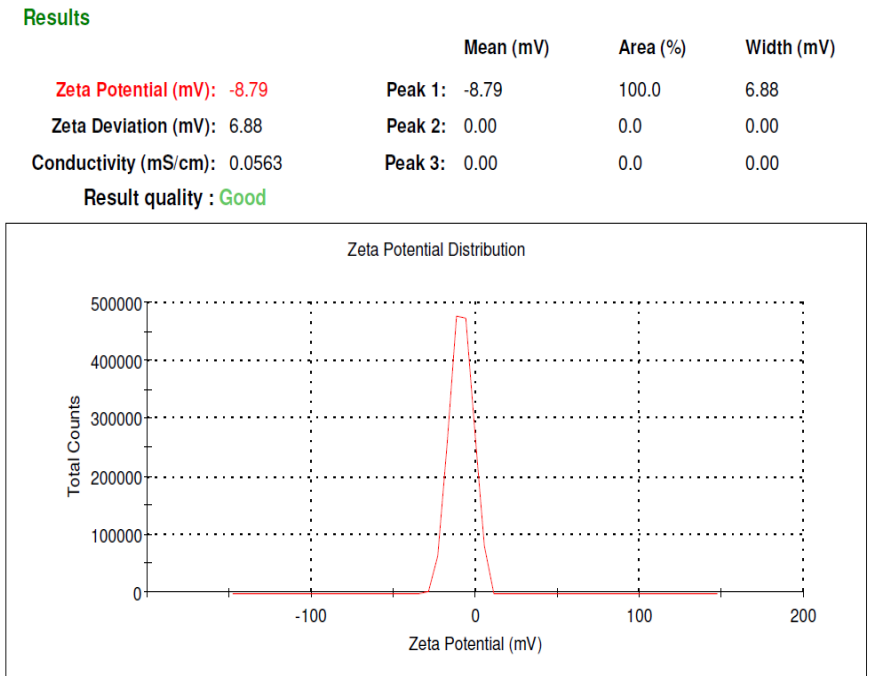

Figure 8: Zeta potential graph of the formulation X-12. Zeta potential found to be -8.79 mV.

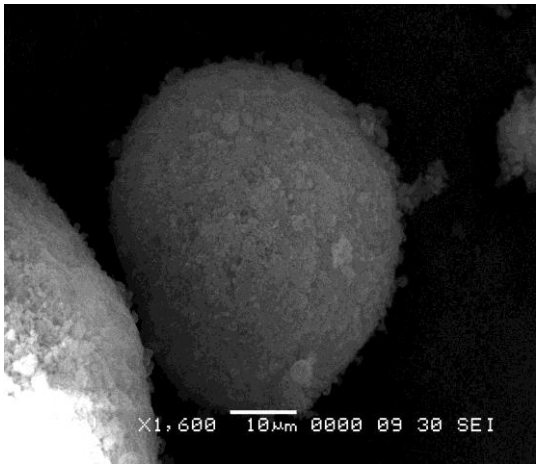

Figure. 9A: Adsorbent (MCC)

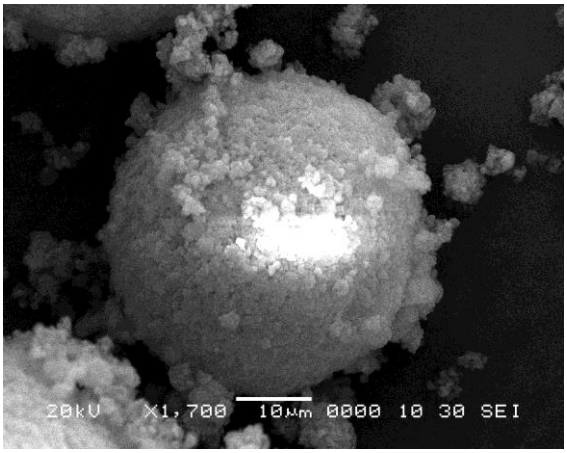

Figure. 9B: S-SNEDDS

Differential Scanning Calorimetry (DSC)

DSC of pure acyclovir and acyclovir loaded S-SNEDDS of formulation X-12 was carried out in order to identify the interactions between excipients and the drug. The melting peak at 252◦C in both the graphs (Figure. 10A, 10B) interprets the melting temperature of acyclovir, indicating no interactions between the drug and excipients[26].

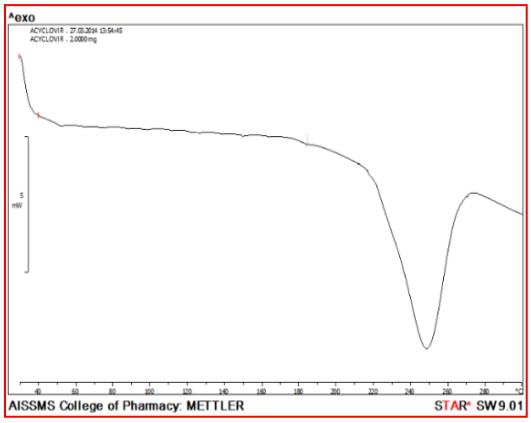

Figure. 10A: DSC of pure drug (acyclovir)

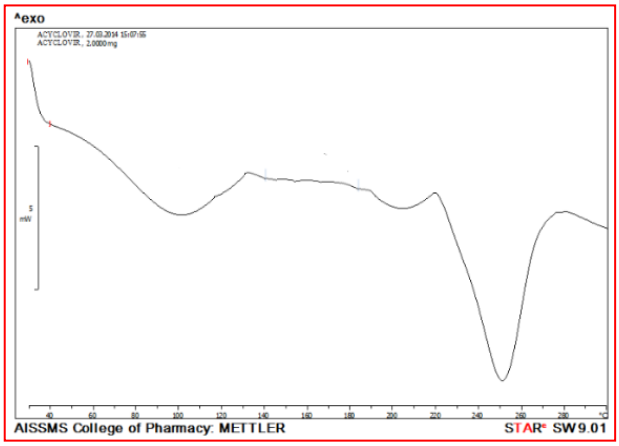

Figure. 10B: DSC of acyclovir loaded S-SNEDDS

***In vitro* drug release studies**

Formulation X-12 shows higher *in vitro* release rate compared to pure drug (acyclovir) as observed in Figure 11A and 11B Extent of acyclovir release from acyclovir loaded S-SNEDDS can be explained by high specific surface area of adsorbents which adsorb L-SNEDDS inside the pores, which limit drug exposure to the surface, thus preventing drug precipitation[42]. It is observed that in both, pH 1.2 buffer medium as well as pH 6.8 buffer medium, there is initially a rapid rate of drug release upto 20 minutes, which is followed by a gradual decrease in the rate of drug release. L-SNEDDS after adsorption can be entrapped in the pores present on the adsorbent, thus lowering the dissolution rate. Gradual decrease in drug release at subsequent time points is due to gradual access of dissolution medium to the L-SNEDDS present in these pores. In pH 1.2 buffer, acyclovir S-SNEDDS showed release of 93% whereas in pH 6.8 buffer, release was 82% in a span of 1 hr.

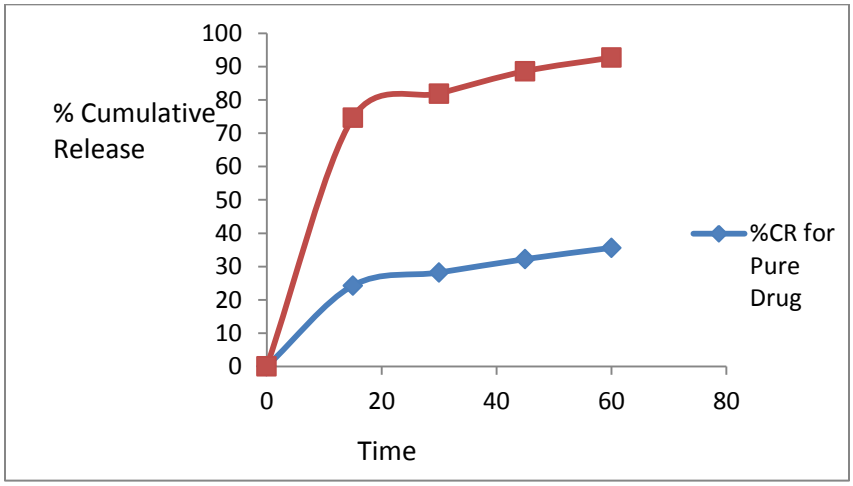

Figure. 11A: Dissolution profile of acyclovir and acyclovir loaded S-SNEDDS in pH 1.2 buffer.

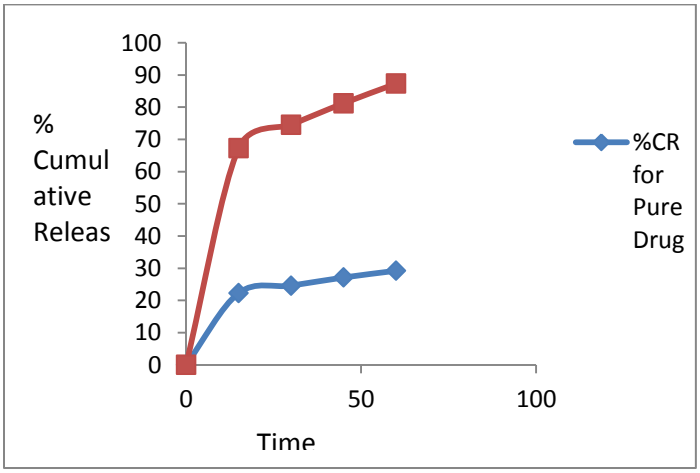

Figure. 11B: Dissolution profile of pure acyclovir and acyclovir loaded S-SNEDDS in pH 6.8 buffer.

Therefore, the presentation of acyclovir at the molecular level in the form of the nano-emulsion formulation led to an increased solubilization and enhanced drug release. This finding also supports the hypothesis that nanosized droplets of emulsion can enhance the release of poorly soluble drugs [19].

Accelerated stability studies

Table 12 shows the insignificant variations in the values of % transmittance and drug content during 6 months storage of the acyclovir loaded S-SNEDDS. No change in the physical appearance of formulation X-12(S-SNEDDS) was observed during the stability studies and the powder remained colourless (white) with no signs of decolouration. This indicated that there was no drug excipient interaction even at accelerated stability conditions (40 ± 2 ◦C/75 ± 5% RH). Also, there was very miniscule variations in values of % transmittance and drug content of the formulation over a period of 6 months, indicating that the formulation remained stable (Table 12).

Table 12: Stability study of formulation X-12 S-SNEDDS at 40 ± 2 ◦C/75% ± 5% RH.

Time (Months)	Appearance	% Transmittance	Drug content (%)
0	White (no decolouration)	95.28	79.61
1	White(no decolouration)	95.28	79.61
3	White(no decolouration)	95.26	79.60
6	White(no decolouration)	95.24	79.59

CONCLUSION

The L-SNEDDS of acyclovir was prepared by incorporating PG-12 as an oily phase, Cremophor RH 40 as surfactant and PEG 400 as co-surfactant. The formulation with 05% oil, 30% surfactant and 55% co-surfactant proportion was optimized utilising the Box- Behnken design. The L-SNEDDS was concerted to S-SNEDDS (powder form) by adsorbing the liquid on MCC. The negative zeta potential of the S-SNEDDS showed the presence of electrostatic repulsive forces. This proved that there was no coalescence within the formulation. The DSC graphs clearly showed the melting peak of the drug, indicating no interactions between the drug and excipients. *In vitro* release studies implied that acyclovir S-SNEDDS formulation showed superior release than the pure drug. The miniscule change in % transmittance and drug content with no change in formulation appearance meant that the formulation remained stable even at accelerated stability conditions.

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