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Original Article

DESIGN AND EVALUATION OF SELF-MICRO EMULSIFYING DRUG DELIVERY SYSTEMS OF ACYCLOVIR

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ABSTRACT

Objective: The aim of this this study was to increase the solubility and permeability of Acyclovir by formulating in to self-microemulsifying drug delivery system (SMEDDS).

Methods: The Saturation solubility of Acyclovir was studied in various oils,surfactants and co-surfactants and pseudo ternary phase diagrams were drawn to identify self-emulsifying region.Based on solubility and phase diagram, prototype formulations were prepared by varying the amount of oils and ratios of surfactantsand co surfactants. The formulations were evaluated for self-emulsification time, drug loading capacity, *in-vitro* diffusion. Based on comparative results the optimum formulation was selected. The optimized formulation was evaluated for globule size,zeta potential and thermal stability.

Result: Tween was selected as surfactant, Transcutol as co surfactant and Oleic acid as oil component based on solubility study. All the prepared formulation exhibited self-emulsification properties. The optimized formulation contained Acyclovir (40 mg), Tween 80 (56.25%), Transcutol (18.75%) and Oleic acid (25%). The *in-vitro* diffusion of SMEDDS was significantly higher as compared to marketed product. No marked changes in physical, emulsification and chemical attributes were observed on stability

Conclusion: Based on this study, it can be concluded the solubility and permeability of Acyclovir can be increased by formulating into SMEDDS.

Keywords: I*n-vitro* diffusion, Zeta potential, Stability, Tween 80

INTRODUCTION

Acyclovir is used orally for the treatment and prophylaxis of initial and recurrent episodes of genital and for acute treatment of herpes zoster and varicella (chickenpox) in immune competent individuals[1,2].Acyclovir is slightly soluble in water and solubility values range from 1.2 to 1.6 mg/mL [3,4].Acyclovir is available in capsules (200 mg), tablets (200, 400 and 800 mg) and ointments; predominately tablets of 200 mg are used five times a day [5]. Long term administration is required in immune compromised patient with relapsing herpes simplex infection [6].

Acyclovir absorption in the gastrointestinal tract is slow, variable and incomplete. The bioavailability of acyclovir after oral administration ranges from 10% to 30%. Approximately 80% of an oral dose is never absorbed and is excreted through faeces. The main excretory organ for acyclovir is the kidney. The plasma half-life of oral acyclovir on average is 3 hours in adults with normal renal function[7].

The poor bioavailability (BA) is considered to be a result of the characteristics of the drug itself and not its delivery vehicle[8]. Its absorption occurs mainly by passive diffusion mechanism and is slow, variable and incomplete. Some studies suggest that increasing doses results in decrease in BA[9] or less than proportional increases in Cmax and it has been suggested that this behaviour may be due to saturable carrier system or limited area for absorption in the gastro-intestinal (GI) tract or to low solubility[10].The Acyclovir undergoes metabolism to a small extent and it is not highly permeable according Caco-2 permeability data and log P_{oct}values[11].

There is a need to develop novel oral formulation of Acyclovir which increases its solubility and enhances permeability across the biological membrane to overcome its poor bio availability.

SMEDDSs are isotropic mixtures of oils, surfactants and co surfactants. These forms emulsion on contact with an aqueous phase under gentle agitation[12].The advantages of these systems are improved drug solubilisation and absorption due to drug present in dissolved form and large interfacial surface area. The SMEDDS form transparent micro emulsion with droplet size lesser than 100 nm. The droplet size of the emulsion is a critical factor in self-emulsification performance because it determines the rate and extent of drug release as well as absorption[13,14]. Upon aqueous dilution the lipid formulation forms lipid droplets ranging from 100 nm (SEDDS) to less than 50 nm (SMEDDS). The optimum concentrations ranges of oil, surfactant and co-surfactant necessary to form self-emulsification is determined by ternary phase diagram[15].

The ability of the drug in solubilized form is influenced by its solubility in oil phase and capacity of surfactant and co-surfactants. The surfactants are amphiphlic in nature and are capable of dissolving and solubilizing high quantities of hydrophobic drugs. The hydrophilic co-surfactants are preferable in lipid formulations which are known to reduce the oil/water interface and allow the spontaneous formation of micro emulsion[16].

The objective of this study was to develop and characterize SMEDDS of Acyclovir for increasing solubility and permeability across the biological membrane to improve the bioavailability, dosing frequency and enhance patient compliance.

MATERIALS AND METHODS

Materials

Acyclovir was received from Macleodspharmaceuticals, Mumbai. All the surfactants and co surfactants were received from Gattefosse and Oleic acid from Lobachemiepvt.ltd. Hard gelatin capsules were received from Associated Capsules, Mumbai, India and all other materials were purchased from SD fine chemicals. All the excipients and reagents were used as received. Double distilled water was prepared freshly and used whenever required.

Methods

Saturation solubility study

Saturation solubility of Acyclovir in various oils, surfactants and co-surfactantswas determined by shake flask method. The list of vehicles used and their solubility results are mentioned in table 1. In this study, an excess amount of Acyclovir (approximately 100 mg) was added to 2 ml of each vehicle in screw-capped glass vials. The mixture was mixed using cyclo mixer to get uniform slurry;vials were fixed in to flask shaker and stirred for 72 hrs. The samples were centrifuged at 4500 rpm for 10 min to separate the supernatant. Aliquots of supernatant were taken, filtered through syringe filter, filtrate was suitably diluted with methanol and drug content was quantified by measuring absorbance at 255 nm using UV spectroscopy.

Table 1: Saturation solubility of Acyclovir in various vehicles

Name of the vehicle	Solubility in mg/mL
Surfactants	
Labrasol	0.04
Gelucire 44/14	0.62
Span 80	6.09
Tween 80	86.14
Co-surfactants	
Transcutol	4.78
Propylene glycol	4.21
Glycerol	7.96
Lutrol E-400	3.28
PVA 1%	1.51
Transcutol:Glycerol (1:3)	20.59
Oils	
Labrafil	0.34
Plurololeique	0.28
Capryol 90	0.36
Eucalyptus oil	25.28
Oleic acid	19.66
Isopropyl myristate	0.38
Castor oil	0.80

Construction of Pseudo ternary phase diagram

The Pseudoternary phase diagrams with oil (Oleic acid), three different ratios of surfactant:co-surfactant (Tween 80:Transcutol) and water were developed using water titration method. The phase diagrams were developed with 1:3, 2:1 and 3:1 ratios of surfactant and co surfactants. The procedure consists of taking specific ratio of surfactant/co-surfactant and adding varying amount of oil mixing well. Each mixture was titrated with water, observe for clarity and record the observation. The appearance of clear solution indicates the formation of emulsion and appearance of hazy solution indicates non-emulsion[17].From the diagram the maximum and minimum ratios of individual component required for getting emulsion region are noted and the same knowledge was used in designing the prototype formulations.

Effect of drug on phase diagram

This was done to investigate the effect of acyclovir on self-emulsifying performance of SMEDDS. Approximately 50 mg of Acyclovir was added to 1 ml ofboundary formulations of SMEDDS and checked for formation of clear solution.

Prototype formulation

Prototype formulations were prepared by varying Oleic acidand ratios of mixture of Tween 80 and Transcutol. The formula composition is mentioned in table 2. In the first trial the Oil was used at 10% and increased by 5% for each subsequent trial up to 40%. The ratio of surfactant to co surfactant was maintained at 3:1.

The process used for preparation of mixture is as follows: Accurate quantity of oil and ratios of surfactant and co-surfactant were taken and mixed well and then drug was added stirred for 15 min. The mixture was heated to 30-40°C till the drug gets solubilized.

The drug loading capacity of each mixture was determined by adding the excess drug to each prototype mixture till the clear solution was obtained. The solution was filtered, diluted and measured the absorbance at 255 nm using UV-Visible spectroscopy. As per drug loading capacity of prototype formulation, calculated quantity of drug was added to each prototype formulations. The preparation containing 40 mg of Acyclovir was filled into capsules and analysed for emulsification and other attributes.

Self-emulsification propertyand self-emulsification time

Few ml of prototype formulation (approximately 1 ml) was added to 250 ml of purified water, stirred gently and checked for clarity of solution. Self-emulsification time of formulation was determined using USP II dissolution apparatus. 1 ml of formulation was added drop wise to 250 ml of purified water at 37˚C, gentle agitation was provided by dissolution paddle rotating at 50 rpm/min.[18].Time taken for formation of clear solution was noted as self-emulsification time and target time was fixed at 1 minute.

In-vitro diffusion

The *in-vitro* diffusion of prototype formulation wasdetermined by dialysis method. The formulation containing 50 mg of Acyclovir was taken in a dialysis tube and immersed in beaker containing 200 ml of 0.07 N HCl. The contents were stirred at 150 rpm to maintain sink condition. The aliquots were taken at predetermined intervals from release medium and replaced with same volume of fresh medium[19]. The samples were filtered through 0.22 µm syringe filter and absorbance was measured at 255 nm to determine the drug content. Similarly in-vitro diffusion of marketed tablet product 200 mg of Acyclovir was studied.

Globule size of analysis[20,21].

The globule size and distribution was determined by dynamic light scattering apparatus (Malvern Zetasizer Nano ZS 170 version 7.02). The optimised SMEDDS formulation was diluted 250 times with 0.1 N HCl / distilled water under gentle stirring. After achieving equilibrium, the emulsions were analysed by Zeta sizer. A laser beam at 632 nm wavelength was used and light scattering was monitored at 25^0c at 90^0.

Zeta-Potential and Conductivity

The surface charge on emulsion droplets and their mean zeta potential were determined using Malvern Zetasizer(Make: Malvern Instruments, UK, Model: ZetasizerVer 7.02). The magnitude of zeta potential gives an indication of potential stability of the formulation.

1 ml of SMEDDS was diluted by 10 times and 100 times with distilled water in beaker with constant stirring on a magnetic stirrer. Zeta-potential and electrophoretic mobility of the formulation was determined.

Stability

The hard gelatin capsules of optimised formulation containing 50 mg of Acyclovir were kept at 40˚C, 25˚C and in refrigerator for stability study. The samples were withdrawn at 15, 30, 45 and 60 days and observed for any physical changes like capsule leaking, colour change, shrinkage and tested for self-emulsification time and in-vitro diffusion and % Assay[22].

RESULT AND DISCUSSION

Saturation Solubility Studies: The saturation solubility of Acyclovir in various oils, surfactants and co-surfactants are tabulated in table 1.Based on the relative solubility data, Oleic acid was selected as oil, Tween 80 as surfactant and Transcutol as co surfactant.

Pseudo ternary phase diagram

The pseudo ternary phase diagrams were constructed to identify the self-emulsifying regions and to optimize the concentration of oil, surfactant and co-surfactant. The series of mixtures were prepared and their self-emulsifying properties were observed visually. The surfactant and co surfactant ratios of 1:3, 2:1 and 3:1 were evaluated and accordingly graphs were drawn as shown in fig 1 to 3. The

emulsification area is denoted by closely drawn rectangles and non-emulsification area by broadly drawn vertical and horizontal lines. The emulsification area obtained with the surfactant to co surfactant ratio of 3:1 was quite larger as compared to other ratios. Hence this ratio was used in the prototype formulation.

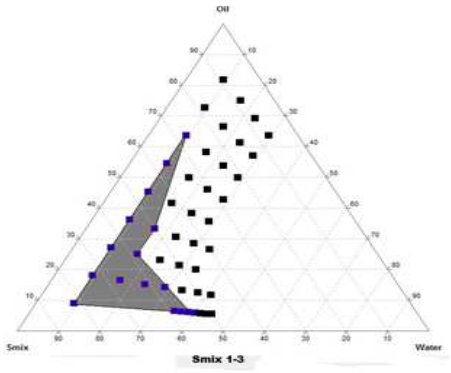

Fig. 1: Pseudo ternary phase diagram (1:3)

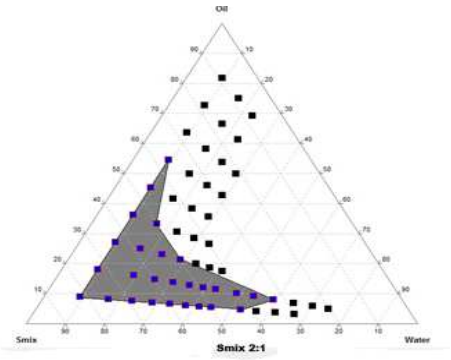

Fig. 2: Pseudo ternary phase diagram (2:1)

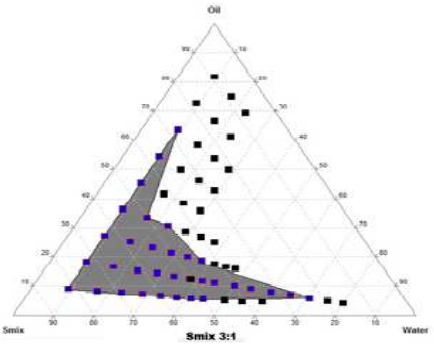

Fig. 3: Pseudo ternary phase diagram (3:1)

Effect of Drug on Phase Diagram

The presence of drug does not showed any marked effect on self-emulsifying performance of the formulations as compared to formulation without the drug.

Prototype formulation

Totally 7 prototype formulations were prepared by varying Oil and ratios of Tween 80 and Transcutol as mentioned in table 2. The formulations were clear and moderately viscous.

Table 2: Formula composition of prototype formulations

Formulation code	Oil (%v/v)	% Tween 80	% Transcutol
F1	10	67.5	22.5
F-2	15	63.75	21.25
F-3	20	60	20
F-4	25	56.25	18.75
F-5	30	52.5	17.5
F-6	35	48.75	16.25
F-7	40	45	15

Evaluation of SMEDDS

Self-emulsification property and emulsification time

All the prototype formulations formed clear solution within a minute.

Drug loading capacity

The drug loading capacity of formulation coded with No. F-4was 40.73 mg/ml and was highest as compared to other formulations; hence this formulation was considered as optimised formulation.

***In-vitro* diffusion**

The in-vitro diffusion of all the prototype formulations was similar (fig 4). The same study was conducted with marketed tablet of 200 mg. The in-vitro diffusion of optimised SMEDDS was significantly higher as compared to marketed product; the graph is shown in fig 5. The faster in-vitro diffusion of the SMEDDS might be due to formation of smaller droplets on contact with aqueous medium.

Globule Size Analysis

The globule size of optimum formulation on addition to water was determined using Zetasizer. The average droplet size range of self-emulsified system was 96.17 nm, the graph is shown infig 6. The result indicates that SMEDDS produced nano emulsion.

Zeta-Potential and conductivity

Zeta potential of the system was -17.6 mV and conductivity of 0.0601 mS/cm. The results of zeta-potential and conductivity are shown in Fig 7.

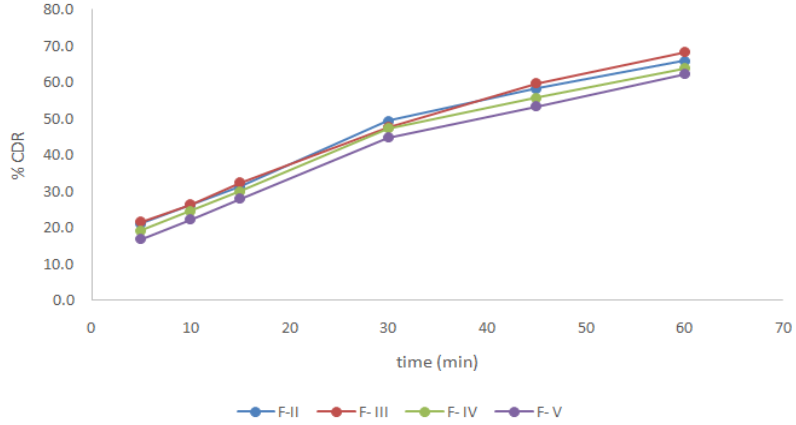

Fig. 4: In-vitro diffusion of prototype formulations

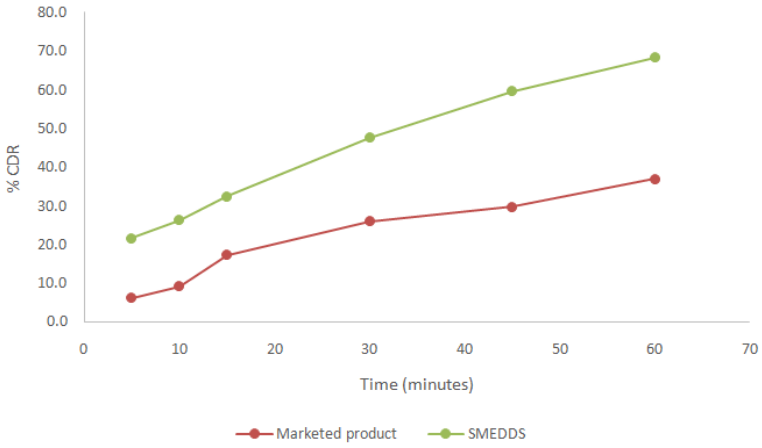

Fig. 5: Comparative in-vitro diffusion of optimised SMEDDS and marketed tablet

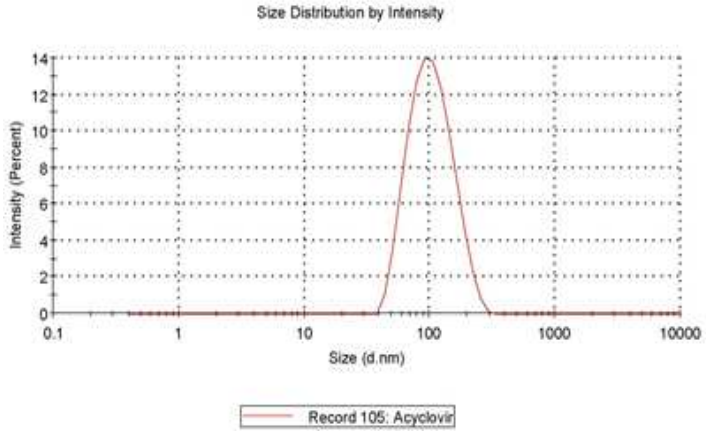

Fig. 6: Globule size analysis of SMEDDS by Malvern

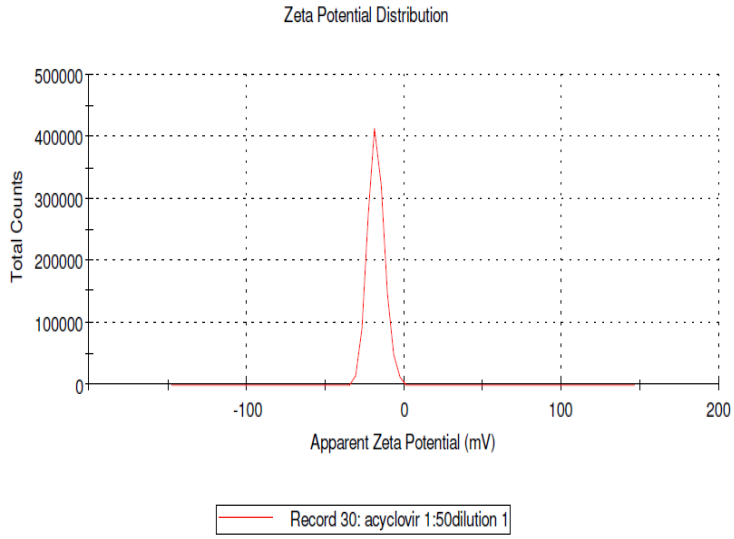

Fig. 7: Zeta potential of SMEDDS by Malvern

Stability

No evidence of phase separation or any flocculation or precipitation was observed andformulation retained the self-emulsification properties and no changes in in-vitro diffusion were observed on stability up to 60 days at 40°C.

CONCLUSIONS

The stable SMEDDS of Acyclovir was prepared to enhance the solubility and permeability to overcome difficulties in clinical usage of the drug. On analysing saturation solubility study result and pseudo ternary phase diagram, the oil, surfactant and co surfactant were selected.All the prepared formulations exhibited the self-emulsification properties. The optimised formulation was evaluated for zeta potential, conductivity, globule size analysis and stability study. The results suggest that the optimised formulation was stable and produced nano emulsion on addition to water. The in-vitro diffusion study of the SMEDDS formulation was higher as compared to marketed product, indicating that the prepared formulation is having higher solubility and permeability. Thus it can be concluded

that micro emulsion formulation can be used as a one of the formulation technique to enhance the bioavailability of the poorly soluble and permeable drugs. The in-vivo study in lab animals shall be carried out to confirm the improvement in the bioavailability.

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