DEVELOPMENT OF AN ORAL FORMULATION CONTAINING POORLY ABSORBED ANTIVIRAL DRUGS FOR IMPROVEMENT OF BIOAVAILABILITY

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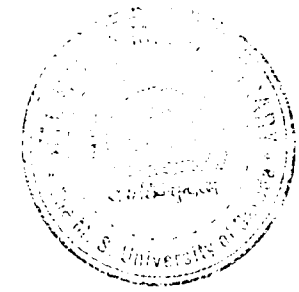

Summary and conclusions

Poorly soluble drugs have motivated the development of drug delivery technologies to overcome the obstacles to their solubilization through either chemical or mechanical modification of the environment surrounding the drug molecule, or physically altering the macromolecular characteristics of aggregated drug particles. These technologies include both traditional methods of solubility enhancement, such as particle size reduction via comminution and spray drying, addition of surfactants and inclusion in cyclodextrin-drug complexes, and the use of more novel mechanisms such as self-emulsifying systems like microemulsion, micronisation via nanoparticles, pH adjustment and salting-in processes.

Anti viral therapy including AIDS is the most frightening health issue being faced in developed/ developing countries and is considered to be the biggest calamity-facing mankind. The disease primarily affecting the immune system. Most of the drug used for the antiviral therapy facing tremendous problem over oral bioavailability and have severe toxic effects like nephrotoxicity, neutropenia, thrombocytopenia, renal impairment etc. .

Acyclovir absorption in the gastrointestinal tract is slow, variable, and incomplete. The bioavailability of acyclovir after oral administration ranges from 10-30%. (C. Fletcher, 1995). Approximately 80% of an oral dose is unabsorbed and is excreted through feces. Efavirenz absorption is also slow and incomplete and also varies in presence of foods. Peak efavirenz plasma concentrations of 1.6-9.1 μM were attained by 5 hours following single oral doses of 100 mg to 1600 mg administered to uninfected volunteers. (Bruce J. Aungst et al, 2002) Dose-related increases in Cmax and AUC were seen for doses up to 1600 mg; the increases were less than proportional suggesting diminished absorption at higher doses.

Microemulsions and self-emulsifying systems have emerged as potential solubility enhancing technologies, whose solubilising and absorption promoting effect is thought to lay in the reactivity of triglycerides and surfactants with the walls of the gastrointestinal tract. Traditionally, long- and medium-chain triglycerides (LCTs and MCTs, respectively) have been employed with surfactants to incorporate drugs into self-emulsifying systems. The growth of self-emulsifying drug delivery systems in recent years has resulted in the optimization of several methods of solubilising active

compounds using novel, synthetic MCTs and co-solvents in addition to non-ionic surfactants. Non-ionic surfactants, such as Tweens (polysorbates) and Labrasol (caprylocapryl macrogol glycerides), with low hyrophile-lipophile balances (HLB) are often used to ensure immediate formation of oil-in-water (o/w) droplets during production. Ampiphillic, non-ionic surfactants allow higher degrees of drug solubilization to occur and may prevent the precipitation of drug out of the microemulsion *in vivo*.

Besides the microemulsion technology, Solid lipid nanoparticles (SLN) show great promise for enhancing the oral bioavailability of some of the most poorly absorbed components and the simultaneous digestion. SLN are an alternative carrier system to polymeric nanoparticles with increasing attention from different research groups SLN combine advantages of polymeric nanoparticles (solid matrix for controlled release), emulsions and liposomes (physiological material of high toxicological acceptance, facility of industrial scale production by high pressure homogenization). The areas of application are very broad. The SLN can be incorporated in topical or ophthalmic formulations or administered perorally for controlled release inside the gastrointestinal tract (GIT). Their matrix can protect drugs against chemical degradation, the general adhesive properties of small particles to the gut wall opens the perspectives of less variable and/or enhanced bioavailability of drugs. Intravenous administration of SLN can be used for the delivery of poorly water-soluble drugs avoiding toxicologically less acceptable solubilize excipients (e.g. Cremophor EL) or in general for drug targeting similar to polymeric nanoparticles.

The present investigations describe the preparation and evaluations of microemulsion and SLN as carriers for anti-viral drugs. The contents of the thesis mainly divided into three parts. Part I consists of introduction, literature review and profile of drugs for the study. Part II involves method development, preparation and characterization of microemulsions containing acyclovir and efavirenz. Part III describes the preparation and characterization of SLN preparation of acyclovir and efavirenz.

Part I: Introduction and Literature review on colloidal drug delivery system and drugs.

Part I of the thesis divided into three chapters. Chapter 1 consists of introduction and proposed plan of work. Chapter 2 shows the literature review on colloidal drug delivery systems mainly on microemulsions and SLN. It also consists of profile of drugs (acyclovir and efavirenz) used in this study.

Part II Method development, preparation and characterizations of microemulsions and SLN:

Part II divided into 6 different chapters

Chapter 3: Method development for acyclovir and efavirenz in formulations and rat plasma.

Chapter 3 describes the development of analytical methods for the estimations of acyclovir and efavirenz in different vehicles by different analytical techniques. Spectrophotometric methods for both drugs used for evaluations of drug content in the drug incorporated microemulsions and SLN. High performance liquid chromatography methods used for estimating the amount of drug present in blood plasma. A selective, sensitive and reproducible high performance liquid chromatographic method was developed for the separation and determination of acyclovir in pharmaceuticals from biological samples and rest of excipients and materials used to prepare the dosage form. The analytical method was subjected to statistical analysis and evaluated for the accuracy and precision. The precision and accuracy of the proposed methods are within the acceptable limits and the quantization limit is also low. The method involves less time consuming sample preparation steps. The mobile phase used for the plasma and tablet sample was 20mM Ammonium acetate with 5mM Octane sulphonic acid sodium salt in water (pH adjusted to 3 by acetic acid) -methanol (98:2, v/v). Flow rate was adjusted to as follows: 1ml/min (0-14min), 2ml/min (16-30 min) and then again 1ml/min (30-50min). Detection wavelength fixed at 254nm. Under these conditions acyclovir eluted at ~10.6minute. Linear relationship was observed in the range between 10ng/ml to 5µg/ml

in water and 100ng/ml to 5μg/ml in plasma and correlation coefficient value was 0.9995 and 1 for water and plasma sample respectively. % Bias of all the sample was <10%, proves the accuracy of the method. This method permits the determination of acyclovir in a small amount of rat plasma without any complication. The method was shown to be highly reproducible and it seems to be adequate for routine therapeutic monitoring. It could be used without any interference form surfactants, tablet excipients and endogenous substances from the plasma samples. Chapter 3 also involves the method development for the estimation of efavirenz in rat plasma. The mobile phase used for the plasma sample was 58% acetonitrile in 0.1% ammonium bicarbonate (NH_4HCO_3), pH 7.4 in water (pH adjusted to 7.4 by 0.1N HCl or by 0.1N NaOH) - Flow rate was adjusted to 1 ml/min. The HPLC effluent was monitored at 247 nm. The column was washed with 90% acetonitrile in NH_4HCO_3 for 3 min at the end of each run. Overall run time was 15 min per injection. The retention time for the efavirenz in mobile phase was found at approx 8.0min. Linear relationship was observed in the range between 100ng/ml to 5μg/ml in mobile phase and in plasma and correlation coefficient value was 0.9999 and 0.9995 for water and plasma sample respectively. These methods can be considered to be of real interest for the rapid and reliable clinical, pharmacokinetic studies of acyclovir and efavirenz.

Chapter 4: Preparation and optimization of microemulsion:

Chapter 4 describes the preparation and optimization of microemulsion containing acyclovir and efavirenz. Solubility of the drug was evaluated in different oil and surfactant. The particular oil and surfactant showing the maximum solubility for the drug was taken for evaluation. After selecting the suitable oil and surfactant, the appropriate surfactant and cosurfactant and their ratio was found out by estimating the interfacial tension using spinning drop tensiometer and by Pseudoternary phase diagram study

For the preparation of microemulsions, ratio of surfactant to cosurfactant (Km) was kept constant. The required quantity of oil phase was taken in screw-capped test tube. Then known quantity of drug was mixed and dissolved into it by vortexing. Then required quantity of surfactant and cosurfactant at a fixed ratio was added into the above mixture, which was followed by through mixing. The resulted mixture was titrated against

distilled water to check the transparency of the system. The mixture was shaken after each addition of water for a short time (about 1 min) by hand or by using a Vortex mixer. The experiment was carried out at room temperature (25 ±2 °C).Different surfactant to cosurfactant ratio (km) like 4:1, 3:1, 2:1, 1:1, 1:2, 1:0 (no surfactant) was tried to evaluate the phase diagram.

For acyclovir microemulsion: Tween 80, Labrasol was used as surfactant, plurol olique, propylene glycol was used as cosurfactant and labrafac was used as oil phase. The effect of Km and labrafac content on the maximum water uptake to form microemulsion was evaluated. The lower the concentration of labrafac, higher water uptake takes place and optimum formulation achieved when Km = 2 and labrafac: STmix= 0.25. From Pseudoternary phase diagram study, it observed that the larger zone of microemulsion was found when Km=3:1, 2:1 as compared 4:1 for Labrasol, plurol olique, labrafac and water system (System A). The similar microemulsion zone was found for System B, when Km is either 3:1 or 2:1, which are greater as compared to when Km=1:1 or 1:2. (Tween 80, propylene glycol, labrafac and water system). For both the system, entrapment of drug was estimated. It was observed that, the stable microemulsion formulation achieved when concentration of acyclovir was 10mg/ml of the formulation,

For efavirenz microemulsion formation takes place when 4:1≥ Km > 1:2 in Labrasol transcutol, labrafil M 1944 CS and water system (System C) and Cremophor RH 40, propylene glycol, labrafil M 1944CS and water system (System D). The greater microemulsion zone was found when Km is either 4:1 or 3:1 as compared to when Km=2:1 or 1:1 or 1:2 for system C. Similarly, the larger zone was found for system D, when Km= 3:1 or 2:1, as compared to when Km=1:1 or 1:2. The stable microemulsion formulation achieved when efavirenz concentration was 20mg/ml of the formulations.

Chapter 5: Microemulsion Characterisations:

Chapter 5 describes physical and chemical characterisations of microemulsions, which are very important for a meaningful comparison of different microemulsion formulations. The prepared microemulsion was characterized for particle size and distribution studies by Zetasizer, viscosity studies by Brookfield viscometer, electrical conductivity by a

conductometer, surface charge by Zetasizer, effect of dilution on particle size, % transmittance and refractive index, pH and morphological behavior by transmittance electron microscopy (TEM) etc.

In case of system A, 100% acyclovir was incorporated into the formulation, whereas 99.03 % entrapment was achieved for system B. For efavirenz, 100% drug entrapment observed for both the system C and D. Percolation behavior observed for System A only where there is a sudden increase of electrical conductivity takes place on water addition. For system A, addition of 10% water increases viscosity from 51.24cp to 60.46cp when Km=4:1, 61.35 to 68.47cp when Km=3:1and from 77.38 cp to 82.42 when Km=2:1. For system B, upon gradual addition of water, viscosity of the system decreases irrespective of Km. Similar observations was made for efavirenz microemulsion for system C and D. From electro-conductivity and viscosity measurement, it was observed that all the prepared microemulsion systems are w/o type.

All the prepared microemulsion shows a particle size of less than 100nm. For acyclovir microemulsion (system A) when Km=3, particle size increases to 458.65 and 463.58nm when the microemulsion pre-concentrate was diluted by 100 times with water and simulated gastric fluid (SGF) respectively. But for system B, the particle size increases to 64.25 and 69.85 nm only, by 100 times dilution with water or SGF respectively. For System C, the value was 389.8 and 414.4nm and for system D the value was 22.4 and 25.5nm. All the prepared microemulsion shows a positive surface charge. Besides this all the prepared microemulsion shows % transmittance > 96% and refractive index close to 1.333, which proves the transparency of the systems. The pH of the system was in the range of 6 -8, which is suitable for oral administrations. The TEM studies show that the nanometric spherical particles are dispersed throughout the system. Stability study of the system was also done by visual observations, centrifugation, and freeze thaw cycle. In all the cases the formulation shows quite stable. It was also observed that the particle size of the formulation was does not increase when it was stored at normal temperature for a period of six months.

Chapter 5 also consists of in-vitro diffusion study of the prepared microemulsions. It was observed that maximum absorption takes place when Km was 4:1 as compared to when

Km=2:1 or 3:1 for system A. After 5 hr of diffusion study only 22.1% of drugs got diffused from pure drug suspension. At similar time 66.5% 96.0, 84.6%, and 80.2% of acyclovir got diffused from tablet, Km=4:1, Km=3:1 and Km=2:1 respectively. For system A, when Km=4, the maximum flux rate (J), permeation constant (P) and diffusion coefficient (D) was observed and the corresponding value was 94.33 ($\mu g\ cm^{-2}\ min^{-1}$), 0.023608 ($cm\ min^{-1}$), 1.51 E-05 ($cm^{-2}\ min^{-1}$) respectively. Whereas as for system B, thé maximum diffusion occurs when Km=2 and thé observes value for J, P and D was 102.03 ($\mu g\ cm^{-2}\ min^{-1}$), 0.025508 ($cm\ min^{-1}$), 1.56 E-05 ($cm^{-2}\ min^{-1}$). For efavirenz microemulsion : in system C maximum diffusion takes place when Km=4 and the obtained value for J, P, D was 117.8($\mu g\ cm^{-2}\ min^{-1}$), 0.02945 ($cm\ min^{-1}$) and 1.49E-05($cm^{-2}\ min^{-1}$) whereas for commercially value obtained was 59.829($\mu g\ cm^{-2}\ min^{-1}$), 0.014957($cm\ min^{-1}$) and 4.80E-06($cm^{-2}\ min^{-1}$).

Chapter 6: Preparation and characterization of SLN:

Taguchi orthogonal experimental design was applied for the preparation of SLN using High Pressure Homogenization (HPH) technique. Taguchi orthogonal experimental design [$L_9(3^4)$] varying four independent variables, type of lipid, concentration of lipid molar ratio, type of surfactant and concentration of surfactant at three levels. Taguchi's signal to noise ratio was used for finding the optimum levels. The concept ANOVA was further used to find the optimum levels of the factors.

Briefly the preparation of SLN as follows: the drug, lipid and surfactant were separately weighed in different ratios in a beaker. Hydrophilic surfactants (Sodium deoxycholate, Polaxomer 188 or their combinations) were separately weighed in another beaker and dissolved in distilled water. The drug and lipid mixture was heated till complete melting of all lipid ingredients. Simultaneously, the aqueous surfactant containing phase was also heated to the same temperature as the melt. The drug containing lipid melt was then added to the hot aqueous phase under high speed stirring to form an initial pre-emulsion. This pre-emulsion was subsequently homogenized in a heated High Pressure Homogenizer maintained at 70-80°C in a water bath. The homogenization was carried out at high pressures of about 10000 psi for three cycles, wherein the pre-emulsion is pushed through a narrow gap (in the range of a few microns). The fluid accelerates on a

very short distance to a very high velocity (over1000 Km/h). Very high shear stress and cavitations forces disrupt the particles down to the submicron range, giving a hot nano-emulsion which contains liquid lipid droplets. This nano-emulsion was allowed to cool down to room temperature which resulted in the re-crystallization of the lipid back to the solid state giving an SLN dispersion containing drug entrapped solid lipid nanoparticles suspended in an aqueous medium.

The process validation of the SLN formulations was carried by changing homogenization pressure, number of homogenization cycle. Optimization of drug loading was checked by changing the drug load on the lipidic core. Reproducibility of the formulation was also carried out by taking similar formula at six different days.

The prepared SLN was evaluated for particle size distribution by Malvern particle size analyzer, drug entrapment efficiency, lyophilization of nanoparticle dispersion with cryoprotectant. The surface morphology was evaluated by SEM. Differential Scanning Calorimetry (DSC) studies were conducted for optimum batches having minimum particle size and maximum entrapment efficiency. The surface charge for the prepared formulations was recorded by Zetasizer.

For Acyclovir SLN:

Stearic acid (SA), glyceryl mono-stearate (GMS) and glyceryl di-stearate (GDS) was taken as lipid. Polaxomer 188 and sodium deoxycholate (SDC) and 1:1 ratio of Polaxomer 188 and SDC was taken as surfactant. The concentration of lipids was varied from 3-5% and concentration of surfactant was varied from 1-3% with respect to the total aqueous phase. All the batches were prepared under similar conditions keeping the all process parameters constant. 25ml distilled water taken as aqueous phase. Homogenization pressure was fixed at 10,000psi and process continued for 3 cycles.

The type and concentration of lipid, concentration of surfactants had a statistically significant ($P<0.001$) influence on the particle size of Acyclovir SLNs. The mean particle size was decrease in case of GDS which was followed by GMS and SA. When concentration of lipid was 4%, the mean diameter of Acyclovir SLN was smallest. The type of different surfactant showed only a slight influence on the size of resultant SLNs but concentrations of surfactant have significant impact on particle size.

From ANOVA it can be concluded that, optimum batch should consist of GDS as lipid at 4% concentration and SDC as surfactant at 2% concentration. The average particle size and entrapment was found 201nm and 70.7% respectively.

The effect of drug load very slightly increases the particle size but drastically decreases the drug entrapment after 20mg of drug loading. Particle size slightly increases to 264nm as compared to 238 when drug load was increased to 30mg from 20mg. But drug entrapment was drastically decreases and it comes to 56.67% from 65.55% when drug load was increased from 20 to 30mg. From SEM, it was found that the SLN had a smooth surface and were spherical in shape. Also, there was a complete absence of any other colloidal species like liposome or micelles. The zeta potential of SLN was found -45.5mV, when dispersion was made in water. In vitro diffusion study revels that, all the SLN formulation having some initial burst release followed by sustained release. Percentage cumulative drug released vs. $\sqrt{t}$ curve shows a straight line with regression coefficient (R^2) greater than 0.94, suggesting that SLN formulation follows Higuchi kinetics. Optimum SLN formulation shows higher flux rate 15.381 ($\mu g\ cm^{-2}\ min^{-1}$) and higher permeation constant 0.015381 ($cm\ min^{-1}$) as compare to the other SLN formulation. On stability, the particle size of SLN was increased from208 nm to 259 nm and 302 nm when stored at 4^0C and 25^0C (dark, amber color bottle), respectively for 6 months. But when stored at clear bottle the particle size increase very fast and it goes upto 2.462μm after 1 month storage.

For efavirenz SLN:

Glyceryl monostearate (GMS), glyceryl di-stearate (GDS) and glyceryl tri-stearate (GTS) was taken as lipid. Polaxomer 188 and sodium deoxycholate (SDC) and 1:1 ratio of Polaxomer 188 and SDC was taken as surfactant. Like acyclovir SLN, the concentration of lipids was varied from 3-5% and concentration of surfactant was varied from 1-3%with respect to the total aqueous phase.

The different type and concentration of lipid as well as surfactants had a statistically significant ($P<0.001$) influence on the particle size. The mean particle size was decreased in case of GTS which was followed by GDS and GMS. With an increasing concentration of lipid, the mean diameter of SLNs decreased significantly. The different type and

concentrations of surfactant have significant impact on particle size. When combination of surfactants (Polaxomer 188: SDC, 1:1) was used in the SLN preparation, best results obtained. It was also observed that, the greater the concentration of surfactant, lesser particle size. From ANOVA, it can be concluded that optimum batch should consist of GTS at 5%) and Polaxomer 188: SDC (1:1) at 3%. The average particle size and entrapment was found 217nm and 97.4% respectively. The drug loading into SLN was neither altered the particle size nor drug entrapment significantly. It was also observed that upto 40mg of efavirenz loading entrapment was found satisfactory (98.23%) but further increase of drug load (50mg) decreased entrapment to 90.84%. The SEM of spray-dried nanoparticles shows spherical nature. The zeta potential value of efavirenz SLN was found -20.5mV in aqueous dispersion. Form in vitro drug diffusion study it was found that, % diffusion of drug hindered by increasing the lipid concentration irrespective of the type and concentration of surfactant and follow Higuchi kinetics. Optimum SLN formulation shows higher flux rate 13.317 ($\mu g\ cm^{-2}\ min^{-1}$) and higher permeation constant 0.013317 ($cm\ min^{-1}$) as compare to the other SLN formulation. The SLN formulations of efavirenz show stable for 6 months when stored at 4^0C.

Chapter 8: In vivo studies of the prepared formulations:

Chapter 8 describes the comparison of pharmacokinetic behavior and biodistribution of pure dug with their respective microemulsion and SLN preparations. Absorption studies were performed in male albino rats. The animals were divided were separate groups each consist of four animals. Different formulations (microemulsion and SLN and commercially available dosage form) were given orally to different groups of rat. The solution of drug was injected intravenously to separate group of rat, to estimate the absolute bioavailability. After suitable time intervals, blood samples were collected and plasma was separated out from the blood. The amount of drug was quantified by HPLC analysis as described in chapter3.

For acyclovir:

It was observed that Cmax of tablet formulation was 0.813mcg/ml achieved after 30miniute where as 1.64 mcg/ml achieved for microemulsion formulation after 2hours. In case SLN formulation release rate is slower as compared to the tablet or microemulsion

formulation. Cmax of the SLN was achieved 1.25mcg/ml after 4 hours. Although Cmax of SLN formulations was not so high as compared to the microemulsion formulation, but total AUC is much higher than the microemulsion formulation. For Acyclovir AUC was 2047.54(µg. hr/ml) as compared to 1067.78 (system A) (for ME04) and 1647.12(µg. hr/ml) (system B).The relative bioavailability of the microemulsion (system B) and SLN were 27.34 and 33.98, respectively, as compare to the commercially available tablet dosage form.

For efavirenz:

In case SLN formulation release rate is slower as compared to the capsule or microemulsion formulation. It was also observed that Cmax of capsule formulation was 3.212mcg/ml achieved after 60miniute where as 2.64 mcg/ml achieved for microemulsion formulation (system C) after 2hours. The total AUC of the SLN formulations was much higher as compare to microemulsion or commercially available capsule dosage form. AUC (µg. hr/ml) of efavirenz was found as 1304.13 (from SLN) as compared to 1094.05 and 1161.90 for system C and D respectively. The relative bioavailability of the SLN formulation was 3.49 times as compare to the commercially available capsule dosage form. Similarly, for microemulsion the relative bioavailability was 3.11 times and 2.93 times for ME13 and ME23 formulations.

Chapter 9: Toxicity studies of the prepared formulations:

Chapter 9 describes the histopatological and biomedical analysis of the free drug and formulations (microemulsion and SLN)

For acyclovir:

Nephrotoxicity study was carried out for different microemulsion and SLN formulations. Blood urea, creatinin and BUN value was estimated for control rat and rat treated with different formulations for 15 days. DAM method was used for the estimation of urea and BUN whereas alkaline picrate method was used for the estimation of creatinin. The animals were then sacrificed and the kidney was examined macroscopically and cross sections of kidney were collected in 5% formalin in saline for histopathological evaluation.

The administration of microemulsion or SLN formulations does not elevate the levels of creatinin, urea or BUN. Also from histopathology evaluation it was observed that necrosis occurred in the tubular epithelial cells located in the outer stripe of the outer medulla. Necrosis was also accompanied by scattered apoptosis, as evidenced by diminished cells when compared to the control rat kidney. Microemulsion and SLN treatment for 15 days had shown not an increase of necrosis on kidney.

For efavirenz:

To check the safety of the different formulations (microemulsion as well as SLN) hepatoxicity tests was carried by estimating SGPT and SGOT on control rat and rat treated with different formulations for 15days. From the histopathology studies, it was clear that the microemulsion or SLN formulation does not show any significant damage irrespective to the formulation and SLN formulation are even less toxic than microemulsion formulations. The hepatic globules with accessories structure such as bile caneli-culi, peripheral and pericentral region clearly visible in SLN formulations. Active drug and microemulsions formulation shows a similar toxic effect.

Conclusion:

The present study demonstrates some new findings which may be helped to improving the therapeutic efficacy of anti-viral drugs using microemulsion and SLN based drug delivery systems. It was found from the study that the reversed phase high-performance

liquid chromatography (HPLC) methods for estimation of drugs involved less time consuming in the sample preparation steps. The methods were shown to be highly reproducible and it seems to be adequate for routine therapeutic drug monitoring. It could be used without any interference from surfactants, tablet or capsule excipients and endogenous substances from the plasma samples. Simple stirring method was adopted for microemulsion formulation whereas for High Pressure Homogenization (HPH) technique utilized for SLN preparation. Pseudoternary phase diagram study and interfacial tension measurement was utilized for the optimization of microemulsion formulation whereas for SLN, Taguchi orthogonal experimental design [$L_9(3^4)$] varying four independent variables, type of lipid, concentration of lipid molar ratio, type of surfactant and concentration of surfactant at three levels were utilized for optimization.

Two different microemulsion formulations of acyclovir were prepared using two different strategies. One utilized the labrasol as surfactant and plurol olique as cosurfactant. In another tween 80 was utilized as surfactant and propylene glycol as cosurfactant. Similarly for efavirenz two different formulations were prepared. One utilizes Labrasol and another cremophor as surfactant. All the systems were characterized by various methods mentioned in regular quality control tests used for ensuring batch to batch reproducibility in the preparation of theses novel drug delivery systems. It includes viscosity, water uptake, electro conductivity, refractive index, % transmittance, particle size, pH, TEM, effect of dilution on particle size, in vitro diffusion study, stability of the formulations etc. Finally in vivo absorption study and toxicity study of the developed formulations was carried out. Oral bioavailability of the developed formulations was much higher than the pure drug or commercially available dosage form. The stability data of microemulsion showed that there was no increase in particle size after storage for 6 months.

Similarly, optimum SLN formulation of acyclovir was made with glyceryl di-stearate as lipid at 4% concentration and sodium deoxycholate (SDC) as surfactant at 2% concentration. The efavirenz SLN was made with glyceryl tristearate as lipid at 5% concentration and Polaxomer 188 and SDC (1:1) at 3% concentration. All the prepared SLN was evaluated for particle size distribution, drug entrapment, SEM, DSC, zeta

potential, in vitro diffusion, stability, toxicity and finally in vivo absorption study. The prepared SLN formulation shows major improvement in bioavailability as compare to the commercially available dosage form.

Thus the study presents some new findings which may be exploited in therapeutic efficacy of acyclovir and efavirenz using microemulsion and SLN as delivery systems. However, extensive clinical trials have to be performed to establish the efficacy and safety of the formulated microemulsion or SLN in clinical practice.

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