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Formulation and characterization of acyclovir loaded solid lipid nanoparticles

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ABSTRACT

The aim of the present study was to prepare, characterize, and evaluate solid lipid nanoparticles (SLNs) containing acyclovir (ACY) to improve therapeutic efficacy and reducing its dose related side effects. Acyclovir solid lipid nanoparticles were prepared by an emulsification and low temperature solidification method by using soya lecithin as surfactant and sodium starch taurocholate as co surfactant and stearic acid as lipid. The results revealed that this method is reproducible, more feasible and led to the entrapment of drug. All ACY-SLNs formulations were in the size range below 1µm with smooth surface which was confirmed by PCS and SEM analysis. The process variables like effect of Stearic acid concentration and effect of dispersion ratio were also optimized with respect to their percentage entrapment and *invitro* release. Optimized formulation of ACY-SLNs (F5) showed maximum percentage drug entrapment (75.61) and in vitro release were about 85.87% after 12hrs which indicated the acyclovir loaded Solid lipid nanoparticles provide sustained release over a period of 12h and release behavior was in accordance with higuchi-equation. The FTIR analysis revealed that there was no known chemical interaction between drug and polymers. The particles remained in their colloidal state during 3 month of storage time at 4˚, 25˚, 35˚C temperature.

Key words: Acyclovir, Solid lipid nanoparticles, *In vitro study.*

1. INTRODUCTION

Acyclovir [9-(2-hydroxyethoxymethyl) guanine] (ACV), a synthetic analogue of 20-deoxiguanosine, is one of the most effective and selective antiviral drugs. Unfortunately, acyclovir has poor oral bioavailability (about 15–30%) due to its low water-solubility. Because of acyclovir short circulation half-life (2.5 h) the oral dosage form must be taken five times daily, which is very inconvenient for patients. [1]

Acyclovir conventional formulations do not allow suitable drug levels at target sites following oral administration due to the low water solubility. This drug is particularly active against herpes labialis (caused by herpes simplex type 1,) and genital herpes (caused by herpes simplex type 2), which remain as common viral infections in humans.[2,3]

Solid lipid nanoparticles (SLN) are colloidal carriers made from solid lipids and are attracting major attention as novel colloidal drug carrier, which combines the advantages of emulsion, liposome, and polymeric micro-and nanoparticles simultaneously and avoids some of their disadvantages.[4,5] The additional advantage of the SLN are the large scale production,good biocompatability of the carriers, possibility of controlled drug release , drug targeting, increased drug stability and high drug pay load.[5,6]

Hence, to overcome these inherent draw backs associated with oral drug delivery of acyclovir an attempt is being made to provide an alternative drug delivery system of acyclovir loaded solid lipid nanoparticles (ACY-SLNs) were successfully developed, and the physicochemical characteristics were investigated.

2. Methodology

2.1. MATERIALS

Acyclovir was obtained from Cipla Pvt. Ltd., Stearic acid was purchased from Merck, EPIKURON 200 ® (containing 95% of soya phos phatidyl choline) was purchased from National Chemicals.Taurocholic acid sodium salts (TK)were purchased from Loba Chemie, Trehalose were purchased from Sigma. The other chemicals were of Analytical reagent grade.

2.2. Preparation of Solid Lipid Nanoparticles

The preparation of Acyclovir SLN was based on emulsification and Low-temperature Solidification Method. lipid phase (0.753%w/w) was prepared by melting stearic acid in ethanol at 80°C, to which the Epikuron 200 (3.9%w/w) was added and stirred with acyclovir in 2ml of DMSO

for 3 min, followed by sonication and known amount of aqueous Sodium taurocholate (13.8%w /w) which maintained at 80°C was added in these mixtures, stirring (Remi-motor ltd, Mumbai) at 3000 rpm for 20-30min, results the formation of O/W emulsion. The warm microemulsion was then transfer into ice cold water (2-3 ^{0}C) drop wise with continuous stirring for 3h in order to form drug loaded SLNs. The ratio between the microemulsion and the dispersion medium was about 1:10, 1:20, and 1:30. The SLN dispersion was then washed twice with double distilled water. The ACY SLNs suspension was stored at (0-4^{0}C) for long term storage.[7]

Table – 1: Composition of stearic acid micro emulsions

FORMULATION CODE	MEX	MEY	MEZ
Ingredients	**Amount for 10 gms**	**Amount for 10 gms**	**Amount for 10 gms**
Stearic acid	0.50%w/w	0.75%w/w	1.0%w/w
Soyalecithin	0.39%w/w	0.39%w/w	0.39%w/w
Sodium taurocholate	1.38%w/w	1.38%w/w	1.38%w/w
Double distilled water	7.53%w/w	7.53%w/w	7.53%w/w
Acyclovir	200mg	200mg	200mg

Table – 2: Preparation of acyclovir SLN from microemulsion

Micro emulsion (ME)	MG: Aqueous Phase	Formulation Code
MEX	1:10	F1
	1:20	F2
	1:30	F3
MEY	1:10	F4
	1:20	F5
	1:30	F6
MEZ	1:10	F7
	1:20	F8
	1:30	F9

2.3. 1.Characterization of SLNs

Prepared ACY SLNs were subjected to light microscope by placing 0.1 ml of dispersion on glass slide and observed immediately to see their visible movement. The Average particle size and size distribution of the ACY SLNs were determined by photon correlation spectroscopy. Surface morphology and internal structure of acyclovir SLN were determined under scanning electron microscopy (SEM). A thin film of aqueous dispersion of nano particles was applied uniformly in to circular aluminum stubs using double adhesive tape, and coated with gold using sputter gold coater and examined in Joel JSM 840, Japan at an acceleration voltage of 10 Kv and a magnification of 5000X. [8]

2.3.2. Fourier Transform infrared (FTIR) spectroscopic analysis

The FTIR spectra of Acyclovir, stearic acid, soyalecithin, sodium taurocholate and formulation were obtained using FTIR spectrophotometer in the range of 4000-400cm-1 and the resolution was 4 cm^{-1}. [9]

2.4. Drug entrapment efficiency

The entrapment efficiency of the compound was determined by measuring the concentration of free acyclovir in the dispersion medium. The SLN suspension was ultra centrifuged at 4000 rpm for 30 minutes at 4°C temperature by using remi cooling centrifuge to separate the free drug. The amount of free acyclovir was determined in the clear supernatant by UV spectrophotometer against blank at 255 nm.The analysis was made in triplicate. The drug entrapment efficiencies was calculated by using following equation . [10]

$$EE = \frac{\text{Amt of drug in SLN}}{\text{Amt of drug added}} \times 100$$

2.5. Evaluation of in vitro release

The drug release studies of SLN were carried out by the dialysis bag diffusion technique.2ml of SLNs formulation free from any un entrapped drug was taken in the dialysis bag (cellulose membrane, molecular weight cut off 12,000 D), hermetically sealed and immersed into 50ml PBS (pH 7.4) at 37±1°C under magnetic stirring at 100rpm throughout the study. Aliquots of the dissolution medium were withdrawn from receptor compartment at different time interval and the same volume of fresh medium was replaced to keep a constant volume at fixed time point. Drug concentration in the samples was analyzed by UV spectrophotometer at 255 nm. The control Nanoparticles without acyclovir were treated similarly and used as blanks for the measurements. [11]

2.6. X- Ray diffraction

PXRD studies were performed on the samples by exposing them to Cukα1 radiation (50 kV, 34 mA) and The scanning rate was 2° /min over a range of 20-80° and with an interval of 0.02.The amount of pure drug taken for PXRD analysis is equivalent to that present in formulations. [12, 13]

2.7. Short-term stability study

The stability of selected SLN formulation was monitored by the time-dependent changes in the physical characteristics, like drug content, changes in nanoparticles average size, at 4°C, room temperature, 45°C temperature for 3 month period time in stability chamber. [14]

3. RESULT AND DISCUSSION

Acyclovir solid lipid nanoparticles were prepared by an emulsification and low temperatur e solidification method. Photon correlation spectro scopy (PCS) the most powerful techniques for routine measurements of particle size. F5 formulat ion size was measured under photon correlation spectroscopy having a mean diameter 144±1.2nm (n=5) Fig 1.The SEM photograph of acyclovir SLNs of formulations F5 was shown in Figure 2. It can be seen that nanoparticles are almost spherical with smooth surface.

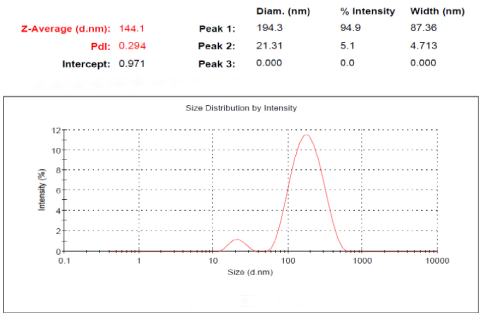

Figure - 1: Average particle size of formulation F5

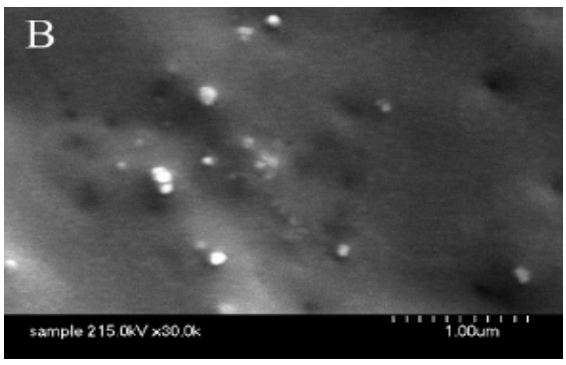

Figure – 2: Scanning electron microscope photo graphs of solid lipid nanoparticles

To ensure the compatibility of the drug with stearic acid pre formulation studies were done using IR spectrum recorded on Perkin Elmer, FT-IR USA, by preparing KBr disk. The results of FTIR spectral studies showed that there was no significant interaction between the drug and stearic acid. This was confirmed by the characteristic peaks of pure drug acyclovir was unaltered in optimized formulation F5 (fig 6).

All batches shows percentage entrapment more than 50% and it is found that entrapment of drug increases with an increase in the amount of the stearic acid in formulations, this may be due to the higher intactness of the lipid. Formulation F5 shows maximum entrapment 75.6%. Where as formulation F1 shows minimum entrapment (51.52%) of the acyclovir in the stearic acid as shown in table 3.

The release profile of nine batches of SLNs was studied for first two hours in stimulated gastric pH using 0.1N hydrochloric acid followed by stimulated Intestinal pH 7.4 (phosphate buffer). The *in vitro* release of all batches showed an interesting biphasic release with an initial burst effect this was followed by a prolonged second phase (zero order) release, which may be due to diffusion of drug through the polymer matrix as the lipid erodes slowly. Cumulative percent drug release for F1, F2,F3,F4 after 12 hrs was found to be 60.41%, 69.09%,64.39%,69.81% (fig.3) and for F5,F6, F7, F8,F9 formulation was 85.87% ,76.57 % , 67.06%,72.73% and 70.42% respectively (fig.4). In all the formulations, with the increase in the lipid concentration, the rate and amount of acyclovir release was found to be decreased, which could be attributed to the greater thickness of lipid. As the concentration of lipid increases, diffusion distance for drug to diffuse out from SLN was increased.

Table – 3: Drug entrapment efficiency and drug content of acyclovir loaded solid lipid nano particles

Micro emulsion	ME: Aqueous Phase	Formulation Code	Drug entrapment Efficiency in % (n=3)	Drug content
EX	1:10	F1	51.52 ±0.42	9.71
	1:20	F2	58.64 ±0.85	9.60
	1:30	F3	56.55 ±1.11	9.79
EY	1:10	F4	63.52 ±0.47	9.62
	1:20	F5	75.60 ±0.69	9.44
	1:30	F6	68.51 ±1.11	9.86
EZ	1:10	F7	58.29 ±0.43	9.81
	1:20	F8	65.25 ±0.87	9.93
	1:30	F9	61.12 ±1.07	9.69

Putting all data in different release kinetics models and comparing the coefficient of determination (r2), it was found all formulation releasing drug by diffusion mechanism confirming Higuchi kinetics, and F1 to F9 fits better with Zero Order kinetic model as compared to first order kinetic as shown in Table 4.The X-ray diffraction patterns of acyclovir and formulation are shown in fig.5 and fig.6 respectively. In X-ray diffraction pattern of formulation, the sharp peaks of pure acyclovir were not observed; indicates that there may be partial or complete transition of crystalline state to amorphous state.

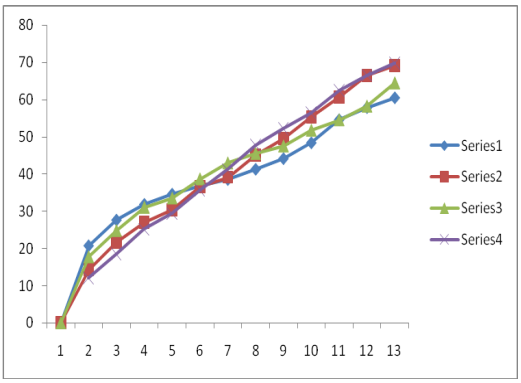

Figure -3: Compartive release profile of formulation F1 to F4

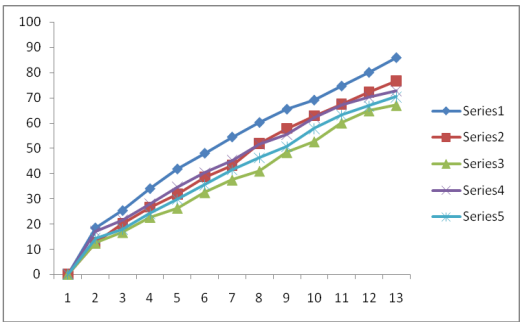

Figure – 4: Comparative release profile of formulation F5 to F9

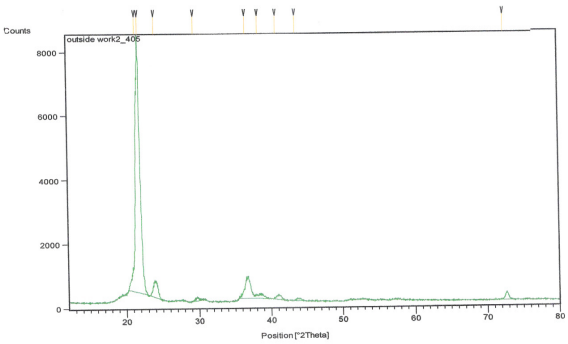

Figure – 5: XRD pattern of acyclovir

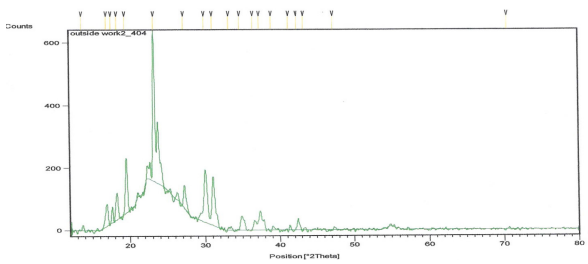

Figure – 6: XRD pattern of acyclovir loaded solid lipid nanoparticles

Table-4:Kinetic parameters of Acyclovir loaded solid lipid nanoparticles

Code	ZERO ORDER	FIRST ORDER	HIGUCHI	PEPPAS (n)	% drug released after 12hrs
F1	0.904	0.628	0.976	0283	60.41
F2	0.981	0.663	0.970	0.453	69.09
F3	0.932	0.649	0.993	0.338	64.39
F4	0.987	0.677	0.967	0.519	69.81
F5	0.970	0.742	0.986	0.464	85.87
F6	0.988	0.703	0.964	0.529	76.57
F7	0.991	0.657	0.949	0.569	67.06
F8	0.976	0.694	0.978	0.495	72.73
F9	0.988	0.675	0.962	0.558	70.42

Table – 5: Drug content of the formulations during stability study periods

Formulations Code	Initial period	One month time period	Second month time period	Third month time period
F1	99.71	99.58	99.42	99.23
F2	99.60	99.39	99.24	99.03
F3	99.79	99.57	99.38	99.16
F4	99.62	99.41	99.35	99.18
F5	99.44	99.22	98.97	98.79
F6	99.86	99.65	99.46	99.24
F7	99.81	99.60	99.36	99.13
F8	99.93	99.72	99.51	99.27
F9	99.69	99.48	99.25	98.97

In stability study, all the formulation showed almost the same drug content which was observed in the initial time. It indicates

formulations are stable. The drug content of all the formulations during the stability period was shown in table 5.

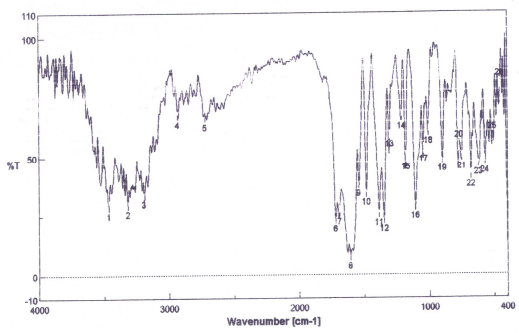

Figure - 6: A .FTIR spectra of Acyclovir pure drug

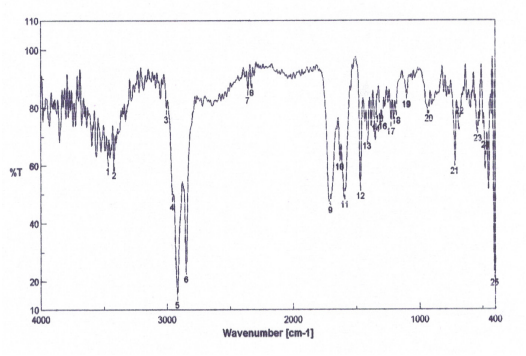

Figure - 6: B. FTIR spectra of stearic acid

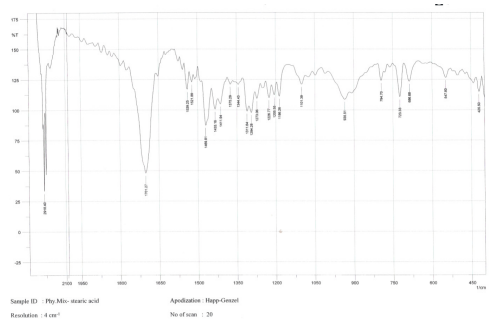

Figure – 7: FTIR spectra of optimized formulation

4. CONCLUSION

In the present study, ACY-SLNs were successfully prepared by an emulsification and Low-temperature Solidification Method using different concentration of stearic acid lipids. The ACY-SLNs were spherically shaped when observed under SEM. The acyclovir -SLN obtained in vitro release experiments exhibited a biphasic release pattern with burst release at the initial phase followed by sustained release. The mean particle size and size distribution, drug entrapment efficiency and in-vitro drug release studies on acyclovir SLN showed that the formulation F5 (1:0.75) was found to be good enough and feasible technique for the formulation. Among the nine formulations, the formulation F5 exhibited significantly optimized release profile. The ACY-SLN formulated with the lipid, stearic acid, may be good choice for the improvement of bioavailability, and reduction in toxicity. However, in vivo studies for acyclovir-SLN should be performed to determine its oral delivery efficacy.

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