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Fabrication and invitro evaluation of acyclovir loaded pH sensitive nano drug delivery systems for effective anti-viral activity

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ABSTRACT: The objective of this research is to formulate and evaluate Solid Lipid Nanoparticles containing Acyclovir by various phospholipids and surfactants using hot homogenization technique. Totally nine batches of SLN was formulated by hot homogenization technique and evaluates for PS,ZP,PI,Drug content, Entrapment efficiency, Invitro drug release studies and Invitro release kinetics studies. From the data's, it was concluded that hot homogenization technique method followed by ultrasonication was an optimized technique for the preparation of SLN nanoparticles containing Acyclovir, which lead to better results like high entrapment efficiency, high drug content and SLS was a better choice of surfactant to reduce the particle size and leads to uniform distribution of SLN in its phase. So, SLN will be an alternative drug delivery system for acyclovir to enhance the bioavailability and therapeutic response of drug.

Key Words: Acyclovir, Solid Lipid Nanoparticles, Invitro evaluation, Particle Size (PS), Polydispersity index (PDI), Zeta potential (ZP) etc.

INTRODUCTION

Solid Lipid Nanoparticles (SLNs) as novel lipid based nanocarrier with size range between 10 to 1000 nm. SLNs were introduced to overcome problems of polymeric nanoparticles (Garud et al., 2012; Mathur et al., 2010; Anu M et al., 2014; Yadav et al., 2012). Solid lipid nanoparticles (SLN) are aqueous colloidal dispersions, the matrix of which comprises of solid biodegradable lipids. SLNs combine the advantages and avoid the drawbacks of several colloidal carriers of its class such as physical stability, protection of incorporated labile drugs from degradation, controlled release, and excellent tolerability. SLN formulations for various application routes (parenteral, oral, dermal, ocular, pulmonary, and rectal) have been developed and thoroughly characterized in vitro and in vivo (Vimal Kumar S et al., 2012; Vijay Kumar S et al., 2011; Faghfouri, 2012; Nerella et al., 2014; Balvinder S et al.,2009). Acyclovir [9-(2-hydroxyethoxylmethyl) guanine], a synthetic purine nucleoside analogue derived from guanine. Acyclovir was the first specific antiviral drug to become widely used against herpes viruses, particularly Herpes Simplex Viruses (HSV) types I and II and Varicella Zoster. It is widely used in the treatment of various ocular viral diseases. The topical application of Acyclovir is limited due to low corneal penetration of the drug and by its poor water solubility. Many strategies have been developed to improve therapeutic efficacy of the Acyclovir such as chemical modification, liposomes, and nanoparticles. However they have not met the therapeutic requirement. Hence there should be a strong delivery system .Pharmacokinetic data ; Bioavailability15–20% (oral); Protein binding 9–33%; Metabolism – Hepatic; Half-life2-4 hours; Excretion Renal (62-90% as unchanged drug) (Sellappan V et al., 2011; Bhosale et al., 2013; Jaymin et al., 2014; Shivprasad H et al., 2012). The objective of this research is to formulate and evaluate Solid Lipid Nanoparticles containing Acyclovir by various phospholipids and surfactant.

MATERIALS

Acyclovir obtained as a gift sample from Microlabspvt ltd. Hosur, Other excipients used in research are obtained from Himedia, Mumbai.

METHODOLOGY

FTIR Studies

Drug and excipients compatibility studies are done by IR spectrophotometer. Infrared spectrum of any compound or drugs gives information about the groups present in that particular compound. A spectrophotometer for recording the spectrum in the infra-red region consist of an optical system capable of providing the monochromatic light in the scanning range was 400-4000 cm-1 and resolution was 2cm-1. FTIR is used to analyze solid samples for figure print region. A drop of SLN sample is directly place on the stage of (ATR) and scanned from 400cm-1 to 4000 cm- 1.The infrared spectrum of the sample was obtained using Bruker FTIR and ATR spectrophotometer (Germany) using opus software

Hot Homogenization Method

Hot homogenization is generally carried out at temperatures above the melting point of the lipid. A pre- emulsion of the drug loaded lipid melt and the aqueous emulsifier phase (same temperature) is obtained by high shear mixing device. The resultant product is hot o/w emulsion and the cooling of this emulsion leads to crystallization of the lipid and the formation of SLNs. Smaller particle sizes are obtained at higher processing temperatures because of lowered viscosity of the lipid phase. However, high temperature leads to the degradation rate of the drug and the carrier. Increasing the homogenization temperature or the number of cycles often results in an increase of the particle size due to high kinetic energy of the particles. Generally, 3-5 homogenization cycles at a pressure of 500-1500 bar are used (Holkar et al., 2012; Surender V et al., 2013; Rahul Nair et al., 2011)

Evaluation of acyclovir SLN Particle size

The particle size and Polydispersity index of SLNs was determined by using Zeta size by dynamic light scattering (Nano ZS -100, Horiba, Japan). Six replicates were measured and values were measured as mean standard deviation (SD) (Rohit and Pal A, 2013)

Scanning Electron Microscopy

Colloidal dispersion of SLNs was deposited on a glass cover slip which was previously adhered to carbon tape attached to metallic stub. This was then air dried and further metalized with gold coating using a vacuum coater. This sample was then analyzed by scanning electron microscopy (SEM; S-3400N, Hitachi) (Madhushri et al., 2013; Jawahar N et al., 2012; Ramteke KH et al., 2012)

Poly Dispersibility Index and Zetapotential

The average diameter (Z-AVE), Polydispersity index (PI) and zeta potential of Acyclovir dry Nanosuspension was determined by photon correlation spectroscopy (PCS) (Zeta-sizer Nano ZS, Malvern Instruments, UK) at room temperature. Nanosuspension was added to the sample dispersion unit (deionized water) and stirred at 2000 rpm with magnet in order to reduce the inter-particulate aggregation, and laser obscuration range was maintained between 10-20 %. The samples were adequately diluted with deionized water and placed in an electrophoretic cell. The average particle size was measured after performing the experiment in triplicates. The mean zeta-potential was calculated from the electrophoresis mobility using the Smoluchowski equation. (Luo Y et al., 2005).

Drug Content

From the prepared SLN formulation 1ml of suspension is dissolved in the 10 ml of 6.8pH PBS buffer and ethanol mixture. The amount of drug was determined using UV spectrophotometer. The placebo formulation prepared similarly to drug loaded SLN is used as blank

Drug Content= (Test absorb/standard absorb) ×100

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 254 nm. The analysis was made in triplicate. The drug entrapment efficiencies were calculated by using following equation.

Entrapment efficiency (EE%) = Amt of drug in SLN X 100

Amt of drug added

In-Vitro Drug Diffusion

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A 4–5 cm long portion of the dialysis tubing was made into a dialysis sac by folding and tying up one end of the tubing with thread. It was then filled up with phosphate-buffered saline pH 6.8 and examined for the leaks. The sac was then emptied and 1 ml of the ACV liquid Nano suspension was accurately transferred into sacs which served as the donor compartments. The sacs were once again examined for leak and then suspended in the glass beakers containing 50 ml phosphate-buffered saline, which become the receptor compartment. At Predetermined time intervals, 3 ml samples were withdrawn from the receptor compartment and analyzed spectrophotometrically at 254 nm. Fresh buffer was used to replenish the receptor compartment at each time interval (Mukherjee S et al 2014; Waghmare AS, 2012).

Invitro Drug Release Kinetics

Different kinetic models such as zero order (cumulative amount of drug released vs. time), first order (log cumulative percentage of drug remaining vs. time), Higuchi model (cumulative percentage of drug released vs. square root of time), korsmeyer-peppas model and Hixson Crowell model were applied to interpret the drug release kinetics from the formulations. Based on the highest regression values for correlation coefficients for formulations, the best-fit model was decided (Mamdouh M et al., 2004; Swamy NGN et al., 2013)

The release rate and mechanism of release of drug from the prepared SLN were analyzed by fitting the release data into

Zero-order equation,

Q = K0 t, Where, Q is the amount of drug release at time, t and K0 is the release rate constant.

First order equation

Log Q = K1 t, Where Q is the percent of drug release at time, t and K1 is the release rate constant.

Higuchi's equation

Q = K2 t ½, Where, Q is the percentage of drug release at time t and K2 is the diffusion rate constant.

Peppa's equation

Mt/M∞ = Ktn, Where Mt/M∞ is the fractional release of the drug, t is the release time, K is a constant incorporating structural and geometric characteristic of the release device, „n" is the release exponent indicative of mechanism of release. For non-Fickian (anomalous/zero order) release, „n" value is between 0.5 to 1.0; for Fickian diffusion, n < 0.5; for zero order release, n = 1; „n" is estimated from linear regression of log (Mt/M∞) Vs logt.

RESULTS AND DISCUSSION

Drug and Excipient Compatiblility Studies – FT IR Studies

The FT-IR spectrum of the Acyclovir pure drug was and best formulation were carried out and shown in Figure 1 – 3 and Table 2. The FT IR Studies shows that the main functional groups as in pure drug are reproducible in the mixture and formulation, it shows that there is no interaction between the drug and phospholipids and other excipients used in the formulations. It confirm that the compatibility between the drug and phospholipids are good enough for formulation and the character of phospholipids and other excipients will not change both physical and Chemical character of Acyclovir. The results are shown in table 2 and figure 1-3.

Particle size

The particle size analysis revealed that, all the SLNs formulation was in the nanometer range. The size of the nanoparticles is based on the product and process variables like Homogenization time, Concentration of Phospholipids and Surfactant. The sizes of the loaded SLNs were found to be between 15.3 nm to 0.5 nm (10 to 1000 nm).By comparing the formulation S7 shown better and desired particle size of about 10.7 nm with Polydispersity index of 0.263. This shows good dispersiblity of particles throughout the phase. The results are shown in Table 3 and figure4.

Zeta Potential

The stability of the formulated SLNs was evaluated by measuring the zeta potential of the SLNs by the Malvern particle size analyzer. The results are shown in Table 3 and figure 5. Zeta potential of Acyclovir loaded formulations was in the range of -41.2mV to -11.4 mV.S7 shown best zeta potential value of – 41.2 mV, which shows good surface charge potential and stability of particle in continuous phase on long storage condition.

Scanning electron microscopy (SEM)

Shape and surface morphology of the Optimized SLNs S7 formulation was observed by scanning electron microscopy. The study revealed that most of the SLNs were fairly spherical in shape, the surface of the particle showed a characteristic smoothness, and that the particle size was in the nanometric range, as depicted bySEM.

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Drug content

The prepared formulations were analyzed for drug content and the data is reported in table no.3. It was observed that the drug content in the prepared solid lipid nanoparticles was satisfactory and the drug was uniformly distributed in all the formulations. The percentage drug content is highest for S7 formulation was about 85.80 ± 1.64% and lowest for S9 was about 69.88 ± 1.46 %.

Entrapment efficiency

There was deviation in the percentage yield of the compound. Especially in the freeze drying process, the percentage yield of the product was calculated after freeze drying the SLN. Entrapment efficiency gives the amount of drug entrapped in the solid lipid nanoparticles. The results are shown in table no.3. The entrapment efficiency was highest for S7 formulation was about 89.46 ± 2.16% due to less particle size.

Invitro Drug Release Study

The Invitro drug release studies for all six formulations of Acyclovir loaded SLNs were carried out in pH 6.8 phosphate buffer using dialysis membrane and Franz diffusion apparatus. The in vitrorelease profile obtained for all ten Acyclovir loaded SLN formulations S- 1 to S-9, are shown in Figure 6. The cumulative percent drug release of Acyclovir loaded SLNs S7 formulation, after 8 hrs was found to be 51.26% for S-1 to S-10 respectively. From the results it was observed that, all the formulation shows better control release of drug from the SLN. But smaller particles leading to faster drug release due to larger surface area. In general the drug release from all formulation followed a steady pattern. It was observed that the drug release from the formulations decreased as in the tween 80 concentration. In the formulations the cholesterol having SLS concentration with 0.5% shows the good release. The drug release may be mainly controlled by drug diffusion through the lipid matrix respectively.

In-vitro drug release kinetics

The release kinetics of Acyclovir loaded SLNs are evaluated by fitting the data into various kinetic models like first order, zero order, Higuchi, Peppas, and Hixon– Crowell equations. The drug release kinetic data of Acyclovir loaded S7 SLNs was respectively shown in Table 4 and figure no 7, which shows Zero order model regression R2 values as 0.9919 respectively. So it was concluded that S7 formulations follow Zero order kinetics, which release the same amount of drug at unit time and it is the ideal method of drug release to achieve pharmacological prolong action. The values of release exponent (n) of all the formulations lies within of n = 0.5- 1 have been observed, which are regarded as Non-fickian diffusion mechanism. Based on the results, the release of Acyclovir from SLNs was best-fitted in Peppas fitting kinetics and the possible mechanisms for the drug release might be diffusion of the drug from the matrix and matrix erosion resulting from degradation oflipids.

Table 1. Formulation of Acyclovir SLN

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Acyclovir	50mg	50mg	50mg	50mg	50mg	50mg	50mg	50mg	50mg
Cholesterol	50mg	100mg	-	-	-	-	50mg	100mg	-
Compritol	-	-	50mg	100mg	-	-	-	-	50mg
Steanc Acid	-	-	-	-	50mg	100mg	-	-	-
Tween80	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	-	-	-
SLS	-	-	-	-	-	-	0.5%	0.5%	0.5%
Water	50ml	50ml	50ml	50ml	50ml	50ml	50ml	50ml	50ml
Homogenization Time	15min	15min	15min	15min	15min	15min	15min	15min	15min
Ultrasonication Time	10min	10min	10min	10min	10min	10min	10min	10min	10min

Table 2. Drug excipients compatibility results - Frequencies of figure print region shown in FTIR

Characteristic Infrared Absorption Frequencies			Sample Frequency Range cm^{-1}		
Bond	Compound Type	Frequency range	Acyclovir (Pure Drug)	Mixture	S7(Acyclovir SLN)
C-H	Alkynes	3333-3267(s) stretch	3322.66	3315.05	3304.94
C-0C	Alkynes	2260-2100(w,sh) stretch	2116.85	2136.14	2116.24
C=C C=C	Aromatic Rings Alkenes	1600, 1500(w) stretch 1680-1640(m,w)) stretch	1638.55	1636.63	1636.52
C-H	Alkynes	700-610(b) bend	611.44	630.62	631.28

Table 3. Characterization of Acyclovir SLN

Formulation	Mean particle size(nm)	Zeta potential (mV)	PDI	Drug content (%)	Entrapment efficiency (%)	Inference
S1	15.3	-16.4	0.134	85.40 ± 1.80	80.01 ± 2.14	Mean particle size is within the range (10-1000nm),but zeta potential is very less (Not upto ± 30 mV)
S2	3.5	-11.4	0.145	74.07 ± 1.42	86.40 ± 2.02	Particle size is not within the range (10-1000nm)
S3	0.5	-12.3	0.155	72.86 ± 1.66	79.10 ± 2.26	Particle size is not within the range(10-1000nm)
S4	0.5	-23.3	0.165	72.25 ± 1.58	72.28 ± 2.42	Particle size is not within the range(10-1000nm)
S5	10.9	-35.6	0.177	71.81 ± 1.44	77.14 ± 2.128	Particle size ,zeta potential is within the range but on long storage condition at 4°C itshows Phase separation
S6	14.4	-36.4	0.180	72.59 ± 1.32	7.94 ± 2.08	Particle size ,zeta potential is within the range but on long storage condition at 4°C it shows Phase separation
S7	10.7	-41.2	0.187	85.80 ± 1.64	89.46 ± 2.16	Particle size ,zeta potential is within the range but PDI is very high but on long storage condition at 4°C it shows Good Stability & dispersibility
S8	1.4	-38.4	0.175	73.06 ± 1.48	74.46 ± 2.14	Particle size not within the range
S9	-	-39.4	0.154	69.88 ± 1.46	80.12 ± 2.08	No mean particle size shown

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Table 4. Release model of formulation S7

Release Model	Regression	Formulation Code
		S7
Zero order	R2	0.9919
First order	R2	0.8782
Hixson Crowell	R2	0.9951
Higuchi	R2	0.9318
Peppas	R2	0.9852
	N	0.891
Best Fit Model		Peppas

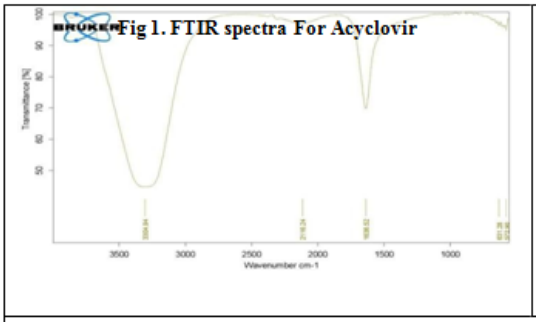

Fig 1. FTIR spectra For Acyclovir

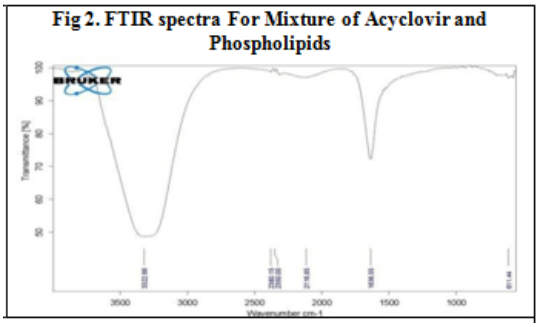

Fig 2. FTIR spectra For Mixture of Acyclovir and Phospholipids

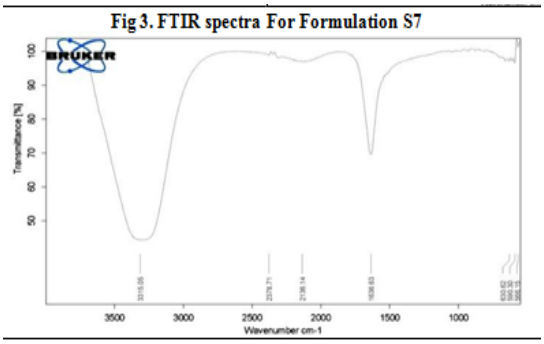

Fig 3. FTIR spectra For Formulation S7

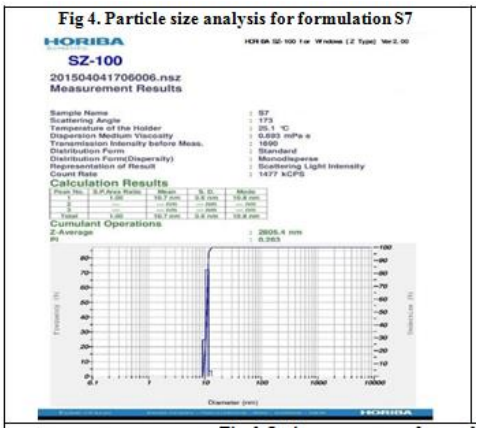

Fig 4. Particle size analysis for formulation S7

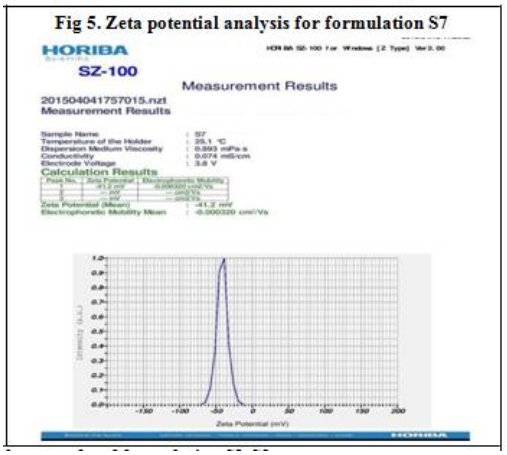

Fig 5. Zeta potential analysis for formulation S7

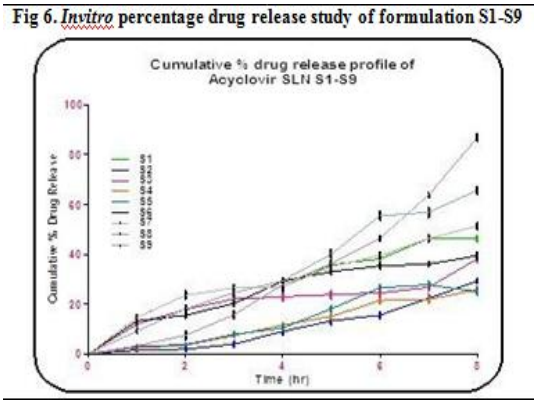

Fig 6. *Invitro* percentage drug release study of formulation S1-S9

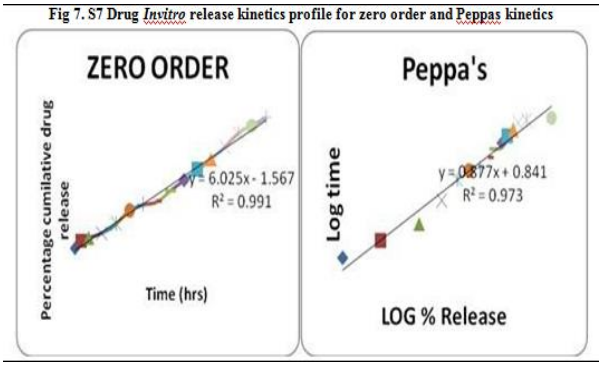

Fig 7. S7 Drug *Invitro* release kinetics profile for zero order and Peppas kinetics

SUMMARY AND CONCLUSION

The particle size analysis revealed that, S7 SLN formulation closer to the lesser nanometer range of 10.7nm which is approximately between the range of (10-1000 nm). The stability of the S7 formulation was evaluated by measuring the zeta potential of the SLNs by Horiba particle size analyzer and found to be -41.2mV, that showing uniformity in surface charge distribution in the particle and it confirms the good dispersibility of the particles in phase and it conclude that the SLN S7 formulation is the good stable formulation. The percentage drug content, entrapment efficiency was found to be very high in S7 formulation. The S7 formulations show good

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stability while comparing to other formulation. It was concluded that hot homogenization technique method followed by ultrasonication was an optimized technique for the preparation of SLN nanoparticles containing Acyclovir, which lead to better results like high entrapment efficiency, high drug content and SLS was a better choice of surfactant to reduce the particle size and leads to uniform distribution of SLN in its phase. So, SLN will be an alternative drug delivery system for acyclovir to enhance the bioavailability and therapeutic response of drug.

CONFLICT OF INTEREST

The authors declare that they have no conflict of Interest.

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