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Fabrication of Nanostructured Lipids carrier for acyclovir and enhancements of its oral bioavailability

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Abstract : Acyclovir is used to treat brain encephalitis caused by Herpes virus. Acyclovir being a hydrophilic drug has poor oral bioavailability (15% - 30%) and its blood plasma to cerebrospinal fluid ratio is 10:1. So, the nanostructured lipids are formulated using polysorbate 80 for targeting brain. So that the concentration of drug in brain can be increased by which the dose of drug can be reduced. In high doses, hallucinations, nephrotoxicity have been reported it may be reduced.

NLC's were prepared by emulsification method. The formulation was optimized by changing different parameters like stirring time, stirring speed, liquid lipid content. The prepared NLC's were evaluated for entrapment efficiency, percentage drug loading, particle size, zeta potential and studied for *in-vitro* dissolution, *in-vivo* bio-distribution studies.

Further the NLC's should be studied for their pharmacodynamic nature, brain toxicity, receptor targeting and long term stability studies. Due to these studies the prepared NLC's can be considered effective in the treatment of encephalitis.

Keywords: Nanostructured Lipids carrier, acyclovir, oral bioavailability.

Introduction:

In the last decade, an emerging interest has been growing towards brain drug targeting and the increasing awareness of the lack of rational and common efforts among different and complementary research areas has pointed out the need for a deeper understanding and a closer collaboration among diverse research experts of the field. Encephalitis is an acute inflammation of the brain. Encephalitis with meningitis is known as meningoencephalitis. Symptoms include headache, fever, confusion, drowsiness, and fatigue. More advanced and serious symptoms include seizures or convulsions, tremors, hallucinations, and memory problems. In India average deaths per year due to meningoencephalitis is 1170. Acyclovir is the first choice of drug but it's very low bioavailability and less penetration capacity through BBB limits its use[1]. Nanostructured lipids (NLCs) are lipid based submicron particulate colloidal carrier systems. They are not readily taken up by the cells of Reticuloendothelial system (RES), bypasses liver and spleen filtration[2], controlled release of incorporated drug[3], excellent reproducibility and are cost effective and ability to prevent chemical, photochemical or oxidative degradation of drug. All these pros make NLCs ideal carriers. Hence, attempts have been made to formulate Acyclovir loaded nanostructure lipids coated with polysorbate 80 to enhance the cerebrospinal fluid

concentration by transferrin receptor mediated absorption in BBB. This may act as a new formulating design for effective treatment of Brain Encephalitis.

Experimental:

Preformulation Studies:

Development of solubility profile of selected drug in various solvents:

Solubility profile of Acyclovir in various solvents and buffers was checked by the general USP method.

Compatibility Study:

Infrared spectra matching approach was used for detection of any possible chemical interaction between the drug, lipid, oil and surfactants. A physical mixture of drug, lipid, oil and surfactants was prepared. It was scanned from 4000 to 400 cm^{-1} in a FTIR spectrophotometer (FTIR 8400 S, Shimadzu). The IR spectrum of the physical mixture was compared with those of pure drug, lipid and surfactants and peak matching was done to detect any appearance or disappearance of peaks.

Partition Coefficient studies:

Partitioning behaviour of Acyclovir was determined in different lipids viz. tristearin and glyceryldibehenate (GDB). 10mg of Acyclovir was dispersed in a mixture of melted lipid (1g) and 1mL of hot phosphate buffer pH 7.4 (PB) and shaken for 30min in an water bath shaker maintained at $10^{o}C$ above the melting point of lipid, the mixture was separated from the lipid by centrifugation at 10000rpm for 20min.

Preparation of Nanostructured Lipid Particles (NLCs):

NLC's were prepared by emulsification and ultrasonification method[4,5]. Three different batches were prepared using GDB as solid lipid and oleic acid as liquid lipid. Amount of lipids taken were solid lipid: liquid lipid shown in table 1.

Table.1 Optimising the lipid quantity

Solid lipid: liquid lipid	Particle size (nm)	Zeta potential(mV)	PDI	Encapsulation efficiency (%)	Drug loading (%)
87.5:12.5	249.0	24.8	0.348	54.3 ± 0.54	8.36 ± 0.37
75:25	272.3	18.38	0.342	69±0.8	10.31 ± 0.21
62.5:37.5	916	19.5	0.311	81 ± 0.56	11.89 ± 0.22

The formulation was optimized by changing different parameters in the preparation like stirring time, stirring speed, liquid lipid content. Evaluation of the NLCs like entrapment efficiency, drug loading capacity, particle size, zeta potential and Polydispersity Index were done. The optimised batches were tested for *in-vitro* dissolution and *in-vivo* bio distribution studies.

Results and discussion:

The solubility of Acyclovir was determined in different buffers viz. acidic pH 1.2, Phosphate buffer pH 4.8, pH 6.8 and pH 7.4. It was concluded that the perfect linearity between the concentration and absorbance was observed when the concentration range was from 5 to 25 µg/ml.

In compatibility Studies, the spectra obtained from IR studies at wavelength from 4000cm^{-1} to 400cm^{-1} was interpreted and compared. It was confirmed that there was no major shifting, loss or appearance of functional peaks between the spectra of drug, lipid, physical mixture of drug and lipid. From the spectra it was concluded that the drug was entrapped into the lipid without any chemical interaction. It was concluded that, the selected lipids were found to be compatible with the selected drug Acyclovir.

Acyclovir is a slightly hydrophilic drug. The partition co-efficient was found in order of tristearin (37.21) <GDB (43.86). GDB is less lipophilic of the 2 lipids and has higher affinity for Acyclovir.

Various batches of solid lipid nanoparticles were prepared by micro emulsion method. The effect of stirring time on particle size revealed that increase in stirring time resulted in decrease in particle size up to 3 hours. When the stirring was continued up to 4 hours there was no significant decrease in particle size where the stirring speed is kept constant at 1000 rpm.

Table. 2 Influence of stirring speed on particle size

Stirring speed (rpm)	Particle Size (nm)	Polydispersity index (PI)
1000	760	0.343
2000	386.3	0.05
3000	249	0.348

The effect of stirring speed on particle size showed the particle size would decrease with increase in stirring speed up to 3000 rpm. And hence, 3000 rpm was taken as the optimised speed for the preparation of further batches.

The zeta potential for the ideal batches was found to be 24.8mV in 12.5% of liquid lipid in formulation and 18.38mV in 25% of liquid lipid in formulation.

The polydispersity index (PI) was measured using Malvern zetasizer. The optimized batches having least particle size (249 nm) had a PI of 0.348 for 12.5% liquid lipid NLC and (272.3 nm) had a PI of 0.342 for 25% liquid lipid NLC.

As observed with NLC with solid lipid: liquid lipid ratio 87.5:12.5 and 75:25 ratio, entrapment efficiency was found to be 54.3±0.54% and 69 ± 0.8% respectively. So the amount of liquid lipid greatly influences the entrapment efficacy of the drug.

The drug content is estimated by the U.V spectrometer. The study explains that the change in the liquid lipid content influence the drug content but the change in the stirring time or the speed has no effect in the drug content in the formulation.

An *in-vitro* release study was undertaken by dialysis bag diffusion method. The cumulative percentage release of drug-loaded batches show a biphasic pattern of initial burst effect and the remaining amount of drug was found to release in a sustained manner. Among the two batches of nanoparticles, batch with 25% of liquid lipid containing 18mg of drug with 145mg of polymer showed a comparatively higher percentage of drug release (99.04%) within 12hours.

The cumulative percentage of drug release from all the drug loaded batches after 24 hours was found to be in the range of 90 % to 100 % which may be considered to be satisfactory. By studying drug release kinetics, the drug release was found to follow fickian diffusion (0.38).

Wistar rats (Male) weighing 200±20g were used for brain bioavailability studies. All animal experiments were approved by Institutional Animal Ethical Committee, JSS College of Pharmacy, Ooty (Proposal no. JSSCP/IAEC/M.PHARM/PH.CEUTICS/01/2013-14). All the rats were fasted for overnight before the experiments but had free access to water.

The drug and the formulation were given to animals at specific time intervals and cerebrospinal fluid was collected at regular intervals. After 24hrs, the animals were sacrificed and heart, kidney, liver were collected separately. Concentration of drug in different organs was estimated. From the result of the biodistribution studies it is known that the concentration of the acyclovir had increased up to 8 times when given in the NLC 25% liquid lipid and 6.8 times when given in the NLC 12.5% liquid lipid form. It is also known that the NLC with 25% liquid lipid shown more release than the 12.5% liquid lipid this may be due to the sustain effect of drug in 12.5% liquid lipid NLC's. When the organ samples were compared the drug distribution do not varied from pure sample to the NLC's. The drug distribution does not change much in the kidney with the change in the formulation. So with this result it is known that, with NLC the CSF concentration increases but not the kidney so the dose can be reduced which reduce the risk of nephrotoxicity caused by Acyclovir.

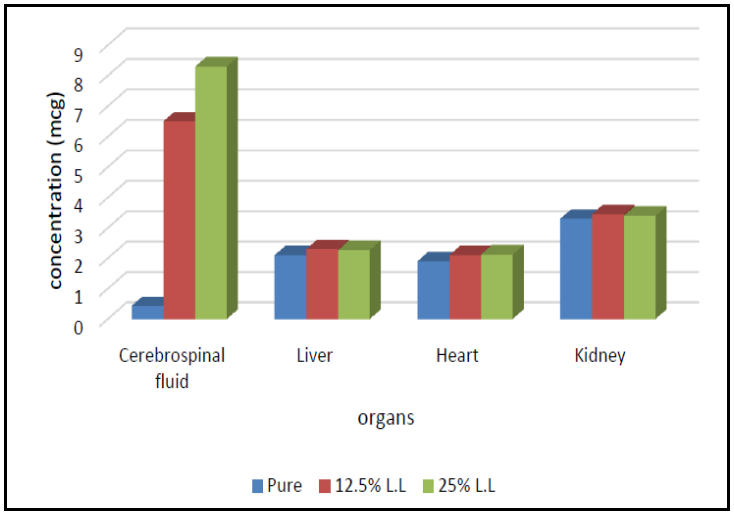

Figure.1 Bio-distribution profile of all formulations

Conclusion:

To conclude, results revealed that microemulsion technique can be used as a suitable method for the lab-scale production of NLC's. The formulated nanostructured lipids showed a significant increase in penetration of Acyclovir through BBB compared to the standard Acyclovir.

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