"Formulation and Evaluation of Nanosuspension of Antiviral Ophthalmic Application''

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ABSTRACT

Formulation and evaluation of Nanosuspension of Acyclovir for ophthalmic application.In this study, an attempt was made to improve the solubility and dissolution characteristics of a poorly soluble drug (Acyclovir) using nanosuspension technology.To improve the solubility. To formulate and evaluate acyclovir loaded nanosuspension Optimization of formulation and process parameters enhanced corneal residence time.To achieve sufficient drug concentration at absorption site, improve Therapeutic effect and lower dose with better patient compliance. Results conclusively prove that Acyclovir nanosuspension prepared by high speed homogenization method. Different excipients are used in formulation like Poloxamer-188, PVP K-90, different concentrations. F5 batch has shown the better results in high speed homogenization Different excipients were shown variation in particle size, DSC study, drug content, entrapment efficiency and in-vitro dissolution study. Optimized formulation was chosen on the basis of results obtained from particle size and entrapment efficiency. The combination of excipients yields nanosuspension with the smallest average particle size and by the transformation of the nanosuspension into the physical stability of this system could be further enhanced.

Keywords: *Nanosuspension, Antiviral ophthalmic application, Acyclovir*

INTRODUCTION

Nanosuspension

Nevertheless, pharmacokinetic studies of BCS class – II drugs showed that they have a low oral bioavailability, which may be due to the poor water solubility of the drug. There are many classical pharmaceutical ways to improve drug dissolution rates such as dissolution in aqueous mixtures with an organic solvent[1] [9], the formation of ß-cyclodextrin complexes[2], solid dispersions[3] and drug salt form[4].

During last 20 years a new technology, reducing drug particle size, has been developed to increase drug dissolution rate. According to Noyes–Whitney equation, drugs with smaller particle size have enlarged surface areas which lead to increase dissolution velocity. Higher the dissolution rate together with the resulting higher concentration gradient between the gastrointestinal lumen and systemic circulation could further increase oral bioavailability of drugs[5]. Nanosuspension is a submicron colloidal dispersion of drug particles which are stabilized by surfactants. A pharmaceutical nanosuspension is defined as very finely dispersed solid drug particles in an aqueous vehicle for oral, topical, parenteral or pulmonary administration. The particle size distribution of the solid particles in nanosuspensions is usually less than one micron with an average particle size ranging between 200 and 600 nm[6]. In nanosuspension

technology, the drug is maintained in the required crystalline state with reduced particle size, leading to an increased dissolutionrateand therefore improved bioavailability. An increase in the dissolution rate of micronized particles (particle size < 10 μm) is related to an increase in the surface area and consequently the dissolution velocity. Nanosized particles can increase solution velocity and saturation solubility because of the vapor pressure effect. In addition; the diffusional distance on the surface of drug nanoparticles is decreased, thus leading to an increased concentration gradient. Increase in surface area, as well as concentration gradient, leading to a much more pronounced increase in the dissolution velocity as compared to a micronized product. Another possible explanation for the increased saturation solubility is the creation of high energy surfaces when disrupting the more or less ideal drug microcrystals to nanoparticles. Dissolution experiments can be performed to quantify the increase in the saturation solubility of a drug when formulated into a nanosuspension[7].The stability of the particles obtained in the nanosuspension is attributed to their uniform particle size which is created by various manufacturing processes.

MATERIAL AND METHOD

MATERIAL

Sr.No.	**List of Chemicals**	**Gifted by**
1	Acyclovir	Ipca Laboratories, Mumbai.
2	Poloxamer 188	Astron Pharma, Ahmedabad.
3	Polyvinyl alcohol	S.D.Fine Chemicals, Mumbai
4	PVP K 90	S.D.Fine Chemicals, Mumbai
5	SLS	LobaChemiee , Mumbai.
6	Mannitol	Merck SpecialitiesPvt. Ltd., India.
Solvents		
7	Methanol (AR grade)	Merck Specialties Pvt. Ltd, India.
8	Ethanol (AR grade)	Merck Specialties Pvt. Ltd, India.
9	Acetone (AR grade)	Merck Specialties Pvt. Ltd, India.

RESULT & DISCUSSION

Pre-formulation of Drug

Identification of drug

The sample of Acyclovir for organoleptic characters and it was found to be almost white crystalline powder with bitter taste.

Identification of drug

Sr. no.	Parameter	Description
1	Color	White to off white
2	Odor	Odorless
3	Appearance	Non-hygroscopic white or whitish crystalline powder

Melting point determination

Capillary Tube Method

Melting point of Acyclovir was found by glass capillary method at 257-258°C and as per reported 256-258°C.

Melting point determination

Sr. No.	Method	Observed Melting Point	As per Standards
1	Capillary tube method	257-258°C	256-258°C

Solubility

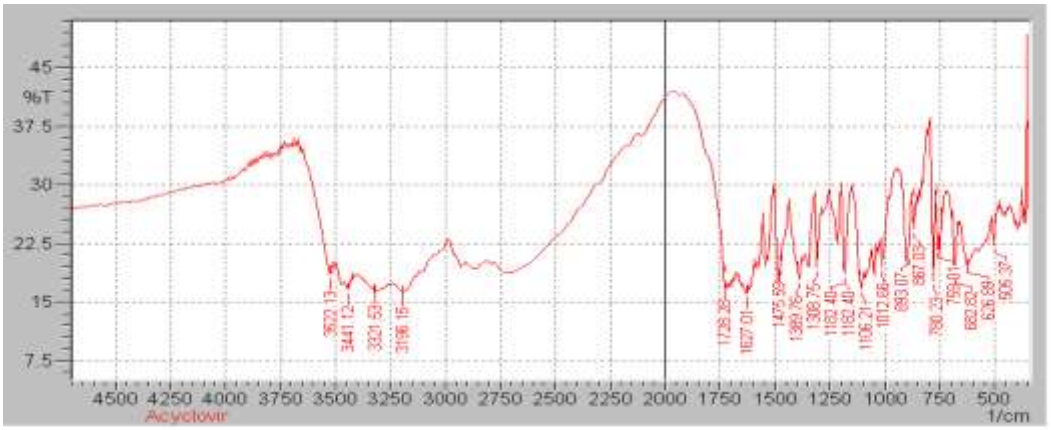
The solubility in Ethanol, pH 7.4 and Distilled water, while optimized formulation is completely soluble in methanol, and poorly soluble in water, Ethanol, pH 7.4. ofpure drug Acyclovir is completely soluble in methanol.

Fourier-transform infrared spectroscopy (FT-IR)

FTIR spectroscopy was conducted using a Shimadzu FTIR 8400 spectrophotometer (Shimadzu, Tokyo, Japan) and the spectrum was recorded in the wavelength region of4000–400 cm-1 as shown in Figure

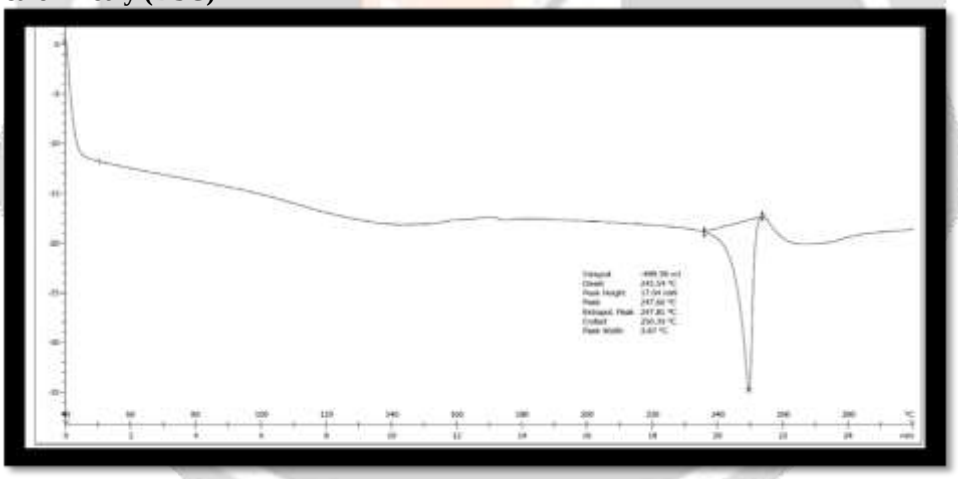
FT-IR Interpretation of Acyclovir

Sr. No.	Observed peaks (cm^{-1})	Reported peaks (cm^{-1})	Interpretation of chemical group	Intensity
1	1627.01	1620-1520	C= O Phenyl	Medium
2	1389.76	1454-1358	C-H	Strong
3	1182.40	1180-1185	C-O	Strong
4	893.07	991-802	-CH	Strong
5	3441.12	3400-3700	O-H	Medium-strong



FT-IR Spectrum of Acyclovir

Differential scanning calorimetry (DSC)



Partition Coefficient

Partition coefficient of acyclovir was found to be 0.023 and as per standard 0.025

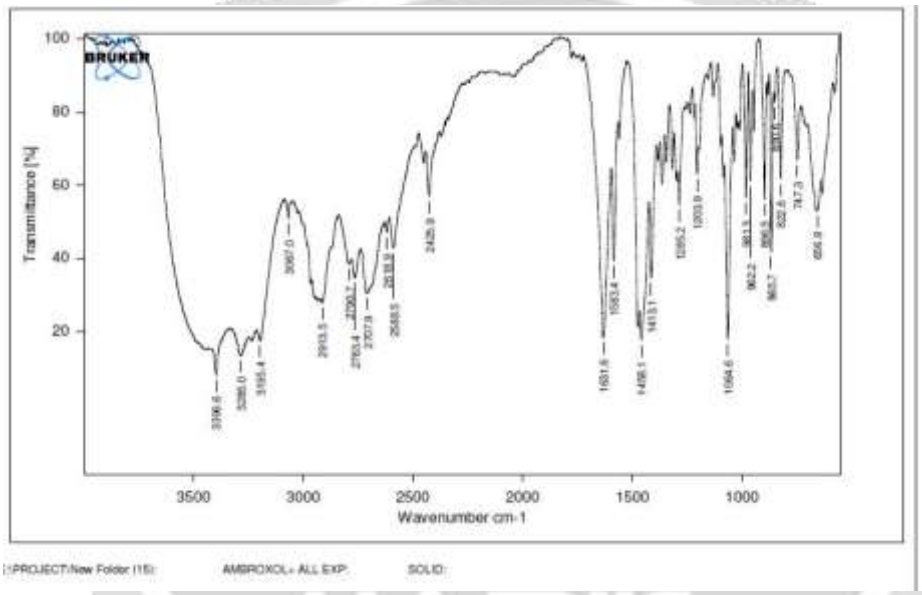
Excipient study

Excipients study (FTIR spectroscopy)

IR interpretation of polymer

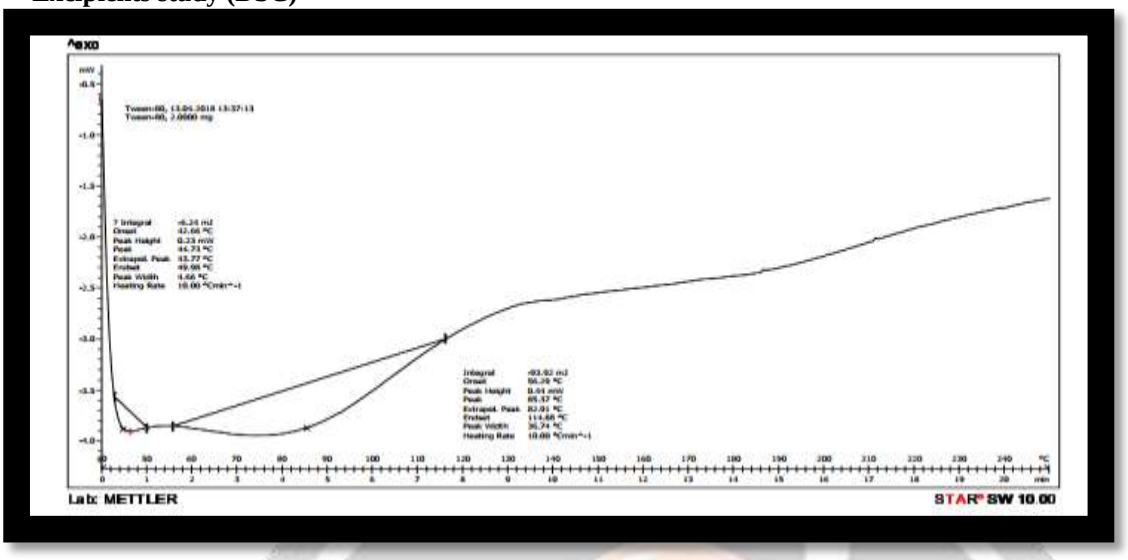
Sr. no.	**Observed peaks (cm^{-1})**	**Reported peaks (cm^{-1})**	**Interpretation of chemical group**	**Intensity**
1	3010.98	3100-300	Alkenes stretch	Medium
2	2872.10	1740-1720	Aldehyde C=O	Strong

3	1724.42	1725-1700	Carboxylic acid O=H	Strong
4	1460.16	1550-1350	Nitro (R-No_2)N=O	Strong
5	1165.04	1350-1000	Amines C=N	Medium-strong
6	869.92	900-690	Aromatic (out of plane)	Medium-wide



-FT-IR spectrum of polymer

Excipients study (DSC)



DSC graph of polymer

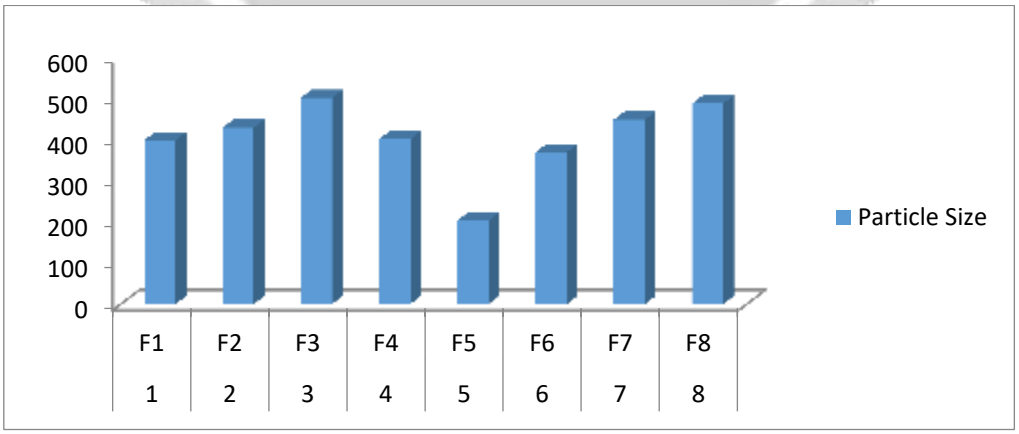
Formulation of Nanosuspension by high speed homogenization

Nanosuspension is prepared by Nanoprecipitation method. 10gm of Acyclovir dissolved in 35ml Methanol. This solution is added in specific gravity bottle or vials. Bottle is place in ultrasonic bath for 12hr. After 12hr the concentrated solution is filter through whatman filter paper. 5gm of poloxamer-188 dissolved in 20 ml of water in another beaker and filter through whatman filter paper. Beaker kept in ice bath for overnight.Polymer beaker place in high speed homogenizer at 1000 rpm. With continue process the collect the sample in 1ml syringe from specific gravity bottle. With continues drop by drop addition in the side of homogenizer beaker. At the continue homogenization for 5 hr drug will precipitate in the form of crystals and nanosuspension Prepared. Suspension is added in epien drop tube for centrifugation for 10 min Separated solution and partials collect in air tight container for further experimental work.

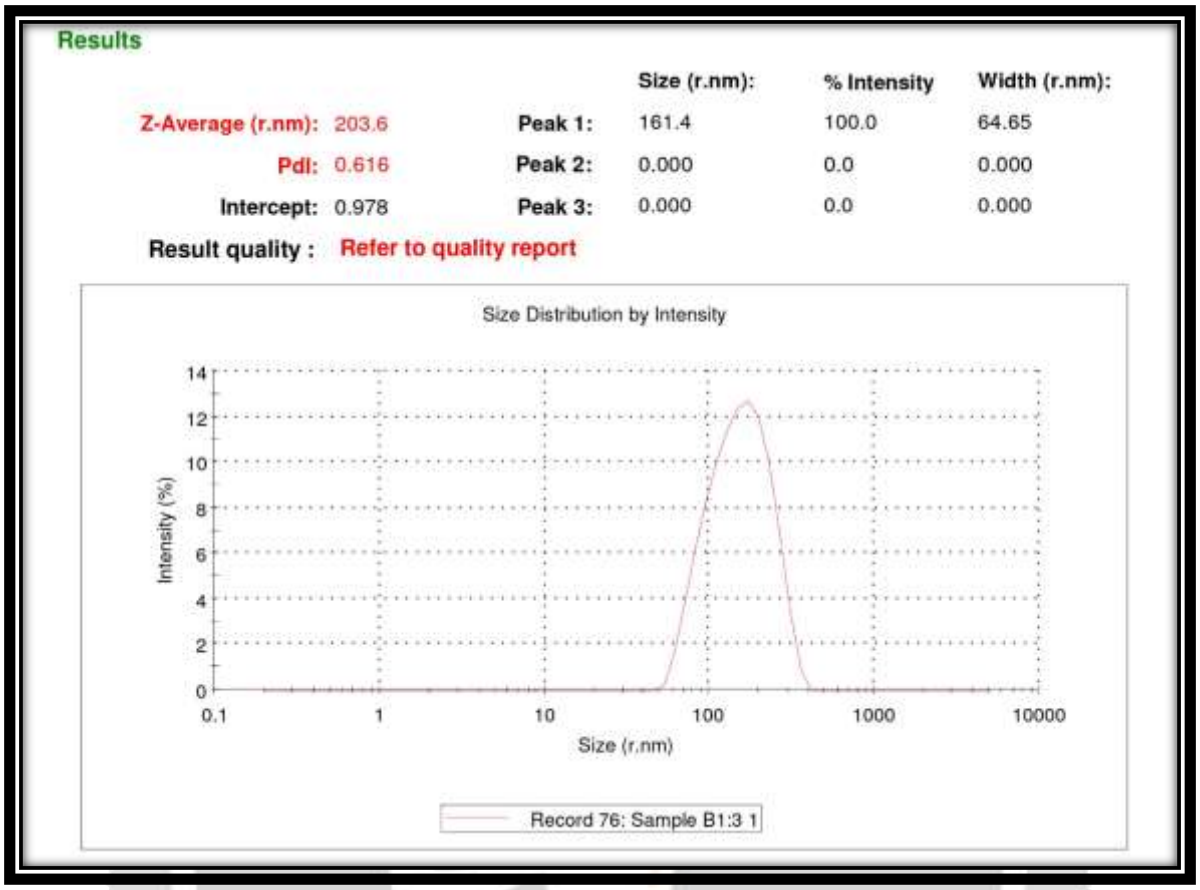
Characterization of Nanosuspension

Particle size analysis

When considering irritation and comfort, the particle size is an important factor in the development of an ocular drug delivery system. The mean particle size of prepared nanoparticle formulae. The particle size varies from 203.6 nm and 502.3.



Particle Size of Nanosuspension Formulations



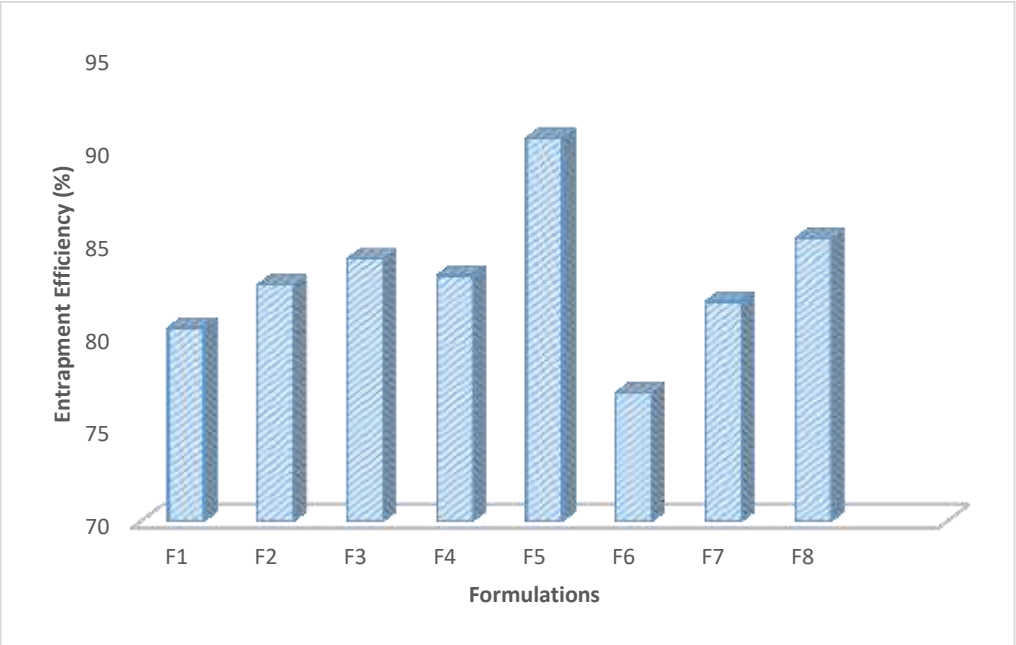
Particle size of Optimized Nanosuspension formulation

Entrapment efficiency (%)

Nanoparticle encapsulation efficiency % ranged between 80.2 to 96.8%. All tested variables have a significant effect on EE%. It was observed that the increase in polymer content resulted in decrease in EE% of the nanoparticles formulation. This result may be attributed to the increasing viscosity of the organic phase upon increasing polymer content.

Entrapment efficiency of Nanosuspension formulation (%)

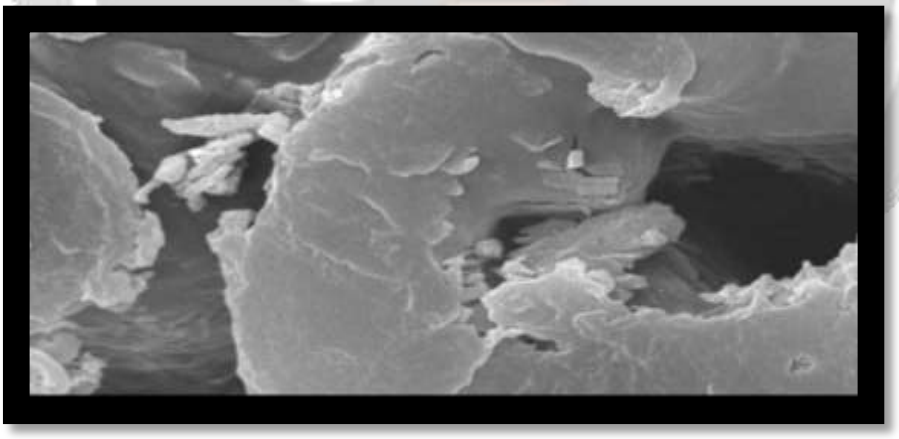
Sr. no.	Formulation	Entrapment efficiency%
1	F1	80.4 ± 0.34
2	F2	82.7 ± 0.30
3	F3	84.7 ± 0.87
4	F4	83.2 ± 0.98
5	F5	90.6 ± 0.12
6	F6	76.9 ± 1.23
7	F7	81.8 ± 1.34
8	F8	85.2 ± 0.78



-Entrapment efficiency of Nanosuspension Formulation

Scanning Electron Microscopy (SEM)

The morphology of drug loaded nanosuspension was accessed using SEM and is shown in Fig. This figure indicates that the nanoparticles were cylindrical in shape and their size was in the nanometer range with smooth surface essential for ocular drug delivery.

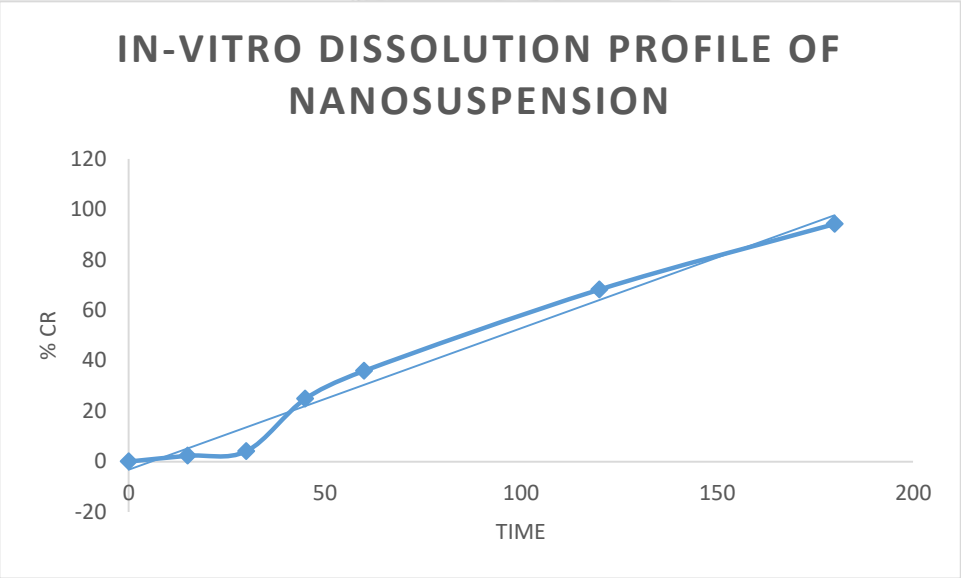


SEM of the optimized nanosuspension formulation

In-Vitro Drug Release Study

Drug release study

Sr.No	Time (min)	% Cumulative Drug Release
1	0	0
2	15	2.30
3	30	4.11
4	45	24.87
5	60	35.95
6	120	68.20
7	180	94.20



In vitro release study of the Optimized nanosuspension formulation

Redispersibility of Nanosuspension

It was found that when using mannitol as cryoprotectants the dispersibility was improved and products were spontaneously dispersed into primary nanosuspension within 1-3 min in both media (0.1 N HCl and phosphate buffer pH 6.8). It is recommended that mannitol in the products would improve the wetting of the hydrophobic drug and accelerate the penetration of water into the products. On the other hand, the products without cryoprotectants could not be dispersed well and transformed into the original nanosuspension within 15 min as expected from their agglomerated structure.

Stability Studies

Three months of stability studies the optimized batch by high speed homogenizer of Acyclovir formulation were selected. The variations between Particlesize and % EE in 3 months of storage were assessed. Respective data are given in table no7.19. The particle size was increased slightly during stability studies. The % EE of the optimized batch after 3 months (75.7%) indicated that the drug was retained within the nanoparticles throughout the stability period. The obtained results showed that there was slightly change in the mean particle size after month of period . Accelerated studies of formulations were conducted using parameter of particle size, entrapment efficiency. There were slight changes in particle size and three month storage from 225.1 ± 0.45 nm to 230.3± 1.23 nm and 250.1 ± 1.09 to 255.5 ± 1.23, respectively. The % EE of the selected formulation initially was found to be 91.7 ± 0.30 % and after three month, it's found to be 59.4 ± 1.08 %. On storage, there was no significant alteration in size, EE (%).

Hence is found to be stable at 70.9± 0.41°C/59.4± 1.08% in a total period of months

Stability Studies of optimized formulations

	ter	**h**	**h**	**h**	**h**
1	Particlesize (nm)	203.2 ± 0.45	204.7 ± 0.12	205.1 ± 0.3	206.6 ± 0.2
2	% Entrapment Efficiency (EE %)	90.6±0.5	88.2 ± 0.30	84.2 ± 0.4	82.2 ± 1.0

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