ORIGINAL ARTICLE

Formulation and evaluation of acyclovir nanosuspension for enhancement of oral bioavailability

Mangesh Ramesh Bhalekar, Prashant Girish Upadhaya, Satish Reddy, Sanjay J. Kshirsagar, Ashwini Raghvendra Madgulkar

Department of Pharmaceutics, AISSMS College of Pharmacy, Pune, Maharashtra, India

Acyclovir, an antiviral drug used against herpes simplex virus and varicella zoster virus. The dose ranges between 200 and 800 mg and the oral bioavailability is 10-20%, which decreases as the dose is increased. The aim of this research work was to formulate and characterize nanosuspensions of acyclovir with an intention to increase the oral bioavailability. Nanosuspensions were prepared by the precipitation-ultra sonication method and the effects of important process parameters i.e., precipitation temperature, stirring speed, end point temperature of probe sonicator, energy input and sonication time were investigated systematically, the optimal nanosuspension (particle size 274 nm) was obtained at values of 4°C, 10,000 rpm, 30°C, 600 Watt and 20 min, respectively. The nanosuspension was lyophilized using different matrix formers and sucrose (100% w/w to drug) was found to prevent agglomeration and particle size upon reconstitution was found to be 353 nm. The lyophilized nanocrystals appeared flaky in scanning electron microscopy images, the X-ray powder diffraction and differential scanning calorimetry analysis showed the nanoparticles to be in the crystalline state. *Ex vivo* permeation study for calculating absorption rate and *in vivo* bioavailability area under the curve both showed three-fold increase over marketed suspension.

Key words: *Differential scanning calorimetry, ex vivo chicken intestine study, precipitation-ultra sonication, probe sonicator, scanning electron microscopy, X-ray powder diffraction*

INTRODUCTION

Bioavailability of drug depends on its solubility and permeability in a given medium. At present, about 40% of drugs in the development phase and approximately 70% of drugs coming from synthesis or high throughput screening are poorly soluble in aqueous media, and many are not soluble in organic solvents as well. The low aqueous solubility of biopharmaceutics classification system (BCS) class II drugs is a major obstacle for their clinical application. However, the problem is severe in case of BCS class IV drugs which suffer low bioavailability because of low solubility as well as low permeability. When these molecules are formulated by conventional methods, the performance of the drug in preclinical screens is oftentimes erratic and highly variable. In general, it is more expeditious and cost-effective to chemically re-design the molecule, than to move a blemished molecule through the development process.

Traditional strategies, such as solubilization using co-solvents, the use of permeation enhancers, oily solutions and surfactant dispersions, cyclodextrin complexes, solid dispersion, which evolved earlier to tackle the formulation challenges, have limited use.[1] Another general approach for solubility enhancement is micronization of drug. Micronization increases the dissolution velocity of the drug due to the increase in surface area, but does not change the saturation solubility. At very low saturation solubility, the achieved increase in dissolution velocity does not lead to a sufficiently high bioavailability.[2] The next development step involved transformation of the micronized drug

Address for correspondence:
Dr. Mangesh Ramesh Bhalekar,
AISSMS College of Pharmacy, Kennedy Road,
Pune, Maharashtra, India.
E-mail: mrbhalekar@gmail.com

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powder to drug nanoparticles[3,4] and these submicron colloidal particle dispersions of drug stabilized by surfactant or mixture of surfactants are known as nanosuspensions.

Nanosuspensions are distinguished from nanoparticles which are polymeric colloidal carriers of drug, and from solid lipid nanoparticles, which are lipidic carriers of drug. The major advantages of nanoparticles are its general applicability to most drugs and its simplicity.

A lot of work regarding improvement of bioavailability of BCS class IV drugs using nanosuspension approach is reported.[5] In the present work, an attempt has been made to improve solubility and permeability by producing acyclovir nanosuspensions using bottoms up method by causing precipitation by temperature change using probe sonicator.

MATERIALS AND METHODS

Materials

Acyclovir (FDC Ltd., Goa, India); poloxamer 188 (SD Fine chemicals Ltd., Mumbai, India); Polyvinylpyrrolidone K30 (Loba Chemicals, Mumbai, India); tocopheryl polyethylene glycol succinate (Isochem Pvt. Ltd, France); dimethyl sulfoxide (DMSO) (Loba Chemicals, Mumbai, India) and all other chemicals were procured from local sources and were of analytical grade.

Methods

Selection of solvent system

The solubility of acyclovir was determined in following solvents

1. Poloxamer 188 dispersed in DMSO
2. Tween 80 dispersed in DMSO
3. Dichloromethane
4. Hot water (90°C).

Formulation of nanosuspension and selection of process parameters

Nanosuspension was prepared by precipitation-ultra sonication technique.[6,7]

The precipitation step was carried out by the addition of saturated drug solution in antisolvent solution (water and surfactant mixture) with stirring for pre-decided time interval subsequently the sample was subjected to probe sonication.

The process parameters viz. precipitation temperature (PT), energy input (EI) in probe sonicator and probe sonication time (PST) were systematically varied keeping stirring speed (SS), stirring time, end point temperature (EPT) of probe sonicator constant [Table 1].

Selection of surfactant system

The selection of surfactants was done by varying the surfactant combinations and drug surfactant ratio in a system using hot water as solvent for acyclovir, PT of 4°C, stirring at 10000 rpm for 10 min followed by 20 min of probe sonication with EI of 600 watt, amplitude 70% and EPT of 30°C. Various surfactants combinations studied are represented in Table 2.

Freeze drying of nanosuspensions

The nanosuspensions were mixed with various matrix formers [Table 3] and subjected to deep freezing near −45°C temperature. After 24 h, it was subjected to lyophilization at room temperature and 0.002 mbar vacuum using Labconco FreeZone 2.5 lyophilizer (USA). The particle size was measured before and after freeze drying.

The resultant lyophilized nanosuspensions were subjected to Fourier transform infrared spectroscopy (FTIR) spectrum analysis using Jasco FTIR spectrophotometer (Jasco FTIR-401, Japan) at transmission mode over wave number range of 4000-400/cm.

Evaluation of nanosuspensions

Particle size analysis

The particle size analysis was performed using Malvern Mastersizer (SM 2000K, Malvern Ltd). The average particle size and size distribution of each nanosuspension was recorded.

Table 1: Optimization of process parameters

Process parameter	Range
PT	4°C to RT
EI in probe sonicator	200-750 watt
PST	15-25 min
SS	10,000 rpm
ST	10 min
EPT of probe sonicator	30°C

RT: Room temperature, PT: Precipitation temperature, SS: Stirring speed, ST: Stirring time, EI: Energy input, PST: Probe sonication time

Table 2: Formulations to study the effect of surfactant system on particle size

Surfactant system	Drug:Surfactant ratio
Tween 80-poloxamer 188	1:2, 1:3
TPGS-poloxamer 188	1:2, 1:3
PVP K30-TPGS	1:2, 1:3
PVA-TPGS	1:2
PVP K30-poloxamer 188	1:2, 1:3

TPGS: Tocopheryl polyethylene glycol succinate, PVP: Polyvinyl pyrrolidone, PVA: Poly vinyl alcohol

Table 3: Ranges of various matrix former concentrations used for freeze drying

Matrix former	Concentration (% w/v) to drug
Sucrose	50, 100, 200
Mannitol	50, 100, 200
MCC	50, 100, 200

MCC: Microcrystalline cellulose

Particle morphology

In the study, lyophilized nanosuspension sputtered with platinum in an ion sputter for 300 s. Images were collected at an acceleration voltage of 15 kV using a backscattered electron detector on scanning electron microscopy (SEM) (Quanta 200, Felin Ltd.). Analysis was performed at 25°C ± 2°C.

Differential scanning calorimetry

The degree of crystallinity was analyzed by Differential scanning calorimetry (DSC) i.e., DSC (DSC823 Mettler Toledo, Mettler Ltd). The freeze dried acyclovir nanosuspension and plain powder was weighed into standard aluminum pans using an empty pan as reference. A heating rate of 5°C/min was applied. The samples were heated from 30°C to 300°C under liquid nitrogen. Inert atmosphere was provided by purging nitrogen gas flowing at 40 ml/min.

X-ray diffraction

X-ray scattering measurements were carried out with an X-ray diffractometer (PW 3710, Philips Ltd). A Cu Ka radiation source was used, and the scanning rate (2 h/min) was 5°C/min. X-ray diffraction (XRD) measurements were carried out on unprocessed acyclovir powder and acyclovir nanosuspension.

Determination of saturation solubility

Excess quantity of the sample was added to 25 ml of distilled water and equilibrated by shaking in thermostatically controlled mechanical orbital shaker (CIS-24, Remi) for 48 h. The samples were filtered through 0.22 µm filter, diluted suitably using distilled water and analyzed spectrophotometrically at 254 nm. In the similar way saturation solubility was determined in buffers of pH 1.2, 6.8 and 7.4. These studies were carried out for bulk drug powder and lyophilized nanosuspension. Each determination was carried out in triplicate.

In vitro dissolution

In vitro dissolution studies were carried out on marketed suspension and freeze dried nanosuspension using USP dissolution apparatus I at 37°C ± 2°C, SS 100 rpm, in 0.1 N HCl 900 ml. The aliquots were collected at 10, 20, 30, 45 and 60 min. Quantification of acyclovir released was performed using ultraviolet spectrophotometer at a wavelength of 254 nm.

Ex vivo permeation study

The *ex vivo*[8] drug absorption study for marketed acyclovir suspension and nanosuspension was performed using everted chicken intestine model [Figure 1].[8] A fresh intestinal segment was clamped to the perfusion apparatus. The total volume of the absorption compartment (tube A and tube B of perfusion apparatus) was 35 mL of Krebs-Ringer solution.[1,5] The apparatus was placed in dissolution medium during the *in vitro* dissolution studies. The drug diffused from dissolution medium (mucosal side) to the serosal side (absorption compartment). The marketed acyclovir suspension was transferred to the dissolution basket of the designed system. The suspension was rotated at 75 rpm speed. The transported drug from the absorption compartment was sampled (1 ml) with replacement (Krebs-Ringer solution) at 5, 10, 20, 30, 45, 60, 70, and 90 min and analyzed spectrophotometrically. The experiment was carried out in triplicate (n = 3) using fresh dissolution medium as well as fresh intestinal segment each time.

In vivo study

The *in vivo* study[9-11] and the protocol was approved by institutional animal ethics committee (CPCSEA) AISSMS College of Pharmacy. The studies were carried out on male Wistar rats weighing 150-200 g, divided into three groups containing six rats each [Table 4]. The animals were fasted for 24 h and formulations were administered using oral feeding tube. Blood sample of 0.5 ml was withdrawn from the retro-orbital plexus at 0, 15, 30, 60, 120, 180, 240, 360 and 480 min time interval and collected in ethylenediaminetetraacetic acid tubes, centrifuged immediately for 10 min at 2500 rpm and the separated plasma was stored at −20°C in screw capped polypropylene tubes until the time of analysis.

The samples were analyzed using high-performance liquid chromatography (Agilent) with Agilent TC-C C_{18} (250 × 4.6, 5 µm) column protected with guard column and mobile phase acetonitrile: Phosphate buffer of pH 2.5 in 5:95 v/v ratio. The parameters for analysis were flow rate: 1 ml/min, detection wavelength 254 nm, sample injector: 20 µl loop at ambient temperature.

RESULTS AND DISCUSSION

Selection of solvent

Acyclovir is soluble in DMSO, but a large amount of DMSO

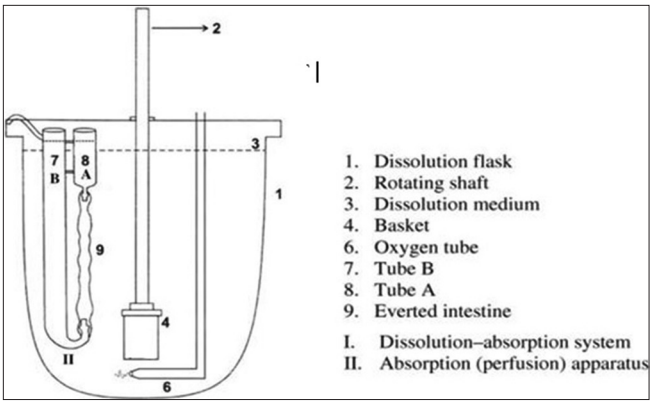

Figure 1: Design for ex vivo absorption study

Table 4: *In vivo* study protocol for animal studies

Group (n=3)	Treatment
Group I	Distilled water
Group II	Acyclovir marketed suspension 80 mg/kg orally
Group III	Lyophilized acyclovir nanosuspension 80 mg/kg orally

is required for drug loading (about 26 ml/g acyclovir). The use of surfactants poloxamer 188 and tween 80 was resorted to increase the solubility, but desired results were not obtained, whereas due to high melting point (198°C) removal of DMSO from the system is difficult. Furthermore, acyclovir is slightly soluble in dichloromethane and isopropyl alcohol. Finally, good solubility was noticed in hot water (90°C) which was about 1 g in 30 ml. The use of water omits need for removal of solvent, hazards of residual solvent and toxicity problems.

Formulation of nanosuspension and selection of process parameters

From solubility studies, it was evident that water at 90°C could be used as solvent and solution of surfactant/stabilizers in water as antisolvent for precipitation. The process parameters for formulation containing surfactants containing tween 80 and poloxamer 188 as stabilizer (1:1:1 ratio) and using hot water (90°C) as solvent. PT, EI in probe sonicator and PST were systematically varied to achieve desired product attribute of least particle size [Table 5].

During probe sonication, electrical energy is converted into shock waves through a series of transformation. These shockwaves produce bubbles which violently explode and collide with particles resulting in generation of heat.[6,12] The EPTs during sonication was fixed to 30°C to avoid dissolution of nanoparticulates.

Precipitation occurs in two steps i.e., initial creation of crystal nuclei and subsequent crystal growth. To ensure smaller particle size initial rate of creation of crystal nuclei should be high and crystal growth rate should be low.

High super saturation ensures the rapid crystal nuclei formation. This condition can be achieved by diluting small amount of drug solution in large amount of antisolvent. To achieve this rapid dilution high speed of stirring is necessary during addition of solvent into antisolvent.[13] Both processes (nuclei creation and growth) are dependent on temperature. The temperature dependence of process can be described by Arrhenius expression.[6,13]

$$K = Ae^{2\frac{-E}{RT}}$$

where *K* is rate of process, to the change in temperature *T*; *E* is energy of activation, *R* is universal gas constant, A is Arrhenius constant. Temperature during preparation should be kept below the optimum temperature required for the growth. This ensures low growth rate and high nucleation rate. Two temperature conditions viz. room temperature (25) and 4C (PF8 and PF 9) were evaluated, considerable reduction in particle size was observed at 4C (858 nm) compared to room temperature (995 nm). It is obvious that particle size decreases with increase in the EI of probe sonicator and sonication time. It was observed from formulation PF_4 and PF_7 that increase in EI above 600 watt did not have a considerable effect on particle size. Hence, EI during probe sonication was fixed to 600 watt. Furthermore, it was observed from formulation PF_{10} and PF_{11} that PST of more than 20 min did

Table 5: Formulations for selection of process parameter

FC	PT (°C)	SS (rpm)	ST (min)	EPT (°C)	EI (watt)	PST (min)	Inference
PF_1	RT	10000	10	30	250	15	Particle size 1340 nm
PF_2	RT	10000	10	30	400	10	Particle size 1325 nm
PF_3	RT	10000	10	30	400	15	Particle size 1134 nm
PF_4	RT	10000	10	30	600	10	Particle size 1224 nm
PF_5	RT	10000	10	30	600	15	Particle size 1035 nm
PF_6	RT	10000	10	30	600	20	Particle size 990 nm
PF_7	RT	10000	10	30	750	10	Particle size 1195 nm
PF_8	RT	10000	10	30	750	20	Particle size 995 nm
PF_9	<4	10000	10	30	600	20	Particle size 858 nm
PF_{10}	<4	10000	10	30	750	20	Particle size 845 nm
PF_{11}	<4	10000	10	30	750	25	Particle size 837 nm

RT: Room temperature, PT: Precipitation temperature, SS: Stirring speed, ST: Stirring time, EPT: End point temperature, EI: Energy input, PST: Probe sonication time, FC: Stands batches, SF: Stands for formulations

Table 6: Formulations for selection of surfactant system

FC	% w/w to drug		Inference
	Stabilizer 1	Stabilizer 2	
SF_1	Tween 80 (100)	Poloxamer 188 (100)	Particle size 995 nm
SF_2	Tween 80 (150)	Poloxamer 188 (150)	Particle size 858 nm
SF_3	TPGS (100)	Poloxamer 188 (100)	Particle size 774 nm
SF_4	TPGS (150)	Poloxamer 188 (150)	Particle size 543 nm
SF_5	PVP K30 (100)	TPGS (100)	Particle size 645 nm
SF_6	PVP K30 (150)	TPGS (150)	Particle size 446 nm
SF_7	PVA (100)	TPGS (100)	Particle size 964 nm
SF_8	PVP K30 (100)	Poloxamer 188 (100)	Particle size 920 nm
SF_9	PVP K30 (100)	Poloxamer 188 (200)	Particle size 274 nm

TPGS: Tocopheryl polyethylene glycol succinate, PVP: Polyvinyl pyrrolidone, PVA: Poly vinyl alcohol, FC: Stands batches, SF: Stands for formulations

not have a considerable effect on particle size. Hence, PST was fixed to 20 min.

Selection of surfactant system

The formulations [Table 6] were prepared by using water (at 90°C) as solvent, PT of 4°C, stirring at 10,000 rpm for 10 min followed by 20 min of probe sonication with EI of 600 watt, amplitude 70%, and EPT of 30°C.

In precipitation which is bottoms up technique nanoparticles are formed by building particles up from molecular stage. During this new surface area (ΔA) is formed, which produces high free-energy (ΔG), leading to agglomeration of particles and subsequent increase in particle size. This tendency can be avoided by addition of surface active agents, which reduces, $\gamma_{s/l}$ and therefore the free surface energy. Hence, during the formulation of nanosuspension the optimization of surface active agents has prime importance.[13]

The formulation _SF_9 displayed least particle size (274 nm), hence was selected for optimization of other parameters.

Freeze drying of nanosuspension

To facilitate the freeze drying[14] and obtain free flowing powder without significant increment in particle size various matrix formers were used in different concentration as tabulated in Table 7.

For oral administration, the rapid dissolution originating from the increased specific surface area of drug nanocrystal is generally regarded as its main advantage. In the suspended state, this can be achieved by the selection of a proper stabilizing system, preventing particle agglomeration. However, further transformation into solid products is often required for physical stability and/or patient convenience reasons. Wetting and disintegration characteristics of the products upon addition of the solid dosage form to water should be good in order to maintain these dissolution characteristics. Therefore, often a matrix former is added to the suspension prior to the drying operation. Higher freezing rates and lower drug concentrations may result in less agglomerated products. Formulations FF_2, FF_3, FF_6 and FF_9 were non-sticky, formulation FF_2 was selected as final formulation as it employed lower amount of matrix former. FTIR spectra recorded were shown in Figure 2.

Table 7: Various matrix former concentrations used for freeze drying

FC	Matrix former	Concentration (% w/v)	Inference
FF_1	Sucrose	50	Sticky powder
FF_2	Sucrose	100	Particle size 353 nm (before lyophilization 274 nm)
FF_3	Sucrose	200	Particle size 342 nm (before lyophilization 274 nm)
FF_4	Mannitol	50	Sticky powder
FF_5	Mannitol	100	Sticky powder
FF_6	Mannitol	200	Particle size 367 nm (before lyophilization 274 nm)
FF_7	MCC	50	Sticky powder
FF_8	MCC	100	Sticky powder
FF_9	MCC	200	Particle size 410 nm (before lyophilization 274 nm)

MCC: Microcrystalline cellulose

The characteristic peaks (values) of the drug are maintained with only minor changes in frequency and height. Thus, FTIR analysis shows no evidence of any significant degradation in drug.

Evaluation of nanosuspension

Particle size analysis

Particle size of unprocessed powder was found to be 5.74 µm while that of optimized nanosuspension was 274 ± 8 before lyophilization and 353 ± 7 post lyophilization. The polydispersity index for these samples was 0.216 and 0.241, respectively which indicates fairly narrow size distribution, whereas a PI value greater than 0.5 indicates a very broad distribution.

Particle morphology (SEM study)

Morphology of acyclovir pure drug showed larger crystals with wide particle size distribution and lyophilized acyclovir nanosuspension showed narrow size distribution Figure 3.

DSC studies

DSC thermogram [Figure 4], shows sharp endothermic peak (250C) ascribed to melting point of bulk acyclovir, whereas the lyophilized nanosuspension melted at 239C which is due to reduced particle size, the sharp melting endotherms indicates crystalline nature of final formulation.

XRD study

Figure 5 shows XRD pattern of unprocessed acyclovir powder and lyophilized acyclovir nanosuspension . The diffractograms showed no changes in crystalline nature upon nanonization and lyophilization. However, the differences in the relative intensities of their peaks might be attributed to the differences in the crystallinity of the samples.

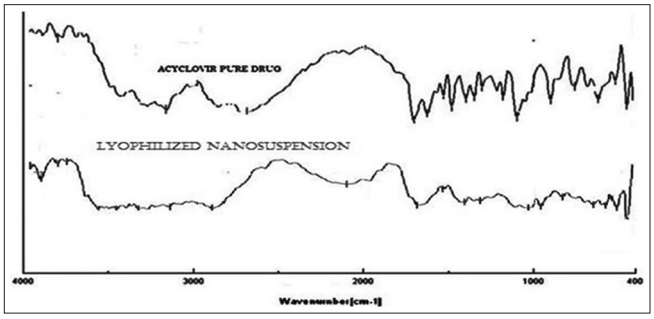

Figure 2: Fourier transform infrared spectroscopy spectra for process feasibility study

Saturation solubility determination

Saturation solubility data obtained for unprocessed acyclovir powder and lyophilized nanosuspension is tabulated in Table 8. Approximately 4 times increase in saturation solubility of lyophilized nanosuspension was observed than that of unprocessed drug.

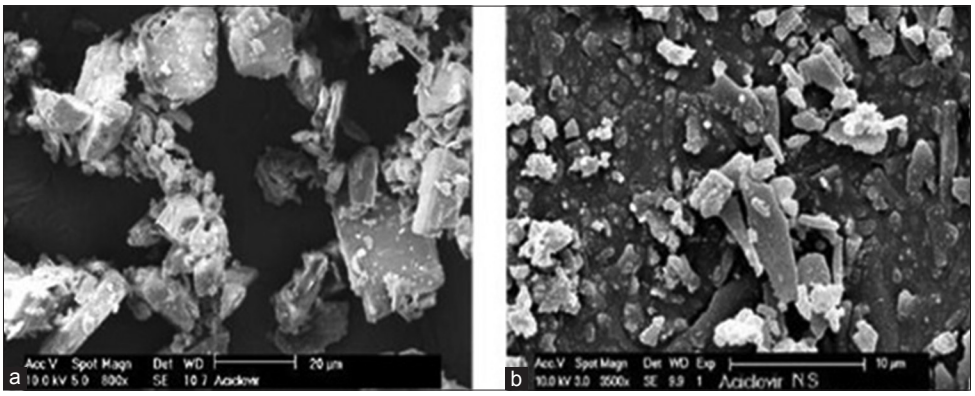

Figure 3: Scanning electron microscopy images of (a) unprocessed acyclovir powder (b) lyophilized nanosuspension powder

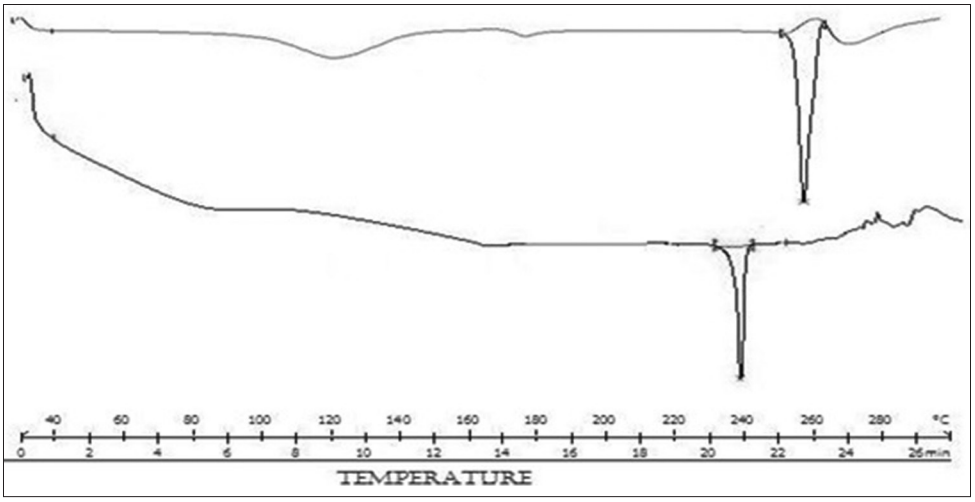

Figure 4: Differential scanning calorimetry thermogram of (a) unprocessed acyclovir powder (b) lyophilized nanosuspension powder

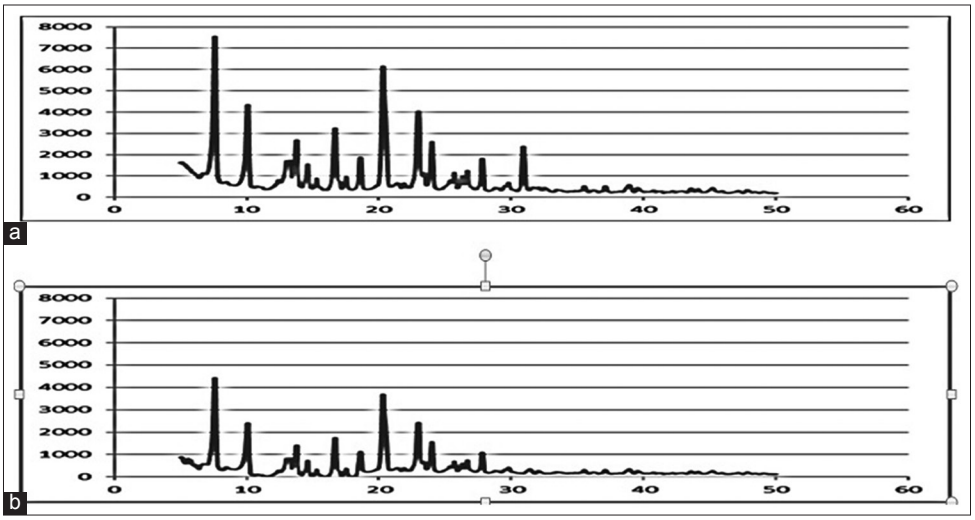

Figure 5: X-ray powder diffraction data of (a) unprocessed acyclovir powder (b) lyophilized acyclovir nanosuspension

The explanation for increase in saturation solubility can be given with Ostwald-Freundlich's equation which is described as follows

$$S = S_{\infty} \exp\left(\frac{2\gamma M}{r\rho RT}\right)$$

where *S* is the saturation solubility, S_{∞} is the solubility of the solid consisting of infinitely large particles, γ is the interfacial tension of substance, *M* is the compound molecular weight, *R* is the gas constant, *T* is the absolute temperature, Þ is the density of the solid and *r* is the radius. This equation is significant below 1 µm. This makes nanosizing is more efficient than micronization.

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. Lyophobic surfaces from the inside of the crystal are exposed to the aqueous dispersion medium during nanosizing. According to Ostwal-Freundlich's equation, *S* is dependent on the interfacial tension γ and subsequently on the interfacial energy *G* (G=γA). Differences in interfacial energy have a profound effect on the saturation solubilities of polymorphic forms of the drug; the same explanation might be valid for the nanosuspension (high energy form = polymorph II = higher *S*) compared to microparticulate suspensions (low-energy form = stable polymorph I = lower *S*).[1,15]

In vitro dissolution study

When compared with marketed suspension, nanosuspension shown 99.56% release in 5 min as against 92.74% release in 60 min for former [Figure 6]. This increase in dissolution can be explained with following theory.[1,15,16]

Table 8: Saturation solubility study

Buffer of pH	Solubility at 37±1°C (mg/ml) (*n*=3, mean±SD)	
	Unprocessed drug	Nanosuspension
1.2	3.6±0.13	14.34±0.35
6.8	2.4±0.18	10.11±0.18
7.4	2.5±0.15	9.70±0.21
Distilled water	2.5±0.22	9.56±0.15

SD: Standard deviation

Nernst-Brunner/Noyes-Whitney equation

The solid drug dissolution rate is directly proportional to surface area available to dissolution, which can be explained by Nernst-Brunner/Noyes-Whitney equation

$$\frac{dx}{dt} = \frac{AD}{h}\left(C_S - \frac{X_d}{V}\right)$$

where, $\frac{dx}{dt}$ is the dissolution velocity, *D* is the diffusion coefficient, *A* is the surface area of the particle, *h* is the diffusional distance, C_s is the saturation solubility of the drug, X_d is the concentration in the surrounding liquid and *V* is the volume of the dissolution medium. It is evident that particle size down to submicron range will further increase dissolution rate due to increase of effective surface area.

Ex vivo permeation study

The *ex vivo* drug permeation from *in vitro* dissolution vessel across everted chicken intestine is compared for marketed and lyophilized suspensions [Figure 7].

Marketed suspension showed 19.22% absorption with absorption rate of 0.0585, while lyophilized nanosuspension showed 67.56% absorption with absorption rate of 1.5354. Increase in absorption rate might be due to increase in dissolution rate resulting in increased concentration gradient and poloxamer 188, which may contribute by modifying the permeability.

In vivo study

The results of *in vivo* studies corroborated the findings of *ex vivo* studies, for the marketed suspension, T_{max} was found to be 30 min and C_{max} was 1125.23 ± 668.3 ng/ml. While for lyophilized nanosuspension T_{max} was reduced to 15 min. C_{max} for lyophilized nanosuspension was found approximately three-fold higher than marketed suspension (3178.54 ± 745.7 vs. 1125.23 ± 668.3 ng/ml). Approximately three-fold increase in area under curve of lyophilized nanosuspension than marketed suspension was observed (20933.07 ± 567.8 vs. 7780.924 ± 457.7 ng h/ml). The difference in T_{max} and C_{max} of acyclovir lyophilized nanosuspension was extremely significant ($P < 0.0001$) when compared with acyclovir marketed suspension. Decrease in T_{max} and increase in C_{max} can be attributed to increase in dissolution rate and absorption

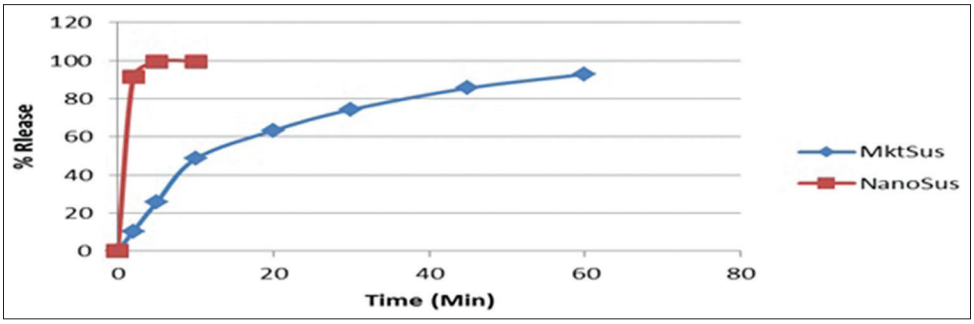

Figure 6: *In vitro* release study

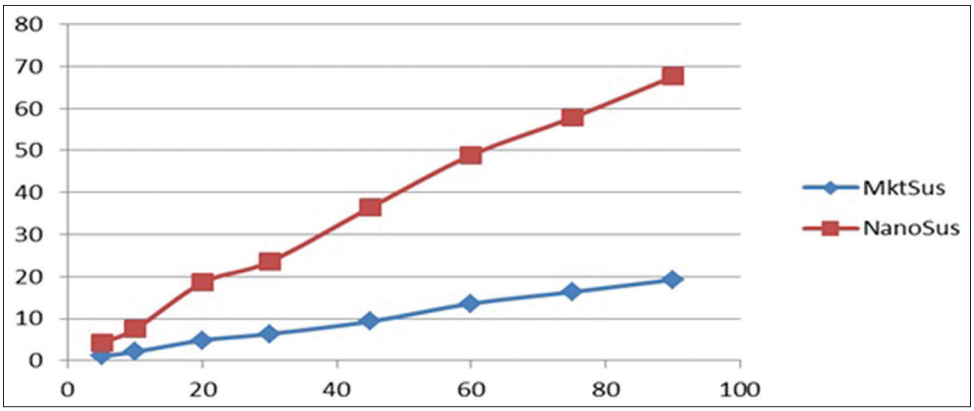

Figure 7: Ex vivo permeation study (x axis = time, y axis = percent permeated)

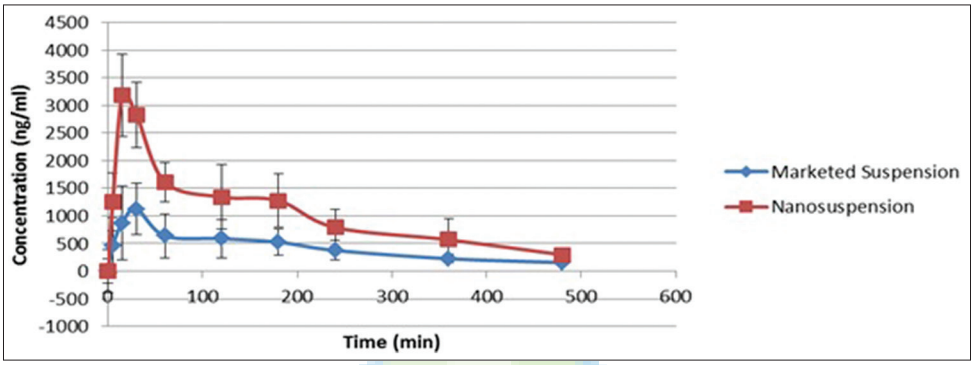

Figure 8: Plasma concentrations profile of acyclovir formulations in rats

rate. Thus, it can be concluded that significant increase in bioavailability of acyclovir is possible with nanosuspension formulation. The increased bioavailability will ultimately result in dose reduction. Eventually reduced dose will lessen the side effects [Figure 8].

CONCLUSION

It was concluded that a simple precipitation-ultrasonication method can be successfully employed to produce stable acyclovir nanosuspensions. The advantage of process lies in it being totally free of organic solvents. The particle size of nanocrystals was highly dependent on process parameters and with careful optimization nanosuspensions with diameter of about 274 nm (±8 nm) could be obtained. The process does not alter crystalline character of bulk acyclovir and marked enhancement of dissolution rate was achieved by the reduction in particle size. The oral bioavailability of acyclovir in Wistar rats resulted from nanosuspension was increased by three-fold compared with the marketed suspension. The significant dose reduction is possible for acyclovir with nanosuspension formulation can enhance patient compliance.

REFERENCES

1. Patravale VB, Date AA, Kulkarni RM. Nanosuspensions: A promising drug delivery strategy. J Pharm Pharmacol 2004;56:827-40.
2. Müller RH, Jacobs C, Kayser O. Nanosuspensions as particulate drug formulations in therapy. Rationale for development and what we can expect for the future. Adv Drug Deliv Rev 2001;47:3-19.
3. Liversidge GG, Cundy KC, Bishop J, Czekai D. Surface modified drug nanoparticles. US Patent No. 5145684; 1991.
4. Muller RH, Becker R, Kruss B, Peters K. Pharmaceutical nanosuspensions for medicament administration as system of increased saturation solubility and rate of solution, US Patent No. 5858410; 1998.
5. Chen Y, Liu J, Yang X, Zhao X, Xu H. Oleanolic acid nanosuspensions: Preparation, *in-vitro* characterization and enhanced hepatoprotective effect. J Pharm Pharmacol 2005;57:259-64.
6. Xia D, Quan P, Piao H, Sun S, Yin Y, *et al*. Preparation of stable nitrendipine nanosuspensions using the precipitation-ultrasonication method for enhancement of dissolution and oral bioavailability. Eur J Pharm Sci 2010;40:325-34.
7. Agnihotri SM, Vavia PR. Diclofenac-loaded biopolymeric nanosuspensions for ophthalmic application. Nanomedicine 2009;5:90-5.
8. Dixit P, Jain DK, Dumbwani J. Standardization of an *ex vivo* method for determination of intestinal permeability of drugs using everted rat intestine apparatus. J Pharmacol Toxicol Methods 2012;65:13-7.
9. Technical Brochure, BASF Corporation. Available from: http://www.pharma-solutions.basf.com. [Last accessed on 2012 Aug 24].
10. Tao Y, Lu Y, Sun Y, Gu B, Lu W, Pan J. Development of mucoadhesive microspheres of acyclovir with enhanced bioavailability. Int J Pharm 2009;378:30-6.
11. Boulieu R, Gallant C, Silberstein N. Determination of acyclovir in human plasma by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 1997;693:233-6.
12. Product manual of probe sonicator VCX750. Available from: http://www.sonics.biz/lp-vibra.htm [Last accessed on 2012 Mar 23].
13. Hany SM. Preparation of hydrocortisone nanosuspension through a bottom-up nanoprecipitation technique using microfluidic reactors. Int J Pharm 2009;375:107-13.
14. Van Eerdenbrugh B, Van den Mooter G, Augustijns P. Top-down production

of drug nanocrystals: Nanosuspension stabilization, miniaturization and transformation into solid products. Int J Pharm 2008;364:64-75.
15. Kesisoglou F, Panmai S, Wu Y. Nanosizing-Oral formulation development and biopharmaceutical evaluation. Adv Drug Deliv Rev 2007;59:631-44.
16. Chingunpituk J. Nanosuspension technology for drug delivery. Walailak J Sci Technol 2007;4:139-53.

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