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ORIGINAL ARTICLE

Preparation and *In Vitro*/*Ex Vivo* Evaluation of Acyclovir-Loaded Polymeric Nanoparticles for Ophthalmic Application

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ABSTRACT
Due to the eye's physiological barriers, ocular bioavailability of drugs from conventional eye drop is very low. Ocular efficacy is usually closely linked to the bioavailability of ocular drugs, which can be improved by increasing the penetration of corneal drugs and prolonging precorneal residence time. The existing study also involves the growth, characterization, and evaluation of biodegradable nanoparticles of acyclovir intended for ocular use. Using Poly D, Lactide, nanoparticles were prepared using nanoprecipitation techniques. 3^2factoral designs were applied to optimize the drug formulation. The effect on entrapment efficiency and particle size of independent variables such as drug ratio and speed of homogenizers was investigated. On the optimized method, further studies such as differential calorimetry scanning, X-ray diffraction and electron microscopy scanning were performed. In-vitro drug release study indicates prolonged drug release. Ocular tolerance was evaluated using the HET-CAM (Hen's Egg Chorioallantoic Membrane) test which showed non-irritant efficacy of the formulation developed. These results suggest the feasibility of encapsulating biologically degradable polymeric nanoparticles of Acyclovir for ocular delivery.
Keywords: *Acyclovir, Poly D,L-lactide, nanoparticle, ocular drug delivery*

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INTRODUCTION

Acyclovir is an antiviral drug with a important and highly selective efficacy against herpes viruses and is commonly used to treat various eye diseases [1].The normal use of acyclovir, now widely available as ointments and drops, is limited by low corneal penetration of the medication, poor bioavailability of the ocular medication, pulse drug entry, systemic exposure due to the drainage of the nasolacrimal duct and decreased entry into the rear segments of the eye due to the lens-iris diaphragm. Hence development of acyclovir-loaded polymeric nanoparticles was undertaken for effective ocular delivery of the drug with improved bioavailability.

The recommended drug delivery method is eyepiece drug delivery method. Ocular drug delivery systems are designed to treat the eye locally, whereas past formulations are designed to achieve systemic circulation and to solve all the drawbacks of conventional dosage types such as ophthalmic solutions [2].The main problem with conventional dosage forms is eye irritation (due to drug particle size and shape) which induces lacrimation i.e. overflow on to lids, tear turn over, and due to pharmacokinetic responses like metabolism, non-specific binding and different mechanisms like diffusion, dissolution and erosion the conventional dosage forms are less advantageous [3].

The eye drop dosage type is easy to instill but suffers from the inherent downside that the bulk of the drug it contains is absorbed instantly in the tear film as soon as the eye drop solution is instilled into the cul-de-sac and is easily drained away from the precorneal cavity by continuous tear streaming, a process that continues more intensively in inflamed eyes than in normal eyes and lacrimal-nasal drainage [4].

Ocular efficacy is typically closely linked to the bioavailability of ocular drugs, which can be improved by increasing penetration of corneal drugs and prolonging precorneal residence time. A variety of eye drug

delivery mechanisms such as implants and collagen shields and colloidal mechanisms such as liposome, nanoparticles and nanocapsules have been developed and investigated for improved ocular bioavailability. The use of nanotechnology based drug delivery systems, such as microemulsions, nanosuspension, nanoparticles, solid lipid nanoparticles, niosomes, dendrimers and liposome, has led to the solution of numerous problems associated with solubility of poorly soluble drugs [5]. Polymeric nanoparticle formulation is one of the strategies currently used to improve drug absorption across biological membranes.

Given the fact that there is a short residence of the dosage form in the ocular cavity, it was proposed that the use of mucoadhesive polymers improve the concentration and residence time of the associated drug[6].Based on literature evidence, the three most commonly used polymers in ophthalmic drug formulations are poly (alkyl cyanoacrylates), polycaprolactone, and poly D, L-lactide. The literature data also reveals that in the case of ocular drug delivery, a suitable particle size and a narrow size range, ensuring short irritation, sufficient bioavailability and compatibility with ocular tissues, should be required for every suspended drug. Among the wide range of mucoadhesive polymers reported in the literature, the Poly D, L-lactide has been selected as a polymer of choice because of its unique properties including acceptable biodegradability, biocompatibility and the ability to increase membrane permeability [7].

The goal of the present research was therefore to develop and characterize biodegradable nanoparticles containing Acyclovir, in order to improve precorneal residence time and eye bioavailability.

MATERIAL AND METHODS

Acyclovir was received from Ipca Laboratories, Mumbai., India as a gift sample. Polyvinyl alcohol (PVA) M. Wt. 22000 Dialysis tubing cellulose membrane which is having molecular weight cut-off 12000-14000 g/mole was purchsed from Sigma Aldrich Pvt Ltd. Mumbai, India. Poly (D,L-lactide) ester having viscosity of 0.34 dL/g was purchased from Sigma Aldrich Pvt. Ltd. Mumbai. All other reagents used were of analytical grade.

Preparation of drug loaded nanoparticles using nanoprecipitation technique

The nanoprecipitation technique was used for the preparation of Acyclovir nanoparticles. Organic solution of biodegradable polymer (PLA) and exact amount of Acyclovir (50 mg) in 10 mL of acetone were prepared. The organic phase was added dropwise into 20 mL of aqueous solution containing PVA (1%) and stirred magnetically. After 30 min of stirring the volume of nanoparticles dispersion was concentrated to 10 mL under reduced pressure using a Rota evaporator with vacuum (KNF, vaccum pumps & system). The aggregates were removed by filtration through a 0.45µm syringe filter. Separation of non-encapsulated drug was performed by ultracentrifugation (Beckman Coulter) at 50,000rpm at 4^0C for 30 min. The supernatant was discarded and separated nanoparticles were washed twice with distilled water to remove excess surfactant. The washed particles were resuspended in 5 mL of water solution containing 5 %(w/v) mannitol as cryoprotectant and freeze dried for 48 hrs. The whole experimental carried out in aseptic area. The nanoparticles were kept at 2 to 8^0C for further investigation.

Design of the experiment

The effect of different parameters on the physicochemical properties of the prepared nanoparticles was studied using 3^2full Factorial design. Concentration of polymer and different speed were selected as the independent variables. The particle sizes of colloid system and encapsulation efficiency of the drug were selected as the dependent variables.

Table I: Experimental plan of the 3^2F1ctorial designs.

Factor	Level		
	++	+	-
Polymer Concentration (mg)	150	100	50
Speed of Homogenizer	1200	1000	800

Table II: Composition of Acyclovir loaded nanoparticles formula prepared according to 3^2Factorial designs.

Formula Number	Drug : Polymer Concentration	Speed
F1	1:1	800
F2	1:2	800
F3	1:3	800
F1	1:1	1000
F5	1:2	1000
F6	1:3	1000
F7	1:1	1200
F8	1:2	1200
F9	1:3	1200

Characterization of the nanoparticles

Determination of the particle size

Photon correlation spectroscopy was used to determine size distribution, average particle size and PDI using Zetaparticle scale, Model Nano ZS. The different nanoparticles were measured followed by distilled water dilution. The particle size and PDI were measured at a scattering angle of 900 and at a temperature of 25 °C. All experiment done in triplicate [8].

Encapsulation efficiency (EE %) measurements

The efficiency of encapsulation of Acyclovir in the polymeric nanoparticle was determined by the extraction and quantification of the encapsulated acyclovir. Acyclovir polymeric nanoparticles were applied to 25 ml methylene chloride and subjected to 12 h room temperature shaking to ensure complete particle dissolution. The resulting solution was diluted with appropriate dilution and the concentration of the drug in methylene chloride was determined by spectrophotometric by measuring UV absorbance at 253 nm [9].

EE of the drug= (amount of encapsulated drug) / (total amount of the drug) X 100

…….Equation No.1

Redispersibility of nanoparticles:

For further analysis, the selected formulation was congeldried to obtain a dry powder. Furthermore, the effect of cryoprotectant on freezing drying and dispensability of prepared nanosuspension was studied. Mannitol is used as a cryoprotectant at a concentration of 5 times the total solid content in formulation. Two samples of nanosuspension each were placed in a flask, the amount of mannitol required added to one and shaken to dissolve, the second sample left with out of cryoprotectants. These flasks were frozen in a deep freezer at −20°C for 12 h for primary freezing. Then the container was attached to the vacuum adapter of the lyophilizer. The solvent sublimed under a pressure of 80 mmHg for 48- 72 h [10].

Swelling Index

The accurately weighed nanoparticles were placed in a glass vial containing pH 7.4 phosphate buffer 10 mL at 37 ± 0.5 °C in incubator and was stirred occasionally. The nanoparticles were periodically removed by blot using filter paper and the change in weight of particulates was measured till equilibration. The weight was recorded after a period of 3 h in triplicate and the swelling ratio (SR) was calculated using formula (Eq. 2) [11].

Swelling index (%) = W1-W2 X 100 / W1

…….. Equation No. 2

Where,

W1 = Weight of nanoparticles after swelling

W2 = Initial weight of nanoparticles

Differential Scanning Calorimetry

Differential scanning calorimetric measurements were carried out by using differential Scanning Calorimeter(DSC DA 60 Shimadzu, Japan)equipped with a liquid nitrogen sub ambient accessory. The DSC was performed for the pure acyclovir, the PLA and the drug loaded nanoparticle formulation. Sample 2 mg were loaded in a flat-bottomed aluminum pan and subjected to a heating cycle from 40 to 400^0C with a heating rate of 10^0 C/min. A stream of nitrogen gas was used to control the heating and cooling rate. The temperature and energy scales of the instrument were calibrated using purified indium as the reference material [12].

2.3.6 X-ray diffraction

X-ray diffraction analysis was employed to identify the crystallinity of the drug in nanoparticle formulation, which was conduct using a Philips PW 3710 x-ray diffractometer (XRD) with a copper target

and nickel filter. Powders were mounted on aluminum stages with glass bottoms and smoothed to a level surface. The XRD pattern of each sample was measured from 10 to 50 degrees 2-theta using a step increment of 0.1 2-theta degrees and a dwell time of 1 second at each step [13].

Scanning electron microscopy

The scanning electron microscopy (JEOL Model JSM - 6390LV) was use to characterize the surface morphology of nanoparticles. The nanoparticles were mounted directly on the SEM stub, using double sided, sticking tape, and platinum coated, and scanned with a directed electron beam in a high vacuum chamber. Secondary electrons were detected, released from the samples, and the picture formed [14].

In vitro release studies

The release study of nanoparticle formulation for the release of Acyclovirfrom polymeric nanoparticles was accepted out using a membrane diffusion technique. In vitro diffusion cell was made using dialysis membrane as a semi permeable membrane. To preserve the nanoparticles as well as to enable the free drug to diffuse freely into the release media a dialysis membrane of 12,000-14,000 Molecular weight cut-off was used. Formulated nanoparticles equivalent to 1 mg of the drug were dispersed in 1 mL of isotonic phosphate-buffered saline at pH 7.4. The nanoparticle dispersions were packed in dialysis membrane secured with two clamps at each end. To maintain sink condition, the dialysis bag was immersed in tightly-capped glass vials (7 × 2.8 cm) containing 10 mL of 0.5 % (w/v) of sodium lauryl sulphate solution in distilled water. The release test was performed by placing the glass vials in a thermostatically-controlled shaking water bath adjusted to 37 ± 0.5 °C with a constant shaking rate of 100 rpm. At predetermined time points, the whole release medium was withdrawn and replaced with fresh release medium. The concentration of the drug released was measured with a UV spectrophotometer. All experiments were carried out in triplicate [15].

Chorioallantoic membrane (HET-CAM) test and irritation score calculation

In the Draize eye irritation test, a chorioallantoic membrane (CAM) test as a mucous membrane discomfort test was conducted. Commercially available fertilized white chicken eggs without micoplasms were used for the test. For the CAM test, the hen's eggs were put in incubator trays with the wide ends up; the trays were put in the incubator, which rotates automatically and was held at the optimal point temperature of 37.5±0.5°C. The eggs were candled on day 5 of incubation and every day thereafter; nonviable embryos were removed. On day 10 of incubation the egg shell was scratched around the air cell by a dentist's rotary saw and then pared off. After careful removal of the inner egg membranes the vascular CAM was exposed. The test sample in volume of 0.2ml was applied on the CAM surface. A series of four eggs was used; two eggs, treated with vehicle only, serve as controls. After the application of the test substance, the CAM, the blood vessels, including the capillary system, and the albumen wereexamined and scored for irritant effects (hyperaemia, haemorrhages, coagulation) at 0.5, 2 and 5 minutes after treatment. The numerical time-dependent scores for hyperaemia, haemorrhages and coagulation (Table III) were summed to give a single numerical value indicating the irritation potential of the test substance on a scale with a maximum value of 21. The mean value of four tests makes possible an assessment by a classification scheme analogous to the Draize categories (Table IV) [16].

Table III: Scoring scheme for irritation testing with the hen's egg chorioallantoic membrane.

Effect	**Score (Time in Second)**		
	0.5	2	5
Hyperemia	5	3	1
Hemorrhage	7	5	3
Coagulation	9	7	5

Table IV: Classification of cumulative scores in the chorioallantoic membrane test.

Cumulative Irritation	**Score assessment**
0-0.9	Practically none
1-4.9	Slight
5-8.9	Moderate
9-21	Strong

Stability Studies:

Stability studies were carried out on optimized formulation at 30 ± 2°C in stability chamber (Thermolab) for 6 months. The optimized formulation stored in the sealed in glass bottle. After 6 months, drug content, particle size and redispersibility studies were carried out [17].

Sterility Testing:
All parenteral preparations should be sterile. Sterility studies were passed out to ensure the sterility of finished product. Since it is administered by parenteral route, direct inoculation method was ideal to carry out sterility testing. In this method, the specified quantity of sample under test was drawn aseptically from the containers and transferred to fluid thioglycollate medium (20 mL) and Soybean-Casein digest medium (20 mL), separately. Mixture of nanoparticles with the medium was incubated for not less than 14 days at 30°C–35°C in case of fluid thioglycolate medium and 20°C–25°C in case of Soybean-Casein digest medium. The growth of any microorganisms in the medium was observed [18].

RESULTS AND DISCUSSION

Experimental Design
Two factors (polymer concentration and homogenizer speed) were calculated on the basis of the experimental and above studies crucial factor for particle size and trap efficiency of prepared polymeric nanoparticles. Polymer concentration has been shown to have a major effect on the efficacy of trapping as the optimal polymer concentration.The speed of the homogenizer is also effect on optimization formulation [19].

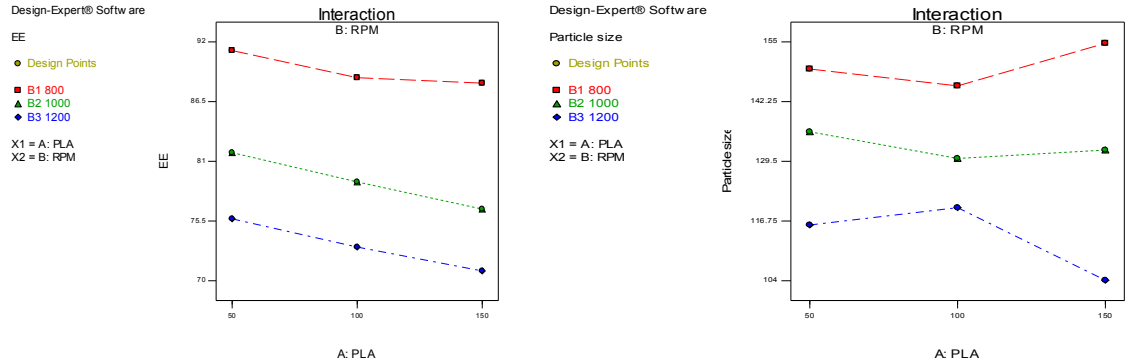

Figure.1: Surface plots showing the effect of (A) Encapsulation Efficiency (B) Particle Size

Preparation of drug loaded nanoparticles:
Acyclovirloaded PLA Polymeric nanoparticles were successfully prepared using the technique of nanoprecipitation because it is fast and simple to conduct. The creation of nanoparticles is one step and the process is instantaneous. When the polymer solution is applied to the non-solvent, nanoprecipitation follows rapid desolation. The polymer precipitates as soon as the polymer containing solvent has diffused into the dispersing medium, causing immediate product treatment. In addition, this method often produces a carrier size within the range of nanometers and uses ingredients with low toxic potential which are suitable for ocular path.

Characterization of nanoparticles

Determination of particle size
The particle size is an important factor in the development of an ocular drug delivery system when considering discomfort and comfort. Figure 2 displays the mean particle size of prepared formulae for the nanoparticles. The size of the particles ranges between 104.1 and 154.7 nm. The effect of various variables of formulation, namely concentration and speed of polymers, had a major impact on particle size. Concerning the effect of polymer concentration Fig. 2 show that the particle sizes obtained by using lower polymer concentration were significantly smaller than those obtained by using higher polymer concentrationThe particle size is also influenced by different speeds, as shown in Fig.2.
Particle sizes obtained by using low velocity were substantially greater than those obtained by using high velocity. Yet it should be noticed that it did not decrease the particle size to the significant extent when speed is increased further.
Particle size distribution represented by polydispersity index (PDI) was measured for all nanoparticles formulations. The mean PDI values ranges from 0.555 to 1.0. The narrow distribution represented by the small PDI values denotes particle size uniformity in the nanoparticle formulae.

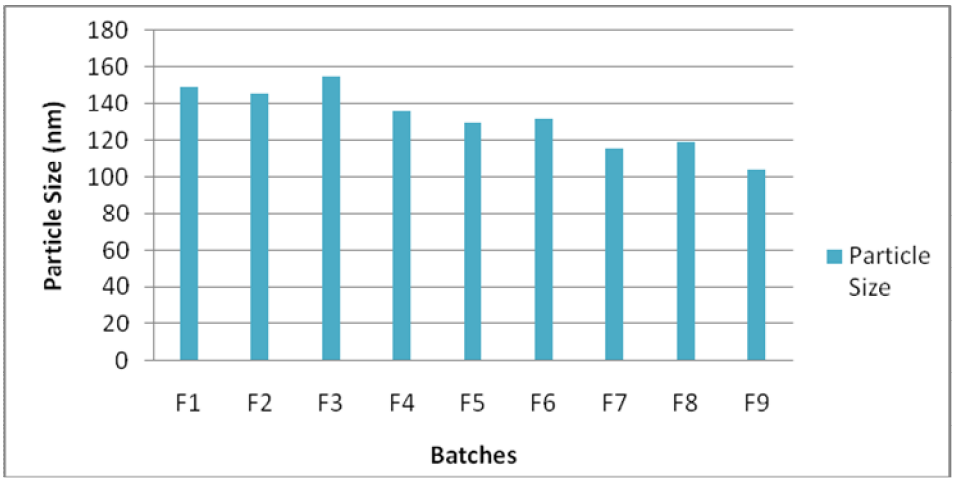

Figure 2: Particle size of the Acyclovir nanoparticles formulations

Encapsulation Efficiency (EE)

The efficiency of encapsulation of nanoparticles ranged from 70.2 to 91.9 per cent. All variables tested have a major effect on the EE rate. It was observed that the increase in polymer content resulted in a decrease in the EE percentage of formulae for nanoparticles. Figure 3 this finding can be due to the increasing viscosity of the organic process as the polymer content increases.

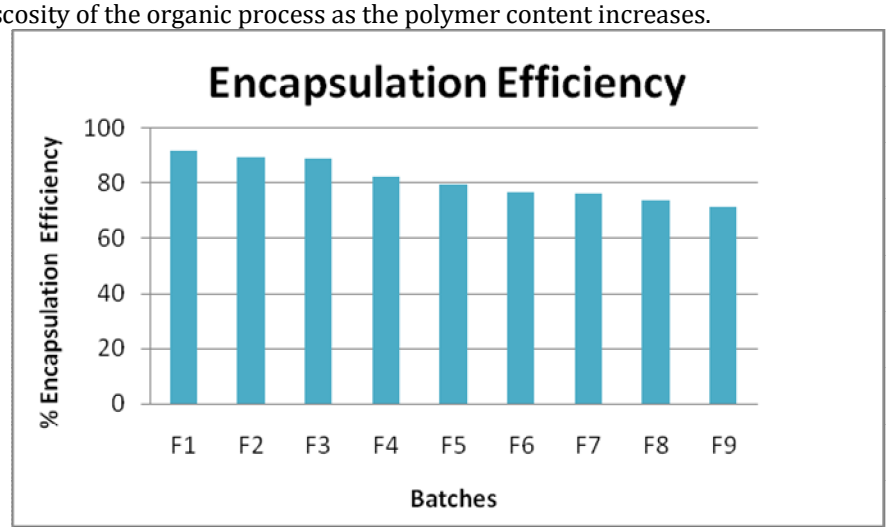

Figure 3: Encapsulation Efficiency of the Acyclovir in nanoparticles

Redispersibility Test:

Dispersibility was found to be increased by spontaneously dispersing mannitol as cryoprotectants and products into primary nanosuspension in both media within 1 to 3 min (0.1 N HCl and phosphate buffer pH 6.8). It suggested that mannitol in the drugs would enhance hydrophobic drug wetting and increase water penetration into the drugs. 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.

Swelling Index:

As polymer concentration is increase from 50 mg to 150 mg exploration increase in swelling index from 58% to 80% indicates that being PLA as hydrophilic polymer uptake excessive amount of water responsible for swelling for polymeric nanoparticles.

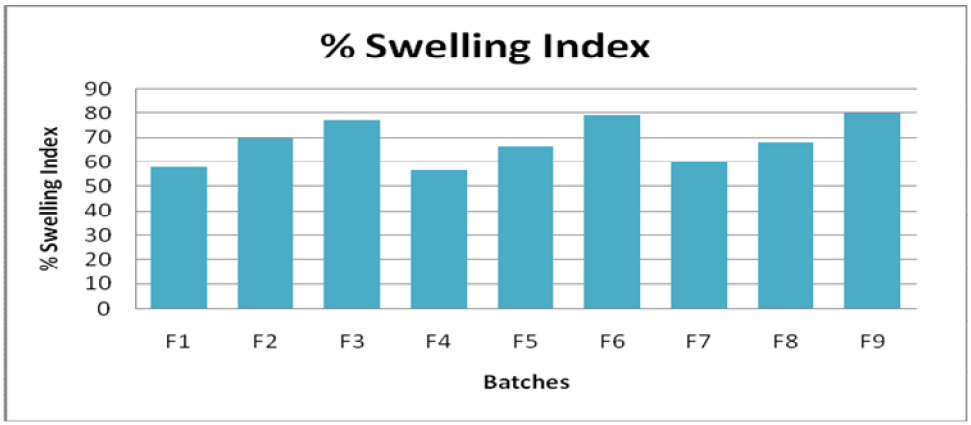

Figure 4: Percentage of Swelling Index of Nanoparticles Formulation.

DSC

To verify the existence in the physical interaction between Acyclovirand excipients, each sample was analyze by differential scanning calorimetry (DSC). DSC thermogram of Acyclovir, PLA and lyophilized drug-loaded nanoparticles is shown in fig 5. In the drug loaded nanoparticles thermogram, the characteristic endothermic peaks at 254^{0}C of the drug disappeared. It could therefore be concluded that Acyclovirwas entrapped in an amorphous or molecular dispersion state in the polymer matrix.

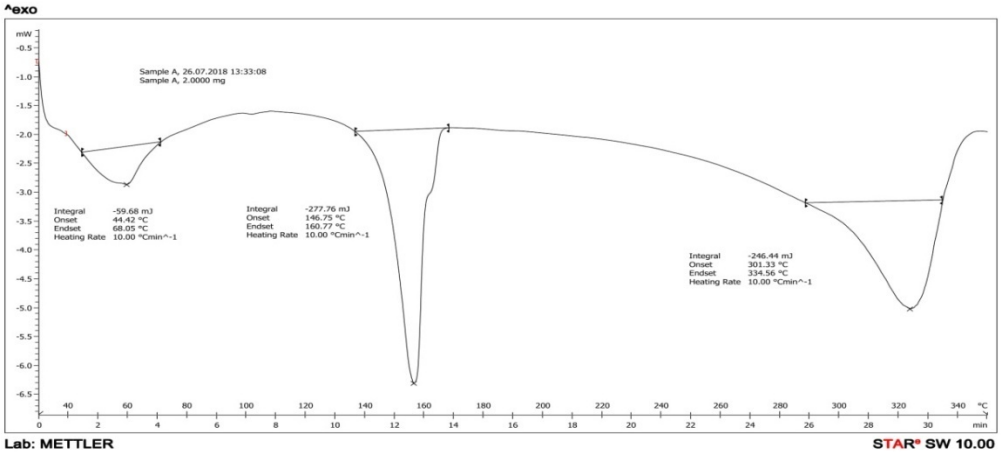

Figure 5: DSC thermogram of the optimized nanoparticles formulation (F1).

XRD

The Acyclovir in crystallinity of the nanoparticles was achieved by powder X-ray diffraction. Fig. 6 shows structured formulation (F1) powder X-ray diffraction patterns. It shows that the drug was present in amorphous state in the nanoparticles, supporting DSC performance.

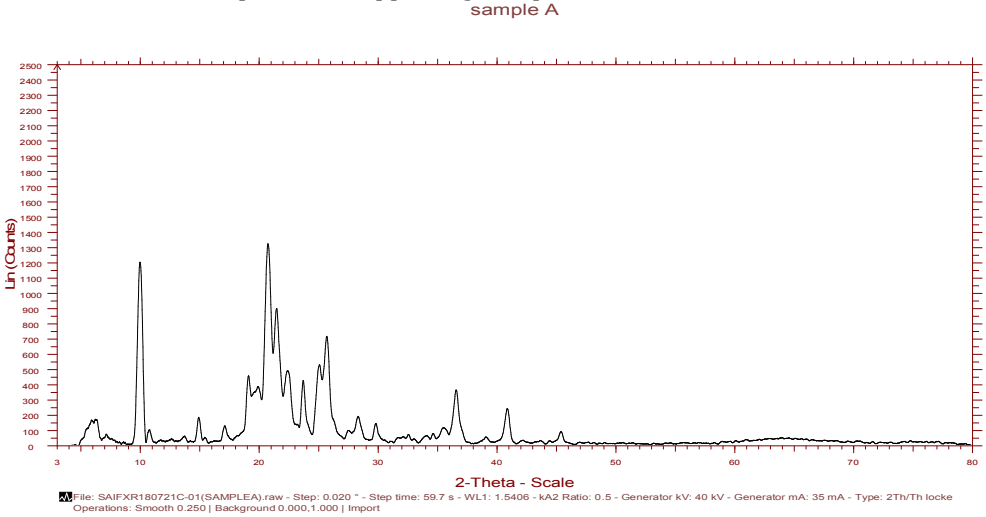

Figure 6: XRD of the optimized nanoparticles formulation (F1).

Scanning Electron Microscopy (SEM):
The morphology of drug loaded nanoparticles (F1) was accessed using SEM and is shown in fig 7. 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.

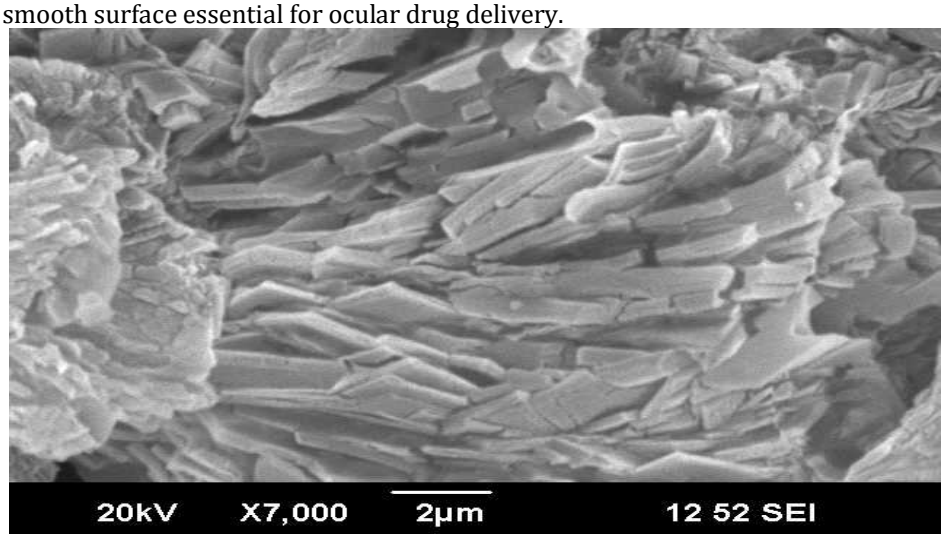

Figure 7: SEM of the optimized nanoparticles formulation (F1).

In vitro drug release from nanoparticles
The drug formulae prepared with nanoprecipitation technique were subjected to in vitro release study. The amount of Acyclovirreleased from nanoparticles was evaluated using a dialysis technique. The release profiles of Acyclovirare shown in Fig 8.
Drug release from PLA based nanoparticles reveals sustained release up to 1 hrs due to wetting followed by immediate release as results of rapid diffusion of drug release from swollen polymeric nanoparticles.

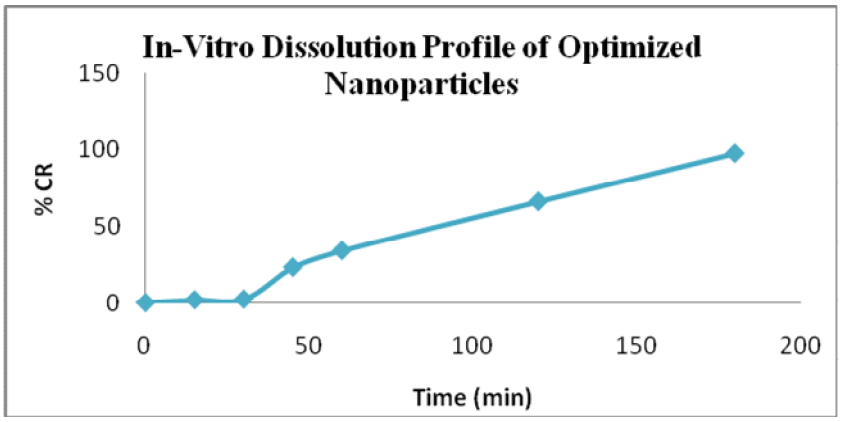

Figure 8: In vitro release study of the Acyclovir nanoparticles formulation.

CAM Test
It is found to that formulation is non irritant as score value for Hyperemia, Hemorrhage and coagulation is zero, as compared to phosphate buffer solution pH 7.4 should coagulation after five minutes (Score value 1.5) which is slightly irritant.

Figure 9: CAM Test of the optimized nanoparticles formulation (F1).

Stability Studies:
Stability tests in stability chamber (Thermolab) for 6 months were performed on optimized formulation a t 30 ± 2 ° C. The optimized formulation contained in aluminum foil in the sealed form. Studies on drug qua lity, particle size, and redispersibility were performed after 6 months.

Table 5: Stability of Acyclovirnanoparticles during storage (F1)

Parameter	**0 Day**	**1 Month**	**3 Month**	**6 Month**
Drug Content	91.2	91.0	90.8	90.1
Particle Size	149.2	151.4	154.7	155.3

3.2.11 Sterility Testing
During sterility testing, we found that there was no evidence of microbial growth when formulations were incubated for not less than 14 days at 30°C to 35°C in case of fluid thioglycolate medium and at 20°C to 25°C in case of Soybean-Casein digest medium demonstrating that formulation passes the test for sterility.

CONCLUSION

Acyclovir was successfully designed to use nanoprecipitation technique in biodegradable nanoparticles. The analysis of formulations using 3^2 Factorial designs is used for achieving the optimum formulation. The drugpolymer ratio and velocity had a major impact on nanoparticle particle size and encapsulation efficiency. The formulated acyclovir nanoparticles have been found to be an effective and potential natural carrier in terms of particle size, drug load efficiency, characteristics of in vitro release redispersibility, sterility and better eye tolerability. The study of acyclovir's stability from nanoparticles showed spectacular results.

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CONFLICT OF INTEREST

There is no Conflict of interest

REFERENCES

1. Rajendran N N, Natrajan R, Siva Kumar R, Selvaraj S. (2010). Acyclovir-loaded chitosan nanoparticles for ocular delivery. Asian Journal of Pharmaceutics., 4(4): 220-226.
2. Lee VHL, Robinson JR. (1986). Topical ocular drug delivery: recent developments and future challenges. Journal of Ocular Pharmacology., 2(1): 67–108.
3. Lang JC. (2002). Ocular drug delivery conventional ocular formulation. Advanced drug delivery review 16(1):39-43.
4. K Basavaraj, Nanjawade, Manvi FV and Manjappa AS.(2007). In situ-forming hydrogels for sustained ophthalmic drug delivery. Journal of Controlled Release., 122(2): 119–134.

5. Sahoo KS, Fahima SAD, Kumar K. (2008). Nanotechnology in ocular drug delivery, Drug delivery today., 13: 144-151.
6. Weidener J. (2003). Mucoadhesive ocular inserts as an improved delivery vehicle for ophthalmic indications. Drug Discovery Today.,8: 906– 907.
7. Himanshu Gupta, Mohammed Aqil, Roop K Khar, Asgar Ali, Aseem Bhatnagar, Gaurav Mittal. (2010). Sparfloxacin-loaded PLGA nanoparticles for sustained ocular drug delivery. Nanomedicine.,6(2): 324–333.
8. Gupta P., Pandit J, Ajay P, Swaroop P. and Gupta S. (2010). Pharmaceutical Nanotechnology Novel Nanoemulsion-High Energy Emulsification Preparation, Evaluation and Application. The Pharma Research. 3:117-138.
9. S.A. Abdel Halim*, S. Salah. (2014). Development of Nanoparticulate formulations for ocular delivery of prednisolone acetate: preparation and characterization. Journal Drug Del. Sci. Tech.,24(2):159-165.
10. V.Viswanath, B. Narasimha Rao, K. Gnana Prakash, S.Rahath Fatima, G. Krishnaveni. (2017). Formulation and evaluation of Terbulatinesulphate loaded inhalation nanoparticles for pulmonary drug delivery system. Int. Journal Pharmacy Sci. Rev.,42(1):256-260.
11. Dillen K.,Vavdervoort J., VandenMooter G., Verheyden L.,Ludwing A. (2004). Factorial design physicochemical characterization and activity of Ciprofloxacin–PLGA Nanoparticles. Int.J. Pharm., 275(1-2), 61-66.
12. Pignatello R, Bueolo C, Spedalieri G, Maltese A, Puglis. (2002). Flurbiprofen-loaded aerylate polymer nanosuspension. Biomaterials.,23: 3247-3255.
13. Junise V, R. Sarawasati. (2015). Development and characterization of chitosan nanoparticles loaded with isoniazid, Journal Pharma Sci.,4(3):190-195.
14. Roya Yadollahi, KrasimirVasilev, Clive A. (2013). Prestidge and Spomenka Simovic. Polymeric Nanosusoension for Enhanced Dissolution of Water Insoluble Drug. Journal of Nanomaterials Article., ID 170201:1-10.
15. Higuchi T. (1961). Rate of release of medicaments from ointment bases containing drugs in suspension, J. Pharma Sci., 50(10):874-875.
16. N. P. Lupepke. (1985). Hen's Egg Chorioallantoic Membrane Test for Irritation Potential. FdChem. Toxk., 23(2):287 291.
17. Drug Stability: (2001). Principle and Practices, third edition, revised and expanded edited by Jens T. Carstensen and C.T. Rhodes, Drug and Pharmaceutical Science, Vol.107, Marcel Dekker New Work.
18. RekhaKhaira, Jyoti Sharma, and Vinay Saini. (2014). Development and Characterization of Nanoparticles for the Delivery of Gemcitabine Hydrochloride. Hindawi Publishing Corporation Scientific World Journal.,Article ID 560962, 1-6.
19. PayalPatil, PoojaWankhede, Mahajan Hitendra,Laxmikant Zawar. (2018). Aripiprazole loaded polymeric micelles: fabrication, optimization and evaluation using response surface method. Recent Patent on Drug Delivery & Formulation. 12: 1-7.