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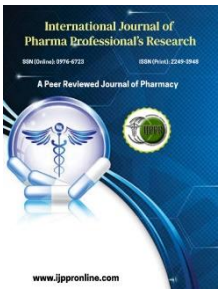

Utilization of ionotropic gelation method for targeting an Anti-viral nanoparticulate gel at the corneal surface for management of Varicella zoster viral infection

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ABSTRACT: The objective of the present work was to develop nanoparticulate gel of Acyclovir to enhance its residence time at ocular surface and release the drug in sustained manner for prolonged period. Initially, nanoparticles of selected drug was prepared. For that, Acyclovir and PLGA (100 to 400 mg) was taken in 1:1 ratio. The prepared nanoparticles were evaluated for entrapment efficiency, particle size & zeta potential, PDI, surface morphology and *in-vitro* drug release. The optimized formulation was then converted into gel by using Poloxamer 407 and Carbopol 940, thermo-responsive gelling agents. It was found that prepared formulation was clear. The pH value of the formulation was found nearly similar with lacrimal environment. The prepared gel formulation showed sustained drug release for a period of 12 h. The formulation was clear with pH value of 7.4. From the stability studies, it was revealed that optimized nanoparticulate gel formulation of Acyclovir (ACT) was remained stable at given conditions of temperature and humidity. Hence the research work, in reference, comprised of quite novel approaches of investigations.

1. Introduction

These days, the market is filled with many ocular drug delivery systems, among which are the widely utilized ophthalmic eye drops. The manufacture of polymeric gels, ointments, suspensions, and emulsions are some others. Of all topical eye preparations, topical eye drops are the most practical, non-invasive, and patient-friendly. When it comes to treatments, eye drops face a few obstacles. According to the study, a significant portion of patients had trouble injecting the drops.[1] In addition, the solution may be lost or diluted due to tear drainage, which rises with the amount of eye drops used. Apart from that, the eye pocket's small capacity makes it impossible to determine the amount of medicine absorbed into the ocular tissue. The ocular medicines' solubility and bioavailability may be improved by the emulsion-based formulation. The (o/w) emulsion is the superior choice because it has a higher ocular tolerance and causes less eye irritation.[2] Ointment is a blend of solid and semisolid hydrocarbons, including

paraffin, that melts at body temperature and doesn't irritate the eyes. Another dosage type for topically administering medication to the eye is ocular gel. Mucoadhesive polymers, one of the components that make up gels, are crucial for the localized distribution of active substances. In order to boost the effectiveness of ophthalmic gels, mucoadhesive polymers have been included. Because the ophthalmic premade gel is available at room temperature as a gel material, it is not as ideal as other dosage forms.[3] Despite the widespread use of traditional topical ocular treatments nowadays, there are still certain issues with their application, effectiveness, and safety. As a result, numerous strategies have been developed and researched. One strategy is to use nanoparticles and nano micelles, which are forms of nanotechnology, in the ocular drug delivery system. Other methods, such liposomes and ocular implants, can potentially be used to enhance the ocular delivery system.[4] Acyclovir is a novel antiviral medication that specifically inhibits the DNA polymerase of herpesviruses. It exhibits strong in vitro action against varicella-zoster and herpes simplex viruses. The medication can be applied externally to the skin, intravenously, or topically to the eyes (at this time, topical and intravenous versions are the only ones available for use). Improved patient compliance and a continuous and regulated release of the medications are two benefits of the gel-based drug delivery method.[5] To meet these evaluation criteria, an antiviral medication-loaded nanoparticulate gel was created and characterized in the current work.

2. Material and Methods

Acyclovir nanoparticulate gel was formulated using a variety of components that were obtained from reputable suppliers.

2.1 Methods

2.1.1 Formulation of Acyclovir (ACT) loaded PLGA Nanoparticles

Utilizing a high-speed homogenization process, PLGA nanoparticles loaded with acyclovir were created. To obtain a clear solution, 100–400 mg of PLGA and acyclovir were dissolved in a 1:1 dichloromethane and methanol solution (Organic phase). Tween 80 was used as surfactant at a concentration of 0.3 to 1.2%. To create a clear solution (Aqueous phase), tween 80 was dissolved in purified water while being constantly stirred. The organic phase was moved to a needle-equipped syringe and gradually introduced to the aqueous phase using a high-speed homogenizer running at 20,000 rpm. To evaporate the entire organic phase, the system was shaken for two to four hours at room temperature. Centrifugation was used to separate the NPs at 10,000 rpm for 30 minutes at a temperature of -10 C. The resultant NPs were utilized in thermoreversible gel after being freeze-dried with trehalose acting as a cryoprotectant to produce a free-flowing powder of ACT-PLGA-NP.[6,7]

2.1.2 Development of ACT-PLGA-NP loaded thermoreversible gel

Using a cold technique, thermoreversible gel was created. In double distilled water (between 3 and 4^0 degrees Celsius), poloxamer 407 was gradually dissolved while being continuously stirred with a magnetic device for four hours. For the polymer to properly soak, this solution was refrigerated overnight at a temperature between 2 and 8^0 C. In double distilled water, carbopol 940 was gradually introduced while being constantly stirred. The polymeric phase was neutralized by adding 0.1 N NaOH solution dropwise. The Carbopol slurry was kept at a final pH of 7-8. The Carbopol slurry was kept at a final pH of 7-8. After being sonicated for ten minutes, the equal amount of optimized ACT-PLGA-NP was distributed in double-distilled water. To ensure that the two phases were properly mixed, this dispersion was added to the poloxamer-Carbopol slurry and stirred continuously for 20 to 30 minutes. The preservative was added to the

polymeric phase containing ACT NPs after being separately dissolved in hot water (60–70 0C) and the mixture was agitated for a further half-hour. After dissolving 0.9% sodium chloride in double-distilled water, the solution was introduced to the polymeric phase that contained ACT NPs. After adjusting the pH to 7.4 with 0.1 N NaOH, the final volume of gel was prepared using double-distilled water. To better characterize the gel, this formulation was used. [8,9]

2.2 Characterisation of NFL-PLGA-NP

2.2.1 Particle Size, Zeta Potential and Polydispersity Index (PDI)

The Malvern particle size analyzer was used to measure the ACT-loaded PLGA NPs' particle size. To ensure that the nanoparticulate powder was distributed uniformly, it was mixed with double-distilled water and sonicated for ten minutes. Particle size, zeta potential, and PDI were measured after the samples were loaded into the particle size analyzer. [10]

2.2.2 Surface Morphology

Scanning electron microscopy was used to define the NPs' surface morphology. Lyophilized NPs powder was sputter coated with gold at 20 kV for 4 minutes after being mounted on an aluminum stub that had been taped down with adhesive tape. To study the surface morphology, pictures were taken at different spots.[11]

2.2.3 Encapsulation Efficiency (EE)

The supernatant solution that was left behind after centrifugation was used to calculate the non-encapsulated ACT. Using a UV visible spectrophotometer, the absorbance of the supernatant solution was determined at each absorption maxima. Using the following formula, the entrapment efficiency of ACT nanoparticles was determined.[12]

%EE = Drug entrapment in Nanoparticles/Total drug used in formulation×100

2.3 *In-Vitro* ACT release from NPs

The *in-vitro* ACT release was determined using dialysis membrane (5 kDa) method in pH 7.4 buffer. Before use of membrane, it was treated as per instructions of manufacturer and soaked in pH 7.4 buffer for 24 hours to saturate the pores of the tubes. ACT-PLGA-NPs equivalent to 50 mg were dispersed in pH 7.4 buffer and placed in dialysis tubing tied on both ends properly. This tube was then placed in 600mL of pH7.4 buffer at 37^0C and kept under magnetic stirring. The samples (2 mL) were withdrawn at predetermined time intervals and replenished with fresh buffer to maintain the sink condition. The samples of each time point were analyzed at 254 nm using U.V visible spectrophotometer.[13,14]

2.4 Characterisation of ACT-PLGA-NP loaded Thermoreversible Gel

2.4.1 Clarity and Visual Appearance

Visual assessment against a black and white backdrop was used to assess the prepared gels' clarity and appearance.[15]

2.4.2 pH of the Gel

Sensitive, calibrated pH meters were used to measure the gel compositions' pH levels. The pH was measured three times, and the average was found.[16]

2.4.3 Gelation Temperature

Three milliliters of refrigerated sample were placed in a test tube that was properly sealed with parafilm to ascertain the gelation temperature of each formulation. The temperature of these test tubes was raised at a pace of 5^0 C per minute while they were in a heating water bath. When the test tube was turned upside down and the gelation temperature was recorded, the temperature was allowed to rise steadily until the

gel did not fall. For every gel formulation, the measurements were made three times.[17]

2.4.4 Viscosity of the Gel Formulations

The viscosities of all the formulations were determined by using cone and plate viscometer (Brookfield viscometer; Model cap 2000 + 2). Few drops of the test formulations were applied to plate of the viscometer using glass rod. The temperature of the system was increased from 25–37^{0}C and apparent viscosity was determined with respect to temperature change.[18]

2.4.5 *In-vitro* diffusion study

The *in-vitro* diffusion study was conducted by utilizing Franz diffusion cell. Cellophane membrane was taken as semi-penetrable membrane for diffusion. The Franz diffusion cell has receptor compartment with an effective volume. The membrane was placed between the donor and the receptor compartment. A 2cm^2 size patch taken and weighed then set on one face of membrane confronting donor compartment. The receptor medium was phosphate buffer pH 7.4. The receptor compartment was encompassed through water casing to keep up the temperature at 37±0.5^{0}C. It was placed on thermostatic hot plate with a magnetic stirrer. The receptor liquid was mixed by Teflon covered magnetic bead which was put in the diffusion cell. Amid each testing interim, samples were taken and replaced by equivalent volumes of fresh receptor liquid on each sampling. The samples withdrawn were analyzed spectrophotometrically at 254 nm.[19]

2.5 Stability study

The stability study was performed on the prepared formulation i.e., ACT-PLGA-NPs gel as per the ICH guidelines at accelerated conditions. (40^0 ±2^{0}C, 75%±5% RH).[20]

1. Results and Discussion

3.1 Nanoparticles Development and Characterization

ACT loaded PLGA nanoparticles were prepared by high speed homogenization method at varying ratio of ACT to PLGA concentration (1:1, 1:2, 1:3 and 1:4) using tween 80 as surfactant. Nanoparticles were formed at 1:2, 1:3 and 1:4 ACT: PLGA ratio. At 1:1 ratio there was no evidence of formation of nanoparticles. The concentration of surfactant was also important for the formation of nanoparticles. The particle size of ACT loaded nanoparticles was found in the range of 110–255 nm. Direct relation was observed between polymer concentration and particle size. Zeta potentials between −30 mV and +30 mV are considered as ideal for stability of the nanoparticulate suspension. The zeta potentials of prepared formulations were found to be in the range of -23.45 to -28.52 mV which can maintain the repulsive force between particles to attain better stability of the nanoparticulate suspension. Another important parameter of nanoparticles is PDI which indicates the homogeneity in particle size of nanoparticles. The PDI was found within acceptable range i.e., less than 0.3. The prepared formulations demonstrated the homogeneity in particle size with PDI less than 0.3. The EE of ACT was found in the range of 89.25 to 92.55% which showed maximum capacity of nanoparticles to encapsulate ACT in matrix system. Surface morphology study showed good spherical, smooth nanoparticles without cracks on the surface. All these physical parameters of the nanoparticles were found highly acceptable for any kind of drug delivery system. The results were represented in table 1.

The F1 formulation consists of 1:1 ratio of ACT and PLGA. For successful formation of nanoparticles sufficient amount of polymer is required to form the matrix embedded with nanoparticles. From observation it may be concluded that the amount of PLGA polymer used in F1 formulation would have not been sufficient for the formation of matrix of nanoparticles. Moreover, Tween 80 is used as surfactant which is responsible for the separation

of particles from each other and prevents the agglomeration. The concentration of Tween 80 in F1 formulation was 0.3% and this concentration was also not sufficient for the preparation of nanoparticles. So, overall, the polymer and surfactant system was not favorable for the generation of nanoparticles. So in F1 formulation nanoparticles were not observed while in rest for the formulations it was generated. F4 formulation even at higher polymer concentration failed to show sustained release pattern as compare to other formulations. The development of in situ thermoreversible gels has attracted so many researchers over the past few years due to various advantages over conventional gelling systems. These gels offers various advantages including increased ocular residence of formulation, release of drugs at controlled rate, ease of application, increased shelf life and preventing wash away effect as seen in normal gels. Nanoparticles due to nanoscale dimension could easily permeate through the corneal membrane. Drug loaded nanoparticles enhances the solubility of poorly soluble drugs; like-wise, the solubility of NFL could have increased and further helped to enhance the permeation through corneal membrane.

Table 1: Physical characterization of ACT loaded nanoparticles

Parameters	**ACT-PLGA Nanoparticles**			
Batch No.	**F1**	**F2**	**F3**	**F4**
ACT-PLGA ratio	1:1	1:2	1:3	1:4
Particle size	NPs not formed	110	125	255
PDI	0.172	0.187	0.191	0.199
Zeta Potential (-mV)	-23.45	-25.12	-26.75	-28.52
%EE	89.25	90.68	91.75	92.55

3.2 *In-Vitro* ACT Release from PLGA nanoparticles

In-vitro ACT release from F1, F2, F3 and pure F4 was determined by dialysis tubing method. F3 formulation showed excellent slow, gradual and sustained release of ACT over the period of 24 hours. This formulation showed nearly 15% burst release within 1 hour followed by sustained release pattern. Other formulations like F3 and F4 showed release for the period of 24 and 12 hours, respectively. Among all these formulations, F3 formulation was considered as optimized formulation considering all physical and chemical parameters. Surface morphology of F3 batch showed that nanoparticles were found to be spherical in shape with rough surfaces (Figure 1). The release pattern of F3 formulation could be helpful to maintain the plasma concentration of ACT within therapeutic window and could show long lasting effect in ocular infections. It was seen that about 94.31% drug was released from optimized batch (F3) in pH 7.4 phosphate buffer while it was about 96.89% in STF (Figure 2).

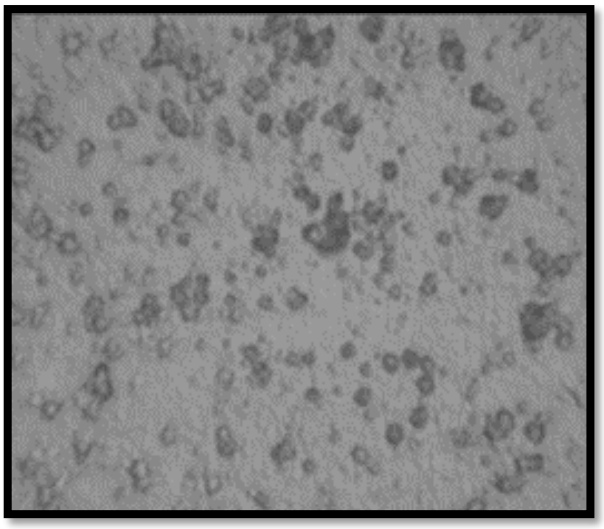

Figure 1: Scanning electron image of optimized batch of Acyclovir nanoparticles

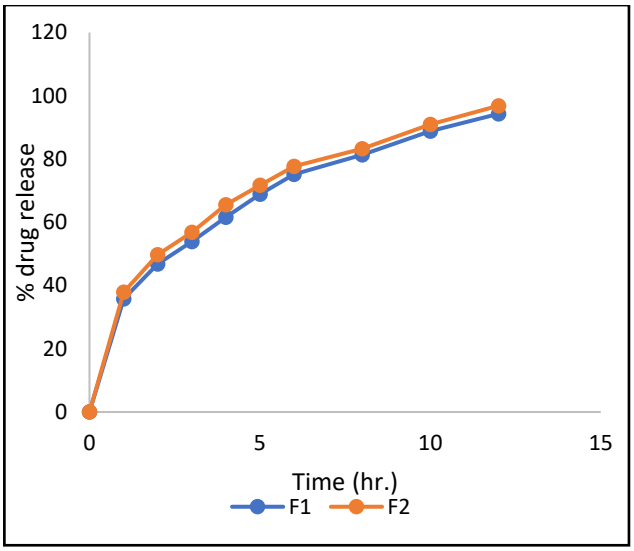

Figure 2: *In–vitro* release study of Optimized batch of Acyclovir nanoparticles (F3) in pH 7.4 & simulated tear fluid (STF)

3.3 Thermoreversible Gel Development and Characterization

In this research work nanoparticles loaded thermoreversible gel was developed using varying concentration of poloxamer 407 and Carbopol 940. Nearly all formulations (FG1, FG2, FG3 and FG4) developed were found to be clear and transparent which is prime requirement of any ophthalmic drug delivery system. The gelation temperature of 37–38^{0}C was desirable for such thermoreversible gels at which drug-polymer solution gets converted to gels. In the present research, FG3 and FG4 formulations showed excellent gelling capacity at body temperature. Among all these formulations, FG4 was considered as optimized due to excellent and desirable physicochemical properties. FG4 formulation showed maximum ACT content (98.25%), gelation temperature of 37^{0}C, greater gelling capacity (+++) and desirable viscosity of 1225 cp. The pH of all formulations ranged between 6.35 and 6.57 which were desirable for ophthalmic formulations. The gelling strength was found to be polymer concentration dependent (Poloxamer and Carbopol). The drug content was varied from 90.75 to 97.88% which was also found to be polymer concentration dependent. Considering all these factors we have developed ideal ophthalmic formulation for the treatment of Varicella zoster viral infection and can retain for maximum time in ocular cavity. The results were depicted in table 2. The results of in-vitro drug release study revealed that about 89.95±1.28% drug was released from ACT-PLGA-NP gel in a sustained manner (Figure 3).

Table 2: Physico-chemical characterization of ACT-PLGA-NP gel

Formulation batch no.	**FG1**	**FG2**	**FG3**	**FG4**
Visual appearance	Non transparent	Transparent	Transparent	Transparent
Clarity	Hazy	Clear	Clear	Clear
pH	6.35	6.40	6.57	6.50
Drug content (%)	90.75	93.29	95.52	97.88

Gelation temperature (^{0}C)	39	37.5	36	37
Gelation capacity	-	++	++	+++
Viscosity (cP)	Not done	Not done	815	1225

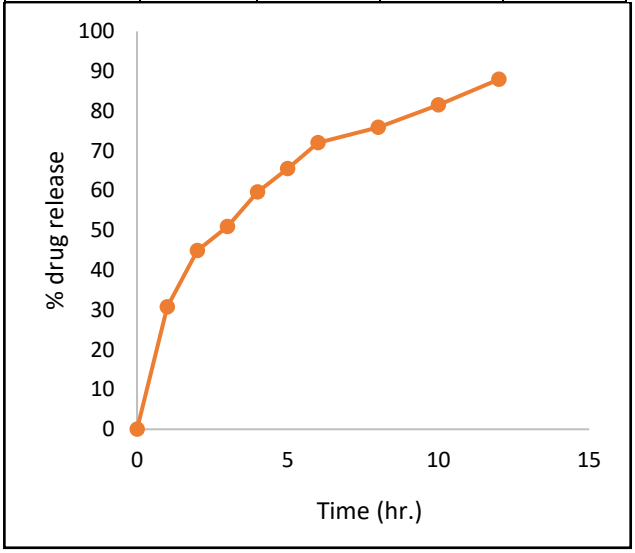

Figure 3: Percent (%) drug release data of ACT-PLGA-NP gel (FG4)

3.4 Stability study

The stability study was performed on the prepared formulation i.e., nanoparticulate gel of Acyclovir as per the ICH guidelines at accelerated conditions. (40^0 ±2^0C, 75%±5% RH) and it showed that the formulation was stable. The dissolution profile of the optimized batch (ACT-PLGA-NPs gel) was studied and it was found that no considerable changes were seen in the release profile.

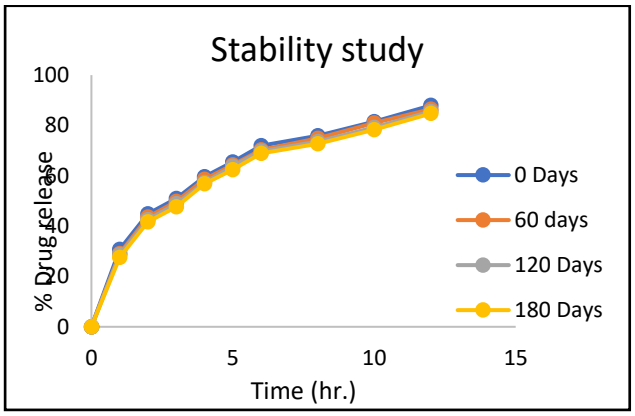

Figure 4: Comparative release profile of optimized batch (ACT-PLGA-NPs gel) at different time intervals (0, 60, 120, 180 days) on stability

2. Conclusion

In this research work we have successfully developed thermoreversible gel containing Acyclovir (ACT) loaded PLGA nanoparticles and demonstrated excellent *in-vitro* results. The ACT loaded nanoparticles showed nano-sized dimensions, smooth spherical surface morphology with slow and gradual release of ACT over the period of 24 hours. The thermoreversible gel developed with these nanoparticles demonstrated desirable physiochemical properties with enhanced corneal membrane penetration. We proposed the use of such novel formulation for the treatment of Varicella zoster viral infection.

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