World Journal of Pharmaceutical research

Volume 3, Issue 1, 1030-1043. Research Article ISSN 2277 – 7105

# DESIGN AND BIOLOGICAL EVALUATION OF ACRYLATE NANOPARTICLES FOR IMPROVED OCULAR BIOAVAILABILITY: AN APPLICATION TO ANTI GLAUCOMA DRUG

***Parmanand Verma[1], Roop Narayan Gupta[1], Arvind Kumar Jha[2]**

[1]Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi 835215, India.

[2]Faculty of Pharmaceutical Sciences, Shri Shankaracharya Group of Institutions, Bhilai 490020, India.

Article Received on
21 October2013
Revised on 23 November 2013,
Accepted on 26 December 2013

***Correspondence for Author:**
**Parmanand Verma**
Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi 835215, India.
parmapharma@gmail.com

## ABSTRACT

Purpose of present study is to develop a novel drug delivery system for improved ocular bioavailability of antiglaucoma drug. Eudragit® RS 100 nanoparticles were prepared by Nanoprecipitation Method. Particles size and zeta potential was measured by Malvern Zetasizer, TEM was used as an aid for visualization of Nanoparticles, drug interaction study was done by FTIR spectroscopy, entrapment efficiency was determined by centrifugation technique, in vitro drug release study was done by using franz diffusion cell, Standardized Riester Tonometer was used to check Intra Ocular Pressure lowering potential of formulations. Short term stability study was done. Particle size measured was between 89.21±3.98 to 134.21±3.43 nm, all the formulations were having positive charge on surface and were almost spherical in shape, no any drug to polymer interaction was seen. Entrapment efficiency was between 52.1±2.4 to 66.1±3.1 %. Drug release was recorded for 8 hrs, means sustained delivery was achieved. The IOP lowering potential of plain drug solution in rabbit's eye was significantly lower than Eudragit nanoparticles (***P<0.001), Formulations were stable for 3 months as seen in stability studies. Eudragit® RS 100 nanoparticles increased the ocular bioavailability of Acetazolamide and sustained the drug release for extended period of time.

**KEYWORDS:** Eudragit® RS 100, Nanoparticles, Antiglaucoma drug, Ocular, Topical, Bioavailability, Intra Ocular Pressure.

## INTRODUCTION

It is estimated that around 5.3 million people worldwide will be blind owing to primary angle-closure glaucoma by 2020[1]. The majority of those affected will be living in Asia. These figures have been calculated using data accumulated from a number of population-based glaucoma surveys conducted in various countries in Asia over the last decade [2-8]. Glaucoma is a term describing a group of ocular disorders with multi-factorial etiology united by a clinically characteristic intraocular pressure-associated optic neuropathy. Increase in IOP > 21 mmHg is most critical risk point of glaucoma [9, 10]. Reduction of IOP is management of glaucoma [11-15]. ACZ is used orally for the reduction of IOP in patients suffering from glaucoma [16-19]. Due to its low solubility and permeability profile, topical administration on ocular site results in poor bioavailability [20-23]. Huge research has been done to increase topical ocular bioavailability of ACZ [16, 22, 24-26]. Nanoparticles (NPs) unique size and other properties make it suitable for ocular delivery. Various polymers are being utilized for preparation of NPs [27, 28]. Eudragit RS 100 is a co-polymer of poly(ethylacrylate), methyl-methacrylate, and chlorotrimethyl-ammonioethyl methacrylate, containing an amount of quaternary ammonium groups between 4.5% and 6.8% [29]. It is insoluble at physiologic pH values and capable of limited swelling, thus representing a good material for the dispersion of drugs. It is a cationic polymer so that capable of mucoadhesiveness at ocular site for longer time. In present study Acetazolamide loaded Eudragit RS 100 nanoparticles are to be prepared with the aim of improved ocular bioavailability and for longer drug release.

## MATERIALS AND METHODS

### MATERIALS

ACZ was obtained as a gift sample from Intas Pharma Pvt Ltd (Dehradun, India), Eudragit® RS 100 was kindly provided as a gift sample by Evonik Röhm GmbH (Darmstadt, Germany). Polyvinyl alcohol (PVA), Acetone, methanol and salts for Simulated Tear Fluid (STF) like Magnesium Chloride, Sodium Chloride, Sodium bi Carbonate and Calcium Chloride were purchased from Sigma Aldrich (India). Dialysis membrane cut off 12000 Da was purchased from Himedia Laboratories (India). Diffusion cell was purchased from Bombay Chemicals (Mumbai, India). Riester Tonometer No. 5113 schiotz C, eye tonometer spec. 3, with inclined scale was purchased from Rudolf Riester GmbH (Jungingen, Germany).

## PREPARATION OF ACETAZOLAMIDE LOADED EUDRAGIT® RS 100 NANOPARTICLES

Acetazolamide Loaded Eudragit Nanoparticles were prepared by Nanoprecipitation method as described by *fessi et al* and other authors [30-32]. Briefly, ACZ and Eudragit® RS100 (1:10) were dissolved in 20 ml portion of various organic phases. This organic phase was poured drop wise into aqueous phase (Containing 1% w/v of PVA) at various ratios with magnetic stirring (9,000 rpm) at room temperature (RT) (Table1). NPs were spontaneously formed and turned the solution slightly turbid. Finally, the organic solvents were evaporated under reduced pressure at 58°C. The resulting particle suspension was filtered through 1.2 µm cellulose nitrate membrane filters in order to remove larger particle aggregates.

**Table: 1 Formulation Code and Composition**

| Formulation Code | Drug Polymer Ratio | Organic Aqueous Phase Ratio | Organic Phase |
|---|---|---|---|
| S1 | 1: 10 | 1:2 | Acetone |
| S2 | 1: 10 | 1:3 | Acetone |
| S3 | 1: 10 | 1:4 | Acetone |
| S4 | 1: 10 | 1:2 | Acetone and Methanol |
| S5 | 1: 10 | 1:3 | Acetone and Methanol |
| S6 | 1: 10 | 1:4 | Acetone and Methanol |
| S7 | 1:10 | 1:2 | Acetone and Ethanol |
| S8 | 1:10 | 1:3 | Acetone and Ethanol |
| S9 | 1:10 | 1:4 | Acetone and Ethanol |

## PARTICLE SIZE ANALYSIS AND ZETA POTENTIAL MEASUREMENT

The size distribution and zeta potential (ZP) of NPs were determined by dynamic light scattering (DLS) using a computerized inspection system (Malvern Inst. UK; Nano ZS). The measurements were conducted in triplicate, in a multimodal mode of 200 s each at medium stable count rate. The ZP was determined by injecting the diluted system into a ZP measurement cell [32].

## TRANSMISSION ELECTRON MICROSCOPY

To visualize the Nanoparticles Transmission Electron Microscope (TEM) (Morgagni 2680 FEI, Holland) was used. Samples were dried on carbon-coated grid and negatively stained

with aqueous solution of phosphotungstic acid. After drying the specimen was viewed under the microscope [31].

## FOURIER TRANSFORM INFRARED SPECTROSCOPY

Any possible drug- excipient interaction, was studied by Fourier Transform Infrared Spectroscopy (FTIR) of ACZ, polymer and one formulation S1. Infrared spectroscopy of the samples was performed in IR-Prestige 21 FTIR Spectrophotometer (Shimadzu, Japan). Eudragit® RS 100, PVA and drug powder were analysed by preparing pellets. Pellets are prepared by taking a few milligrams of the samples were ground in a mortar with about 100 times the quantity of potassium bromide (KBr). The finely ground powder was introduced into a stainless steel die. The powder was then pressed in the die between polished stainless steel anvils (Pressure: about 9 t / $in^2$). The IR spectrum of the liquid formulation (NPs suspension) was recorded in DLS Mode by direct introduction [33].

## DRUG ENTRAPMENT EFFICIENCY

Centrifugation method was employed to determine drug loading EE of ACZ-E-NPs was determined by using cooling centrifuge [Remi instrument Ltd. Mumbai, India]. NPs suspension (10 ml) was taken and centrifuged at 10,000 rpm for 1 hr, after centrifugation supernatant was collected from tube by decantation method. After proper dilution, the concentration of ACZ was measured at 292 nm using UV Visible double beam spectrophotometer 1800 (Shimadzu, Zapan). The EE was calculated using formula:

$$EE= [Qt-Qs/Qt]*100$$

EE is the entrapment efficiency, Qt is amount of ACZ in suspension added, and Qs is amount detected only in the supernatant [34].

## *IN VITRO* DRUG RELEASE STUDY

In vitro drug release from ACZ-E-NPs was studied using Franze Diffusion Cells, whereby a dialysis membrane with a molecular weight cut off of 12,000 Da (Himedia Laboratories, India) separated the acceptor from the donor compartment, consisting of 20 ml of formulation. The acceptor compartment was filled with 20 ml STF and stirred magnetically at 200 rpm. Temperature was maintained at 37 ± 0.5°C. At regular time intervals within 24 hours, samples of 1 ml were withdrawn from the acceptor compartment and replaced by the same volume of fresh STF solution and analyzed by UV spectroscopy method as described earlier [34].

### *IN VIVO* STUDIES

To compare the IOP lowering potential of prepared Eudragit RS 100 Nanoparticles with plain drug solution, adult male albino rabbits (3 - 4 kg) were used to measure IOP. All the rabbits were provided balanced diet pellets and kept at 20$^{o}$C to 24$^{o}$C before the experiments. The experiments were done according to Animal ethical guidelines approved by Rungta College of Pharmaceutical Science and Research (Bhilai, India). The IOP was measured using a standardized Riester Tonometer. The rabbits were divided into 3 groups, each consisting of 6 rabbits: Group I received 0.5 % ACZ solution; Group II and Groups III received freshly prepared formulations E3 and E8 respectively. Formulations were so diluted that all the rabbits received dose equivalent to 0.5 % ACZ. All the rabbits were given dose in right eye and left were treated as a control. In this way the possible intersubject variability may be minimised. After administration of doses, IOP readings in both eyes of each rabbits were measured immediately before drug administration (zero reading), 30 minutes after instillation of the different drug formulations, and then every half an hour for a period of 8 hours (since biological half life of ACZ is 1.5 hr). All the measurements were done in triplicate. Variables like time of start of experiment and investigator was kept constant. The ocular hypotensive activity is expressed as the average difference in IOP (ΔIOP) between the treated and control eye of the same rabbit, according to the following equation [16, 24]:

ΔIOP = IOP Control eye - IOP Treated eye

### STATISTICAL ANALYSIS

The statistical significance of differences in IOP lowering effect between drug solution and various formulations was tested by two way analysis of variance (ANOVA) followed by multiple comparison with Bone ferroni post test using GraphPad Prism 6.0 software (GraphPad Software, Inc, California) [31].

### SHORT TERM STABILITY STUDY OF ACZ-E-NPS

Physical stability of prepared NPs was assessed by keeping one formulation (S3) at RT (25$^{0}$C) and freeze temperature (FT) (4$^{0}$C) for 3 months in glass vials. 500 µl samples were withdrawn after every 15 days interval and analysed for PS, surface charge, surface morphology and EE as described earlier [32].

## RESULTS

### PARTICLE SIZE ANALYSIS AND ZETA POTENTIAL MEASUREMENT

The size of particles to be applied onto the eye should be < 10 µm [35]. All the NPs prepared in this study were <200nm in size which is suitable for ophthalmic use. All the formulations showed positive surface charge (Table 2).

**Table: 2 Average particle size, poly dispersity index and zeta potential of formulations**

| F | d (nm) ±SD | PI ±SD | Zeta Potential (mV) |
|---|---|---|---|
| S1 | 134.21±3.43 | 0.876 ±0.23 | +17.4±0.12 |
| S2 | 121.23±3.32 | 0.698±0.21 | +16.9±0.19 |
| S3 | 111.12±2.12 | 0.765± 0.12 | +16.23±0.13 |
| S4 | 98.23±3.31 | 0.562± 0.13 | +15.32±0.15 |
| S5 | 92.32±4.23 | 0.452± 0.32 | +15.98±0.23 |
| S6 | 89.21±3.98 | 0.410± 0.23 | +16.31±0.12 |
| S7 | 122.21±4.34 | 0.421 ±0.23 | +17.32±0.12 |
| S8 | 112.32±2.43 | 0.764 ±0.23 | +14.12±0.15 |
| S9 | 100.43±2.45 | 0.342 ±0.23 | +14.2±0.14 |

Values are mean ± SD (n=3)

### TRANSMISSION ELECTRON MICROSCOPY

The NPs were negatively stained and images were taken at various magnifications. The images revealed that all the NPs prepared were almost spherical in shape. None of any perforations or imperfections was seen in on the surface of NPs. It is good to have uniform surface properties of all the NPs because it may be helpful in topical application of drug onto the eye and proper dosing of drug (Figure 1{a} and {b}).

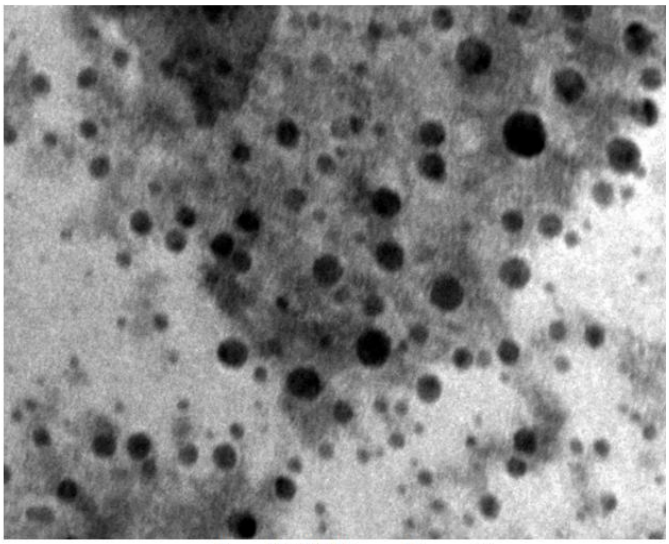

Figure 1(a). TEM Image of Nanoparticles

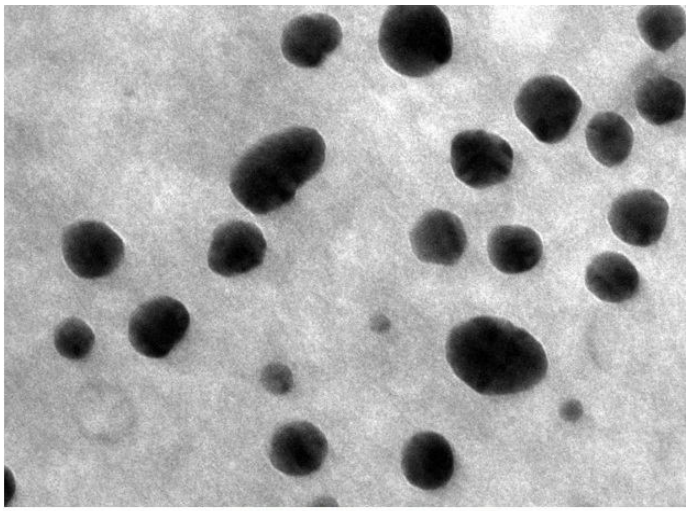

Figure 1(b). TEM Image of Nanoparticels

## FOURIER TRANSFORM INFRARED SPECTROSCOPY

Any possible drug polymer interactions were checked by FTIR spectroscopy. ACZ showed peaks at 3587.60 $cm^{-1}$ (-$NH_2$ str), 1157.29 (C=N Str), 1654.92 (C=O Str), 1382.96 (-$CH_3$ Str), (C-S Str), 1037.70 (O=S=O Str), Eudragit® RS 100 showed peaks at 1654.92 (C=O Str), 1382.96 (-$CH_3$ Str), 2133.27 $cm^{-1}$ ( $4^0$ ammonium compund) and PVA showed peaks at 3639.68 ( -OH str), 3043.67 ( C-H str), 1126.43, (C-Cstr). Formulation S1 showed peaks at 3587.60 $cm^{-1}$ (-$NH_2$ str), 2133.27 $cm^{-1}$ ( $4^0$ ammonium compund), 1654.92 (C=O Str), 1382.96 (-$CH_3$ Str), 871.82 (C-S Str), 1037.70 (0=S=0 Str), 1111 (C-N Str), 1157.29 (C=N Str) (Figure 2).

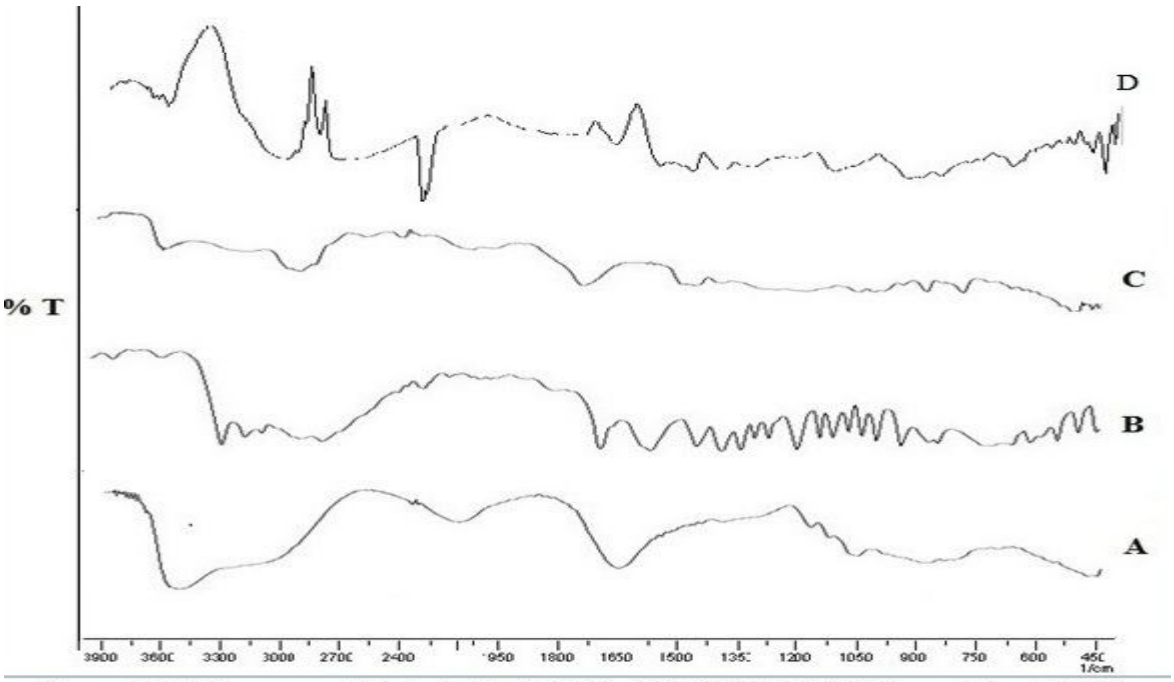


Figure 2: FTIR spectra of Eudragit RS 100 (A), ACZ (B), PVA (C), Formulation S1 (D)

## DRUG ENTRAPMENT EFFICIENY

EE is drug loading capacity of any formulations. In present study centrifugation method was used to determine EE [34]. The composition of organic phase has a marked influence on drug loading capacity of NPs. The EE was highest in case when acetone and methanol were used to form organic phase. Acetone and ethanol give intermediate, which in turn was higher than acetone alone (Figure 3).

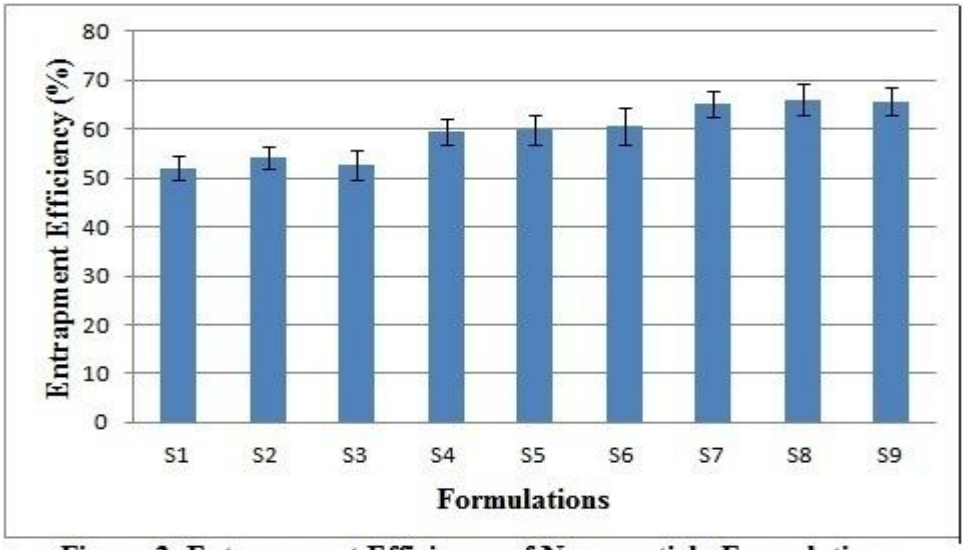


**Figure 3. Entrapement Efficiency of Nanoparticle Formulations**

## *IN VITRO* DRUG RELEASE STUDY

Diffusion cell was used to study the drug release profile of formulations. Cumulative % drug release was recorded up to 8 hr. Drug content had a direct influence on drug release of all the formulations (Figure 4).

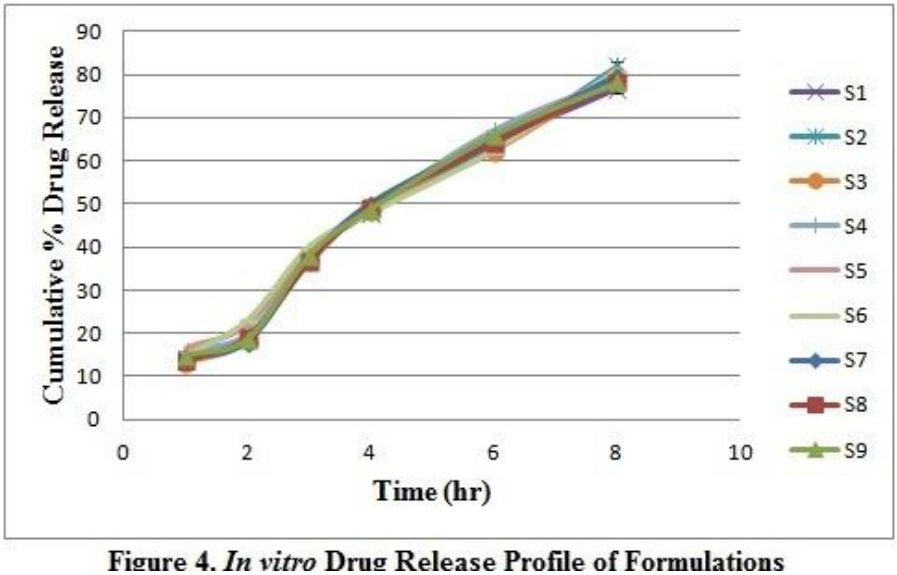


**Figure 4. *In vitro* Drug Release Profile of Formulations**

### *IN VIVO* STUDIES

After administration of drug doses, ΔIOP was measured Riester Tonometer in rabbit's eye. IOP lowering potential of ACZ-E-NPs was compared with plain ACZ solution. Plain drug solution lowered the IOP for a very less period of time and reduction of IOP was also not so high (Max ΔIOP = 2.98 mm Hg) whereas ΔIOP was remarkably higher in case of ACZ-E-NPs (E3 and E8). The effect was seen for a long time (up to 8 hr) (Table 3). The IOP lowering potential of plain drug solution was lower significantly ($P<0.001$) as compared to ACZ-E-NPs.

**Table 3: Data of i*n vivo* studies**

| Time (Hr) | ΔIOP±SD After Topical Administration (mmHg) | |
|---|---|---|
| | Acetazolamide Solution | S5 |
| 0.5 | 2.23±0.09 | 2.41±0.06 |
| 1 | 2.43±0.11 | 3.01±0.05 |
| 2 | 2.98±0.11 | 3.46±0.21 |
| 3 | 2.56±0.08 | 3.97±0.14 |
| 4 | 0.43±0.12 | 4.74±0.14 |
| 5 | 0.43±0.11 | 4.99±0.12 |
| 6 | 0 | 5.32±0.06 |
| 7 | 0 | 5.16±0.09 |
| 8 | 0 | 5.04±0.03 |

Values are mean ± SD (n=6)

### SHORT TERM STABILITY STUDY OF NANOSUSPENSION

The Formulation (S3) was kept at RT and FT for 3 months (t=3 months), and was evaluated for Particle Size (PS), surface charge, surface morphology and EE. Data is shown in table 4.

**Table: 4: Data of stability studies**

| Parameters | S3 (t=0 Months) | S3 (t=3 Months) |
|---|---|---|
| Particle Size (nm) | 111.12±2.12 | 106.23±1.32 |
| Zeta Potential (mV) | +16.23±0.13 | +14.11±0.09 |
| Change in Surface Morphology | No Change was observed | |
| Entrapment Efficiency (%) | 52.7±1.9 | 51.2±2.3 |

Values are mean ± SD (n=3)

## DISCUSSION

ACZ-E-NPs were prepared by Nanoprecipitation method [30-32]. Organic to aqueous phase ratio and composition of organic phase were altered to study the effect of these variables on physicochemical properties of NPs. As the aqueous phase volume was increased, PS decreased. This is due to higher diffusion of non solvent into the aqueous phase. Because as the aqueous phase volume is increased it becomes dilute. So diffusion increased and results into smaller PS. This result was in accordance with our earlier studies and studies done by Das et al. [32]. NPs prepared with acetone (E1-E3) as solvent system were largest, NPs prepared with acetone and methanol (E4-E6) were smallest and intermediate in the case of acetone and ethanol (E7-E9) as solvent phase. A correlation between Dielectric constant (DC) and PS has been noted in this study. PS increased with lower DC of solvent systems [(DC of Acetone - 20.7, Ethanol- 24.6, Methanol 32.7) (Table 2)]. Mechanism of correlation is not fully understood, the results confirm our previous study [36]. Almost all formulations showed the Poly dispersity index (PI) less than 0.7, which indicates the homogeneity of formulations. Positive surface charge noted for the NPs were due to polymer. It is a cationic polymer having quaternary ammonium groups. Positive surface is essential for mucoadhesion of NPs onto the corneal surface of eye. Surface morphology was done by TEM. All the particles were almost spherical in shape which is good for the topical administration. Any possible drug excipient interaction was checked by FTIR spectroscopic method. Presence of all important peaks of ACZ, Eudragit® RS 100 and PVA in FTIR peak of formulation E1 advocates no interaction between them. Calibration curves for estimation of ACZ have been prepared in distilled water and STF which are to be used in EE study and *in vitro* drug release study respectively. The method used was validated by determining various parameters of validation. The EE of drug in ACZ-E-NPs was determined by centrifugation method. Solubility of drug has a marked influence in EE. In present study three different solvent systems (Acetone alone, Mixture of acetone and methanol and mixture of acetone and ethanol) were taken as organic phase. Differences in EE data were noted with change in solvent systems. EE was higher in solvent systems in which the drug is highest soluble. ACZ solubility is 8.3 mg/ml in acetone. But as methanol and ethanol were added as co-solvents, the solubility rises to 11.3 mg/ml and 10.1mg/ml respectively. So that the EE was highest in case when mixture of acetone and methanol was taken as organic phase, lowest in case when acetone alone was taken as organic phase and intermediate in case when acetone and ethanol was taken as organic phase. The *in vitro* drug release study was done by using diffusion cells. As the drug content in formulation was higher, the release rate also increased. This may be

due to saturation of quaternary ammonium group present in Eudragit® RS 100 polymer by drug molecules, occurred at a high drug content, which results in increased drug release from the formulations. None of the formulations showed burst release pattern. This indicates that the drug is homogeneously dispersed in polymer matrix. Drug release data was fitted to various kinetic models. Higuchi Model best described the drug release kinetics of our study. This is due to Eudragit matrix which releases the drug by diffusion. As the polymer came into the contact of tear fluid it started swelling. As the polymer swells the drug come out of it by diffusion. Further Korsemeyer Peppas Model indicated the Fickian Diffusion pattern. IOP lowering potential of formulations was compared with plain drug solution. The study was conducted for 8 hr. This is done by considering half life of ACZ i.e. 1.5 hr. IOP lowering potential of plain drug solution was for a very short duration and also the magnitude was low as compared to ACZ-E-NPs. Both the facts were due to the cationic NPs. Because of positive charge of polymer the ACZ-E-NPs stayed on corneal surface for a long time which allows it to cross the cornea in higher amount. The ACZ solution results in low ocular bioavailability because of the drainage system of eye, low solubility and low permeability of drug across the cornea (BCS class IV). But in case of ACZ-E-NPs (E3 and E8) the magnitude of reduction of IOP was significantly higher ($P<0.001$) and the time of action was also longer as compared to ACZ solution. Short term stability study revealed the stability of ACZ-E-NPs after 6 months. Stability study was done for one formulation out of nine formulations because the composition of all the formulations was same. No signs of any damage to NPs were noted.

## ACKNOWLEDGMENTS

After GOD and parents authors are thankful to Head of Department of Pharmaceutical sciences and Central Instrument facility (CIF), Birla Institute of Technology, Mesra Ranchi for providing sufficient research facility to carry out this study. Authors are also thankful to AIIMS New Delhi for providing TEM facility. We are also thankful to Dr Ravishankar Pandey, Assistant Professor, Guru Ghasidas University, Bilaspur for his kind suggestions.

## DECLARATION OF INTEREST

The authors report no declarations of interest.

## REFERENCES

1. Quigley HA, Broman AT. (The number of people with glaucoma worldwide in 2010 and 2020). *Br J Ophthalmol,* 2006; 90:262–67.

2. Foster PJ, Baasanhu J, Alsbirk PH. Glaucoma in Mongolia. (A population-based survey in Hovsgol province, northern Mongolia). *Arch Ophthalmol*, 1996; 114:1235–41.
3. Foster PJ, Oen FT, Machin D. (The prevalence of glaucoma in Chinese residents of Singapore: a cross-sectional population survey of the Tanjong Pagar district). *Arch Ophthalmol*, 2000; 118:1105–11.
4. Dandona L, Dandona R, Mandal P. (Angle-closure glaucoma in an urban population in southern India: the Andhra Pradesh Eye Disease Study). *Ophthalmology*, 2000; 107:1710–16.
5. Bourne RR, Sukudom P, Foster PJ. (Prevalence of glaucoma in Thailand: a population based survey in Rom Klao district, Bangkok). *Br J Ophthalmol*, 2003; 87:1069–74.
6. Ramakrishnan R, Nirmalan PK, Krishnadas R. (Glaucoma in a rural population of southern India: the Aravind Comprehensive Eye Survey). *Ophthalmology* 2003; 110:1484–90.
7. Rahman MM, Rahman N, Foster PJ. (The prevalence of glaucoma in Bangladesh: a population based survey in Dhaka division). *Br J Ophthalmol*, 2004; 88:1493–97.
8. Vijaya L, George R, Arvind H. (Prevalence of angle-closure disease in a rural southern Indian population). *Arch Ophthalmol*, 2006; 124:403–9.
9. Osborne NN. (Pathogenesis of ganglion “cell death” in glaucoma and neuroprotection: focus on ganglion cell axonal mitochondria). *Prog Brain Res*, 2008; 173: 339-52.
10. Osborne NN. (Mitochondria: their role in ganglion cell death and survival in primary open angle glaucoma). *Exp Eye Res*, 2010; 90:750-57.
11. Weber PA. (Neovascular glaucoma, Current management). *Surv Ophthalmol* 1981:149-53.
12. Behki R, Damji KF, Crichton A. (Canadian perspectives in glaucoma management: the role of central corneal thickness). *Can J Ophthalmol*, 2007; 42: 66-74.
13. Liang SYW, Lee GA, Shields D. (Self-tonometer in glaucoma management—past, present and future). *Surv Ophthalmol*, 2009; 54: 450-462.
14. Arthur S, Cantor LB. (Update on the role of alpha-agonists in glaucoma management). *Exp Eye Res*, 2011; 93: 271-283.
15. Eid TM. (Primary lens extraction for glaucoma management: A review article). *Saudi J Ophthalmol*, 2011; 25: 337-45.
16. Kaur IP, Smitha R, Aggarwal D, Kapil M. (Acetazolamide: future perspective in topical glaucoma therapeutics). *Int J Pharm*, 2002; 248: 1-14.

17. Sabri K, Levin AV. (The additive effect of topical dorzolamide and systemic acetazolamide in pediatric glaucoma). *J Ame Ass Ped Ophth Strab*, 2006; 10: 464-68
18. Costa VP, Braga MEM, Duarte CMM, Alvarez-Lorenzo C, Concheiro A, Gil MH, de Sousa HC. (Anti-glaucoma drug-loaded contact lenses prepared using supercritical solvent impregnation). *J Supercrit Fluids*, 2010; 53: 165-173.
19. Sharan S, Dupuis A, Hébert D, Levin AV. (The effect of oral acetazolamide on weight gain in children). *Can J Ophthalmol*, 2010; 45: 41-45.
20. Lindenberg M, Kopp S, Dressman JB. (Classification of orally administered drugs on the world health organization model list of essential medicines according to the biopharmaceutics classification system). *Eur J Pharm Biopharm*, 2004; 58: 265-78.
21. Mora MJ, Longhi MR, Granero GE. (Synthesis and characterization of binary and ternary complexes of diclofenac with a methyl-β-CD and monoethanolamine and *in vitro* transdermal evaluation). *Eur J Med Chem*, 2010; 45:4079-88.
22. Palma SD, Tartara LI, Quinteros D, Allemandi DA, Longhi MR, Granero GE. (An efficient ternary complex of acetazolamide with HP-ß-CD and TEA for topical ocular administration). *J Controlled Release*, 2009; 138:24-31.
23. Zakeri-Milani P, Barzegar-Jalali M, Azimi M, Valizadeh H. (Biopharmaceutical classification of drugs using intrinsic dissolution rate (IDR) and rat intestinal permeability). *Eur J Pharm Biopharm*, 2009; 73:102-106.
24. Kaur IP, Singh M, Kanwar M. (Formulation and evaluation of ophthalmic preparations of acetazolamide). *Int J Pharm*, 2000; 199: 119-127.
25. Aggarwal D, Pal D, Mitra AK, Kaur IP. (Study of the extent of ocular absorption of ACZ from a developed niosomal formulation, by microdialysis sampling of aqueous humor). *Int J Pharm*, 2007; 338:21-26.
26. Granero GE, Maitre MM, Garnero C, Longhi MR. (Synthesis, characterization and *in vitro* release studies of a new acetazolamide–HP-β-CD–TEA inclusion complex). *Eur J Med Chem*, 2008; 43: 464-470.
27. Baba K, Tanaka Y, Kubota A, Kasai H, Yokokura S, Nakanishi H, Nishida K. (A method for enhancing the ocular penetration of eye drops using nanoparticles of hydrolyzable dye). *J Controlled Release*, 2011; 153: 278-287.
28. Sánchez-Martínez M, Martins R. Da C, Quincoces G, Gamazo C, Caicedo C, Irache JM, Peñuelas I. (Radiolabeling and biodistribution studies of polymeric nanoparticles as adjuvants for ocular vaccination against brucellosis). *Rev Esp Med Nucl Imagen Mo*, 2013; 32 (2): 92-97.

29. Pignatello R, Bucolo C, Spedalieri G, Maltese A, Puglisi G. (Flurbiprofen-loaded acrylate polymer nanosuspensions for ophthalmic application). *Biomaterials,* 2002; 23:3247-55.
30. Fessi H, Puisieux F, Devissaguet J, Ammoury N Benita S. (Nanocapsule formation by interfacial polymer deposition following solvent displacement). *Int J Pharm,* 1989; 55: R1-R4.
31. Dillen K, Vandervoort J, Mooter G, Ludwig A. (Evaluation of ciprofloxacin loaded Eudragit® RS100 or RL100/PLGA nanoparticles). *Int J Pharm,* 2006; 314:72–82.
32. Das S, Suresh PK, Desmukh R. (Design of Eudragit RL 100 nanoparticles by nanoprecipitation method for ocular drug delivery). *Nanomed Nanotechnol* 2010; 6: 318-23.
33. Smith BC. Fundamentals of Fourier transform infrared spectroscopy. CRC Press Boca Raton 1996.
34. Aksungur P, Demirbilek M, Denkbaş EB, Vandervoort J, Ludwig A. (Development and characterization of Cyclosporine A loaded nanoparticles for ocular drug delivery: Cellular toxicity, uptake, and kinetic studies). *J Controlled Release,* 2011; 151: 286-294.
35. Zimmer AK, Kreuter J. (Microspheres and Nps used in ocular drug delivery systems.) *Adv Drug Deliv Rev,* 1995;16:61-73.
36. Verma P, Gupta RN, Jha AK, Pandey RS. (Development, in vitro and in vivo characterization of Eudragit RL 100 nanoparticles for improved ocular bioavailability of acetazolamide). *Drug Deliv,* 2013; 20: 269-276.