

Preparation of Poly- ϵ -Caprolactone Nanoparticles by Double Emulsion Solvent Evaporation Technique for Ocular Delivery

SATISH KV^{*1}, KUMAR GS², AND JAYAVEERA KN³

¹Department of Pharmaceutics, Sri Krishna Chaithanya College of Pharmacy, Madanapalle, Chittoor, Andhra Pradesh-517325 (India).

²Department of Life Sciences, School of Pharmacy, International Medical University, Jalan Jallil ,19 Perkasa, Bukit Jalil-57000. Kuala Lumpur (Malaysia)

³Science tech foundations, Sanath Nagar, Hyderabad, Telangana-500018 (India).

ABSTRACT

Poly- ϵ -caprolactone (PCL) is a biocompatible member of the polyester family of biodegradable polymers. PCL has long been a popular choice for drug delivery applications, since it is already FDA-approved for use in the human body as drug delivery device, suture or adhesion barrier. It is being investigated as a scaffold for tissue repair via tissue engineering. Hydrophobic and hydrophilic drugs are encapsulated in PCL particles via single or double-emulsion technique. Briefly, the drug is dissolved with polymer or emulsified with polymer in an organic phase that is then emulsified with the aqueous phase. After the solvent has evaporated, particles are washed and collected via centrifugation for further studies. The present work aims to prepare ketorolac tromethamine encapsulated poly- ϵ -caprolactone nanoparticles by double emulsion solvent evaporation technique and study the effect of various formulation variables on particle size, ζ potential, entrapment efficiency, and *in-vitro* release. Developed nanoparticles were spherical in shape with mean size of 243 ± 18.38 and ζ potential of -30.42 ± 1.41 . The percentage entrapment efficiency of drug loaded nanoparticles was found to be between 12-36 %. *In vitro* drug release studies suggest that all the formulation showed extended drug release profile. *Ex-vivo* corneal permeability was found to be comparable with that of marketed formulation across isolated goat cornea, indicating suitability of nanoparticle formulation in ophthalmic delivery of ketorolac tromethamine.

Keywords: Ketorolac tromethamine, nanoparticle, Poly- ϵ -caprolactone, hydration test, trans-corneal permeation.

Introduction

Despite eyes are among the most readily accessible organs in the body, good ocular bioavailability is still a challenging task. Only 1–2% of drug is available in eye for therapeutic action, rest of the drug is drained out through nasolachrymal drainage system and other ocular physiological barriers when the drug is administered in solution dosage form. The anatomy, physiology and biochemistry of eye render this organ highly impermeable to most of the foreign substances. A significant challenge to the formulators is to circumvent protective barriers of this organ without causing permanent tissue damage [1]. Significant efforts have been made over decades to improve the ocular bioavailability of administered drug, which are inserts, collagen shields and colloidal systems such as liposome's, nanoparticles and nanocapsules. Among all, nanoparticles come out to be the most promising application

in ocular drug delivery [2]. Treatment with nanoparticles system increases bioavailability, reduces administration frequency and promotes drug targeting [3]. Pharmaceutical nanoparticles are submicron-sized, colloidal vehicles that carry drugs to the target site or release drugs in a controlled way in the body. After preparation, nanoparticles are usually dispersed in liquid. Such a system can be administered to humans for example, by injection, or used in ointments and ocular products. Alternatively, nanoparticles can be dried to a powder, which allows pulmonary delivery or further processing to capsules or tablets. Nanoparticles prepared from the biodegradable and biocompatible polymers like polylactide (PLA), polyglycolide (PLG) or their copolymers polylactide co glycolide (PLGA), are the most intensively investigated polymers in novel drug delivery [4, 5, 6]. The choice of a particular method for encapsulation is mainly determined by solubility and molecular stability of the drug. The commonly used techniques for preparation of nanoparticles are nanoprecipitation [7], the emulsification

*Address for correspondence: satikv@gmail.com

solvent evaporation [8], emulsification solvent diffusion technique [9, 10] and double emulsion solvent evaporation technique (DES-E) [11, 12]. These methods suffer from low encapsulation efficiency for the low molecular weight hydrophilic compounds for manufacturing of nanoparticles [13].

Different polymers were used in the preparation of nanoparticles such as, poly-lactide-co-glycolide (PLGA) [14, 15, 16], poly (lactide-co-glycolide-leucine) (PLDL) [17], Eudragit RL-100 [18, 19], Eudragit RS-100 [20, 21, 22], Eudragit E-100 [23], Eudragit S-100 [24], Chitosan [25]. Biodegradable polymer nanoparticles have great potential as drug delivery devices for the eye. The formulation of biodegradable polymers as colloidal systems holds significant promise for ophthalmic drug delivery [17]. Among these poly-ε-caprolactone may serve as superior polymer systems for ocular drug delivery [26, 27]. Marchal-Heussler *et al* compared nanoparticles prepared by using poly acryl cyanoacrylate, poly-ε-caprolactone, and polylactic-co-glycolic acid with betaxolol as model drug. It was shown that the poly-ε-caprolactone nanoparticles yielded the highest pharmacological effect. This was believed to be due to the agglomeration of these nanoparticles in the conjunctival sac and is suitable for controlled delivery of drugs. Poly-ε-caprolactone is a biocompatible polymer which has a slow *in-vivo* biodegradation, slower than the one of poly (L-lactic acid) (PLLA), due to its high crystallinity and hydrophobic character [28]. Furthermore, it has a relatively low melting point and low melt viscosity, making it easy to process.

Ketorolac tromethamine is a non steroidal anti-inflammatory drug approved by FDA for seasonal allergic conjunctivitis, post-cataract inflammation, and ocular discomfort after refractive surgery [29]. A recent review of NSAIDs suggests that ketorolac can be used to treat postoperative inflammation, cystoid macular edema following surgical procedures and allergic conjunctivitis and to relieve discomfort and pain after ocular surgery and trauma [30].

The main aim of the work was to encapsulate ketorolac tromethamine in a biodegradable polymer poly-ε-caprolactone, to study the affect of different formulation variables such as concentration of drug, polymer and

stabilizing agent on particle size, encapsulation efficiency and *in-vitro* release profile.

Materials and Methods

Materials

Ketorolac tromethamine was obtained as a gift sample from Divis Pharmaceutical PVT. LTD., India, Poly-ε-caprolactone (Sigma Aldrich chemicals, U.S.A), Polyvinyl alcohol (Loba Chemie PVT. LTD. India), polaxomer 188(Sigma Aldrich chemicals, U.S.A) and remaining chemicals were analytical grade.

Methods

Nanoparticles Preparation [31]

Ketorolac tromethamine nanoparticles were prepared with slight modification of previously reported technique [31]. Different batches of nanoparticles were prepared by emulsifying 1ml of drug solution in 3ml of Dichloromethane containing poly- ε-caprolactone and polaxmer- 188, by sonication using probe sonicator (QSONICA, model number: CL-18) at 20 % amplitude for 90 seconds. Then the so prepared w/o primary emulsion was then added to 10 ml distilled water and was sonicated for 90 seconds at 50 % amplitude. When poly vinyl alcohol (PVA, MW 1, 15, 000, Loba Chemie PVT. LTD. India) was used as a stabilizer; it dissolved in inner and external aqueous phases, 2 % w/v and 1.5 % w/v, respectively. Organic solvent was evaporated by stirring the emulsion on magnetic stirrer over night with constant magnetic stirring speed of 1500 rpm approximately. The resulting nanoparticles dispersion was collected and subjected for purification and concentration.

Purification of nanoparticles [32]

Nanoparticles were purified by taking 10 ml of the preparation in centricon tubes with cut off size of 10,000 and centrifugation of the same at 18000 rpm speed in cooling centrifuge (C-24BL, REMI, Mumbai, India) for 20 minutes. The concentrated nanoparticles were washed three times by adding doubled distilled water to the centricon tubes and subjected to centrifugation. Purified nanoparticles were taken and again re-suspended in purified water containing 0.5% w/v polyvinyl alcohol and then used for further studies.

Table - 1
Composition of Developed Nanoparticles

Ingredients	EP1	EP2	EP3	EP4	EP5	EP6	EP7	EP7	EP8	EP9	EP10
Drug(% w/v)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
poly-ε-caprolactone	0.2	0.4	0.6	1	2	1	1	1	1	1	1
Polaxomer-188	3	3	3	3	3	0.5	1	2	-	-	-
PVA	-	-	-	-	-	-	-	-	2	3	-
8 Tween	-	-	-	-	-	-	-	-	-	3	-

Characterization of Nanoparticles

Determination of drug entrapment efficiency

Entrapment efficiency of nanoparticles was determined spectrophotometrically by measuring the absorbance of liquid collected during purification of nanoparticles at 324 nm using UV/Visible Double beam spectrophotometer (UV-10, Thermo scientific). The entrapment efficiency of nanoparticles was determined by subtracting free drug amount from initial added amount of drug. The entrapment efficiency (EE %) could be calculated by following equation [33].

$$\text{ENTRAPMENT EFFICIENCY(EE\%)} = \frac{\text{Initial Drug-Free Drug}}{\text{Initial Drug}} \times 100$$

Particle size and Zeta potential measurement

The mean particle size for the formulations was determined by photon correlation spectroscopy (PCS) with a Zetasizer Nano ZS-90 (Malvern Instruments Ltd, Worcestershire, UK). The reading was carried out at 90° angle to the incident beam at 25°C using sample properly diluted with filtered water (0.5 micrometer filter). The conductivity of all samples was fixed to 2.43 ms/cm. The Zeta potential was determined by a laser Doppler anemometer coupled with Zetasizer Nano ZS-90 (Malvern Instruments Ltd.; UK).

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was performed for morphological evaluation of the nanoparticles. The nanoparticle specimen was properly mounted before loading them onto the specimen stage (F E I Quanta FEG 200 - High Resolution Scanning Electron Microscope, SAIF, IIT, Madras).

Fourier Transform Infrared spectroscopy (FTIR)

The FTIR spectra of Ketorolac tromethamine, poly-ε-caprolactone, physical mixture in the 1:1 drug to polymer ratio and freeze dried nanoparticles were carried out in the range of 4000-400 cm⁻¹ by FTIR spectroscopy (Perkin Elmer Spectrum 1 FT-IR, SAIF, IIT, Madras) using potassium bromide (KBr) pellet technique.

Differential scanning calorimetry (DSC)

DSC studies of ketorolac tromethamine, poly-ε-caprolactone, physical mixture in the 1:1 drug to polymer ratio and freeze dried nanoparticles were carried out. Samples were separately sealed in aluminum cells and set NETZSCH DSC 204 apparatus (SAIF-IIT, Madras) between 50°C and 350°C. Thermal analysis was performed at a heating rate maintained at 10°C per minute in a nitrogen atmosphere. An empty alumina pan was used as reference in each case.

In- vitro drug release

The *in vitro* drug release study of nanoparticles was performed in modified USP dissolution apparatus 1 having a glass cylinder which was open on both sides. A pre-soaked dialysis membrane (cut off 12000-14000, Himedia, Mumbai) was fixed to the terminal portion of glass cylinder.

Nanoparticles (2ml) were accurately placed into glass cylinder from open side and cylinder was fixed to the burette stand. The cylinder was placed in 100 ml dissolution simulated tear fluid (pH 7.4) medium maintained at 37°C ± 5°C at 100 rpm on a magnetic stirrer, so that the dialysis membrane fixed cylinder end just touched the receptor medium surface [34]. The samples were withdrawn at specified time intervals with volume replacement. The withdrawn samples were analyzed for drug content by measuring absorbance at 322 nm in UV/visible spectrophotometer.

Ex- vivo trans-corneal permeation study

Ex-vivo trans-corneal permeation studies were carried out by putting nanoparticles/ marketed eye drop (1ml) on freshly excised goat cornea. The fresh whole eyeballs of goats were obtained from a local butcher's shop and transported to the laboratory in cold condition in normal saline (4°C). The cornea was then carefully excised along with 2 to 4 mm of surrounding scleral tissue and was washed with normal saline till the washing was free from protein. The excised cornea was fixed between clamped donor and receptor compartments of an all glass modified Franz diffusion cell in such a way that its epithelial surface faced the donor compartment. The corneal area available for diffusion was 0.50 cm². The receptor compartment was filled with 10 ml freshly prepared simulated tear fluid (pH 7.4), and all air bubbles were expelled from compartment. The aliquot (1 ml) of prepared nanoparticles/ marketed eye drop was placed on cornea and the opening of the donor cell was sealed with a glass cover slip; the receptor fluid was kept at 37°C with constant stirring using teflon coated magnetic stirrer bead [34]. The permeation study was carried out for 6 hours, and samples were withdrawn from receptor and analyzed for drug content by measuring absorbance at 276 nm in UV/visible spectrophotometer.

Corneal hydration levels

Corneal hydration levels (HL) were calculated by gravimetric method. At the end of experiment, each cornea (freed from adhering sclera) was weighed (W_w), soaked in 1 ml methanol, dried overnight at 80°C, and reweighed (W_d). From the difference weight, corneal hydration was calculated [37]. The corneal HL was thus defined as:

$$\frac{(W_w - W_d)}{W_d} \times 100$$

Results and Discussion

In the preparation of Ketorolac Tromethamine loaded poly-ε-caprolactone nanoparticles different drug: polymer ratios were evaluated by keeping the drug concentration constant i.e. 20 mg and varying the polymer amount i.e. 20, 40, 60, 100, and 200 mg. The nanoparticles were then evaluated for particle size, ζ potential, entrapment efficiency and nanoparticles recovery. (Table 1) Particle size is an important parameter in developing ocular drug delivery. It is also an important parameter to assess the ocular tolerance. Larger particle size will induce rapid tear production which lead to the rapid drainage of the instilled dose and therefore reduced bioavailability. Optimized formulations prepared

Table 2
Evaluation of diclofenac sodium loaded nanoparticles

Formulation-Code	Drug polymer ratio, W/W	Particle size (nm ± SE)	Polydispersity index (nm ± SE)	Drug entrapment (% ± SE)	Nanoparticle recovery (%)	Z Potential (mV ± SE)
EP1	1:1	215.6913.68±	0.152 ±0.003	12.09 ± 2.98	28	-29.79 ±1.56
EP2	1:2	229.1919.59±	0.179 ±0.009	19.01 ± 5.91	56	-30.65 ±1.19
EP3	1:3	246.5±31.19	0.139 ±0.008	27.57 ± 3.93	69	-31.29 ±1.24
EP4	1:5	243±18.38	0.106 ±0.007	35.90 ± 2.09	71	-30.42 ±1.41
EP5	1:10	354.92±19.08	0.181 ±0.01	36.39 ± 4.51	74	-30.67 ±1.3

in laboratory showed a mean particle size below 250 nm, which is considered suitable for the ocular delivery [35], Figure 1.

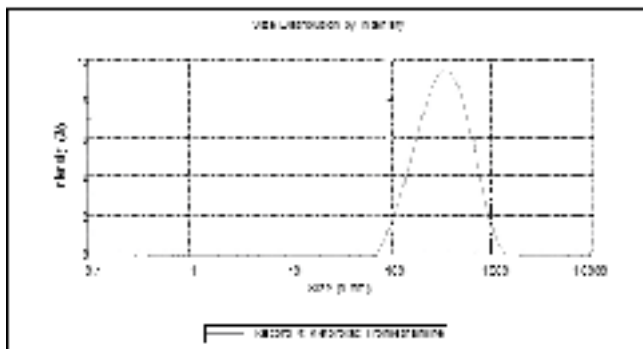


Fig.1: Showing particle size distribution of optimized formulation

The particle size distribution is narrow as the formulation has poly dispersity index (PI) near to 0.1, which corresponds to a mono disperse system. The optimized formulation was selected on the basis of small particle size, less PI and high entrapment efficiency with maximum nanoparticle recovery. Entrapment efficiency and nanoparticle recovery of the optimized formulation (EP4) was found to be 35.90 % and 71%, respectively, therefore EP4 was selected for further studies. ζ Potential is an important parameter to analyze the long-term stability of the nanoparticles. Generally higher ζ potential values, both (+) or (-) indicate long-term stability because of electrostatic repulsion between particles with same charges avoids aggregation [36]. ζ Potential of developed nanoparticles was found to be around -30 mV which is essential for long stable formulation. Negative charge on poly- ϵ -caprolactone nanoparticles is due to the ionization of carboxylic end group. Morphology of the nanoparticles was assessed by SEM. SEM gives information about the structure and size of the nanoparticles. Prepared nanoparticles were found to be spherical in shape as shown in Figure 2.

Fourier Transform Infrared spectroscopy (FTIR)

The FTIR spectrum of pure Ketorolac tromethamine shows a peak at 3448 cm^{-1} which is attributed to the N-H and NH₂ stretching and peaks at 1478 cm^{-1} , is due to C=C aromatic stretching, peak at 1378 cm^{-1} is due to C-N

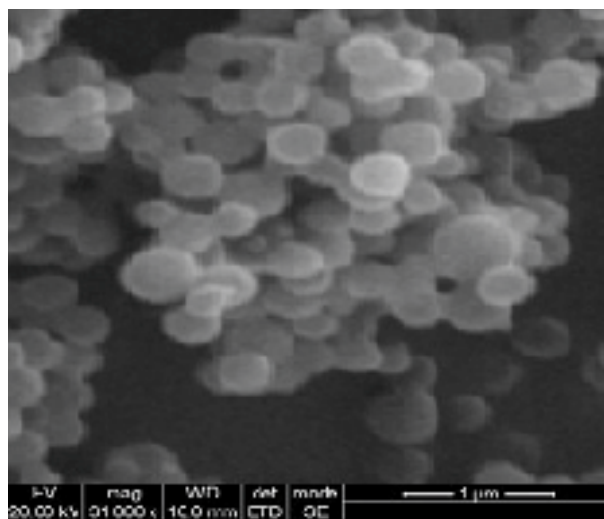


Fig.2 : Scanning electron micrograph images of optimized nanoparticles.

vibrations, peak at 10508 cm^{-1} is due to OH bending confirms presence of alcoholic group, peaks at 704, 780 and 798 cm^{-1} confirms the C-H bending (aromatic). Hence it confirms the structure of drug Ketorolac tromethamine (Figure 3). Whereas characteristic peak of poly- ϵ -caprolactone at 2945 cm^{-1} , 2871 cm^{-1} due to CH stretching cm^{-1} and 1692 cm^{-1} due to carbonyl adsorption (Fig. 4). FTIR study concluded that drug, polymer, physical mixture and nanoparticles exhibited the characteristic bands which confirm no interaction between ketorolac tromethamine and poly- ϵ -caprolactone (Fig.5, 6).

Differential scanning calorimetry (DSC)

Degradation endotherm of ketorolac tromethamine was observed at 168.89°C, in DSC of pure drug and physical mixture of drug with poly- ϵ -caprolactone. (Fig. 7) However, no distinct peak is observed in the EP4 formulation because of entrapment of drug in the polymer matrix and owing to decreased crystallinity in the formulation.

In vitro release

The formulation EP4 showed cumulative release (%) i.e. 94.37 ± 0.21 at the end of 24 hours as compared with other nanoparticles formulations. The release profile (Fig. 8) showed a biphasic release pattern, initial fast release

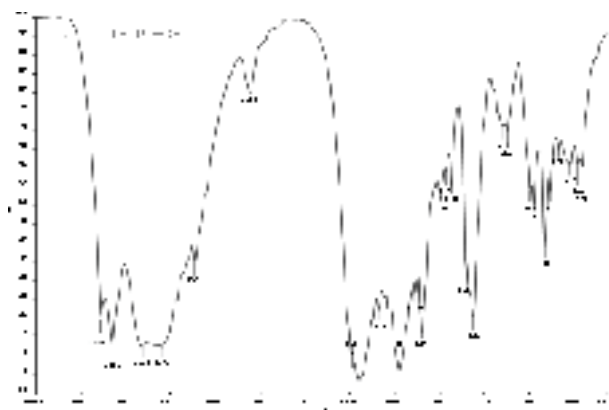


Fig. 3: FTIR spectra Ketarolac tromethamine

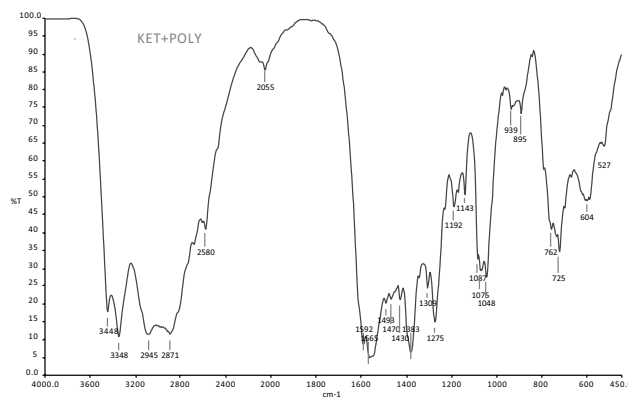


Fig.4: FTIR spectra Ketarolac tromethamine and poly-ε-caprolactone physical mixture

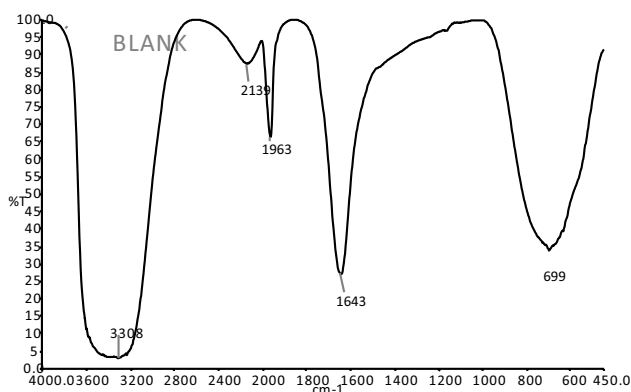


Fig.5: FTIR Spectra of Blank Nanoparticles

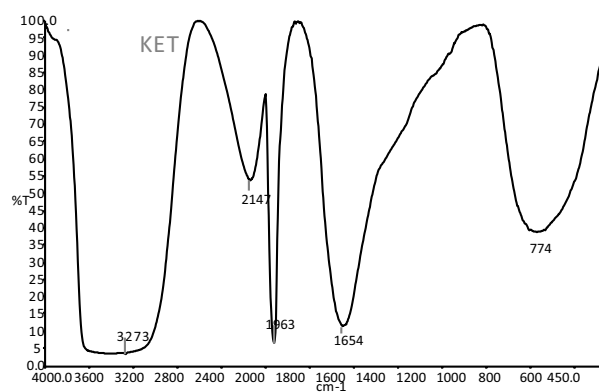


Fig. 6: FTIR Spectra of Drug loaded Nanoparticles

due to fraction of drug which is weakly absorbed or bound to the surface of nanoparticles followed by a slow release phase (extended release) which is due polymer entrapped fraction of drug. The effect of polymer also contributed the extended release of drug from nanoparticles. An initial fast release pattern is beneficial in terms of analgesic activity as it helps to achieve the therapeutic concentration of drug in minimal time followed by slow release to maintain sustain and controlled release effect. The in vitro release study suggests that the EP4 formulation provide higher drug release

profile as compared to other formulations. This might be due to smaller particle size with lower polydispersity index which could help to enhance dissolution and permeation profile.

In vitro release kinetics

The drug release data obtained from various in vitro release experiments were subjected to various kinetics equations to evaluate the drug release mechanism and kinetics [38]. The kinetics models used were zero order (as cumulative amount of drug release versus time), first order (as log cumulative percentage of drug remaining versus time),

Table - 3
Release kinetics of in-vitro drug release from ketorolac tromethamine loaded nanoparticles

Formulation Code	r ²				n vaue
	Zero-order	First- order	Higuchi	Korsemyer- Peppas	
EP1	0.516	0.610	0.799	0.945	0.4985
EP2	0.644	0.871	0.878	0.957	0.5533
EP3	0.625	0.778	0.874	0.954	0.5258
EP4	0.728	0.894	0.929	0.995	0.6073
EP5	0.750	0.943	0.944	0.954	0.5562

Higuchi model (as a cumulative percentage of drug released versus square root of time) and Korsmeyer-Peppas (as log cumulative percentage of drug release versus log time). In vitro release kinetics of all the ketorolac tromethamine loaded poly- ϵ -caprolactone formulations was studied. The regression coefficients (r^2) for all the batches using different kinetics equations are listed in Table 3. The kinetics data showed that in vitro release from ketorolac tromethamine loaded poly- ϵ -caprolactone nanoparticles is best explained by Korsmeyer-Peppas (K-P) model ($R=0.995$), with value of $n \sim 0.6073$). The n values for all nanoparticles batches as per K-P model was found between 0.49 and 0.61. This indicates the drug release from nanoparticles follows anomalous behavior, where swelling, diffusion and erosion may play an important role.

Ex -vivo trans-corneal permeation

Trans-corneal permeation ability of the substance to permeate the corneal barrier depends on various factors *i.e.* chemical nature of substance, size and conformation, partition co-efficient and degree of ionization etc. Epithelium, which is lipid in nature, is main barrier for hydrophilic drug whereas the aqueous stroma, which constitutes bulk of cornea, is the major rate limiting barrier for hydrophobic agent [39]. Trans-corneal permeation profile of ketorolac tromethamine nanoparticles is compared with marketed formulation. The graph is shown in Figure 9. Ketorolac tromethamine from marketed formulation was permeated 36.9% in 4 h whereas ketorolac tromethamine from poly- ϵ -caprolactone nanoparticles was permeated 47.43% in 4 h. Drug loaded nanoparticles shows a significantly ($P > 0.05$) higher permeation capability as compared to marketed eye drops [27]. This increased permeation through nanoparticles across the cornea could be attributed to the agglomeration of nanoparticles as depot near the cornea from which the drug is slowly delivered to the precorneal area. Corneal hydration

is generally used as an important parameter to evaluate damage to the corneal tissue. Generally, a normal cornea has a HL of 76–80% [3]. An alteration in this normal level by 3–4% shows damage to the epithelium or endothelium. HL found for marketed formulation and developed nanoparticles was found to be 76.91% and 77.12%, respectively, which indicates that formulations did not cause any damage to corneal tissue.

Conclusion

Ketorolac tromethamine loaded poly- ϵ -caprolactone nanoparticles were developed using double emulsion solvent diffusion technique. The optimum particle size and higher zeta potential values indicates that the formulations were quite stable with satisfactory drug release. The *in-vitro* drug release profile from all formulations shows biphasic in nature, *i.e.* first immediate release followed by extended release. The DSC and FTIR studies in were support to provide evidence of absence of drug polymer interaction. The optimized formulation of ketorolac tromethamine nanoparticles showed maximum in vitro trans-corneal permeation through freshly excised goat cornea. Finally it was concluded that ketorolac tromethamine loaded poly- ϵ -caprolactone nanoparticles can be used as promising ocular drug delivery carrier. The optimized formulation is hence suitable for sustained ocular drug delivery and warrants clinical evaluation and application.

Acknowledgements

The authors are thankful to Principal, Management, teaching and non teaching staff of Sri Krishna Chaithanya College of Pharmacy, Madanapalle for their constant encouragement and support. The authors are thank full to Divis Pharmaceutical PVT. LTD. for providing a gift sample of drug.

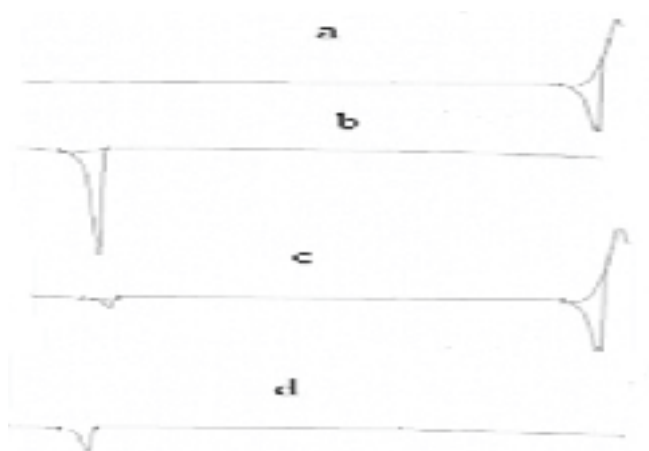


Fig.7: Differential scanning calorimetry thermograms.
a). Ketorolac tromethamine, b) Poly- ϵ -caprolactone, c) Physical mixture of drug and polymer, d) Drug loaded nanoparticles.

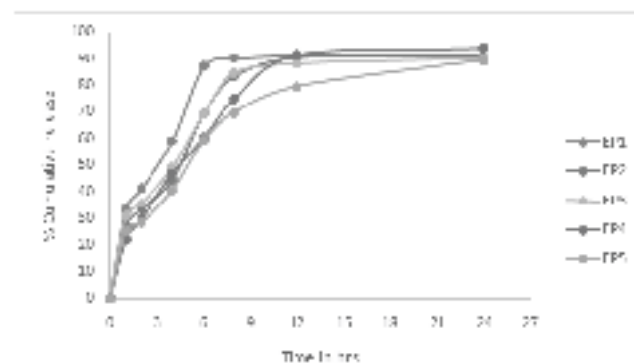


Fig. 8 : In-vitro release profile of different nanoparticle formulations

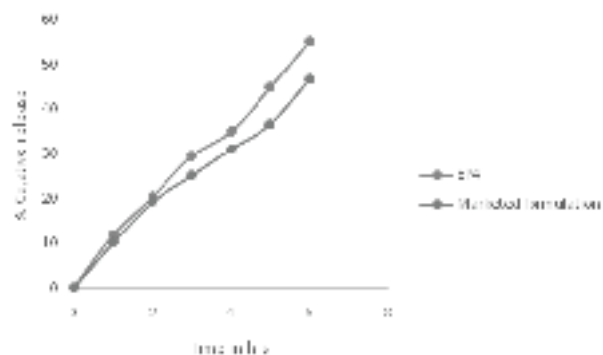


Fig.9 : Ex-vivo Trans-corneal permeation study for the marketed eye drops and optimized nanoparticles.

References

- Ashim KM, University of Missouri-Kansas City, Ophthalmic Drug Delivery System. 2nd ed., Revised and Expanded, Kansas City, U.S.A.; Marcel Decker; 2003.
- Mandal B, Halder KK, Dey SK, Bhounik M, Debananth MC, Gosh LK. Development and physical characterization of chloramphenicol loaded biodegradable nanoparticles for prolonged release. *Pharmazie*, 2009; 64: 445-449.
- Vega E, Gamisans F, Garcia ML, Chauvet A, Lacoulonche F, Egega MA. PLGA nanospheres for the ocular delivery of flubiprofen drug release and interactions. *J Pharm Sci*, 2008; 97: 5306-5317.
- Kingsley JD, Dou H, Morehead J, Rabinow B, Gendelman HE, Destache CJ, Nanotechnology: a focus on nanoparticles as a drug delivery system. *J. Neuroimmune. Pharmacol*, 2006; 1 (3): 340-350.
- Pridgen EM, Langer JD, Farokhzad OC. Biodegradable polymeric nanoparticle delivery systems for cancer therapy. *Nanomedicine*, 2007; 2 (5): 669-680.
- Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE. Biodegradable polymeric nanoparticles as drug delivery devices. *J. Control. Release*, 2001; 70 (1-2): 1-20.
- Fessi H, Devissaguet JP, Puisieux F, Thies C, U.S. patent, US 5118528, 1992.
- Janderhoff JW, El-Aasser MS, Ugelstad J. Polymer emulsification process, U.S. Patent, US 4177177, 1979.
- Niwa T, Takeuchi H, Hino T, Kunou N, Kawashima Y. Preparations of biodegradable nanospheres of water-soluble and insoluble drugs with d,l-lactideglycolide/copolymer by a novel spontaneous emulsification solvent diffusion method, and the drug release behavior. *J Control Release*, 1993; 25 (1-2): 89-98.
- Quintanar GD, Allemann E, Fessi H, Doelker E. Pseudolatex preparation using a novel emulsion-diffusion process involving direct displacement of partially water-miscible solvents by distillation. *Int J Pharm*, 1999; 188 (2): 155-164.
- Ogawa Y, Yamamoto M, Okada H, Yashiki T, Shimamoto T. A new technique to efficiently entrap leuprolide acetate into microcapsules of polylactic acid or copoly (lactic/glycolic) acid. *Chem Pharm Bull (Tokyo)*, 1988; 36 (3): 1095-1103.
- Zambaux MF, Bonneaux F, Gref R, Maincent P, Dellacherie E, Alonso MJ, Labrude P, Vigneron C. Influence of experimental parameters on the characteristics of poly(lactic acid) nanoparticles prepared by a double emulsion method. *J Control Release*, 1998; 50 (1-3): 31-40.
- Quintanar GD, Allemann E, Fessi H, Doelker E. Preparation techniques and mechanisms of formation of biodegradable nanoparticles from preformed polymers. *Drug Dev Ind Pharm*, 1998; 24 (12): 1113-1128.
- Aksungur P, Demirbilek M, Denkbaz EB, Vandervoort J, Ludwig A, Unlu N. Development and characterization of cyclosporine A loaded nanoparticles for ocular drug delivery: cellular toxicity, uptake, and kinetic studies. *J Control Release*, 2011; 151(3): 286-94.
- Javadzadeh Y, Ahadi F, Davaran S, Mohammadi G, Sabzevari A, Adibkia K. Preparation and physicochemical characterization of naproxen-PLGA nanoparticles. *Colloids Surf B Biointerfaces*, 2010; 1:81(2): 498-502.
- Yoncheva K, Vandervoort J, Ludwig A. Development of mucoadhesive poly (lactide-co-glycolide) nanoparticles for ocular application. *Pharm Dev Technol*, 2011; 16(1): 29-35.
- Agnihotri SM, Vavia PR. Diclofenac-loaded biopolymeric nanosuspensions for ophthalmic application. *Nanomedicine*, 2009; 5(1): 90-95.
- Pignatello R, Ricupero N, Bucolo C, Maugeri F, Maltese A, Puglisi G. Preparation and characterization of eudragit retard nanosuspensions for the ocular delivery of cloricromene. *AAPS Pharm Sci Tech*, 2006; 24:7(1): E27.
- Mandal B, Alexander KS, Riga AT. Sulfacetamide Loaded Eudragit RL 100 Nanosuspension with Potential for Ocular Delivery. *J Pharm Pharmaceutical Sci*, 2010; 13(14): 510-523.
- Adibkia K, Siahishadbad MR, Nokhodchi A, Javadzede A, Barzegar-Jalali M, Barar J, Mohammadi G, Omidi Y. Piroxicam nanoparticles for ocular delivery: physicochemical characterization and implementation in endotoxin-induced uveitis. *J Drug Target*, 2007; 15(6): 407-416.
- Das S, Suresh PK. Nanosuspension: a new vehicle for the improvement of the delivery of drugs to the ocular

- surface. Application to amphotericin B. *Nanomedicine*, 2011; 7(2): 242-247.
22. Pignatello R, Bucolo C, Ferrara P, Maltese A, Puleo A, Puglisi G. Eudragit RS 100 nanosuspensions for the ophthalmic controlled delivery of ibuprofen. *Eur J Pharm Sci*, 2002; 16(1-2): 53-61.
 23. Eerikainen H, Kauppinen El. Preparation of polymeric nanoparticles containing corticosteroid by a novel aerosol flow reactor method. *Int J Pharm*, 2003;16; 263(1-2):69-83.
 24. Ahuja M, Dhake AS, Sharma SK, Majumdar DK. Diclofenac-loaded eudragit S100 nanosuspension for ophthalmic delivery. *J Microencapsul*, 2011; 28: 37-45.
 25. Agnihotri SA, Aminabhavi TM. Chitosan nanoparticles for prolonged delivery of timololmaleate. *Drug Dev Ind Pharm*. 2007; 33: 1254-62.
 26. Losa C, Marchal-Heussler L, Orallo F, Vila JL, Alonso MJ. Design of new formulations for topical ocular administration: polymeric nanocapsules containing metipranolol. *Pharm Res*, 1993; 10(1):80-87.
 27. Marchal-Haussler L, Fessi H, Devissaguet JP, Hoffman M, Maincent P. Colloidal drug delivery systems for the eye. A comparison of the efficacy of three different polymers: polyisobutylcyanoacrylate, polylactic-coglycolic acid, poly-epsilon-caprolactone. *Pharm. Sci*.1992; 2: 98.
 28. Anle Y, Renjie W. Mechanical Properties and Interfacial Interaction of a Novel Bioabsorbable Chitin Fiber Reinforced Poly(e-caprolactone) Composite. *J Mat Sci Letters*, 2001; 20: 977-979.
 29. Kim, Stephen J, Allan J. Flach, Lee M.J. Nonsteroidal anti-inflammatory drugs in ophthalmology. *Survey of ophthalmology*, 2010; 55(2): 108-133.
 30. www.akorn.com/documents/catalog/sell_sheets/17478-209-19.pdf
 31. Zambaux MF, Bonneaux F, Gref R, Maincent P, Dellacherie E, Alonso MJ, Labrude P, Vigneron C. Influence of experimental parameters on the characteristics of poly(lactic acid) nanoparticles prepared by a double emulsion method. *J Control Release*, 1998; 50 (1-3):31-40.
 32. Angshuman B, Bhattacharjee SK, Mahanta Ritab, Biswanath M, Bandyopadhyay SK, alginate based nanoparticulate drug delivery for anti hiv drug lopinavir. *Journal of Global Pharma Tech*, 2010; 2(3): 126-132.
 33. Vandervoort J, Ludwig A. Preparation and evaluation of drug-loaded gelatine nanoparticles for topical ophthalmic use. *Eur J Pharm Biopharm*, 2004; 57: 251-61.
 34. Meetalı M, Pravin K.P, Preparation and In Vitro/ Ex Vivo Evaluation of Moxifloxacin Loaded PLGA Nanosuspensions for Ophthalmic Application. *Sci. Pharm*. 2013; 81(2):591-606.
 35. Schoenwald RD, Stewart P. Effect of particle size on ophthalmic bioavailability of dexamethasone suspensions in rabbits. *J Pharm Sci*, 1980; 69: 391-394.
 36. Feng S, Huang G. Effects of emulsifiers on the controlled release of paclitaxel (Taxol) from nanospheres of biodegradable polymers. *J Control Release*, 2001; 71:53-69.
 37. Gupta H, Aqil M, Khar RK, Asgar Ali1, Aseem B, Gaurav Mittal. Biodegradable levofloxacin nanoparticles for sustained ocular drug delivery, *Journal of Drug Targeting*, 2011; 19(6): 409-417.
 38. Peppas NA. Analysis of fickian and non- fickian drug release from polymers. *Pharm Acta Helv*, 1985; 60: 110-111.
 39. Chairini A, Tartarini A, Fini A. pH solubility relationship and partition coefficient for some anti-inflammatory arylaliphatic acid. *Arch Pharm*, 1984; 317: 268-273.

