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Research Article

IMPROVED CIPROFLOXACIN PENETRATION IN GOAT EYES USING COMPLEXATION TECHNIQUE

DURGA PANDEY*, DEEPTI JAIN

Department of Pharmaceutics, School of Pharmaceutical Sciences, Rajiv Gandhi Proudyogiki Vishwavidyalaya, Bhopal, Madhya Pradesh, India. Email: durga.pandey9@gmail.com

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ABSTRACT

Objective: The objective of the present work was to improve the retention and penetration of ciprofloxacin (CF) ion pair entrapped within submicron emulsion (SE) in goat eyes and characterize the SE for improvement of ocular activity. The developed delivery system resulted with prolonged drug release as compared to the conventional dosage form.

Methods: SE prepared by high-energy emulsification and sonication to obtain uniform globule size. Ion-pair complex is prepared by precipitation method.

Results: Average internal droplets size of the optimized formulation was 0.300 µm, pH of the optimized formulation was 6.4±0.7 (average of three determinations) and viscosity 3.2±0.3 cP suitable for ocular use. Entrapment was 92.12%. *In vitro* drug release pattern in dialysis membrane showed sustain release of CF, a cumulative percent release of CF was found 77% in 10 h. Scanning electron microscopy showed spherical shape and size within 1 µm. *In vitro* release in goat eyes was found 35.86 % for optimized formulation compared to market, 26.83% in 60 min.

Conclusion: Developed optimized formulation can be a good candidate for ocular drug delivery in severe ocular infections where frequent dosing required such as endophthalmitis, corneal ulcer, and penetrating trauma.

Keywords: Ocular, Submicron emulsion, Ion-pair complex, Prolonged drug release, Ciprofloxacin.

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INTRODUCTION

When a traditional eye drop instilled in the ocular area, there are many factors such as dilution of the drug by overflow, tear renewal, nasolacrimal drainage, conjunctival absorption, and enzyme metabolism affect the drug penetration to the eyes, leading to poor ocular bioavailability [1]. Enzymes present in ocular tissue may metabolize drugs during absorption [2]. Thus, frequent instillation of drug is required to achieve desired therapeutic effect which results with systemic absorption of drug and may result in undesirable effects [3]. Absorption of a drug takes place in corneal and non-corneal route. The non-corneal route involves absorption across the sclera and conjunctiva, which restrains the entry of drugs into the aqueous humor. Only corneal absorption is productive because available in aqueous humor [4]. Advantages of using the controlled ocular delivery system are as follows: (a) Overcoming the side effects of pulsed and frequent dosing of the traditional delivery systems, (b) providing sustained and controlled drug delivery, and (c) increases the ocular bioavailability of drug by increasing precorneal cornel contact time; this can be achieved by effective adherence to the corneal surface [5].

Various approaches to improve ocular bioavailability are such as viscosity enhancement, use of mucoadhesive [6] *in situ* gelling [7] particulate drug delivery, prodrugs [3], and other controlled systems, like Ocuserts [8], are being explored to overcome constraint associated with conventional drug administration. Submicron emulsion (SE), nanoemulsion [9], and miniemulsion have shown potential as an ocular drug delivery vehicle. Various ocular diseases such as endophthalmitis and bacterial keratitis required frequent dosing; in such cases, high concentrations of drug required in the aqueous humor, such problem can be overcome by increase retention in ocular globe through vesicular system [10].

Submicron formulations are nanometer droplet size range and natural biodegradable. Side effects of the drug is reduced with SE formulation. Sustain release of drug is possible by entrapment in the internal phase of oil in water submicron emulsion. Excipients used in the preparation are relatively biocompatible and non-toxic, withstand sterilization [11-14].

Many drugs are weak acid or bases that are ionized at normal physiological pH and in these conditions, they are not well absorbed by the biomembrane. Ion pairing involves the addition of an oppositely charged counterion to form a neutral ion pair, increase lipophilicity, and hence, membrane permeability is increased [15]. The ion pair diffuses into the stratum corneum and dissociates to form the parent compound when it reaches the viable endodermis layer. The ion pairing is a better technique than other as no chemical modification is required as in the prodrug approach, and no external driving force, such as an applied current in iontophoresis, is used. Carteolol bioavailability enhanced when it was ion paired with sorbate [16].

Rationale of the work was to improve precorneal retention and penetration of positively charged SE contained ciprofloxacin (CF) in ion paired form. The positive charge on the droplet surface was induced by chitosan (CN) which interacts with negatively charged corneal surface which enhances the adherence of the formulation to the corneal surface. Cornea is negatively charged due to a layer of the glycoprotein mucin secreted by goblet cells at the conjunctival surface and is the adjacent to corneal surface [17,18]. Novelty is that CF in ion pair form is more effective than alone CF and the developed delivery system can be beneficial in the treatment of deep ocular infections caused by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. These two selected organisms are most perilous ocular pathogens instigating bacterial infection of the human cornea such as keratitis, corneal ulceration, and endophthalmitis [19,20].

MATERIALS AND METHODS

Materials

Poloxamer 188 (polyoxyethylene-polyoxypropylene), egg lecithin, and soya oil were purchased from Sigma-Aldrich (MO, USA) and potassium sorbate (PS) (Sorbic acid potassium salt) from Sigma-Aldrich (MO, USA). All other ingredients used were of pharmaceutical grade. CF was kindly provided by Brooks Laboratory (Baddi, India) as a gift sample.

Animal study

Whole eyeball of goat was collected from local slaughterhouse.

Identification of drug

Identification of drug done by ultraviolet (UV)-visible spectroscopy (Shimadzu 1700 Corporation, Japan) and high-performance liquid chromatography (HPLC) (Shimadzu, Japan) was used. Shimadzu HPLC with an attached UV/visible detector, a stainless steel C_{18} column (2.5×4.6 mm) was used for drug analysis.

Partition coefficient of drug

The drug was added in equal volume of aqueous/octanol mixture in a test tube and was allowed to stirrer for a period of 6 h in orbital shaker and then kept in separating funnel overnight for partitioning. After complete partition, the mixture was separated into two phases and the concentration of CF was determined by UV.

Preparation and optimization of complex

The ionic complexes of CF with PS were prepared by dissolving appropriate quantities of PS in deionized water gradually added the PS solution in CF solution. The formation of ionic complex was optimized by preparing complex at different pH and taking different molar ratio of both components [21,22]. Different molar ration 0.25–8 and pH 3.6, 7.4, and 9.2 were used to prepare complex.

Partition coefficient of complex

Again, partition coefficient determined to check lipophilicity of drug after ion pairing. Complex was added in equal volume of aqueous/octanol mixture and the concentration of CF in both phases was determined by UV at 273 nm. The partition coefficient calculated by subtracting concentration of drug in aqueous phase from total drug.

Formulation

Different batches of SE were prepared with standard procedure with minor modifications. SE was prepared using high-energy emulsification technology [23]. The oil phase contained lecithin was prepared by heating soya oil at 60°C. CF-PS ionic complex (equivalent to 0.3% CF) was added to the oil mixture. The oil phase was added gradually to aqueous phase containing poloxamer 188 and magnetically stirred for 30 min. The crude emulsion was prepared by stirring for 30 min using a high shear mixer at the speed of 24,000 rpm and subsequent emulsification was accomplished by sonication [24] using ultrasonic probe for 5 min. In case of cationic emulsion, SE-CN-CF-PS, the aqueous phase was prepared by dispersing CN in 5% aqueous solution of sorbitol to maintain isotonicity and 2% solution of lactic acid. The pH of the resulting solution was adjusted to 6 to avoid any flocculation of CN, then oil phase as mentioned above added dropwise in aqueous phase with continuous stirring.

Characterization of SE

- Size and size distribution: The average particle size of the SE dispersion was determined using a Zetasizer (Malvern Instruments, UK).
- Zeta potential: Zeta potential was determined by Malvern Zetasizer (Malvern, UK).
- pH Emulsion: pH was recorded at given time intervals using a digital pen pH meter.
- Viscosity measurement: The viscosity of the emulsions was measured and analyzed using Brookfield viscometer (DV2T cone and plate USA).
- Scanning electron microscope (SEM): This analysis was performed, on SEM model Ultra Plus using software ZEISS (Germany).
- Entrapment efficiency: The SE was centrifuged (REMI centrifuge) at ×18,000 g and 4°C for 30 min to separate the incorporated drug from the free drug. The supernatant was analyzed by UV for the free drug concentration to determine the entrapped percentage from total amount of drug. The entrapment efficiency calculated as follows [25]:

$$E.E.\ (\%)=(A1-U2)/A1\times100$$

Where, U2=Amount of unentrapped drug and A1=Total amount of drug taken.

In vitro drug release using dialysis membrane

The *in vitro* release profile of SE was performed using bulk equilibrium reverse dialysis bag technique. The SE within the dialysis bag (Sigma, MWCO: 12000) immersed into 100 ml of phosphate buffer solution, pH 7.4 at 37°C and magnetically stirred at 50 rpm. Aliquots were withdrawn from the release medium at different time duration, namely, 0, 0.25, 0.5, 1, 2, 4, 6, 8, and 10 h and replaced with same amount of the phosphate buffer to maintain sink condition. The concentration of released CF was determined by UV [6,26].

In vitro drug release using goat cornea

Whole eyeball of goat was collected from local slaughterhouse. The cornea was carefully excised along with 2–4 mm of surrounding scleral tissue and was washed with cold normal saline. Isolated cornea was tied to the donor compartment with the help of thread such that surrounding scleral tissue clamped with donor compartment to fix the membrane at lower end. The cornea was tied in such a way that its epithelial surface faced the donor compartment. The corneal area available for diffusion was 0.785 cm^2. The receptor compartment was filled with freshly prepared simulated tear fluid (pH 7.4). About 1 ml of test formulation was placed on the cornea. The temperature of the medium was maintained at 34°C±0.1°C. About 1 ml of sample was withdrawn at various time intervals up to 120 m and same volume of fresh medium was replaced. The withdrawn samples were diluted and analyzed by UV spectrophotometer at 273 nm.

RESULTS AND DISCUSSION

In the current work, drug identification is done by UV and HPLC method. UV spectroscopy gives maximum drug absorption (λ_{max}) at 273 nm and HPLC showed retention time 6.0 (Fig. 1).

Ion pairing of drug

CF is commercially available as CF hydrochloride form and its solubility depends on pH of solvent. Its solubility or retention in oil phase is very low. At acidic pH, it exists in protonated form, which can form ion-pair complex with negative charge counter ion; therefore, more yield found in acidic pH. Based on this assumption, we have prepared CF ionic complexes with another molecule having an opposite charge like PS and subsequently lyophilized.

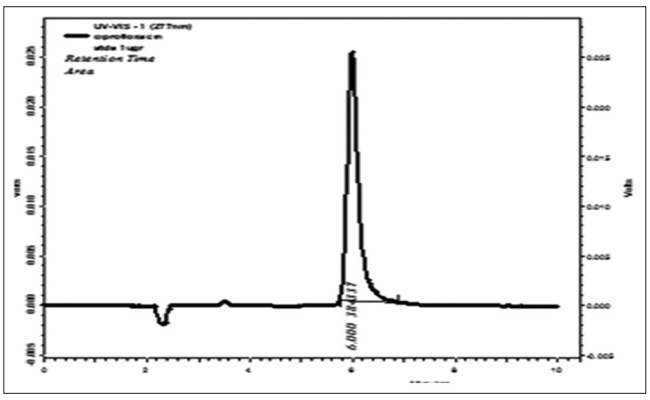

Fig. 1: High-performance liquid chromatography chromatogram showing retention time of ciprofloxacin (R_T=6.0 min)

Ionic complex prepared at 1:1 molar ratio observed as precipitate in sample. Partition coefficient of CF alone was 0.4808 and CF-PS was 2.871, indicated increased lipophilicity.

The amount of free CF recovered in supernatant at 1:1 molar ratio of CF:PS at different pH was studied. The free CF recovery was lower at acidic pH 3.6 which gradually increases as pH of the buffer approached to 7.4 and basic pH 9.2. The effect of a change in a molar ratio on the formation of the complex was studied by observing changes in solution turbidity. The value of transmittance decreases as the molar ratio of PS:CF increases. A complete clear solution (100% transmittance) was observed at 8 M with PS. This clearly indicates saturation of binding sites of CF with negatively charged sorbet. Thus, 1:1 ratio found best for preparation.

Formulation considerations

Various batches prepared by changing the composition of excipients and optimized formulation are selected in Table 1. Lecithin used as emulsifier and polaxamer-188 as a coemulsifier which prevents unfavorable interactions between free fatty acids present in soya oil and cationic agents. It has been reported that polaxamer-188 in optimized concentration is necessary to obtain a CN emulsion with sufficient stability [12].

Cationic polymer CN interacts with the negatively charged corneal mucin of the eyes and improves its retention [27]. Viscosity and pH required for ocular route were achieved 3.2±0.3 cP and 6.4±0.7, respectively (Table 2).

Table 1: Composition of optimized formulation

Excipients	SE-CF	SE-CF-PS	SE-CN-CF-PS
Soya oil	10	10	10
Poloxamer 188	2.5	2.5	2.5
Egg lecithin	1.25	1.25	1.25
CF	0.3	-	-
CF-PS	-	0.3	0.3
CN	-	-	0.5
Water	100	100	100

PS: Potassium sorbate, CF: Ciprofloxacin, SE: Submicron emulsion, CN: Chitosan

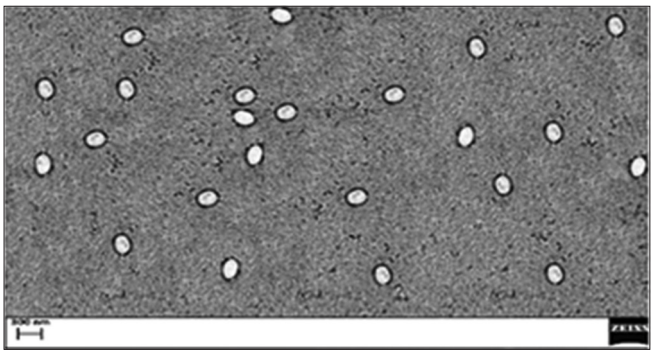

Fig. 2: Scanning electron microscope of submicron emulsion-chitosan-ciprofloxacin-potassium sorbate (spherical shape and size 300 nm)

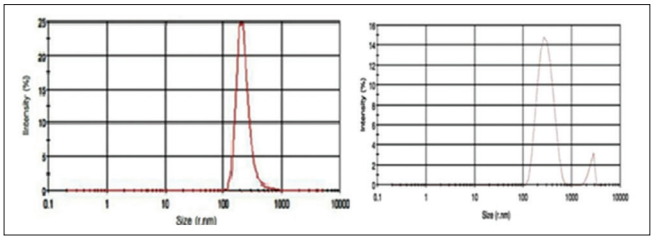

Fig. 3: Particle sizes of different formulations

Particle size, morphology, and surface charge

SEM confirms that droplets of SE are <1 μm and shape of droplets was found as spherical globules (Fig. 2). The zeta potential of the formulations was determined to assess the contribution of cationic inducer on charge distribution. In a blank SE formulation, reversal of zeta potential (−23.5±2.2–+35.7±4.2 mV) was observed when CN (0.5%, w/w) was added to lipid emulsions. CN contributes a positive charge in the formulation due to the presence of amino groups which results in reversal of charge in formulations [28]. Higher concentration of CN resulted in unstable formulation. Average globule size of optimized formulations was in the range of 300 nm, Fig. 3 and Table 2.

% Entrapment efficiency

The emulsion was centrifuged at ×18,000 g and 4°C for 60 min in an ultracentrifuge. The amount of free drug was measured by UV at 273 nm. Drug concentration was determined by comparing the peak areas of CF to the standard. More than 90% entrapment resulted with CN emulsion because CN forms a polymer coat around oil droplet which results in retention of hydrophobic ionic complex (Table 2).

***In vitro* drug release**

For the estimation of the *in vitro* drug release from the emulsion, CF was used as a model drug. The cumulative release of drug from emulsion indicates controlled release of the drug for a longer period time. SE-CF-PS gave a cumulative release of 82.50% in 24 h and SE-CN-CF-PS gave a cumulative release of 77.90% within 10 h, which shows the sustained release behavior of the formulation as compared with control (Fig. 4). The SE-CN-CF-PS shows control release profile among all which could be due to the formation of polymer coating around oil droplet and increase in viscosity. CF-ionic complex diffuses out slowly from the oil phase to external aqueous phase [29-31].

***In vitro* drug release in goat eyes**

In vitro release on goat eyes showed more permeation of optimized formulation, i.e., 20.50% from SE-CN-CF-PS and 12.12% in 15 min from

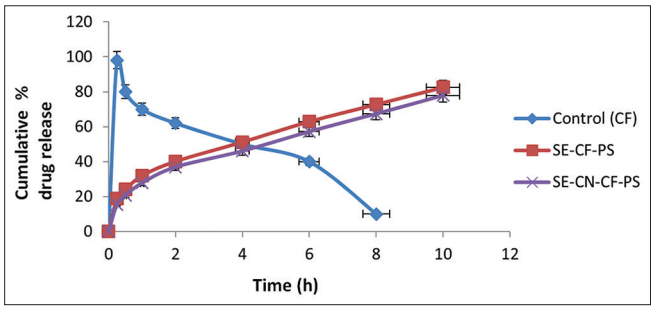

Fig. 4: *In vitro* release profile of ciprofloxacin-potassium sorbate-loaded submicron emulsion formulations carried out in dialysis bag technique at 37°C. The data are represented as mean average value of three separate experiments (n=3)

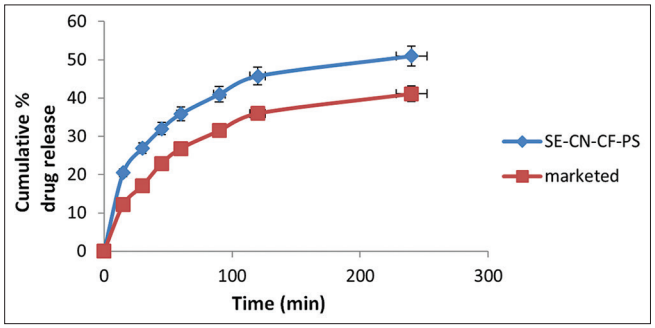

Fig. 5: *In vitro* drug permeation through goat eyes. The data are represented as mean average value of three separate experiments (n=3)

Table 2: Physicochemical characterization of different formulations

Formulation	Average globule size (nm)	Viscosity (Cp)	pH	Zeta potential	% entrapment
SE-CF	234	2.4±0.7	7.2±0.4	−12.37	29.65
SE-CF-PS	238	2.6±0.3	7.2±0.2	−23.5	80.43
SE-CN-CF-PS	300	3.2±0.3	6.4±0.7	+35.7	92.12

PS: Potassium sorbate, CF: Ciprofloxacin, SE: Submicron emulsion, CN: Chitosan

marketed formulation (Fig. 5). The reason behind the more absorption in goat eyes that drug retains for long time in precorneal area which correlated to *in vitro* release study in dialysis membrane, in which prolonged drug release observed for optimized formulation than marketed one.

CONCLUSION

Three tier objectives were successfully completed, first CF-PS complex preparation, second due to this enhancement of drug lipophilicity as PC value for complex was found 2.871, entrapment of CF in internal phase was ≥90%, and third prolonged drug release behavior 77.90% in 10 h with improved drug penetration as compared marketed formulation of the same drug. Most ocular infections are treated by topical application of antimicrobial solutions administered as aqueous eye drops. CF is the most widely used oldest and effective drug still in the first choice for various types of eye infections. A low bioavailability is observed due to rapid and extensive precorneal loss by conventional eye drop solutions.

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AUTHORS' CONTRIBUTIONS

Design of the study, all experimental works, and data compilation were done by author Durga Pandey under the guidance of Dr. Deepti Jain.

CONFLICTS OF INTEREST

Declared none.

REFERENCES

1. Del Amo EM, Rimpelä AK, Heikkinen E, Kari OK, Ramsay E, Lajunen T, *et al.* Pharmacokinetic aspects of retinal drug delivery. Prog Retin Eye Res 2017;57:134-85.
2. Ramesh Y, Kothapalli CB, Reddigari JR. A novel approach on ocular drug delivery. J Drug Deliv Ther 2017;7:117-24.
3. Taskar P, Tatke A, Majumdar S. Advances in the use of prodrugs for drug delivery to the eye. Expert Opin Drug Deliv 2017;14:49-63.
4. Mahor A, Prajapati SK, Verma A, Gupta R, Iyer AK, Kesharwani P, *et al.* Moxifloxacin loaded gelatin nanoparticles for ocular delivery: Formulation and *in-vitro*, *in vivo* evaluation. J Colloid Interface Sci 2016;483:132-8.
5. Brannigan RP, Khutoryanskiy VV. Synthesis and evaluation of mucoadhesive acryloyl-quaternized PDMAEMA nanogels for ocular drug delivery. Colloids Surf B Biointerfaces 2017;155:538-43.
6. Romero GB, Keck CM, Müller RH, Bou-Chacra NA. Development of cationic nanocrystals for ocular delivery. Eur J Pharm Biopharm 2016;107:215-22.
7. Cao Y, Zhang C, Shen W, Cheng Z, Yu LL, Ping Q, *et al.* Poly(N-isopropylacrylamide)-chitosan as thermosensitive *in situ* gel-forming system for ocular drug delivery. J Control Release 2007;120:186-94.
8. Mealy JE, Fedorchak MV, Little SR. *In vitro* characterization of a controlled-release ocular insert for delivery of brimonidine tartrate. Acta Biomater 2014;10:87-93.
9. Singh Y, Meher JG, Raval K, Khan FA, Chaurasia M, Jain NK, *et al.* Nanoemulsion: Concepts, development and applications in drug delivery. J Control Release 2017;252:28-49.
10. Sharma A, Taniguchi J. Review: Emerging strategies for antimicrobial drug delivery to the ocular surface: Implications for infectious keratitis. Ocul Surf 2017;15:670-9.
11. Solans C, Izquierdo PJ, Nolla J, Azemar N, Garcia-Celma MJ. Nano-emulsions current opinion in *colloid* and *interface science*. J Colloid Interface Sci 2005;10:102-10.
12. Tamilvanan S, Benita S. The potential of lipid emulsion for ocular delivery of lipophilic drugs. Eur J Pharm Biopharm 2004;58:357-68.
13. Sari F, Sinaga KR, Donald S. Formulation and evaluation of red palm olein nanoemulsion. Asian J Pharm Clin Res 2018;11:237-40.
14. Khiljee T, Akhtar N. Development and *in vitro* evaluation of a new topical o/w Emulgel from fruit extract of *Pyrus communis*. Int J Pharm Pham Sci 2019;11:75-9.
15. Hingorani T, Gul W, Elsohly M, Repka MA, Majumdar S. Effect of ion pairing on *in vitro* transcorneal permeability of a Δ(9) tetrahydrocannabinol prodrug: Potential in glaucoma therapy. J Pharm Sci 2012;101:616-26.
16. Higashiyama M, Tajika T, Inada K. Improvement of the ocular bioavailability of timolol by sorbic acid. J Ocul Pharmacol Ther 2006;22:333-9.
17. Zhigaltsev IV, Maurer N, Edwards K, Karlsson G, Cullis PR. Formation of drug-arylsulfonate complexes inside liposomes: A novel approach to improve drug retention. J Control Release 2006;110:378-86.
18. Uivarosi V. Metal complexes of quinolone antibiotics and their applications: An update. Molecules 2013;18:11153-97.
19. Dillen K, Vandervoort J, Van den Mooter G, Ludwig A. Evaluation of ciprofloxacin-loaded eudragit RS100 or RL100/PLGA nanoparticles. Int J Pharm 2006;314:72-82.
20. Ravikumar S, Krishnan RG, Selvanathan K, Selvam S. Antibacterial activity of metal oxide nanoparticles against ophthalmic pathogen. Int J Pharm Res Dev 2011;3:122-7.
21. Jena SK, Singh C, Dora CP, Suresh S. Development of tamoxifen-phospholipid complex: Novel approach for improving solubility and bioavailability. Int J Pharm 2014;473:1-9.
22. Devrim B, Bozkir A. Design and evaluation of hydrophobic ion pairing complexation of lysozyme with sodium dodecyl sulphate for improved encapsulation of hydrophilic peptides/protein by lipid-polymer hybrid nanoparticles. J Nanomed Nanotechnol 2015;6:1-5.
23. Ellbogen MH, Olsen KM, Gentry-Nielsen MJ, Preheim LC. Efficacy of liposome-encapsulated ciprofloxacin compared with ciprofloxacin and ceftriaxone in a rat model of pneumococcal pneumonia. J Antimicrob Chemother 2003;51:83-91.
24. Loftsson T, Brewster ME. Cyclodextrins as functional excipients: Methods to enhance complexation efficiency. J Pharm Sci 2012;101:3019-32.
25. Leong TS, Wooster TJ, Kentish SE, Ashokkumar M. Minimising oil droplet size using ultrasonic emulsification. Ultrason Sonochem 2009;16:721-7.
26. Yasir M, Sara UV. Solid lipid nanoparticles for nose to brain delivery of haloperidol: *In vitro* drug release and pharmacokinetics evaluation. Acta Pharm Sin B 2014;4:454-63.
27. NCCLS. National Committee for Clinical laboratory Standard Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically. 5th ed. Villanova, PA, USA: NCCLS, (Approved Standard M-7A5); 2000.
28. Li J, Tan G, Cheng B, Liu D, Pan W. Transport mechanism of chitosan-N-acetylcysteine, chitosan oligosaccharides or carboxymethyl chitosan decorated coumarin-6 loaded nanostructured lipid carriers across the rabbit ocular. Eur J Pharm Biopharm 2017;120:89-97.
29. Jafari SM, He Y, Bhandari B. Nanoemulsion production by sonication and microfluidization a comparison. Int J Food Properties 2006;9:475-85.
30. Oyedele AO, John OO, Ogungbemi HO, Olateju SO. Ocular tolerance and *in vitro* release of chloramphenicol in prospective eye ointment bases. Int J Pharm Pharm Sci 2015;7:306-11.
31. Kandav G, Bhatt D, Jindal DK. Formulation and evaluation of allopurinol loaded chitosan nanoparticles. Int J Appl Pharm 2019;11:49-52.