DOI: 10.21276/sb

Scholars Bulletin
(A Multidisciplinary Journal)
An Official Publication of "Scholars Middle East Publishers",
Dubai, United Arab Emirates
Website: http://scholarsbulletin.com/

ISSN 2412-9771 (Print)
ISSN 2412-897X (Online)

Lipid based systems for ocular drug delivery: Effect of surface modification

Thirupathi G[1], Ramesh B[1]*
[1]Associate Professor, SRR College of Pharmaceutical Sciences, Warangal, Telangana, INDIA

***Corresponding Author:**
Ramesh B
Email: rameshbomma010@gmail.com

Abstract: Significant challenges for pharmaceutical scientists still exist despite numerous technological advancements in the field, and the efficient ocular drug delivery remains elusive. Exclusive and exquisite barrier characteristics, unique physiology make the ocular milieu impervious to xenobiotics. Precorneal residence, mucoadhesion and transmembrane permeation needs to be improved using topical non-invasive strategies across ocular surface show promise for the intraocular delivery. Viable advantages such as higher drug loading, tailored drug release, bio-compatibility and mucoadhesive properties can improve delivery of lipophilic drug using colloidal nanoparticulate frameworks. Lipid based systems comprise solid lipid nanoparticles (SLNs) and Nanostructured lipid carriers (NLCs). Nanoparticles are better internalized in ocular epithelial tissues by endocytosis/transcytosis mechanisms and diffuse rapidly owing to their nano size. Penetration and retention characteristics of ocular small molecule therapeutics can be dramatically improved by the surface functionalization of lipid nanoparticles. This review summarizes scientific results such as articles and patents that involve the potential application of lipid nanoparticles as platform for the ocular drug delivery.

Keywords: Ocular delivery, lipid based systems, surface functionalization, nanoparticles, solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), transcorneal permeability

INTRODUCTION

The ocular globe comprises two anatomical segments. The crystalline and transparent lens demarcates the anterior and posterior segments. Anterior segment constitutes cornea, iris ciliary, aqueous humor tissues whereas posterior segment includes the sclera, conjunctiva, retina and choroid tissues. The cornea is non-vascularized structure featuring alternatively polarized ocular layers namely epithelium (lipophilic), stroma (hydrophilic) and the endothelium (lipophilic barrier). The stroma constitutes 90% of corneal thickness. The corneal epithelium is the rate limiting barrier for lipophilic drugs whereas subsequent stromal layer is the compromising barrier for hydrophilic drugs. However, drugs should possess optimal physico-chemical properties to partition through these barriers and need to formulate in promising delivery platforms for effective delivery. The conjunctiva is vascularized mucus membrane lining the eye lids and the scleral surface except the cornea. The conjunctival membrane, which is devoid of tight junctions, is more permeable and approximately 20 times larger when compared to cornea. The penetration of drugs across scleral membrane is independent of drug lipophilicity and molecular weight enabling the possibility of trans-scleral-conjunctival delivery when compared to corneal route [1-3]. Ocular complications range from inflammation/infection to several sight threatening disorders including but not limited to glaucoma, age related macular degeneration, diabetic retinopathy, retinitis pigmentosa, uveitis, cytomegalovirus retinitis and endophthalmitis. Potential therapeutic routes for ocular drug delivery include topical, local ocular (i.e., subconjunctival, intravitreal, retrobulbar, intracameral), and systemic delivery. Currently, intravitreal drug delivery (injection of drugs into vitreous body) is the most invasive route for achieving highest bioavailability in the ocular posterior segment. However, this route presents several adverse effects such as cataract, retinal detachment, vitreous hemorrhage, and endophthalmitis [4-8]. Topical administration is most preferred route due to ease of application, low cost, safety and patient compliance/adherence. However, less than 5% of instilled dose reaches inner ocular tissues post topical application of conventional formulations like solutions. Topical ocular delivery of drugs is challenging task due to the anatomical, physiological and metabolic constraints put forth by the ocular globe. Ocular physiological and dynamic barriers confer low pre-corneal volume and residence, lacrimation and blinking, tear dynamics, nasolacrimal drainage. Also, ocular efflux barriers and alternatively polarized ocular tissues severely limit the penetration of therapeutic agents [9-12]. Several approaches improving pre-ocular residence time and the corneal penetration of the applied drugs have been reported such as solutions,

suspensions, hydrogels, *in situ* gelling systems, micro particles and colloidal carriers [13-15]. The use of lipid systems as ophthalmic platforms presents unique set of advantages such as tailored release, biocompatibility and biodegradability; high drug loading and encapsulation efficiency. Numerous reports have tested the efficacy of antibiotics, plasmids, anti-inflammatory and immunosuppressive agents for the treatment of ophthalmic disorders exploiting lipid nanoparticles [16, 17]. Nanoparticles coated, dissolved or suspended in mucoadhesive polymers/*in situ* gelling polymers further increase mucoadhesivity and pre-corneal residence time thus promoting ocular bioavailability. Literature suggests that surface functionalization of lipid nanoparticles with different polymeric agents such as poloxamers, chitosans and polyethylene glycols further improved the penetration and retention characteristics of the drug molecules. Furthermore, functionalization of polyethylene glycols/chitosan moiety end chains with certain ligands/cell penetrating peptides for targeted deliver is been reported. This review aims to focus on the latest trend and developments in the non-invasive lipid based systems for anterior and posterior segment ocular delivery [15, 18].

Colloidal lipid nanoparticulate carriers

Lipid nanoparticle systems made up of biocompatible solid/ liquid lipids or both with appropriate compositions, externally stabilized using surfactant/co-surfactants. Lipids used in the formulations are generally recognized as safe (GRAS) substances. The lipid based systems protect the drug molecules from chemical degradation, impart long-term stability and controlled drug release properties. The other advantages provided includes drug targeting, improved bioavailability, scale-up and economic production which eliminates organic solvent processing. Solid lipid nanoparticles (SLNs) and the nanostructured lipid carriers (NLCs) are lipid based platforms widely used in the ocular delivery. NLCs emerged as second generation platforms of SLNs to offer advantages such as high drug loading, higher encapsulation efficiency and on-storage stability. Incorporation of spatially incompatible liquid lipids into solid lipid matrix creates more imperfect crystal lattices, which can accommodate enhanced drug loading [19]. SLNs and NLCs demonstrate ocular surface mucoadhesivity and interaction with the epithelium, depending on the morpho-metrical and surface charge characteristics. The charge on the nanoparticulate surface widely influences its affinity with the ocular mucosa. Literature suggests that positively charged particles interpenetrate mucus layers by charge-charge interactions and mucoadhesive properties. Pre-ocular residence time of nanoparticles is prolonged by surface adsorption/grafting with cationic polymers. Lipid nanoparticles prepared with cationic lipids and/or surfactants promotes the electrostatic interaction and thereby higher intra-ocular bioavailability [20-22]. High pressure homogenization (hot/cold), ultra-sonication, microemulsion and ultrasound techniques commonly used to prepare lipid nanoparticles. The wide spectrum of characteristics and properties of the lipid nanoparticles can be dramatically changed and controlled by production method and therein involved critical manufacturing conditions. Hot high pressure homogenization method is the most efficient technique for the production of lipid nanoparticles in terms of achieving desired particle size characteristics and batch to batch reproducibility. Physicochemical and thermodynamic stability of SLN and NLC dispersions could be obtained using suitable surfactants, lipids, bile salts and biocompatible non-ionic molecules (e.g., ethylene oxide/propylene oxide copolymers, sorbitan esters, and fatty acid ethoxylates). These surfactants adsorb onto the solid lipid particles at the liquid interface and stabilize the dispersion [23-25]. Cytotoxicity studies of SLNs and NLCs performed in various cell lines demonstrated that these systems are well tolerated and non-irritant to the ocular tissues. Cytotoxicity of the NLCs is reported with surfactants of charged species and hence the non-ionic are preferred due to their lower toxicity and irritation potential. All ophthalmic products need to be sterilized prior to pre-clinical/clinical use. Sterilization of SLNs/NLCs is widely reported in the literature with methods which include filtration, moist heat sterilization and gamma radiation. Stabilizers/surfactants used in the lipid dispersion attain cloud points under higher temperature during the sterilization conditions, desorb form the nanoparticle and render them vulnerable for aggregation and thereby instability. The physico-chemical characteristics of the nanoparticle formulations vary slightly/totally change during the terminal moist heat sterilization depending upon the nature of lipids and surfactant frameworks. Hence, sterilization by filtration/gamma radiation is the appropriate methods for lipid nanoparticle sterilization [26-28].

Recent trends in lipid based platforms for ocular delivery

There have been recent developments in the ocular delivery which demonstrated the significance of ligands/agents in improving cellular internalization and corneal penetration from the lipid based frameworks. Various lipid nanoparticles were surface functionalized with agents such as PEG, chitosan, biotin, peptide, Tween 80, D-α-tocopherylpoly (ethylene glycol 1000) succcinate, Eudragit RS 100 (Novel nanostructured lipid carrier-based inserts for controlled ocular drug delivery: evaluation of corneal bioavailability and treatment efficacy in bacterial keratitis). The recent studies are compiled in this review article and discussed hereunder.

Indomethacin (IN) loaded lipid based formulations such as IN SLNs, IN chitosan coated SLNs and IN NLCs were developed in one study. Indomethacin loaded SLNs coated with chitosan chloride improved transcorneal penetration and in vivo ocular tissue distribution, particularly in retina, when compared to uncoated SLNs [29]. In other study, PEGylation demonstrated that surface functionalization of Ciprofloxacin loaded NLCs is viable attempt to improve transcorneal permeation and in vivo ocular disposition [30]. Seyfoddin *et al.* developed chitosan coated NLCs for ocular delivery, where the antiviral efficacy of entrapped acyclovir (ACV) was enhanced by ~3.5-folds after 24 hrs of exposure. Corneal bioavailability of ACV with NLCs enhanced by ~4.5-folds in comparison to marketed ACV ophthalmic ointment [31]. Zhang *et al.*. formulated cationic Eudragit RS 100 coated genistein NLCs for improved ocular bioavailability. Eudragit coated NLCs enhanced AUC (area under the curve) by 1.22-fold in tears compared to uncoated NLCs [32]. In a study chitosan coated NLCs loaded with flurbiprofen were prepared for ocular drug delivery with Chitosan of molecular weight from 3000-6000kDa. After coating, the clearance of the formulations labeled with radioactive 99(m) Tc-DTPA was significantly delayed and the AUC of the surface modified formulation showed ~7.7-fold increase compared to uncoated formulations. Transcorneal permeability was enhanced by ~2.4-fold following the inclusion of chitosan [33]. Liu *et al.* reported curcumin loaded NLCs functionalized by surface adsorption using N-acetyl-L-cysteine grafted chitosan copolymer (CS-NAC). Zeta potential increased from -20.38 ± 0.39 mV to 22.51 ± 0.34 mV. The modification enhanced transmembrane penetration characteristics of NLCs. *In vivo* imaging technique and ocular pharmacokinetic studies demonstrated reduced clearance and prolonged retention in ocular tissues following surface modification of NLCs [34]. Tian Fu *et al.* developed chitosan coated amphotericin B (AmB) loaded NLCs for prolonged ocular application. AmB NLCs exhibited particle size of 185.4 nm, a zeta potential of 27.1 mV, and an entrapment efficiency of 90.9%. *In vitro* release of AmB *demonstrated sustained release profile*. The corneal penetration study showed that the AmB NLCs could penetrate into the cornea without irritation to the rabbit eyes. The *in vivo* studies indicated improved bioavailability of AmB from NLCs [35]. Chitosan lactate modified NLCs loaded with ofloxacin (OFX) were prepared by nehlisan *et al.* The transcorneal permeability from NLCs obtained was significantly higher compared to the control solution. Incorporation of chitosan polysaccharide in NLCs improved preocular residence time and controlled drug release and thereby enhanced intraocular bioavailability [36]. Pandian *et al.* formulated resveratrol (RES) loaded pegylated chitosan nanoparticles for the treatment of glaucoma. It was observed that Particle size and poly dispersity increased and entrapment efficiencies decreased as function of higher concentrations of PEG used in the formulations. Formulations exhibited iso-osmolarity with tears and non-irritation to ocular tissues. The studies demonstrated that chitosan restricted the drugs to corneal membrane whereas PEG delivered the drug into retinal choroid tissues. Also, RES loaded PEGylated chitosan nanoparticles reduced the intra-ocular pressure (IOP) by 4.3 ± 0.5 mm Hg up to 8 h in normotensive rabbits [37]. In other study, RES and quercetin (QUR) were co-encapsulated using chitosan and PEG modified chitosan nanoparticles for the IOP reduction by Natesan *et al.* PEG-chitosan modified carriers lowered IOP with the synergistic effect resulting from RES and QUR. Ex vivo corneal permeation of RES from the co-encapsulated nanoparticles than RES control nanoparticles/dispersion. PEG modified chitosan nanoparticles resulted in prolonged lowering of IOP by 5.5 ± 0.5 mm Hg in normotensive rabbits [38]. NLC loaded with alpha mangostin (AP) was functionalized using mucoadhesive ligand namely oleoyl-quaternized-chitosan (CS) for efficient ocular delivery. These results indicate that CS-NLC shows enhanced cellular uptake and internalization which could be due to mucoadhesive and barrier modulating properties of chitosan moiety [39]. In another study, Cationic lipid nanoparticles using cetyl trimethyl ammonium bromide (CTAB) were developed for testing ocular safety as a function of higher concentration of CTAB ranging from 0.25 to 1% wt. The toxicity testing predicted that 0.5% wt CTAB are safe for ocular use [40]. Battaglia formulated chitosan coated lipid nanoparticles of cyclosporine for improved ocular delivery. Lipid nanoparticles exhibited particle size characteristics and poly dispersity indices in range of 286-487 nm and 0.086-0.139. Chitosan coated lipid systems demonstrated greater permeation and accumulation when compared to neutral, anionic lipid nanoparticles, emulsions and suspensions tested. This could be due to the phagocytic uptake of nanoparticles by corneal epithelial cells [41].

CONCLUSION

SLN and NLC are basic frameworks which could be exploited for modulation of the release profile, imparting enhanced transmembrane penetration and ocular retention characteristics with wide classes of drugs/drug candidates such as anti-infectious, anti-inflammatory, anti-glaucoma agents. Additional added advantages such as good ocular tolerance, bio-compatibility, drug protection, scale-up/production efficiency, little to no organic solvents make the platform more facile to operate with. However, the research investigating the effect of surface functionalization on lipid systems is carried out for other applications, only a few studies are reported for ocular administration. This review brings out the recent

reports on surface modified/surface functionalized lipid nanoparticles as efficient ocular delivery system. This field needs to be explored with integrating multi-disciplinary approaches to advance the lipid systems for effective non-invasive topical delivery.

REFERENCES

1. Hosoya, K. I., Lee, V. H., & Kim, K. J. (2005). Roles of the conjunctiva in ocular drug delivery: a review of conjunctival transport mechanisms and their regulation. *European journal of pharmaceutics and biopharmaceutics*, *60*(2), 227-240.
2. Adelli, G. R., Hingorani, T., Punyamurthula, N., Balguri, S. P., & Majumdar, S. (2015). Evaluation of topical hesperetin matrix film for back-of-the-eye delivery. *European Journal of Pharmaceutics and Biopharmaceutics*, *92*, 74-82.
3. Adelli, G. R., Balguri, S. P., & Majumdar, S. (2015). Effect of cyclodextrins on morphology and barrier characteristics of isolated rabbit corneas. *AAPS PharmSciTech*, *16*(5), 1220-1226.
4. Patel, A., Cholkar, K., Agrahari, V., & Mitra, A. K. (2013). Ocular drug delivery systems: an overview. *World journal of pharmacology*, *2*(2), 47.
5. Raghava, S., Hammond, M., & Kompella, U. B. (2004). Periocular routes for retinal drug delivery. *Expert opinion on drug delivery*, *1*(1), 99-114.
6. Sahoo, S. K., Dilnawaz, F., & Krishnakumar, S. (2008). Nanotechnology in ocular drug delivery. *Drug discovery today*, *13*(3), 144-151.
7. Adelli, G. R., Balguri, S. P., Punyamurthula, N., Bhagav, P., & Majumdar, S. (2014). Development and evaluation of prolonged release topical indomethacin formulations for ocular inflammation. *Investigative Ophthalmology & Visual Science*, *55*(13), 463-463.
8. Balguri, S. P., Adelli, G. R., Tatke, A., Janga, K. Y., Bhagav, P., & Majumdar, S. (2017). Melt-Cast Noninvasive Ocular Inserts for Posterior Segment Drug Delivery. *Journal of Pharmaceutical Sciences*.
9. Short, B. G. (2008). Safety evaluation of ocular drug delivery formulations: techniques and practical considerations. *Toxicologic pathology*, *36*(1), 49-62.
10. Rhone, M., & Basu, A. (2008). Phytochemicals and age-related eye diseases. *Nutrition reviews*, *66*(8), 465-472.
11. Ahuja, M., Dhake, A. S., Sharma, S. K., & Majumdar, D. K. (2008). Topical ocular delivery of NSAIDs. *The AAPS journal*, *10*(2), 229.
12. Al-Halafi, A. M. (2014). Nanocarriers of nanotechnology in retinal diseases. *Saudi Journal of Ophthalmology*, *28*(4), 304-309.
13. Almeida, H., Amaral, M. H., Lobao, P., Frigerio, C., & Manuel Sousa Lobo, J. (2015). Nanoparticles in ocular drug delivery systems for topical administration: promises and challenges. *Current pharmaceutical design*, *21*(36), 5212-5224.
14. Caramella, C., Ferrari, F., Bonferoni, M. C., Rossi, S., & Sandri, G. (2010). Chitosan and its derivatives as drug penetration enhancers. *Journal of Drug Delivery Science and Technology*, *20*(1), 5-13.
15. Morrison, P. W., & Khutoryanskiy, V. V. (2014). Advances in ophthalmic drug delivery. *Therapeutic delivery*, *5*(12), 1297-1315.
16. Battaglia, L., Serpe, L., Foglietta, F., Muntoni, E., Gallarate, M., Del Pozo Rodriguez, A., & Solinis, M. A. (2016). Application of lipid nanoparticles to ocular drug delivery. *Expert opinion on drug delivery*, *13*(12), 1743-1757.
17. Chime, S. A., & Onyishi, I. V. (2013). Lipid-based drug delivery systems (LDDS): Recent advances and applications of lipids in drug delivery. *African Journal of Pharmacy and Pharmacology*, *7*(48), 3034-3059.
18. Adelli, G. R., Balguri, S. P., Bhagav, P., Raman, V., & Majumdar, S. (2017). Diclofenac sodium ion exchange resin complex loaded melt cast films for sustained release ocular delivery. *Drug Delivery*, *24*(1), 370-379.
19. Hippalgaonkar, K., Adelli, G. R., Hippalgaonkar, K., Repka, M. A., & Majumdar, S. (2013). Indomethacin-loaded solid lipid nanoparticles for ocular delivery: development, characterization, and in vitro evaluation. *Journal of Ocular Pharmacology and Therapeutics*, *29*(2), 216-228.
20. Sultana, Y., Jain, R., Aqil, M., & Ali, A. (2006). Review of ocular drug delivery. *Current drug delivery*, *3*(2), 207-217.
21. Lim, S. B., Banerjee, A., & Önyüksel, H. (2012). Improvement of drug safety by the use of lipid-based nanocarriers. *Journal of controlled release*, *163*(1), 34-45.
22. Balguri, S. P., Adelli, G. R., Janga, K. Y., Bhagav, P., & Majumdar, S. (2017). Ocular disposition of ciprofloxacin from topical, PEGylated nanostructured lipid carriers: Effect of molecular weight and density of poly (ethylene) glycol. *International Journal of Pharmaceutics*, *529*(1-2), 32-43.
23. Martins, S., Sarmento, B., Ferreira, D. C., & Souto, E. B. (2007). Lipid-based colloidal carriers for peptide and protein delivery–liposomes versus lipid nanoparticles. *International journal of nanomedicine*, *2*(4), 595.
24. Müller, R. H., Radtke, M., & Wissing, S. A. (2002). Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. *Advanced drug delivery reviews*, *54*, S131-S155.
25. Balguri, S. P. (2017). *Lipid Based Frameworks And Topical Ocular Inserts For The Delivery Of Small Molecule Therapeutics To The Posterior Segment*

Of The Eye (Doctoral dissertation, The University of Mississippi).
26. Pozzi, D., Colapicchioni, V., Caracciolo, G., Piovesana, S., Capriotti, A. L., Palchetti, S., ... & Laganà, A. (2014). Effect of polyethyleneglycol (PEG) chain length on the bio–nano-interactions between PEGylated lipid nanoparticles and biological fluids: from nanostructure to uptake in cancer cells. *Nanoscale*, *6*(5), 2782-2792.
27. Woodle, M. C., Newman, M. S., & Cohen, J. A. (1994). Sterically stabilized liposomes: physical and biological properties. *Journal of drug targeting*, *2*(5), 397-403.
28. Hui, P., & Diluzio, W. (2002). *U.S. Patent Application No. 10/102,228*.
29. Balguri, S. P., Adelli, G. R., & Majumdar, S. (2016). Topical ophthalmic lipid nanoparticle formulations (SLN, NLC) of indomethacin for delivery to the posterior segment ocular tissues. *European Journal of Pharmaceutics and Biopharmaceutics*, *109*, 224-235.
30. Balguri, S. P., Adelli, G., Bhagav, P., Repka, M. A., & Majumdar, S. (2015). Development of nano structured lipid carriers of ciprofloxacin for ocular delivery: Characterization, in vivo distribution and effect of PEGylation. *Investigative Ophthalmology & Visual Science*, *56*(7), 2269-2269.
31. Seyfoddin, A., Sherwin, T., V Patel, D., N McGhee, C., D Rupenthal, I., A Taylor, J., & Al-Kassas, R. (2016). Ex vivo and in vivo evaluation of chitosan coated nanostructured lipid carriers for ocular delivery of acyclovir. *Current drug delivery*, *13*(6), 923-934.
32. Zhang, W., Li, X., Ye, T., Chen, F., Yu, S., Chen, J., ... & Pan, W. (2014). Nanostructured lipid carrier surface modified with Eudragit RS 100 and its potential ophthalmic functions. *International journal of nanomedicine*, *9*, 4305.
33. Luo, Q., Zhao, J., Zhang, X., & Pan, W. (2011). Nanostructured lipid carrier (NLC) coated with Chitosan Oligosaccharides and its potential use in ocular drug delivery system. *International journal of pharmaceutics*, *403*(1), 185-191.
34. Liu, D., Li, J., Pan, H., He, F., Liu, Z., Wu, Q., ... & Yang, X. (2016). Potential advantages of a novel chitosan-N-acetylcysteine surface modified nanostructured lipid carrier on the performance of ophthalmic delivery of curcumin. *Scientific reports*, *6*, 28796.
35. Fu, T., Yi, J., Lv, S., & Zhang, B. (2016). Ocular amphotericin B delivery by chitosan-modified nanostructured lipid carriers for fungal keratitis-targeted therapy. *Journal of liposome research*, 1-6.
36. Üstündağ-Okur, N., Gökçe, E. H., Bozbıyık, D. İ., Eğrilmez, S., Özer, Ö., & Ertan, G. (2014). Preparation and in vitro–in vivo evaluation of ofloxacin loaded ophthalmic nano structured lipid carriers modified with chitosan oligosaccharide lactate for the treatment of bacterial keratitis. *European Journal of Pharmaceutical Sciences*, *63*, 204-215.
37. Pandian, S., Jeevanesan, V., Ponnusamy, C., & Natesan, S. (2016). RES-loaded pegylated CS NPs: for efficient ocular delivery. *IET nanobiotechnology*, *11*(1), 32-39.
38. Natesan, S., Pandian, S., Ponnusamy, C., Palanichamy, R., Muthusamy, S., & Kandasamy, R. (2017). Co-encapsulated resveratrol and quercetin in chitosan and peg modified chitosan nanoparticles: For efficient intra ocular pressure reduction. *International Journal of Biological Macromolecules*.
39. Yostawonkul, J., Surassmo, S., Iempridee, T., Pimtong, W., Suktham, K., Sajomsang, W., ... & Ruktanonchai, U. R. (2017). Surface modification of nanostructure lipid carrier (NLC) by oleoyl-quaternized-chitosan as a mucoadhesive nanocarrier. *Colloids and Surfaces B: Biointerfaces*, *149*, 301-311.
40. Fangueiro, J. F., Andreani, T., Egea, M. A., Garcia, M. L., Souto, S. B., Silva, A. M., & Souto, E. B. (2014). Design of cationic lipid nanoparticles for ocular delivery: development, characterization and cytotoxicity. *International journal of pharmaceutics*, *461*(1), 64-73.
41. Battaglia, L., D'Addino, I., Peira, E., Trotta, M., & Gallarate, M. (2012). Solid lipid nanoparticles prepared by coacervation method as vehicles for ocular cyclosporine. *Journal of Drug Delivery Science and Technology*, *22*(2), 125-130.