

Invasive Modalities in Ocular Drug Delivery: Emphasis on the Posterior Segment

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Article History

Received: 31.08.2017

Accepted: 09.09.2017

Published: 30.09.2017

DOI:

10.21276/sb.2017.3.9.1



Abstract: Ocular drug delivery to the scientists is a fascinating field because of challenges and exquisite barriers encountered in the ocular milieu. Till now, non-invasive approaches in ocular delivery are not yet successful with respect to intervention/treatment of long term ocular diseases. In spite of adverse effects with invasive techniques, they have been demonstrated promise in the treatment of sight threatening complications. The use of intravitreal injections or intraocular implants is gaining momentum with paramount progress in design, safety and efficacy from last two decades. Various intravitreal injections and ocular implants were developed with a wide array of therapeutic application potentials targeting drug localization for an extended period of time. Miniaturization of implants for their direct injection eliminating surgical process is currently being explored. In this review, various modalities comprising these invasive techniques targeting posterior ocular tissues namely retina and vitreous humor are summarized.

Keywords: Invasive ocular delivery, Intravitreal (IVT) Injections, Intraocular implants, Vitreous humor, Retinal delivery, Posterior ocular complications

INTRODUCTION

The anatomy of the eye makes it a challenge to deliver therapeutic agents. The eye is resistant to xenobiotics due to the blood barriers – aqueous and retinal, which compartmentalizes the eye, along with the eye wall itself (cornea and sclera). In regard to

posterior segment ocular diseases, a common culprit in the progression of vision loss is the presence of retinal and/or choroidal neovascularization (CNV). The U.S FDA has since approved several anti-VEGF therapeutics, including pegaptanib, ranibizumab and aflibercept (along with the off label use of bevacizumab), to treat neovascular eye diseases, namely CNV secondary to age-related macular degeneration (AMD), diabetic macular edema (DME) and retinal vascular occlusions (RVO). However, all of these therapies involve treatment via intravitreal injections every month or two for a prolonged period of time. Advances in biomaterials and nanotechnology have led to major growth in research for treatments utilizing permeation-enhancing liposomes and emulsions, biodegradable micro- and nanoparticles, and thermoresponsive hydrogels, all of which may contain ocular pharmacologic agents thereby providing improved delivery of a variety of medications [1, 2]. Oral or systemic administration of therapeutic entities is not effective because of blood-aqueous (BAB) and blood-retinal barriers (BRB), limiting the passage of drugs into the eye from the systemic circulation [3]. Intravenous administration is effective to maintain the drug concentrations in the posterior tissues relatively at high doses but pose adverse effects and systemic toxicity. Intravitreal injection (i.e., direct injection of a drug into the vitreous body) is reported to be the most promising and unique method of delivering a drug to ocular posterior segments. Currently, periocular and intravitreal routes of administration serve as viable platforms for the delivery of drugs to posterior tissues [4-6]. Depending upon the ophthalmic disease state, complexity and origin delivery systems may vary from simple topical solutions to advanced formulations, such as intraocular implants, intravitreal injections [7-9]. In the following review, we present recent advances in ocular drug delivery, with a focus on the posterior segment, through invasive delivery modalities.

INVASIVE OCULAR TECHNIQUES

Recent technological advances in the field of biomedical engineering and ocular surgery fortified the insight of design and development of sustained-release drug delivery implants and intravitreal injections for the treatment of various clinical ophthalmic complications [10]. The implant devices can be implanted in subconjunctival, episcleral, intravitreal, intracameral regions. Biodegradable solid implants are fabricated using poly lactic acid (PLA), poly glycolic acid (PGA), and poly lactic-co-glycolic acid (PLGA) polycaprolactones (PCL) poly anhydrides which does not need post treatment surgical removal unlike non-biodegradable implants, but can cause erratic drug release profiles [11]. Kochinke et.al patented the fabrication of dexamethasone monolithic ocular implant using polyester of lactic and glycolic acid with hydroxypropyl methyl cellulose which could deliver drug for the period of 3 days. This implant can be inserted into various sites of the eye depending upon the ailment and condition to be treated [12]. Rahimy *et al.* conducted studies to

investigate the polysulfone capillary fiber (PCF) as drug delivery device for intraocular applications. Carboxyfluorescein (CF) was used as the model drug for these studies and the subsequent release kinetics of CF from the the PCF device was monitored *in vivo* in the rabbit eyes. PCF dye device was implanted in the vitreous cavity, and fluorophotometry from the retina to the anterior chamber was performed at various times up to 45 days to quantify fluorescein level. At the conclusion of the study, eyes were enucleated and examined for histopathology. The time-course study showed fluorescein level for up to 45 days in the vitreous and further histological examination of the eyes implanted with PCF or PCF-dye device showed no sign of ocular toxicity. Overall, these results may imply that the PCF device is biocompatible and may be useful for the extended release of drugs in the posterior segment of the eye [13]. Kim *et al.* attempted to deliver gadolinium-pentetic acid (Gd-DTPA) to the posterior segment by episcleral sustained release implant and the release rate of the episcleral implant was compared with intravitreal implant (*in vivo*). Episcleral implants delivered 2.7 µg in to the vitreous cavity comprising only 0.12% of the drug in the implant and there were no significant amounts of the drug in the posterior chamber. Intravitreal implants delivered the drug in to the vitreous humor and posterior segments of the eye. The concentration of the drug in the vitreous was 30 times higher (*ex vivo*) when compared to episcleral implant. Author hypothesized that three dimensional MRI and the data would be useful to study the ocular disposition mechanisms in the eye [14, 15]. In other study Kim YM *et.al* delivered the triamcinolone acetonide into posterior segment of the eye using the intrascleral implant. The implant was made of poly (D, L-lactide) comprising 6.4 mg of triamcinolone acetonide(TA) . Sustained release of triamcinolone acetonide for 90 days was observed in the *in vitro* studies and significant levels of TA in aqueous humor until 4 weeks were detected and in retina-choroid until 8 weeks after implantation, but in the vitreous cavity TA was found over 12 weeks. There were no signs of retinal detachment or toxicity in the *in vivo* studies [16]. Okaba *et.al* prepared and evaluated biodegradable scleral implant for the sustained release of steroid betamethasone phosphate to the posterior segments of the eye (*in vitro*). The implant with dimensions of 0.5 mm thick and 4mm in diameter made up of poly (DL-lactide) was inserted in to scleral pocket of the rabbit's eye. The drug levels in the posterior tissues like vitreous and retina-choroid (RC) was maintained constant for 8 weeks. The drug concentration in the RC was significantly greater than in the vitreous humor. Drug levels in the aqueous humor were below limit of detection. The implant exerted good compatibility in eye and there was no significant toxicity to the retina during the experimental studies [17]. Silvia *et.al* formulated dexamethasone intravitreal implants made up of polymer poly (ε-caprolactone) which is suitable for the long term sustained release drug delivery to the vitreous humor. Characteristics of the poly (ε-caprolactone) device, feasibility and intravitreal release of dexamethasone were extensively studied in this experiment. *In vitro* release of dexamethasone was determined and interaction between the drug and the polymer was evaluated by the differential scanning calorimetry. Poly (ε-caprolactone) device provided the sustained release of dexamethasone since it releases 25% of the drug loaded in 21 weeks. There were no significant changes during the experimental studies with morphology, toxicity, and other biological characteristics [18]. Carcabosa *et.al* attempted to deliver topotecan to the posterior segment of the eye for the treatment of intraocular retinoblastoma. Episcleral Implant was developed to control and sustain the delivery of topotecan in to the posterior segment. Implants released 30% to 50% of the drug within 48 hours and 45% to 70% by 10th day (*in vitro*). *In vivo*, topotecan lactone was highly accumulated in ocular tissues such as (sclera, choroid, retina) over 48 hours with all the formulations studied. Low vitreous topotecan lactone levels were detected with high drug load implants [19, 20]. Ganciclovir loaded biodegradable donut shaped minitabket was developed by choonara *et al.* for the treatment of human cytomegalovirus retinitis. Specialized tablet tooling equipment was used to manufacture the device composed of poly (lactic-co-glycolic acid). Device was implanted through parsplana/peripheral retina of rabbits and the left eyes were used as control. The minitabket was well tolerated up to 72 days in supratemporal quadrant of the eye. The device exhibited the control release of ganciclovir at the constant rate of 2.02 µg/hour throughout the experimental studies [21]. Iluvien is sustained release flucinolone acetonide formulation undergone phase 3 clinical trials for treatment of diabetic macular edema (DME). It's an injectable, non-erodible intravitreal implant for the treatment of DME. Iluvien is designed for sustained release of the formulation for over three years. Implant is injected into back-of-the eye using 25G needle creating self-healing hole which is very similar to intravitreal injection. Currently, the only FDA approved method for treating DME involves laser photocoagulation therapy which can leave irreversible blind spot. Retisert[®] is (flucinolone acetonide (FA) intravitreal implant) for treatment of chronic, noninfectious posterior uveitis. Retisert is surgically implanted into vitreous humor by 3-4 mm incision containing 0.59 mg of flucinolone acetonide which delivers the medicament up to 2.5 years. Retisert[®] implant is composed of a central core consisting of FA compressed into a 1.5 mm diameter. Durasert[™] technology system uses a drug core with one or more surrounding polymer layers, and delivers drugs for predetermined periods of time ranging from days to years [22]. Using the Durasert[™] system, an antiviral drug, ganciclovir (GCV)-loaded intravitreal implant has been developed for the treatment of AIDS related cytomegalovirus retinitis. The implant is made of EVA and PVA releases GCV locally to the site of infection for 6–8 months. Ozurdex[®] intravitreal implant contains 0.7 mg of Dexamethasone composed of Poly lactic-Glycolic acid (PLGA) for the treatment of chronic uveitis and macular edema. Moritera *et al.* formulated the microspheres of 5-fluorouracil (5-FU) with biodegradable polymers of lactic acid (PLA) or copolymers of polylactic-co-glycolic acid (PLGA). Poly (lactic acid) microspheres released 70-85% of total 5-FU over 7 days. The intravitreal kinetics of the microspheres was studied in ten rabbits *in vivo*. A suspension of

microspheres was injected into the vitreous cavity of five normal eyes and five vitrectomized eyes. Following 48 ± 5.2 days after injection, the microspheres disappeared from the vitreous cavity in the five normal eyes. Clearance from the vitreous cavity was accelerated in the five rabbits that underwent vitrectomy (14 ± 2.4 days; $P < 0.001$). This study suggests that microspheres as potential drug delivery systems to back of the eye [23]. Bourges *et al.* showed that an intravitreal injection of Poly lactide(PLA) nanoparticles resulted in trans-retinal movement, with a preferential localization in the retinal pigment epithelium (RPE). The presence of the nanoparticles within the RPE cells for 4 months after a single injection shows that a continuous and specific delivery of drugs can be achieved. Histology demonstrated anatomic integrity with no signs of toxicity [24]. Zhang *et al.* reported that Intravitreal injection of dexamethasone (DEX)-loaded poly (lactic acid-co-glycolic acid) nano particles sustained DEX concentrations for a long time in the posterior chambers thus can be used for the treatments of posterior segment diseases [25]. Nano particles prepared by using sialyl-Lewis X conjugated liposome as a site-directed delivery system containing dexamethasone showed selective targeting to the autoimmune uveo-retinitis [26]. Koirala *et al.* reported that Subretinal injections of rhodamine labeled nanoparticles using an RPE-specific reporter vector (VMD2-eGFP) can efficiently deliver genes to the retinal pigmental epithelium and thus can be employed in the retinal gene therapy [27, 28]. Albumin nanoparticles are an interesting delivery system for intravitreal drug administration that has shown controlled drug release and degradation to safe products. *In vivo* rat studies demonstrated their localization in the vitreous cavity and ciliary body for at least two weeks after a single intravitreal injection [29]. Ying *et al.* developed the submicron sized lipid emulsion for intraocular delivery using eye drops. In the study coumairn-6 was used as a model drug with fluorescent marker, and fluorescence was observed in the retina after administration of the lipid emulsion. The fluorescence intensity in the retina increased by surface modification using a positive charge inducer and the functional polymers chitosan (CS) and poloxamer 407. Surface-modified lipid emulsions serve as potential formulation for delivery of hydrophobic drugs to the ocular posterior segment [30]. Polyion complex micelle system was reported which incorporates a dendritic phtalocyanine photosensitizer, tested in rats for its efficacy in photodynamic therapy of choroidal neovascularization. The micellar system exhibited absorption at 650 nm, which is advantageous for the treatment of deep lesions. The formulation may prolong the retention in the blood circulation and achieve a selective accumulation in the choroidal neovascularized lesions, but these aspects require further development [31-33]. Cheng *et al.* attempted to deliver the antiviral drugs to posterior segment of the eye namely ganciclovir and cidofovir in the form of crystalline lipid prodrug hexadecyloxypropyl-phospho-ganciclovir (HDP-P-GCV) and hexadecyloxypropyl-cyclic cidofovir (HDP-cCDV). In this study these lipid prodrugs were administered into rabbit eyes and their vitreal kinetics were determined. Microfluidized particles of HDP-P-GCV showed an increased drug release rate compared with the large-particle drug formulation, with area under concentration-time curve (AUC) of 219.8 ± 114.1 (n=3) versus 108.3 ± 47.2 (n=3) for unmodified HDP-P-GCV during the 12-week period after a $2.8 \mu\text{mol}$ intravitreal injection. There was a 103% increase of the drug released from the micro fluidized formulation of HDP-P-GCV versus the unmodified formulation. Following $100 \mu\text{g}$ eye injections, vitreous HDP-cCDV levels were at $0.05 \mu\text{mol}$ at week 5, which declined to $0.002 \mu\text{mol}$ at week 8. The concentration at week 8 ($0.002 \mu\text{mol}$) remained above the IC50 for cytomegalovirus ($0.0003 \mu\text{mol}$). The pretreatment study demonstrated an antiviral effect that lasted 100 days after a single intravitreal injection [34]. Manuel Diaz *et al.* delivered ganciclovir (GCV) intravitreal for the treatment of retinitis by cytomegalovirus (CMV) in AIDS patients. In the study intravitreal application of liposomal-entrapped GCV kinetics were compared with the intravitreal injection of free GCV and the results suggest that of liposomal-encapsulated GCV showed no retinal toxicity, and therapeutic levels were detected up to 14 days after injection. Author concluded that intravitreal injection of liposomal-encapsulated GCV increases the time period required for reinjections in the treatment of CMV retinitis [35]. Zeng *et al.* formulated amikacin encapsulated liposomes for the delivery to vitreous body to treat bacterial endophthalmitis. The liposome-encapsulated amikacin was prepared by reverse-phase evaporation method and intravitreal kinetics of the liposomes was compared with amikacin in PBS by fluorescence polarization immunoassay. Results suggest that the liposome-encapsulated amikacin prolonged half-life of the drug in vitreous and pharmacokinetic analysis suggested that in endophthalmitis, especially in severe cases, the liposomes may be preferable to conventional preparation [36]. In one study Abrahamic *et al.* delivered bevacizumab loaded liposomes in to vitreous humor of the eye to treat ocular complications. Bevacizumab was encapsulated into liposomes via the dehydration-rehydration method. The free drug concentration in aqueous humor and vitreous samples at Days 3, 7, 14, 28, and 42 after the injection was determined by enzyme-linked immunosorbent assay. Mean concentration of free bevacizumab in the eyes that received liposomal bevacizumab compared with the eyes injected with soluble bevacizumab was 1 and 5 times higher at days 28 and 42, respectively. The results suggest that liposomal formulations can employed to prolong the residence time of bevacizumab in the vitreous body [37, 38]. Fishman *et al.* studied the effect of liposomal encapsulation on the pharmacokinetics of gentamicin, after injection in rabbits. Intravitreal injection of 100 mg liposome-encapsulated gentamicin or 100 mg gentamicin in 0.1 mL of phosphate-buffered saline was administered to each rabbit. The peak free drug concentration in the vitreous was significantly greater for liposome-encapsulated gentamicin than for gentamicin at 24, 72, 120, and 192 hours respectively. The areas under the drug concentration-time curve for the total drug and for the free drug in the case of liposome-encapsulated gentamicin were two fold and 1.5-fold higher, respectively, than those for gentamicin [39, 40].

Carmen *et al.* developed liposomal formulation of foscarnet for the treatment of Cytomegalovirus retinitis. Foscarnet inhibits replication of herpes viruses, including CMV. Liposomes were prepared by reverse-phase evaporation method and pharmacokinetic parameters in vitreous humor were evaluated. Results suggested that liposomal formulation achieves stable and durable therapeutic levels in retina for 72 hours reaching the vitreous humor with adequate levels to accomplish the aims of intravitreal therapy [41]. Coco *et al.* determined the pharmacokinetics governing the distribution and elimination of intravitreal injected vancomycin in normal and infected rabbit eyes. The half-lives were 69 hours in normal vitreous and 14.53 hours in infected vitreous. Therapeutic drug levels were present in the vitreous 84 hours post-injection in all eyes; they were detected from 2 to 48 hours in normal vitreous but at lower levels in the infected ones [42]. In one study Kawakami attempted to deliver O-palmitoyl prodrug of tilisolol-encapsulated liposome to improve the retention time of tilisolol in the peroneal area and vitreous body. The liposomes were administered topically, as well as intravitreal to the rabbit eye. Following topical administration, very low retention of O-palmitoyl tilisolol in the tear fluid was observed even when it was applied as liposomal formulation. The researchers significantly increased the retention property of liposomes by adding 2% of Carmel lose sodium which acted as a reservoir for liposomes. In case of intravitreal administration, o-palmitoyl tilisolol-encapsulated liposomes achieved higher drug concentration in the vitreous body compared to free tilisolol [43]. In a study tacrolimus encapsulated liposomes were formulated and subsequently evaluated for efficacy and safety following intravitreal injection in rats. The vesicles were prepared by reverse phase evaporation technique. Significant changes in the retinal function were not observed in the liposome-treated rats. Histo-pathological examination revealed reduced inflammatory response in comparison to free drug. Liposomes were able to maintain the vitreous concentration more than 50ng/mL for 2 weeks after single administration. Investigators concluded that tacrolimus-loaded liposomes were more effective in the treatment of uveoretinitis [44]. Gupta *et al.* attempted to deliver fluconazole-encapsulated liposomes to vitreous body of rabbit eyes. Entrapment of fluconazole into liposomal cavity significantly reduced clearance of free fluconazole after intravitreal injection with higher fluconazole concentration in the vitreous. The liposomes showed longer half-life (23.40h) in comparison to free fluconazole (3.08h) [45].

CONCLUSION

Ocular drug delivery to the posterior segment is elusive due to the nature of the blood ocular barriers. Delivery modalities in this review have their own advantages and disadvantages. Penetration can be enhanced with periocular depot injections of micro particulate encapsulated drugs, the use of colloidal carriers or via transcleral iontophoresis. These techniques are less invasive than current conventional intravitreal injections; however, thus far they are not as effective as delivery via intravitreal injections or implants. While the challenges pose to be significant, the data presented hold promise for new models in dosing posterior segment drugs. Novel technologies needs to be primarily designed and to be developed to provide sustained action, enhance bioavailability, improved patient safety and minimal adverse effects. In addition, intravitreal injection and implants are to be significantly developed to minimize adverse effects and accommodate the increasing number of patients requiring long term progressing treatments. Advancements in fields of nanotechnology and clinical science could help explore new avenues for drug delivery to the ocular posterior segment in the near future.

REFERENCES

1. Le Broulais, C., Acar, L., Zia, H., Sado, P. A., Needham, T., & Leverage, R. (1998). Ophthalmic drug delivery systems—recent advances. *Progress in retinal and eye research*, 17(1), 33-58.
2. 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.
3. Cunha-Vaz J. The blood-ocular barriers. *Survey of ophthalmology*. 1979 Mar 1;23(5):279-96.
4. Duvvuri, S., Majumdar, S., & Mitra, A. K. (2003). Drug delivery to the retina: challenges and opportunities. *Expert opinion on biological therapy*, 3(1), 45-56.
5. Saha, P., Uchiyama, T., Kim, K. J., & Lee, V. H. (1996). Permeability characteristics of primary cultured rabbit conjunctival epithelial cells to low molecular weight drugs. *Current eye research*, 15(12), 1170-1174.
6. 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.
7. Hsu, J. (2007). Drug delivery methods for posterior segment disease. *Current opinion in ophthalmology*, 18(3), 235-239.
8. Del Amo, E. M., & Urtti, A. (2008). Current and future ophthalmic drug delivery systems: a shift to the posterior segment. *Drug discovery today*, 13(3), 135-143.
9. 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.

10. 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.
11. Miller, R. A., Brady, J. M., & Cutright, D. E. (1977). Degradation rates of oral resorbable implants (polylactates and polyglycolates): rate modification with changes in PLA/PGA copolymer ratios. *Journal of Biomedical Materials Research Part A*, 11(5), 711-719.
12. Wong, V., & Kochinke, F. (1999). *U.S. Patent No. 5,869,079*. Washington, DC: U.S. Patent and Trademark Office.
13. Smith, T. J., Pearson, P. A., Blandford, D. L., Brown, J. D., Goins, K. A., Hollins, J. L., ... & Ashton, P. (1992). Intravitreal sustained-release ganciclovir. *Archives of ophthalmology*, 110(2), 255-258.
14. Kim, H., Robinson, M. R., Lizak, M. J., Tansey, G., Lutz, R. J., Yuan, P., ... & Csaky, K. G. (2004). Controlled drug release from an ocular implant: an evaluation using dynamic three-dimensional magnetic resonance imaging. *Investigative ophthalmology & visual science*, 45(8), 2722-2731.
15. 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).
16. Kim, Y. M., Lim, J. O., Kim, H. K., Kim, S. Y., & Shin, J. P. (2008). A novel design of one-side coated biodegradable intrascleral implant for the sustained release of triamcinolone acetonide. *European Journal of Pharmaceutics and Biopharmaceutics*, 70(1), 179-186.
17. Okabe, J., Kimura, H., Kunou, N., Okabe, K., Kato, A., & Ogura, Y. (2003). Biodegradable intrascleral implant for sustained intraocular delivery of betamethasone phosphate. *Investigative ophthalmology & visual science*, 44(2), 740-744.
18. Fialho, S. L., Behar-Cohen, F., & Silva-Cunha, A. (2008). Dexamethasone-loaded poly (ϵ -caprolactone) intravitreal implants: A pilot study. *European Journal of Pharmaceutics and Biopharmaceutics*, 68(3), 637-646.
19. Carcaboso, A. M., Chiappetta, D. A., Opezzo, J. A., Höcht, C., Fandino, A. C., Croxatto, J. O., ... & Chantada, G. L. (2010). Episcleral implants for topotecan delivery to the posterior segment of the eye. *Investigative ophthalmology & visual science*, 51(4), 2126-2134.
20. 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.
21. Choonara, Y. E., Pillay, V., Carmichael, T. R., Meyer, L. C., Du Toit, L. C., Naylor, S., & Wanblad, C. (2011). In vivo evaluation of a biodegradable donut-shaped minitablet for prolonged posterior segment drug delivery in the rabbit eye model. *Journal of pharmaceutical sciences*, 100(5), 1819-1832.
22. Wesoly, M., Kluk, A., Sznitowska, M., Ciosek, P., & Wróblewski, W. (2016). Influence of Experimental Conditions on Electronic Tongue Results—Case of Valsartan Minitablets Dissolution. *Sensors*, 16(9), 1353.
23. Moritera, T., Ogura, Y., Honda, Y., Wada, R., Hyon, S. H., & Ikada, Y. (1991). Microspheres of biodegradable polymers as a drug-delivery system in the vitreous. *Investigative ophthalmology & visual science*, 32(6), 1785-1790.
24. Bourges, J. L., Gautier, S. E., Delie, F., Bejjani, R. A., Jeanny, J. C., Gurny, R., ... & Behar-Cohen, F. F. (2003). Ocular drug delivery targeting the retina and retinal pigment epithelium using polylactide nanoparticles. *Investigative ophthalmology & visual science*, 44(8), 3562-3569.
25. Zhang, J., Guan, P., Wang, T., Chang, D., Jiang, T., & Wang, S. (2009). Freeze-dried liposomes as potential carriers for ocular administration of cytochrome c against selenite cataract formation. *Journal of Pharmacy and Pharmacology*, 61(9), 1171-1178.
26. Hashida, N., Ohguro, N., Yamazaki, N., Arakawa, Y., Oiki, E., Mashimo, H., ... & Tano, Y. (2008). High-efficacy site-directed drug delivery system using sialyl-Lewis X conjugated liposome. *Experimental eye research*, 86(1), 138-149.
27. Koirala, A., Makkia, R. S., Cooper, M. J., & Naash, M. I. (2011). Nanoparticle-mediated gene transfer specific to retinal pigment epithelial cells. *Biomaterials*, 32(35), 9483-9493.
28. 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.
29. Irache, J. M., Merodio, M., Arnedo, A., Camapanero, M. A., Mirshahi, M., & Espuelas, S. (2005). Albumin nanoparticles for the intravitreal delivery of anticytomegaloviral drugs. *Mini reviews in medicinal chemistry*, 5(3), 293-305.
30. Ying, L., Tahara, K., & Takeuchi, H. (2013). Drug delivery to the ocular posterior segment using lipid emulsion via eye drop administration: effect of emulsion formulations and surface modification. *International journal of pharmaceutics*, 453(2), 329-335.
31. Jang, W. D., Nakagishi, Y., Nishiyama, N., Kawauchi, S., Morimoto, Y., Kikuchi, M., & Kataoka, K. (2006). Polyion complex micelles for photodynamic therapy: incorporation of dendritic photosensitizer excitable at long wavelength relevant to improved tissue-penetrating property. *Journal of Controlled Release*, 113(1), 73-79.

32. Usui, T., Sugisaki, K., Amano, S., Jang, W. D., Nishiyama, N., & Kataoka, K. (2005). New drug delivery for corneal neovascularization using polyion complex micelles. *Cornea*, 24(8), S39-S42.
33. Ideta, R., Tasaka, F., Jang, W. D., Nishiyama, N., Zhang, G. D., Harada, A., ... & Kataoka, K. (2005). Nanotechnology-based photodynamic therapy for neovascular disease using a supramolecular nanocarrier loaded with a dendritic photosensitizer. *Nano letters*, 5(12), 2426-2431.
34. Cheng, L., Hostetler, K. Y., Lee, J., Koh, H. J., Beadle, J. R., Bessho, K., ... & Freeman, W. R. (2004). Characterization of a novel intraocular drug-delivery system using crystalline lipid antiviral prodrugs of ganciclovir and cyclic cidofovir. *Investigative ophthalmology & visual science*, 45(11), 4138-4144.
35. Díaz-Llopis, M., Martos, M. J., España, E., Cervera, M., Vila, A. O., Navea, A., ... & Romero, F. J. (1992). Liposomally-entrapped ganciclovir for the treatment of cytomegalovirus retinitis in AIDS patients. *Documenta ophthalmologica*, 82(4), 297-305.
36. Zeng, S., Hu, C., Wei, H., Lu, Y., Zhang, Y., Yang, J., ... & Song, B. (1993). Intravitreal pharmacokinetics of liposome-encapsulated amikacin in a rabbit model. *Ophthalmology*, 100(11), 1640-1644.
37. Abrishami, M., Ganavati, S. Z., Soroush, D., Rouhbakhsh, M., Jaafari, M. R., & Malaekeh-Nikouei, B. (2009). Preparation, characterization, and in vivo evaluation of nanoliposomes-encapsulated bevacizumab (avastin) for intravitreal administration. *Retina*, 29(5), 699-703.
38. 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.
39. Fishman, P. H., Peyman, G. A., & Lesar, T. (1986). Intravitreal liposome-encapsulated gentamicin in a rabbit model. Prolonged therapeutic levels. *Investigative ophthalmology & visual science*, 27(7), 1103-1106.
40. 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*.
41. Claro, C., Ruiz, R., Cordero, E., Pastor, M. T., López-Cortés, L. F., Jiménez-Castellanos, M. R., & Lucero, M. J. (2009). Determination and pharmacokinetic profile of liposomal foscarnet in rabbit ocular tissues after intravitreal administration. *Experimental eye research*, 88(3), 528-534.
42. Coco, R.M., López, M.I., Pastor, J.C., & Nozal, M.J. (1998). Pharmacokinetics of intravitreal vancomycin in normal and infected rabbit eyes. *Journal of ocular pharmacology and therapeutics*, 14(6), 555-563.
43. Kawakami, S., Yamamura, K., Mukai, T., Nishida, K., Nakamura, J., Sakaeda, T., ... & Sasaki, H. (2001). Sustained ocular delivery of tilisolol to rabbits after topical administration or intravitreal injection of lipophilic prodrug incorporated in liposomes. *Journal of Pharmacy and Pharmacology*, 53(8), 1157-1161.
44. Zhang, R., He, R., Qian, J., Guo, J., Xue, K., & Yuan, Y. F. (2010). Treatment of experimental autoimmune uveoretinitis with intravitreal injection of tacrolimus (FK506) encapsulated in liposomes. *Investigative ophthalmology & visual science*, 51(7), 3575-3582.
45. Gupta, S. K., Velpandian, T., Dhingra, N., & Jaiswal, J. (2000). Intravitreal pharmacokinetics of plain and liposome-entrapped fluconazole in rabbit eyes. *Journal of ocular pharmacology and therapeutics*, 16(6), 511-518.