**Oil-in-water nanoemulsions for glaucoma treatment: An insight into the ‎latest trends**

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**Table S1:** Safety concerns of ophthalmic nanoemulsions

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| **S. No.** | **Safety Issues** | **Considerations** | | **References** |
| 1. | Eye Irritation | **Surfactant effect:** Surfactants are frequently used in nanoemulsions to stabilize the formulation. To prevent eye irritation, the concentration and type of surfactants should be properly selected. | | 1 |
| 2. | Toxicity of the Cornea and Conjunctiva | **Cellular damage:** This can be checked by studying the interaction between nanoemulsion, conjunctival cells, and corneal cells. | | 2 |
| 3. | Stability of the Tear Film | Nanoemulsions may affect the consistency of the tear film, causing dryness or discomfort. The droplet size, surface charge on droplets, and constitution of nanoemulsions can all have an impact on this. | | 3 |
| 4. | Bio-distribution & Elimination | *Systemic Absorption* | Determine the extent to which nanoemulsion components are absorbed by the eyes. Understanding biodistribution is critical for assessing potential systemic effects. | 4 |
| *Clearance Mechanisms* | Examine the pathways by which nanoemulsions are cleared from the ocular surface to ensure that they are not accumulating in the eye or adjacent tissues. | 4 |
| 5. | Disturbing Vision | Eye vision or visual acuity should not be affected by nanoemulsions. It should be described in such a way that it should not affect or minimally affect normal sight. | | 5 |
| 6. | IOP (intraocular pressure) | Glaucoma Risk: Assessment of nanoemulsion impact on IOP is necessary, as it can change IOP, which may be linked to glaucoma or other ocular conditions. | | 6 |
| 7. | Contact Lens Compatibility | *Lens Material Interaction* | Compatibility must be checked between nanoemulsions and lens materials because many people wear them daily. The formulation should not deteriorate or irritate the lens materials. | 6 |
| 8. | Reactions to Allergens | *Sensitization and allergenicity* | Assess the possibility of allergic reactions or sensitization to nanoemulsion components such as surfactants or other excipients. | 7 |
| 9. | Long-Term Application | Chronic exposure: Evaluate the safety of using ophthalmic nanoemulsions over an extended period. Research over a long period should be conducted to assess potential cumulative effects. | | 8 |
| 10. | Compliance with regulations | Regulatory criteria: Ensure adherence to the ophthalmic product regulatory criteria. Appropriate records and safety facts are required for regulatory approval. | | 9 |
| 11. | Microbial contamination and sterility | *Microbial safety* | Because eyes are susceptible to infections, guaranteeing the sterility of ophthalmic nanoemulsions is crucial for preventing microbial contamination. A complete preclinical study comprising *in vitro* and *in vivo* investigations, and clinical trials, is needed to address these safety concerns. To guarantee the safety of ophthalmic nanoemulsions, researchers should follow regulatory requirements and screen for any negative outcomes during the research and post-marketing phases. | 10 |

**Table S2:** Safety concerns of ophthalmic nanoemulsions

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| **S. No.** | **Toxicity assessments** | **Considerations** | | **Reference** |
| 1. | *In vitro* studies | *In vitro*, experiments employing ocular cell lines should be conducted to determine the influence of nanoemulsions on the viability of cells and their morphological and potential cytotoxic effects.  Inflammatory reactions: Assessment of inflammatory reactions in ocular cell cultures by monitoring the secretion of proinflammatory cytokines and other indicators. | | 11 |
| 2. | Ocular irritation investigation | Corneal and conjunctival irritation | Use *of in vitro* models to assess the possibility of corneal and conjunctival irritation. Changes in the longevity of cells, morphology, and responses to inflammation should be evaluated. | 12 |
| 3D tissue models | Three-dimensional tissue models can be used to better replicate the complex structure of ocular tissues and evaluate the impact of nanoemulsions. | 13 |
| 3. | *Ex vivo* Research | *Ocular tissue permeation* | *Ex vivo* models, such as isolated corneas or eye tissue, can be used to explore the penetration and distribution of nanoemulsions within ocular tissues. | 14 |
| *Tissue Compatibility* | Determine whether nanoemulsions are compatible with ocular tissues such as the cornea, sclera, and conjunctiva. | 14 |
| 4. | *In vivo* studies | Using animal models, *in vivo* studies evaluate the ocular tolerability, bio-distribution, and systemic absorption of nanoemulsions, analyze indications of irritation, redness, and discharge, and determine possible absorption potential. | | 15 |
| 5. | Studies on repeat dose and chronic toxicity | Repeat-dose toxicity studies investigate the effects of extended ophthalmic nanoemulsion exposure, whereas organ histopathology examines alterations in ocular tissues after repeated delivery. | | 16 |

**Table S3:** Research done since 2017, including nanoemulsion or nanoemulsion-based *Insitu* gels

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| **S. No.** | **API & Year of research** | **Surfactants Used** | **Method of Preparation** | **Objective of Study** | **In-Vitro In-Vivo Studies** | **Results** | **References** |
| 1 | Betaxolol Hydrochloride | Pluronic F127, Span 60, Tween 20. | Modified two-step emulsification method aided by sonication method. | To formulate reverse micelle-based double nanoemulsion (W/O/W) loaded by Betaxolol Hydrochloride for improving corneal permeability and IOP reducing effect of this drug. | * *In-vitro* drug release * *Ex-vivo* permeation * *in-vivo* performance | * optimized formulation shows sustained release behaviour for up to 7 hours. * Non-irritating, compatible and safe formulation. * 2.5 times Higher ocular permeation and significant ocular pressure decrement as compared to marketed eye drops. | 17 |
| 2 | Wild olive oil (Acebuche) (2023) | Tween 80 | Solvent displacement method | To develop a wild olive (Acebuche) oil-based nanoemulsion, for ocular application as a local treatment to combat hypertension-induced oxidative damage. | * *In vivo* | * formulation shows promise for treating ocular diseases linked to arterial hypertension. | 18 |
| 3 | Dorzolamide hydrochloride  (cationic nanoemulsion) (2022) | Isopropyl myristate, Tween 80, cetyl trimethyl ammonium bromide | High-speed homogenization followed by ultrasonication. | To synthesize cationic nanoemulsions (cnes) containing dorzolamide hcl (DRZ) for improved ocular drug delivery, by using the Box-Behnken design | * *In vitro* * *in vivo* | **In-vitro:**   * compared to a normal dorzolamide solution, the formulation exhibited a prolonged release pattern governed by a non-Fickian diffusion mechanism. * Dilution-resistant, thermodynamically stable, and stable for one month following preservation at ambient (25 °C) and refrigerated (4 °C).   **In-vivo:**   * When compared to ordinary DRZ and commercialized DRZ eye drops, the extended reducing impact of intraocular pressure (IOP) was observed. | 19 |
| 4 | Timolol maleate  (Nanoemulsion insitu gel) (2022) | Tween 80 | Sonication Method | To increase the corneal permeability and bioavailability | * *In vitro* * *In vivo* | **In-vitro:**   * In situ nanoemulsion gel dynamics were zero-order. * The release mechanism was non-Fickian diffusion.   **In-vivo:**   * compared to Timolet OD eye drops, the nanoemulsion in situ gel offered a more prolonged release of the medication. | 20 |
| 5 | Latanoprost  (Nanoemulsion) (2022) | One ophthalmic solution containing benzalkonium chloride,  One nanoemulsion containing a soft preservative (potassium sorbate) |  | To assess the cytotoxicity of this formulation and compare it to a BAK-containing ophthalmic solution | * Cytotoxicity test by MTT assay | * nanoemulsion containing soft preservative less cytotoxic on human ocular cells. | 21 |
| 6 | Brinzolamide (BZ)  (Insitu gel Nanoemulsion) (2021) | Tween 80 as a surfactant, and Transcutol®P as a cosurfactant | Spontaneous emulsification | To assess the transcorneal-permeation of nanoemulsions as compared to already available suspension | * *In vitro* * *ex vivo* Evaluation | * When compared to the marketed BZ suspension, NEs penetrated the isolated bovine cornea better. * HET-CAM and BCOP tests showed that nanoemulsion caused no discomfort and was thus deemed safe for ocular use. | 22 |
| 7 | Latanoprost (2021) | --------- | ----------- | To determine the efficacy and acceptability of a novel latanoprost 0.005%  nanoemulsion that is devoid of benzalkonium chloride (BAK). | * Ocular surface damage * Ocular Surface Disease Index (OSDI) * conjunctival hyperemia * Schirmer I test * tear film break-up time (BUT) * corneal epithelial fluorescein staining, and * tear meniscus height | * The novel nanoemulsion maintains the reduced intraocular pressure impact while improving symptoms and signs of ocular surface disease significantly. | 23 |
| 8 | Δ9 ‑tetrahydrocannabinol‑ valine‑hemisuccinate (NB1111)  (Nanoemulsion) (2021) | Tween 80, Poloxamer 188 | Homogenization followed by ultrasonication | To evaluate the effect of concentration of surfactant and sterilization on the intraocular pressure-lowering activity of Δ9 ‑tetrahydrocannabinol‑ valine‑hemisuccinate (NB1111) | * *In-vivo* | * In the test model, the formulation displayed a considerably superior IOP reduction profile than the marketed ophthalmic solutions examined. | 24 |
| 9 | Brinzolamide  (In situ gelling ophthalmic nanoemulsion) (2020) | Polyoxyl 35 and polysorbate 80 | High-speed high-pressure homogenization technique | To develop an in situ gelling ocular nanoemulsions of brinzolamide for glaucoma treatment that provides sustained release and a longer therapeutic effect | * *In vivo* | * The nanoemulsion formulations tested were very well tolerated and significantly reduced IOP compared to saline and placebo controls (p <0.005). | 25 |
| 10 | Travoprost  (Nanoemulsion) (2020) | Tween 80 | Emulsion inversion point (EIP) low energy method | To enhance the absorption and bioavailability of the drug and its comparison with marketed preparation. | * *In vivo* | * *In vivo*, studies revealed that travoprost nanoemulsion absorbs better than the commercially available eye drops Travatan®, as evidenced by the former's higher Cmax and AUC and longer time to reduce intraocular pressure. | 26 |
| 11 | Brinzolamide  (in situ gel nanoemulsion) (2020) | Transcutol and tween 80, pluronic-407 & poloxamer-188 |  | The possibilities for eye damage and therapeutic efficacy were investigated. | * In-vitro MTT test * ocular toxicity potential | * MTT, HET-CAM, and Draize results show that two final selected formulations of in situ gel nanoemulsion containing brinzolamide have no toxic or irritant effects on ocular tissue. Furthermore, when compared to suspension, the developed formulations have a better rate of IOP reduction. | 27 |
| 12 | Brinzolamide  (Nanoemulsion) (2019) | Four non-ionic surfactants (Brij 35, Labrasol, Tyloxapol, and Cremophor RH40) and Transcutol P as a co-surfactant | Spontaneous emulsification method | To study the ocular penetration of brinzolamide nanoemulsions and assessment of their *in vitro* and *Ex vivo* irritancy potential | * *Ex vivo* transcorneal permeation * cell viability * ocular irritation tests | * Seven brinzolamide nanoemulsions excelled over the marketed brinzolamide suspension in terms of diffusion across the isolated bovine cornea. * Transcutol P, triacetin cremophor RH40, and were found to be the least harmful excipients in a cell viability assay on the cells of the retina. | 28 |
| 13 | Brimonidine tartrate  (nanoemulsion) (2018) | Pluronic f-68 | The modified high-shear homogenization method | 1. To increase the permeability of the drug through the ocular barrier  2. A quicker commencement of the action and a more therapeutic impact | * *In vitro* | * Brimonidine tartrate nanoemulsion was effectively made utilizing castor oil, Lipoid S75, Lipoid E80, and PF68 in a 33-factorial design. | 29 |
| 14 | Acetazolamide (Nanoemulsion-based electrolyte triggered in situ gel) (2017) | Tween 80 and/or cremophor EL and Transcutol P as a co-surfactant | Sonication & Homogenization | Formulation and development of Nanoemulsion-based electrolyte triggered in situ gel for ocular delivery of acetazolamide and its comparison to available marketed formulation | * *In-vitro* release * eye irritation test | * Various acetazolamide-loaded nanoemulsion formulations were developed by combining peanut oil, tween 80, and/or cremophor EL as surfactants with transcutol P or propylene glycol as cosurfactants. * In comparison to the nanoemulsion, the in situ gels based on nanoemulsion demonstrated much longer drug release. | 30 |

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