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Nanoemulsions for the ocular delivery of anti-infectives: challenges, advances and prospects in treating infectious eye diseases

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Infectious diseases constitute a significant health burden globally, especially with the widespread emergence of antimicrobial resistance. Infectious eye diseases, usually characterised by inflammation, redness of the eye, ocular tissue destruction and vision impairment, may be caused by bacteria, viruses, fungi or parasites. Early diagnosis and effective treatments are necessary to prevent severe visual impairment and blindness in patients. The conventional treatment strategy is using topically or systemically administered antimicrobial agents alone or in combination with steroidal agents. However, the bioavailability of drugs administered topically or systemically is very low due to the barriers presented by the anatomy and physiology of the eye, which militate against the penetration of drugs into intraocular tissues. Local injections directly into intraocular tissues improve the bioavailability of drugs in the eye. Nonetheless, this approach may lead to ocular tissue damage and secondary infections. Nanoemulsions have shown prospects as an effective strategy to overcome the drawbacks of conventional ocular delivery systems. This review discusses various infectious eye diseases and the challenges encountered in their treatments. The formulation techniques and recent studies on the use of nanoemulsions in the treatment of infectious eye diseases are also discussed. Finally, the challenges and recent technological advancements in nanoemulsion design are highlighted. Nanoemulsions, as novel nanomedicines, have a lot of potential in ocular drug delivery, offering advantages such as increased solubility, enhanced residence time on the ocular surface, and improved penetration through the ocular barriers. Nanoemulsions show great potential in treating infectious eye diseases and reducing the incidence of avoidable vision impairment and blindness.

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1. Introduction

The introduction of infectious organisms into the eyes exogenously (after trauma or surgery) or endogenously (*via* hematogenous spread of microbes from an infected part) may lead to eye diseases. Based on the anatomical location, eye diseases include blepharitis, dacryocystitis, conjunctivitis, keratitis, uveitis, endophthalmitis and retinitis (Fig. 1). Most eye infections occur after an event that disrupts the protective epithelial barrier of eyes, allowing microbial invasion. The predominant risk factors include wearing contact lens, trauma, surgery, and chronic ocular diseases.[1,2] In response to infection and injury, the body initiates intracellular signalling pathways that stimulate responses to heal the affected tissues. These signals produce inflammatory markers and cells that induce inflammation. The immune cells release reactive oxygen species, leading to oxidative stress. Thus, a combination of anti-inflammatory agents, antioxidants, and anti-infectives is used to manage infections.

Ocular infections range from mild self-limiting diseases to severe vision-threatening infections that lead to blindness and/or enucleation. Eye infections are generally characterised by itching, redness, inflammation, ocular discharge, photosensitivity, tearing and vision impairment. Conjunctivitis, keratitis and endophthalmitis are the most common infections of the eye. Trachoma and corneal scarring (mainly caused by eye infections) are among the major causes of blindness globally.[3] Microbial eye diseases are commonly treated with topical and systemic antimicrobial agents. However, the traditional drug delivery systems have failed to combat these diseases due to

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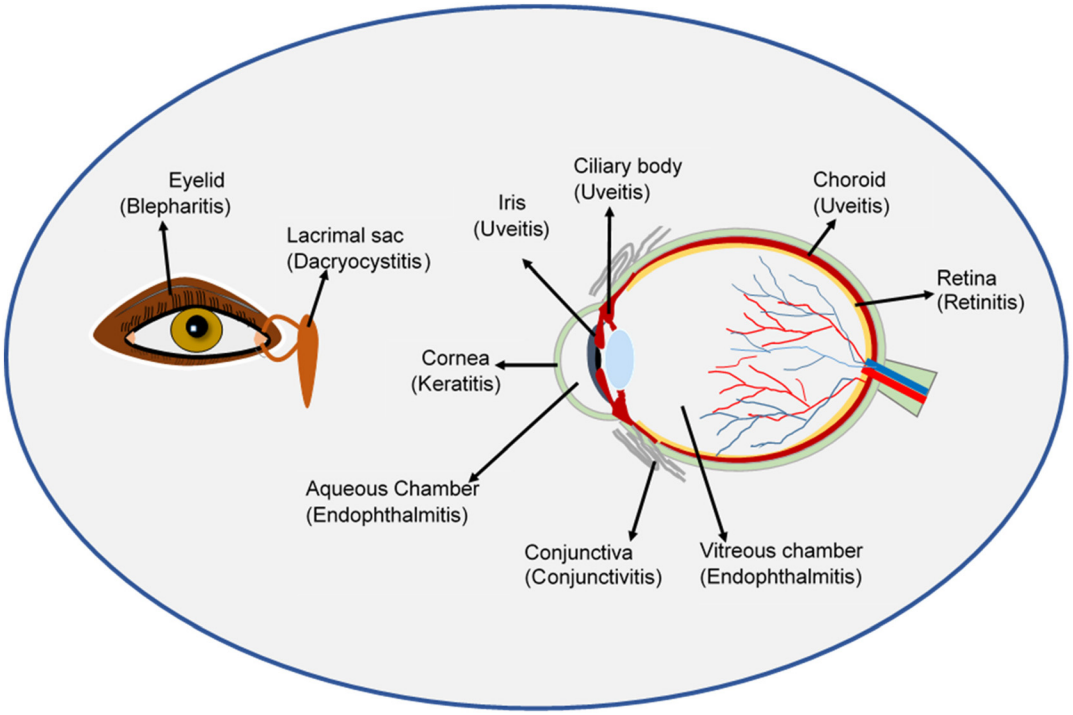

Fig. 1 Infectious eye diseases based on the anatomical location.

their low ocular residence time, poor penetration, low bioavailability, development of resistance, adverse reactions, poor drug targeting at the infection site, and low patient adherence due to drug-related toxicities and extended therapeutic regimens.[4] Therefore, new therapies for these diseases are needed to reduce the incidence of vision impairment, blindness, and loss of eye and to improve the quality of life. Thus, finding an effective drug delivery system for ocular drug delivery is an area of research interest. Stem cell therapy, bacteriophage therapy, photodynamic therapy (PDT) and nanomedicine have been investigated as alternatives to traditional therapy. Stem cell therapy is a promising strategy for treating infectious diseases due to its self-renewal and regenerative attributes.[5] In ophthalmology, stem cell therapy helps repair tissue damage and inflammation associated with infections by regulating the immune response and hastening tissue renewal. It is an attractive strategy in ocular infectious disease management to replace damaged ocular tissues and stop inflammation. However, its limitations such as cost and ethical issues need to be addressed. Bacteriophage (phage) therapy is another area that is growing as an effective alternative to treating infections. Phages are viruses that infect and replicate within bacterial cells. They possess bactericidal and anti-inflammatory activities that are beneficial in infectious disease management. Their lytic effect stems from their ability to attach to specific receptors on the host cells, disrupting the cell structure and function, which results in cell death. They reduce the generation of reactive oxygen species by phagocytes, suppress the NF-κB signalling pathway and inhibit inflammatory cell recruitment.[6,7] However, although the superiority of bacteriophage therapy in terms of higher specificity and relatively low possibility of developing resistance is well established, it presents poor stability, low *in vivo* retention, limited host range, possibility of resistance and interaction with the host immune system.[8] Photodynamic therapy is a treatment approach that combines light and a chemical photosensitiser. The photosensitiser becomes cytotoxic when activated by light in the presence of oxygen molecules in the tissue.[9] PDT has been researched for many decades and has shown potential for treating several malignancies and infectious diseases, including ocular diseases.[10–12] Compared with conventional antibiotics, PDT rapidly kills microbes relatively selectively and has a reduced tendency to develop resistance.[13] Several non-toxic photosensitisers have been in clinical use, while some are under clinical trials, suggesting their potential use as an alternative antimicrobial platform.[14,15] However, the choice of suitable photosensitizers, poor light penetration into tissues, and side effects limit the use of PDT[16]

Nanosystems have been the subject of intensive research over the past few decades, resulting in the approval of many therapeutic and diagnostic agents.[17,18] The use of nanomedicines for eye infections is a promising approach due to their high corneal penetration and prolonged ocular residence.[19–22] Encapsulation of drugs in nanocarriers allows drug targeting at the infection sites and sustained drug delivery, thereby reducing the dosing frequency and increasing patient compliance. Nanocarriers can be engineered to release drugs in the presence of certain triggers and provide temporal control of drug exposure. Nanocarrier surfaces can also be modified with sugars, polymers, peptides, enzymes, and other ligands that

bind to infected tissues or microbes to improve targeting.[23,24] Due to the enhanced nanocarrier permeability at infection sites compared with uninfected tissue, enhanced drug uptake, on-target drug accumulation and reduced off-target toxicity are achieved. Finally, the entrapment of drugs in nanocarriers may protect susceptible medicines (such as proteins) under harsh conditions such as low pH and the presence of enzymes.

Nanoemulsions are colloidal liquid-in-liquid dispersions stabilised by emulsifying agents with droplet sizes in the range of 20 nm to 500 nm. A typical nanoemulsion is composed of oil, water and an emulsifier. Adding emulsifiers is crucial for forming tiny nanosized droplets and achieving nanoemulsion stability through repulsive electrostatic interactions and steric hindrance. Hence, they are kinetically stable systems. Microemulsions, unlike nanoemulsions, are thermodynamically and kinetically stable systems which form spontaneously and exhibit lower particle sizes up to 100 nm.[25] Both nanoemulsions and microemulsions are isotropic dispersions of two immiscible liquids with a high potential to increase the permeation of drugs through the eye. Due to their small droplet size, nanoemulsions have a large surface area and are optically transparent with tunable rheology and stability. They are excellent options for delivering hydrophilic and lipophilic drugs as eye drops, improving the solubility and stability of the entrapped drug, enhancing the corneal permeability, increasing the retention time of the drug, and as a result, require less frequent administration.[26] Interestingly, with the application of nano-/micro-emulsions as eye drops, the side effects caused by systemically administered drugs can be significantly alleviated. Nano-/micro-emulsions can be modified to improve their pharmacokinetic profiles by enhancing mucoadhesion and penetration. Generally, *in situ* gelling systems or cationic nanoemulsions are the most interesting modified systems for ocular drug delivery against various ocular diseases such as dry eye disease (DED), glaucoma and corneal neovascularisation.[26,27]

In this review, the common ocular infections of the eye are discussed, together with the challenges hindering their therapy. We also discuss nanoemulsions as novel drug delivery systems, their formulations, and the recent studies on their use in the treatment of infectious eye diseases. We provide insight into the challenges, technological advances, and prospects of the use of nanoemulsions, hence providing direction for future research. Overall, novel nanoemulsions are a great, promising arsenal in the fight against infectious eye diseases.

2. Ocular infections and treatment challenges

The common ocular infections and the challenges hindering their treatments are presented in the following subsections.

2.1 Common ocular infections

Invasion of ocular tissues by microorganisms can lead to mild, severe or vision-threatening diseases. The eyelids, tear sac, conjunctiva, cornea, uvea, intraocular fluids and retina could be infected, leading to blepharitis, dacryocystitis, conjunctivitis, keratitis, uveitis, endophthalmitis and retinitis, respectively. Some ocular infections, such as trachoma, cytomegalovirus, endophthalmitis, uveitis, keratitis and those caused by rubella, *Acanthamoeba*, shingles, syphilis, toxoplasmosis and histoplasmosis, lead to vision loss and blindness if not treated properly. This subsection discusses the most common ocular infections such as conjunctivitis, keratitis, uveitis and endophthalmitis. A summary of the description, causative agents, symptoms and current treatment strategies of different ocular infectious diseases is presented in Table 1.

2.1.1 Conjunctivitis. Conjunctivitis is an ocular infection primarily characterised by inflammation of the conjunctiva. Globally, in ophthalmology clinics, conjunctivitis is an occurring ailment and the most common cause of red eyes.[28] Common symptoms of conjunctivitis are inflammation of the conjunctival mucosa, erythema, pain, and ocular discharge. In addition to the conjunctiva, conjunctivitis may extend to the surrounding eyelids (blepharoconjunctivitis) or cornea (keratoconjunctivitis). Viruses are the most common cause of infectious conjunctivitis. Compared to bacterial conjunctivitis, viral conjunctivitis is more frequent, self-limiting and unresponsive to antibiotic therapy.[28] Conjunctivitis caused by fungi or parasites is very rare. Conjunctivitis is usually not vision threatening; however, corneal scarring and vision loss may result if not adequately treated.[29] Some conjunctivitis of great importance are epidemic keratoconjunctivitis, methicillin-resistant *S. aureus* conjunctivitis, trachoma, ophthalmia neonatorum (neonatal conjunctivitis), and inclusion conjunctivitis. Though self-limiting, epidemic keratoconjunctivitis (also known as adenoviral keratoconjunctivitis) is a highly contagious inflammatory disease of the conjunctiva and cornea. It causes irreversible changes in the ocular surface, including corneal scarring and long-lasting visual impairments.[30] Also, trachoma is the leading infectious cause of blindness and is common in poor hygienic areas.[3] It is caused by *Chlamydia trachomatis* and presents as a mucopurulent discharge, followed by scarring of the eyelids, conjunctiva, and cornea. Ophthalmia neonatorum is another common conjunctivitis transmitted from a mother to a baby during birth. The principal causative agents are *Chlamydia trachomatis* and *Neisseria gonorrhoea*. Infection is acquired from infected mothers during passage through the birth canal. Other bacteria, such as *Streptococcus pneumonia*, *Staphylococcus aureus*, and *Haemophilus influenzae*, and viruses such as herpes simplex virus and adenovirus are also implicated in ophthalmia neonatorum.[31] Another conjunctivitis of importance is inclusion conjunctivitis, a sexually transmitted infection caused mainly by *Chlamydia trachomatis*, which is common in young adults. Herpes simplex virus and adenovirus, though rare, are also implicated in this disease.[28] Most cases of these infections are unilateral with concomitant genital infection.

2.1.2 Keratitis. Keratitis is inflammation of the cornea and can be caused by bacterial, viral, fungal or parasitic invasion of the eye through trauma. Though keratitis is less common

Table 1 Description, causative agents, symptoms and current treatment strategies for different ocular infectious diseases

Disease	Description/symptoms	Common causes/causative agents	Current treatment options
Blepharitis	Inflammation of the eyelid.	Bacteria such as *Staphylococcus aureus*, *S. epidermidis*, coagulase −ve staphylococci, *Propionibacterium* and *Corynebacterium macginleyi*	Hygiene, topical and oral antibiotics
	Symptoms-crusting of the eyelids, red, swollen eyelids, itching, burning sensation, foreign body sensation, trichiasis, chalazion, dry eye, and seborrhoeic dermatitis.	Viruses such as herpes simplex and varicella zoster	Oral antiviral agents Oral antiparasitic agents Topical corticosteroids
Conjunctivitis	Inflammation of the conjunctiva.	Viruses such as adenoviruses, herpes simplex virus and varicella zoster virus	Oral and topical antibiotics
	Symptoms-swelling of the blood vessels, pain, and ocular discharge	Bacteria such as *Staphylococcus aureus*, *Haemophilus influenza*, *Chlamydia trachomatis*, *Moraxella* spp, *Neisseria gonorrhoeae*, *Bacillus*, *Enterobacteriaceae* spp, *Pseudomonas aeruginosa*, and *Streptococcus pneumonia*	Artificial tears
Keratitis	Inflammation of the cornea	Bacteria such as coagulase-negative Staphylococci, *Staphylococcus aureus*, *Streptococcus* spp., *Pseudomonas aeruginosa*, *Enterobacteriaceae* spp., *Corynebacterium* spp. and *Propionibacterium* spp.	Topical antibiotics
	Symptoms-severe eye pain, redness, blurred vision, tearing and light sensitivity	Virus such as herpes simplex virus, varicella zoster virus	Topical and oral antiviral agents
		Fungi such as *Aspergillus and Fusarium* species	Topical antifungals
		Parasites such as *Acanthamoeba spp*	Artificial tear Topical Corticosteroids
Uveitis	Inflammation of the uvea (iris, ciliary body, choroid)	Parasites-*Toxoplasma gondii*	Topical and oral corticosteroids
	Symptoms-eye pain, redness, blurred vision, floaters and light sensitivity	Virus-Cytomegalovirus (CMV), Herpes simplex virus (HSV), varicella zoster virus, dengue, chikungunya and Zika.	Specific antimicrobial agents
		Bacteria – *Mycobacterium tuberculosis*, *Treponema pallidum*	Cycloplegic agents
		Fungi – *Candida* spp and *Aspergillus* spp	Surgery
Endophthalmitis	Severe inflammation of the interior of the eye	Post-surgical or post-trauma	Intravitreal antibiotics (*e.g.* vancomycin ceftazidime)
	Symptoms-severe eye pain, redness, swelling, vision loss and discharge	*Staphylococcus aureus*, *Bacillus cereus*, *Staphylococcal epidermidis*, *Propionibacterium acnes*, Coagulase-negative Staphylococcus and Streptococci species, *Klebsiella pneumonia*, *Haemophilus* and *Pseudomonas aeruginosa*	Systemic antibiotics
		Fungi such as *Aspergillus sp*, *Fusarium sp* and *Candida*	Vitrectomy
Dacryocystitis	Inflammation of the lacrimal sac Symptoms-pain, redness, swelling near the inner cornea of the eye and discharge	Bacteria such as *Staphylococcus aureus*, *Staphylococcus pneumoniae*, *Haemophilus influenzae*	Systemic antibiotics Surgical drainage or dacryocystorhinostomy for chronic or severe cases
Retinitis	Inflammation of the retina	Virus such as cytomegalovirus, herpes simplex virus, varicella zoster virus	Systemic or intravitreal antivirals such as ganciclovir, forscarnet.
	Symptoms-floaters, vision loss, photophobia and blurred vision	Autoimmune causes	Pathogen-specific antimicrobials Systemic corticosteroids

than conjunctivitis, it is a more severe infection that needs prompt treatment to prevent vision loss. For instance, infectious keratitis is a significant contributory factor to corneal blindness. It is the fifth leading cause of blindness globally.[32,33] Delayed diagnosis, antimicrobial resistance and a limited number of antimicrobial agents for ocular use are the significant challenges in treating infectious keratitis.[34] Interestingly, natamycin is the only approved antifungal agent for fungal keratitis. If left untreated, keratitis can cause blindness. Common symptoms of keratitis are red eye, foreign body sensation in the eye, ocular pain, photosensitivity, excessive tearing and impaired vision. The most common causative agents of bacterial keratitis are coagulase-negative Staphylococci, *Staphylococcus aureus*, *Streptococcus* spp., *Pseudomonas aeruginosa*, *Enterobacteriaceae* spp., *Corynebacterium* spp. and *Propionibacterium* spp.[35–37] However, these organisms cannot penetrate an intact cornea except for *Neisseria*, *Corynebacterium*, *Haemophilus*, and *Listeria* species. Thus, infections usually occur due to conditions that disrupt the cornea epithelium, such as trauma, surgery, prolonged use

of contact lenses, and ocular and systemic diseases. Fungal keratitis is one of the major causes of infectious keratitis that may lead to corneal blindness. It accounts for 45% of cases of microbial keratitis and is a major cause of corneal blindness worldwide.[38] Fungi can spread from the corneal surface to the inner intraocular tissues, leading to endophthalmitis and eventual blindness and enucleation of the eyeballs. Up to 60% of fungal keratitis cases are caused by the entry of fungal fragments or spores in the eye *via* antecedent trauma. Hence, there is a high incidence of this disease in developing countries where agricultural trauma is common.[39] Conversely, the use of contact lenses is the most important contributory factor for fungal keratitis in developed countries.[40] *Acanthamoeba* keratitis is a rare parasitic eye infection caused by the *Acanthamoeba* genus. It is a severe corneal disease that can result in vision loss, irreversible blindness, and keratoplasty.[41] It primarily affects contact lens users but may also result from corneal trauma in non-contact lens wearers. Thus, the early diagnosis and treatment of infectious keratitis are essential to prevent long-term complications, including blindness.

2.1.3 Endophthalmitis. Endophthalmitis is a rare but severe sight-threatening condition caused by the introduction of pathogens into the anterior or posterior chamber of the eye or both.[42] Endophthalmitis is a medical emergency requiring immediate treatment to prevent vision loss and enucleation.[43] It causes inflammation of the intraocular tissues and fluid. It most commonly occurs as a complication following eye surgeries, trauma, or intraocular injections. Symptoms of endophthalmitis include intense pain, decreased vision, and sometimes a cloudy appearance in the eye. Endophthalmitis can be classified as endogenous if it is caused by pathogens spreading systemically through the bloodstream to the eye. Exogenous endophthalmitis results from the introduction of pathogens *via* trauma, surgery, or an infected cornea from the external environment to the ocular surface. Based on the risk factors, exogenous endophthalmitis can be further classified as postoperative endophthalmitis, bleb-associated endophthalmitis, post-intravitreal endophthalmitis, and post-traumatic endophthalmitis.[44,45]

Management of endogenous endophthalmitis includes a combination of systemic and intravitreal antibiotics or antifungal agents.[42] Total capsulectomy and removal of intraocular lenses may be considered for recurrent cases of delayed-onset postoperative endophthalmitis. Bleb-associated endophthalmitis requires more aggressive management, and thus Pars Plana Vitrectomy has been suggested. However, visual outcomes of endophthalmitis are often poor despite management, and the use of Pars Plana Vitrectomy is still controversial.[46,47]

2.1.4 Uveitis. Uveitis is inflammation of the uvea (middle layer of the eye comprising the iris, ciliary body and choroid) and the surrounding tissues, such as the retina, optic nerve, and vitreous humour, which is caused by trauma, surgery, infection, autoimmune diseases or systemic inflammatory disorders. Uveitis accounts for 10% to 25% of blindness globally, and it is the leading cause of blindness in people less than 40 years.[48,49] Common causes of infectious uveitis are toxoplasmosis, herpes simplex virus (HSV), cytomegalovirus and varicella-zoster virus.[50] Emerging viruses such as dengue, chikungunya and Zika have been reported to cause uveitis.[51] Based on anatomy, uveitis is classified into anterior uveitis, intermediate uveitis, posterior uveitis, and panuveitis. Although anterior uveitis (also called iritis) is the most common, posterior uveitis is the most severe. Posterior uveitis can involve only the choroid (choroiditis) or both the choroid and retinitis (chorioretinitis). Chronic or recurrent uveitis can lead to complications such as glaucoma, cataracts, cystoid macular oedema, keratopathy, hypotony, and optic nerve oedema.[52] Vision loss in patients with uveitis is mainly related to the severity, location, and duration of the inflammation and the development of severe complications.

Although there is no standard treatment for uveitis, treatment is based on the anatomic position, cost, availability of drugs, patient's preferred choice, and efficacy. Infectious uveitis, such as toxoplasmosis, requires specific antimicrobials, while autoimmune uveitis is treated with corticosteroids and immunosuppressants.[53] In rare, severe cases, vitrectomy is used.

2.2. Challenges in the treatment of infectious eye diseases

The primary objective of any ocular drug delivery system is to overcome dynamic and static barriers, attain adequate therapeutic drug concentrations at the target sites and minimise frequent dosing. Some of the challenges encountered in designing and delivering anti-infective agents to the eye are summarised in Fig. 2 and briefly discussed in this section.

2.2.1. Ocular barriers. After topical or systemic administration, therapeutic molecules face tissue barriers that significantly affect their ocular bioavailability. Anatomically, the eye has three adjoining tissue layers, each of which has a structure that shields the eye from systemic circulation. Also, the complicated ophthalmic environment, which includes tear film and other precorneal barriers, cornea, conjunctiva, sclera, blood-aqueous barrier, blood-retinal barrier and efflux pumps, are significant challenges in ocular drug delivery.

The tear film offers the first resistance to the topical delivery of drug molecules to the eye. The tear film is a thin, transparent fluid composed of an outer hydrophobic lipid layer, a middle aqueous layer and an inner mucous layer. The outer lipid and aqueous layers prevent the ocular absorption of hydrophilic and hydrophobic molecules into the cornea.[54] The mucous layer protects the eyeball and clears the eye of debris and pathogens. The tear film is also continuously drained into the nasolacrimal duct; hence, the retention of topically administered drugs is significantly impaired, resulting in their low bioavailability (<5%).[55] Lacrimal secretions also reduce the retention time, and consequently the bioavailability of ocular preparations.

The cornea, a five-layered transparent and avascular tissue, is the main drug absorption route after topical administration. It is comprised of the corneal epithelium, Bowman's layer, Descemet's layer, stroma and corneal endothelium. Drug per-

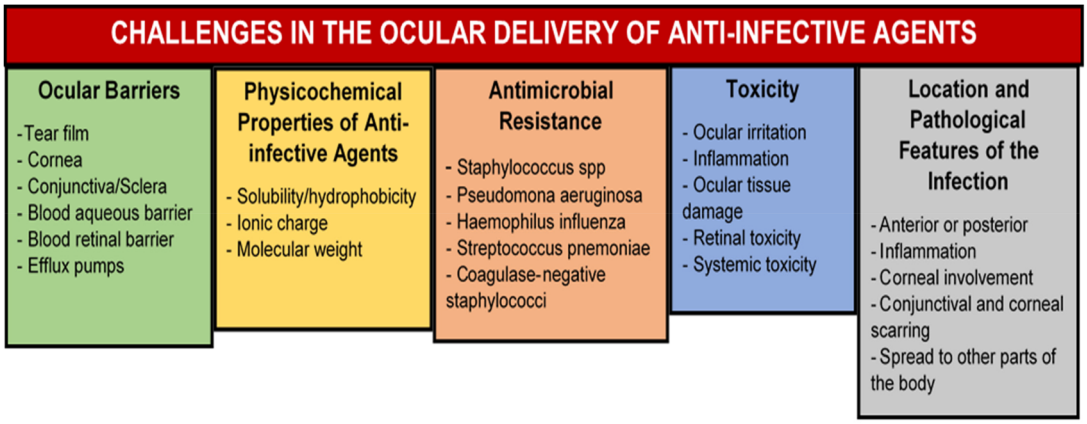

Fig. 2 Challenges in the ocular delivery of anti-infective agents.

meation through the corneal layers physiologically depends on molecular weight and hydro-phobicity/-philicity.[55] The cellular layers (epithelium and endothelium) allow permeation through the paracellular or transcellular routes based on the physicochemical properties of the molecules. Large molecular weight hydrophilic compounds (exceeding 2000 Da) are significantly limited from traversing the cellular layers of the cornea. Alternatively, hydrophobic compounds, with molecular weights up to 600 Da, can partition into the lipid layer of the cell membrane, facilitated through the paracellular or transcellular routes to the underlying tissues.[56] The stromal layer, composed of collagen fibres in a hydrated lamellar matrix, is also a barrier to hydrophobic molecules.

The conjunctiva is a thin mucous membrane that covers the inside of the eyelids and the white of the eye (the sclera) and is an alternative route for drug absorption after topical administration. The conjunctiva has a larger surface area (17 times) than the cornea and is more permeable to hydrophilic and large molecules.[54,57] However, the conjunctiva is also highly vascularised; thus, large concentrations of topically administered drugs enter the systemic circulation. As a consequence, administered drugs have low retention in the ocular tissues and poor bioavailability in the intraocular tissues, necessitating frequent instillation and poor patient compliance. The sclera is a large collagenous structure covering the outer layer of the eyeball. Drug absorption through the sclera is a function of the molecular size of the therapeutic agent rather than its lipophilicity.[58]

The blood-aqueous barrier (BAB) consists of endothelial cells, the iris, the ciliary muscle, and pigmented and non-pigmented epithelium cells. It is the main barrier to the absorption of drug molecules in the anterior segment of the eye. The BAB preferentially allows the permeation of lipophilic and small molecules rather than hydrophilic and large molecules.[54] The blood-aqueous barrier protects the intraocular environment as well as acts as a selective barrier for the transport of solutes across the eye.[55] This barrier only permits the entry of molecules with a low molecular weight. Also, due to the continuous drainage of lacrimal secretions, the bioavailability of molecules is further affected.[59]

The blood-retinal barrier (BRB) is the main barrier to drug permeation in the posterior eye. It prevents water, plasma components, and toxic substances from entering the retina.[60] The BRB also prevents the permeation of hydrophilic drug molecules and expresses drug efflux proteins that lower the bioavailability of the administered drug.[55] The blood-retinal barrier favours the transport of small lipophilic molecules as well as controls the passage of molecules due to its tight intermolecular junctions.

Efflux proteins are present in various ocular tissues, including the conjunctival membrane, corneal endothelium, non-pigmented ciliary epithelium and retinal endothelium. Efflux proteins are responsible for modulating or attenuating ocular bioavailability after the topical administration of a drug. P-glycoprotein is a major efflux protein in the eye and acts as an efflux pump, preventing the entry of lipophilic and hydrophilic molecules. Also, the multidrug-resistant protein (MRP) present in various ocular tissues, which is a membrane-bound efflux transporter.[61]

2.2.2 Physicochemical properties of anti-infective agents. The efficacy of ocular antimicrobial treatments is significantly influenced by the physicochemical properties of the anti-infective agents. These properties play a crucial role in determining the ability of a drug to penetrate ocular tissues and maintain therapeutic concentrations at the site of infection. The structural layers of ocular tissue comprise diverse cellular components and demonstrate differential permeability to hydrophobic, lipophilic, cationic, and anionic molecules.[55] Hence, medications and formulation systems needed for inner eye distribution must be amphipathic and biphasic in solubility.[62] Most antifungals composed of an azole group are hydrophobic and have poor aqueous solubility. Some agents, such as fusidic acid and quinolones, can penetrate the cornea, whereas some other agents, such as bacitracin and polymyxin B, are poorly penetrating and restricted to the treatment of superficial ocular infections.[63]

Studies indicate that anionic molecules are unable to traverse the lens capsule, while cationic molecules get immobilised in the vitreous humour, suggesting that the overall surface charge of a molecule plays a crucial role in its distribution throughout ocular tissues.[55] Likewise, cationic compounds are more retained on the ocular surface given that they interact electrostatically with the negatively charged mucin layer.

Molecular weight is an essential factor to consider in ocular drug delivery systems because the epithelial cells of the eye will only permit molecules with radii less than 5.5 Å or a molecular weight of 500 Da to pass through the cornea.[59] In a study that involved the preparation of a budesonide suspension with varying physicochemical properties (size and viscosity), the results from *in vivo* studies revealed a significant difference in the bioavailability of each preparation. Thus, particle size and formulation viscosity changes can significantly affect ocular drug delivery.[64] The impact of the physicochemical properties of drugs is well pronounced with antifungal agents. The delivery of antifungal agents is a unique challenge due to their large molecular weight and poor aqueous solubility.[65,66] Large molecular weight compounds (>500 Da), such as amphotericin B (924.10 Da), natamycin (665.75 Da), and itraconazole (706 Da), barely penetrate the intact corneal epithelium.[67] Interestingly, due to their limitations, natamycin is the only approved drug for the topical treatment of fungal keratitis.

2.2.3 Antimicrobial resistance. The development of resistance to antimicrobial agents is a global issue that affects the therapy of infectious diseases. Antimicrobial–resistant organisms are becoming more frequent in ocular infections, tremendously impacting cost and treatment outcomes. Common pathogens of ocular diseases have been reported to develop resistance to first-line antibiotics, especially fluoroquinolones and azoles.[68] *Staphylococcus aureus*, which is implicated in many ocular infections, such as keratitis, conjunctivitis and endophthalmitis, has shown high resistance to methicillin and azithromycin.[69] *Pseudomonas aeruginosa*, a common causative agent of bacterial keratitis, exhibits high fluoroquinolones resistance. Although *Haemophilus influenzae* is susceptible to many antibiotics, its resistance to fluoroquinolones and azithromycin has been reported. Other ocular pathogenic agents that have shown increasing resistance to mainstay antibiotics are coagulase-negative staphylococci (CoNS) and *Streptococcus pneumonia*e.[70]

2.2.4 Toxicity. The toxicity of antifungal agents poses significant barriers that impair their clinical utility. Polyenes and azoles are the standard classes of antifungals used in managing ocular diseases due to their broad-spectrum activity. However, these antifungals often exhibit toxicity, which results from their non-specific interaction with both ergosterol and cholesterol in fungal and human cells, respectively.[71] These toxic effects include ocular irritation, inflammation and ocular tissue damage. Triazole antifungal agents such as voriconazole cause visual disturbance given that they directly inhibit the ion channels found in the retina.[72] In two independent toxicity studies, intravitreally administered amphotericin B was found to cause retinal toxicity, loss of retinal ganglion cells and significant pathologic changes.[73,74] Similar retinal toxicity was reported for fluconazole and other quinolones, aminoglycosides and chloramphenicol.[72,75] In addition, many ocular infections require chronic management, increasing the risk of secondary contamination and toxicity. Long-term use of antifungals in ocular disease management impairs the barrier function of the epithelial cells of the cornea.[76] Alternatively, side effects such as hepatotoxicity, nephrotoxicity, skin rashes, adrenal insufficiency, hypogonadism, and GIT upset have been reported with the systemic administration of antifungal agents.[77]

2.2.5 Anatomical location and pathological features of the infection. The anatomical position and pathological features of an eye disease pose a significant challenge in treatment. Although infections that occur in the anterior eye system are easier to access, the treatment of both anterior and posterior eye infections is hindered by the structure and inherent barriers of the eye. Nonetheless, posterior eye infections face greater challenges in achieving a therapeutic drug concentration in the affected tissues if the topical or systemic administration route is used. Hence, intravitreal injections are usually utilised to target the site of infection. However, intravitreal injections can only be administered by skilled personnel and have many complications, such as retinal detachment and endophthalmitis that can worsen the patient's condition and lead to vision loss.[78]

The successful treatment of eye infection depends on its pathological characteristics such as inflammation, scarring, corneal ulcer and opacity, haemorrhages and spread of the infection to other parts of the body. Most anterior eye segment infections affect the cornea, resulting in ulcers, opacity and stromal infiltrates, which are challenging to treat and contribute significantly to vision impairment and blindness.[79] For example, trachoma and *Acanthamoeba keratitis* are difficult to treat due to their pathological features. Symptoms such as conjunctival scarring, trichiasis, corneal opacity and ulcer seen in trachoma make its treatment difficult given that they are irreversible and may require surgery.[80] *Acanthamoeba* forms dormant cysts that are resistant to antimicrobial agents. In addition, they produce proteases that cause extensive cornea damage.[81] Furthermore, some ocular infections such as orbital cellulitis, severe keratitis and ocular syphilis can spread to the brain and other parts of the body, thereby complicating treatment.

3. Nanoemulsions as novel drug delivery systems

Emulsions are colloidal systems comprised of oil and water, which are stabilised by an emulsifying agent. Microemulsions and nanoemulsions are well-known classes of emulsions employed as drug delivery systems. Nanoemulsions can be differentiated from microemulsions based on their droplet size and physical stability features.[82] Nanoemulsions are novel

drug delivery systems made up of emulsified oil and water systems with average droplet diameters in the range 50 of 1000 nm, and their droplets basically range from 20 to 500 nm in size.[83] In comparison, microemulsions are clear, isotropic, thermodynamically stable colloidal systems comprising droplets (5–200 nm in size) distributed in a medium.[55] Considering their stability, microemulsions are thermodynamically stable systems, while nanoemulsions are not, but can be made kinetically stable by the presence of emulsifying agents/surfactants.[84] Although microemulsions form spontaneously if the immiscible liquids and the emulsifying agents are in the right concentration, nanoemulsions require high-energy techniques to overcome the large free energy of the system.[85] Nanoemulsions and microemulsions are usually described as similar systems given that they are both low-viscosity colloidal dispersions formulated using similar ingredients. These systems are grouped into three categories based on their oil and water composition. They are oil-in-water (O/W) nanoemulsions, in which oil droplets are distributed in a continuous aqueous phase, water-in-oil (W/O) nanoemulsions in which water droplets are scattered in a continuous oil phase, and bi-continuous nanoemulsions, in which oil and water microdomains are inter-dispersed across the system.[86,87] The structure of oil/water and water/oil nanoemulsions is shown in Fig. 3. In ocular drug delivery, o/w nano/microemulsions are used more frequently given that they can easily mix with aqueous fluids of the eye and enhance the bioavailability of drug.[55]

3.1 Benefits of nanoemulsions in the ocular delivery of drugs

3.1.1 Improved drug solubility and delivery of hydrophobic drug as eye drops. Nanoemulsions have the most potential as a colloidal drug delivery systems in enhancing the solubility and bioavailability of hydrophobic drugs and other bioactive functional compounds.[88,89] They have been used to deliver hydrophobic drugs as eye drops.[90] Their biphasic composition aids in solubilising both hydrophilic and hydrophobic drugs. For instance, lutein, a poorly water-soluble drug, showed enhanced solubility and was delivered as eye drops when incorporated into a nanoemulsion.[89]

3.1.2 Enhanced stability. Microemulsions have excellent thermodynamic stability, which protect drugs from degradation reactions (such as hydrolysis and oxidation), prolonging their shelf life. Although nanoemulsions are not thermodynamically stable, they exhibit long-term physical stability partly due to their small droplet size. Hence, physical instabilities such as sedimentation, flocculation and coalescence seen with conventional dispersions are minimised. The polymers or surfactants used in nanoemulsion formulations also contribute to their stability *via* steric stabilisation.

3.1.3 Improved precorneal retention and transcorneal penetration. The small droplet size and presence of a surfactant in nanoemulsions enhance the mixing of the carrier system with the precorneal constituents, resulting in an adequate dispersion of the drug over the cornea. These surfactants also facilitate the rapid transcorneal transport of drugs into deeper ocular layers *via* the reversible disruption of the tight junctions. Furthermore, cationic nanoemulsions interact electrostatically with the negatively charged ocular surface, resisting nasolacrimal drainage and promoting the retention of the carriers on the eye. Chitosan is commonly used to induce a positive charge on nanoemulsions due to its mucoadhesive and biocompatibility features.[91,92] The extended contact of nanomedicines with the cornea and enhanced corneal permeability lead to sustained drug release, improvement in their ocular bioavailability, and improved efficacy and therapeutic outcomes.[82]

3.1.4 Targeted drug delivery. In addition to sustained drug delivery, nanoemulsions have been explored for targeted drug delivery. For instance, L-carnitine-modified nanoemulsions

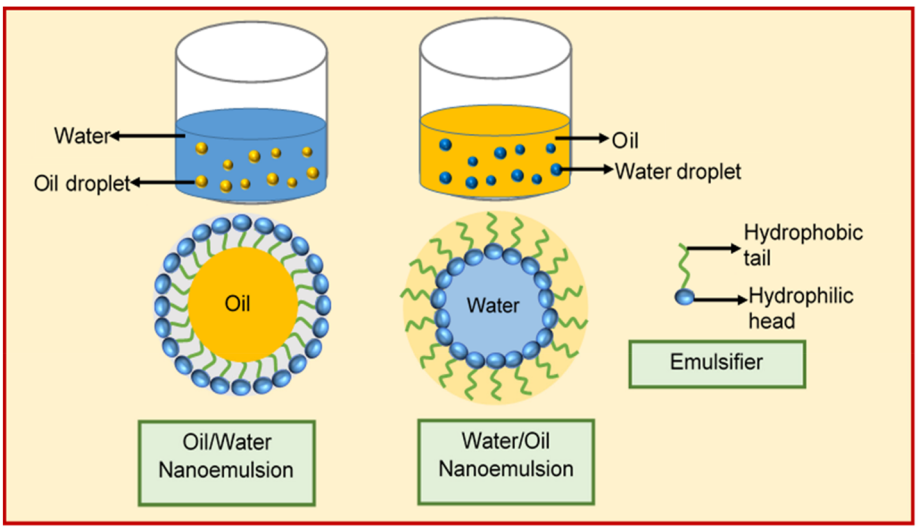

Fig. 3 Structures of oil/water and water/oil nanoemulsions.

were recently explored to target novel organic cation/carnitine transporter II and basic amino acid transporter B, given that they are expressed on the corneal epithelium and endothelium.[93]

3.1.5 Improved dosage regimen and patient compliance. Lastly, compared with conventional eye drops, nanoemulsions may require a simplified dosing regimen and less frequent dosing, resulting in lower side effects and increased patient adherence.[94]

3.2. Composition of nanoemulsions

Nanoemulsions are a heterogeneous mixture of aqueous and oily phases, with a surfactant as an emulsifier. The choice of excipients is critical in ensuring the optimal product given that the physicochemical properties of excipients influence their stability, toxicity and pharmacokinetic profile.[95] The materials commonly used in the formulation of nanoemulsions are presented in Table 2.

3.2.1 Active pharmaceutical ingredients. Nanoemulsions have been used to encapsulate various lipophilic and lipophobic drugs across different pharmacological classes, including antibacterial, antifungal, antiviral, antiparasitic, anti-inflammatory, antioxidant and anti-glaucoma agents for the treatment of ocular diseases.[96–99] Bioactive compounds such as fatty acids, carotenoids, antioxidants, and phytosterols have also been incorporated into nanoemulsion systems to enhance their solubility.[88,100,101]

3.2.2 Oil phase. The oil phase of nanoemulsions generally consists of a carrier oil in which a lipophilic functional compound or active pharmaceutical ingredient is dissolved. The oil phase of nanoemulsions may include free fatty acids, triglycerides, oils and free fatty acids. Fatty acids and monoglycerides such as α-linolenic acid, lauric acid, myristic acid and oleic acid are widely used in nanoemulsions for infectious diseases given that they have been reported to have antimicrobial activity.[102,103] Medium- and long-chain triacylglycerols, synthetic lipids and their combinations are often used in the formulation of nanoemulsions.[104,105] The selection of a suitable oil or lipid is based primarily on the solubility of the drug in the oil, given that the type of oil determines the bioavailable fraction of the drug molecule. Other factors such as viscosity, interfacial tension, refractive index, phase behaviour and density are also considered.[84,95,105] A correlation exists between the viscosity of the dispersed oil phase and the mean droplet size in O/W emulsions. An investigation by Maa and Hsu found that smaller droplets are produced with low-viscosity oils.[106] Low-viscosity oils exhibit a more rapid response to external energy inputs, resulting in quicker disruption. Additionally, the reduced interfacial tension between water and oil enhances the size reduction process, given that this condition necessitates a lower energy expenditure.[84]

3.2.3 Aqueous phase. The aqueous phase of a nanoemulsion predominantly consists of a polar solvent, primarily water, co-solvents such as alcohols and polyols, surfactants and co-surfactants. This aqueous phase has an effect on the physicochemical behaviour of the nanoemulsion, given that it significantly influences the polarity, interfacial tension, refractive index, rheology, pH, and ionic strength of the system.[105]

Surfactants are amphipathic molecules that stabilise nanoemulsions by reducing the interfacial tension and preventing droplet aggregation.[107,108] The surfactants mostly used in drug delivery systems include non-ionic surfactants (sorbitan esters and polysorbates), anionic surfactants (potassium laurate and sodium lauryl sulphate), cationic surfactants (quaternary ammonium halide), and zwitterions surfactants (quaternary ammonium halide).[109] They commonly adsorb at the oil–water interface to form a monomolecular flexible film. This leads to a reduction in the surface tension and surface free energy, in turn decreasing the possibility of coalescence of the droplets and providing steric or electrostatic stabilisation.[110] The choice of surfactant type influences the size and stability of the nanoemulsion. It is also thought to determine the toxicity, pharmacokinetic and pharmacodynamic character of the nanoemulsion. Lecithin (phosphatidylcholine), obtained from

Table 2 Materials commonly used in the formulation of nanoemulsions for ocular drug delivery

Material	Common examples
Active pharmaceutical ingredient	Pharmacological agents – antimicrobial agents (itraconazole, ofloxacin, gatifloxacin, azithromycin, natamycin, amphotericin B, fluconazole, Acyclovir, ganciclovir, rifampicin), anti-inflammatory agents (diclofenac, ketorolac, prednisolone, dexamethasone, and loteprednol), and anti-glaucoma (travoprost, latanoprost brinzolamide, dorzolamide and betaxolol) Phytochemicals-curcumin, isoliquiritigenin, lycopene, α-tocopherol, β-carotene, glycyrrhizin, thymoquinone, and eugenol
Oils/lipid	Oil-Triacetin, ethyl oleate, α-linolenic acid, castor oil, egg lecithin, isopropyl myristate, and oleic acid Cationic lipids-(1,2-dioleoyl-3-trimethylammoniumpropane) (DOTAP), 1,2-dioleoyl-*sn-glycero*-3-phosphocholine (DOPC), cetyltrimethylammonium bromide, and stearylamine
Surfactants	Tween 80, Tween 20 (PEG-20 sorbitan monolaurate), Tween 65 (PEG-20 sorbitan tristearate), poloxamer 407, poloxamer 188, span 20 (sorbitan monolaurate), Cremophor EL (polyoxyl-35 castor oil), Cremophor RH 40, Soluphor® P, lecithin, sodium deoxycholate (bile salt), β-lactoglobulin, sodium dodecyl sulfate, sodium lauryl sulfate, Cerex ELS 250 (PEG-25 hydrogenated castor oil), Brij-30 (PEG-4 lauryl ether), glyceryl caprylate, Labrafil M 2125 CS (PEG-6 corn oil), Labrafac CM 10 (PEG-8 caprylic/capric glycerides), Emulphor El-620 (ethoxylated castor oil), polyoxyethylene hydrogenated castor oil, and glyceryl monooleate
Co-surfactants	Propylene glycol, glycerol, ethylene glycol, Transcutol P
Viscosity modifiers	Chitosan, methylcellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose, carrageenan, xanthan, alginate, pectin, polyvinyl alcohol, polyvinyl pyrrolidone, glycerol

egg yolk or soybean, is commonly used as a surfactant in ocular nanoemulsions. Other commonly used surfactants include sucrose esters, sorbitan fatty acid esters, glycerol fatty acid esters (polyglycerols) and polyoxyethylene ether surfactants.[107] Polysorbate 80, poloxamers, tyloxapol, vitamin E TPGS, bile salts (sodium deoxycholate) and Cremophor EL (polyoxyl-35 castor oil) have been used in commercial formulations.[104,111]

Generally, a combination of surfactant and co-surfactant is required to optimise the formation of a nanoemulsion. Co-surfactants are also amphipathic molecules with an active surface and small size that complement surfactants in maintaining the molecular interfacial film. Co-surfactants enhance the fluidity of the interfacial film of droplets and reduce the concentration of surfactants required, thereby minimising the risk of potential toxicity.[112] The commonly used co-surfactants are propylene glycol, polyethylene glycol (PEG), ethanol, Transcutol P, glycerine and ethylene glycol.

Ocular nanoemulsions require the inclusion of auxiliary substances such as preservatives, tonicity modifiers, buffering agents, viscosity enhancers, antioxidants, and API solubilisers to preserve and enhance their stability as well as biocompatibility.[113]

3.3 Formulation techniques

The preparation methods of nanoemulsions are classified into high-energy and low-energy methods based on the energy requirements. The high-energy method uses mechanical devices like high-pressure valve homogenisers, microfluidisers and ultrasonicators to generate large disruptive forces.[114] The large forces generated by these devices disrupt the dispersed phase into small droplets of nanoemulsions. High-energy methods, although effective at reducing droplet size, are incompatible with labile drugs and macromolecules such as proteins and nucleic acids.[115] High-energy methods include high-speed homogenisation/high shear stirring, high-pressure homogenisation, ultrasonication and microfluidization. Low-energy methods are spontaneous processes that utilise the internal chemical energy of a system to produce nanodroplets. Low-energy methods are considered to be energy-efficient processes and affected by physicochemical factors such as temperature, solubility and composition.[116,117] The production of nanoemulsions using low-energy methods also requires high surfactant concentrations. Low-energy methods include spontaneous emulsification, phase inversion and microemulsion dilution. Low-energy techniques are currently being explored owing to their non-destructive nature, lower energy consumption, greater efficiency, and no need for sophisticated instruments. However, high-energy methods often require lower surfactant concentration(s) than low-energy methods.[110]

Conversely, microemulsions are thermodynamically stable ($\Delta G < 0$) systems that form spontaneously on mixing. Nonetheless, energy input is often applied to overcome kinetic barriers or speed up the rearrangement of surfactant molecules.[84] The methods for the preparation of microemulsions are low-energy methods, such as low-energy emulsification and phase inversion temperature methods. The advantages and limitations of the different nanoemulsion formulation techniques are presented in Table 3.

3.3.1. High shear stirring. High-shear stirring involves the use of high-energy mixers and rotor-stator devices to vigorously mix components of a nanoemulsion.[87] The shear forces are applied to break up coarse dispersion into small-sized droplets. The process is often carried out at room temperature, and the size of droplets can be minimised by increasing the intensity of the devices.[118] Compared to other methods, the high shear stirring technique is preferred because it is the only method with considerably high energy for nanodroplet generation, with only a slight temperature rise.[119] Nonetheless, this method is limited given that ultra-nanosized droplets (<200 nm) are challenging to obtain, and the processing of highly viscous media is inefficient.[120]

3.3.2 High-pressure homogenisation. This technique uses a high-pressure homogeniser to produce nanoemulsions of very low droplet sizes (up to 1 nm).[104] Nanoemulsions are produced by forcing preemulsions through a tiny orifice in a piston homogeniser at a high pressure between 500 and 5000 psi.[104,111] The crucial process parameters in this technique that need to be optimised are the number of passes, applied pressure, and process temperature, while the critical formulation variables are the type and concentration of surfactant. High pressure homogenisation is a highly efficient technique that is routinely applied in laboratory and industrial settings, but it requires a high energy input. It produces uniform-sized droplets with narrow particle size distributions. However, a drawback of high-pressure homogenisation is that it is not suitable for thermolabile formulations, given that an increase in temperature during processing may degrade the constituents of the system.[119]

3.3.3 Ultrasonication. Ultrasonication methods use high-frequency sound waves (>20 Hz) to form nanoemulsions. When two immiscible phases in the presence of a surfactant are subjected to high-frequency waves, emulsion droplets are formed by cavitation.[121] Various process and formulation variables are considered in the optimisation of ultrasonication. Variables such as sonication time, input power, dissolved gas, hydrostatic pressure, apparatus configuration and temperature influence the outcome of nanoemulsification.[111] Also, formulation variables such as oil and surfactant concentrations are significant. A study by Moghimi *et al.* reported that reducing the amount of oil and/or increasing the surfactant concentration reduces the droplet coalescence rate during sonication, producing smaller droplets.[122] Overall, ultrasonication is a favoured technique due to its ease of operation, high energy efficiency, and cost-effectiveness, requiring low surfactant concentrations and demonstrating dispersion stability. However, it may be limited by its long processing times and unsuitable for large volume batches and low energy due to the small surface area of the sonicator tip.[110] Also, there is a possibility of contamination by the sonicator probe.[108]

3.3.4 Microfluidization. Microfluidization, also known as direct emulsification, employs the mechanism of flow through microchannels with dimensions ranging from 50 to 300 μm to

Table 3 Advantages and limitations of different nanoemulsion formulation techniques

Formulation method	Working principle	Advantages	Limitations
High-energy methods			
High-shear homogenization	Involves the use of high-shear forces to break up, emulsify, homogenise and disperse materials into micron-sized droplets.	Useful in the production of large volumes of emulsions. High preference for the creation of formulations with thermolabile constituents.	Preparation of nanoemulsions with droplet sizes of <200–300 nm is difficult. The efficiency of high-shear mixers decreases with the increased viscosity of emulsions.
High-pressure homogenization	Involves the application of high pressure (500–5000 psi) to force a coarse dispersion through a small orifice, generating nanoemulsions.	Short process time. Production of nanoemulsions with low droplet sizes (up to 1 nm).	Poor scalability to high energy requirements. Not suitable for thermolabile agents.
Ultrasonication	Involves using ultrasonic waves and acoustic cavitation forces to create nanosized droplets.	Ease of operation. High energy efficiency. Low emulsifier requirement.	Not suitable for large-volume preparation. Longer processing times.
Microfluidization	Involves the collision of fluids forced through microchannels under high pressure (500–20 000 psi) to form an emulsion.	Production of nanoemulsions with greater stability and narrower particle sizes than homogenisers.	Expensive technology. Can result in blocked microchannels, thereby reducing the efficiency.
Low-energy methods			
Spontaneous emulsification	Dispersion occurs due to changes in interfacial tension and surface area when oily and aqueous phases are mixed.	Ease of scalability. Low energy requirement.	Only suitable for preparing emulsions with a low fraction of the internal phase.
Phase inversion composition	Involves the inversion of a W/O or O/W emulsion by modifying the component concentrations.	Low production costs. Thermodynamic stability of the formed nanoemulsions.	Production of larger and more polydisperse droplets.
Phase inversion temperature	Involves inversion of the initial O/W system to a W/O nanosystem upon temperature change.	Formation of kinetically stable emulsions with small droplet size and monodispersity.	Less applicable with ionic and nonionic surfactants whose hydrophilic-lipophilic balance is less sensitive to temperature changes.

produce emulsion droplets of ultrafine nanosize.[111,119] Microchannels are generally micro-sized channels that permit mixing at the micron-sized level. During the process, fluid is forced through the microchannels at high pressure (500–20 000 psi), and nanoemulsions with submicron sizes are formed. Uniform nanoemulsions can be obtained by repeating the process and varying the operating pressure to get the desired particle size. Microfluidization produces smaller droplet sizes than homogenisation processes. Also, nanoemulsions prepared by microfluidization are stable at low surfactant concentrations.

A major limitation of this process is that microchannels may become clogged, leading to a loss of efficiency. Also, the increased emulsification time causes the coalescence of the emulsion droplets.[123]

3.3.5 Spontaneous emulsification. In spontaneous emulsification, nanoemulsions are achieved under ambient conditions without special equipment or energy input. The process is based on the movement of water-miscible components such as solvent, surfactant and co-surfactant from an organic phase into the aqueous phase.[108,114] The migration of the oily components into the aqueous phase causes interfacial turbulence, leading to an increase in the oil–water interfacial area and the production of nanosized droplets. The droplet size and droplet size distribution have been shown to depend on the interfacial tension, viscosity and oil and surfactant concentration.[118]

3.3.6 Phase inversion temperature (PIT). Phase inversion temperature exploits the characteristics of non-ionic surfactants to alter their affinities to oil and water phases based on temperature changes.[118] The transitions are caused by shifts in the hydrophilic-lipophilic balance of emulsifiers and surfactants in the system. At room temperature, oil, water, and surfactants are mixed to form an initial oil-in-water (O/W) emulsion. With an increase in temperature, the polyethylene oxide groups in the surfactant become dehydrated, making it more lipophilic. Consequently, phase inversion occurs through intermediate structures such as liquid crystalline or bicontinuous phases, eventually forming a water-in-oil (W/O) nanoemulsion. The mixture is then rapidly cooled in an ice bath to stabilise the final nanoemulsion.

The PIT technique produces nanoemulsions with a low polydispersity index and also demonstrates a high emulsification efficiency compared to other methods, such as the phase inversion composition.[114] The drawbacks of the PIT method are that nanoemulsions formulated with this method are temperature-sensitive around their PIT, and therefore require larger amounts of surfactants or co-surfactants to stabilise them. In addition, it may be unsuitable for thermosensitive drugs given that it requires heat input.[108]

3.3.7 Phase inversion composition. Phase inversion composition is a transitional phase inversion method for producing nanoemulsions by altering the system composition rather

than temperature.[117] It is considered an extended form of spontaneous emulsification and operates by modifying the hydrophilic-lipophilic behaviour of surfactant. This methodology involves systematically incorporating an aqueous phase into an oil-surfactant mixture (or *vice versa*) under ambient conditions with controlled agitation. During this process, the system undergoes a structural transformation through intermediate phases, including bicontinuous and lamellar configurations.[110] When the critical transition composition is exceeded, a spontaneous modification occurs in the surfactant interfacial layer, transforming from a zero-curvature configuration to a highly positive curvature, facilitating the formation of a nanoemulsion. The phase inversion phenomenon can also be induced through the manipulation of various compositional parameters, including electrolyte concentration and pH.[110,117]

Phase inversion composition can regulate the droplet size distribution and produce stable nanoemulsions with droplet sizes as low as 50 nm. The nanoemulsions prepared using this method also require high surfactant concentrations.

The choice of formulation methods depends on factors such as the intended use, nature of excipients, desired properties and cost. A critical understanding of the benefits and limitations of the different methods is necessary for an informed decision.

3.4. Modifications of nanoemulsions for enhanced ocular drug delivery

The conventional nanoemulsions are relatively low viscosity nanosystems composed of an oily phase, aqueous phase, and surfactant, with or without an active pharmaceutical agent. The low viscosity of nanoemulsions can lead to reduced contact time with the ocular surface due to nasolacrimal drainage. Thus, different approaches have been employed to modify the conventional nanoemulsions to increase the bioavailability of intraocular drugs. These modified nanoemulsions include cationic and mucoadhesive nanoemulsions, nanoemulsion-based *in situ* gels, microneedles and nanoemulsion-laden contact lenses. They increase the bioavailability and therapeutic efficacy of drugs by prolonging the precorneal retention time and enhancing the corneal penetration.

3.4.1 Cationic nanoemulsions. Cationic nanoemulsions are composed of positively charged oil droplets evenly distributed in an aqueous continuous phase. The positively charged oil droplets result from the localisation of a cationic surfactant on the oily interface. This system offers great benefits in ocular delivery by enhancing its electrostatic interaction with the negatively charged ocular surface, prolonging the residence time, and reducing drug clearance from the ocular surface. The enhanced precorneal residence time resulting from the positive charge and the nanosize range of the nanoemulsion improves drug penetration across the cornea, conjunctiva and adjacent tissues, hence improving the bioavailability and therapeutic outcomes. In addition, cationic nanoemulsions exhibit enhanced physical stability given that the positively charged oil droplets repel each other and prevent coalescence.[124]

Many cationic compounds have been described in the literature for the formulation of nanoformulations, including quaternary ammonium compounds (*e.g.* benzalkonium chloride, cetylpyridinium, hexadecyltrimethylammonium bromide and polyquaternium), stearylamine, oleylamine, poly(L-lysine) and poly(ethylenimine).[125–127] Although some quaternary ammonium compounds such as benzalkonium chloride and cetalkonium chloride have passed the regulatory requirement for use in ophthalmic products, their use in ophthalmic preparations is still limited by the toxicity, stability and regulatory issues. Alternatively, due to their bactericidal property, they have been used as a preservative at very low concentrations in conventional eye drops. However, even at these low concentrations (0.02%), many researchers have reported serious adverse effects.[128] They are more toxic than anionic and non-ionic surfactants, and can damage the epithelial cells lining the ocular surface by disrupting the cell membranes.[129,130] Hence, employing them as surfactants to design cationic nanoemulsions is challenging. Nonetheless, their safety as cationic agents at low concentrations in nanoemulsions has been reported by many researchers. Several patents have also been granted regarding the safe use of these compounds in ophthalmology.[131]

Novasorb® is patented Santen Pharmaceutical technology based on a cationic nanoemulsion and has been used to produce marketed eye drops such as Ikervis® and Verkazia®. Ikervis®, a cationic nanoemulsion, was approved for the therapy of severe keratitis in adult patients with keratoconjunctivitis sicca. It consists of cyclosporine A dispersed in medium-chain triglycerides and emulsified using tyloxapol and poloxamer 188. Cetalkonium chloride, a derivative of benzalkonium chloride, is incorporated in the formulation to impart a positive charge to the nanoemulsions. As a result, there is an electrostatic interaction between the nanoemulsion and the mucins on the ocular surface. This interaction and the two-fold higher dose of Ikervis® explain the once daily dosing.[132] Verkazia® is an emulsion preparation with a similar composition to Ikervis®, given that it is composed of cyclosporine A, medium-chain triglycerides, cetalkonium chloride, glycerol, tyloxapol, and poloxamer 188.[133] Ikervis is used one drop, once daily at bedtime in adults with severe keratitis that has not improved despite treatment with tear substitutes. In contrast, Verkazia® is used to treat vernal keratoconjunctivitis in children and adolescents, and it is dosed one drop up to four times daily.

Despite the benefits of cationic nanoemulsions, the major challenge in their development is the choice of an appropriate cationic agent. An ideal agent should possess sufficient lipophilicity to be able to be localised exclusively in the oil phase with no free molecules in the aqueous phase. This localisation improves its zeta potential, safety and stability. Cationic lipids such as (*N*-(1-(2,3-dioleoyloxy)propyl)-*N*,*N*,*N* trimethylammonium) chloride (DOTAP) have also been investigated in this regard.[131] They possess a fatty acid chain and a positively charged polar group. However, these agents are chemically unstable and must be stored at a very low temperature.

3.4.2 Mucoadhesive nanoemulsions. Another strategy to improve the ocular bioavailability of topically administered drugs is the use of mucoadhesive polymers. Mucoadhesive nanoemulsions are formulated incorporating these polymers to prolong the contact time of the formulation on the precorneal area. These polymers, including chitosan, hyaluronic acid, cellulose derivatives, hypromellose, xanthan gum, gellan gum, polyacrylate and hydroxypropyl guar gum, adhere to the ocular surface and interact with mucin (viscoelastic gel on mucosal surfaces) on the precorneal surface *via* hydrogen, hydrophobic, ionic and other van der Waals interactions depending in the properties of the nanocarrier and the polymer.[134,135] The first step in mucoadhesion is the contact or wetting stage, whereby the polymer forms intimate contact with the mucous layer, spreading the formulation across the ocular surface. The consolidation phase follows and involves strengthening the polymer-mucin interaction through interpenetration of the polymer chain into the mucous layer and forming bonds.[134] Chitosan has been extensively investigated as a mucoadhesive agent in ophthalmic preparations owing to its biocompatibility, biodegradability and non-toxicity profile.[91,92,135] In addition, it has inherent wound healing and antimicrobial properties, which are desirable in treating ocular infections and diseases. The positively charged amino groups in chitosan interact electrostatically with the negatively charged sialic residues of mucins to exert its mucoadhesive effect. Also, chitosan acts through hydrogen bond formation.[136] Furthermore, chitosan enhances paracellular drug transport by reversibly disrupting tight junctions. In the first reported study on the use of cationic nanoemulsions for ocular tuberculosis, rifampicin-loaded nanoemulsions employing chitosan as a mucoadhesive agent were developed.[96] The chitosan-modified rifampicin nanoemulsions exhibited a zeta potential of +51.3 mV, enhancing their electrostatic interaction with the anionic charged ocular surface.[96] Similarly, Choudhari *et al.* investigated three types of ganciclovir microemulsions, *i.e.*, oil-in-water type (o/w), water-in-oil type (w/o), and chitosan-coated microemulsion. Notably, the chitosan-coated microemulsions had the highest corneal permeation through the excised goat cornea compared to the conventional ganciclovir solution. The improved performance of this system was attributed to the prolonged residence time of the colloidal system at the surface of the eyes and its nanoscale particle sizes.

It should be noted that adding mucoadhesive agents could inhibit the diffusion of the drug through the nanocarrier. Reports show that an ocular mucoadhesive effect may not significantly change the corneal permeability of the drug. Moxifloxacin mucoadhesive nanoemulsions prepared using hydroxypropyl methylcellulose showed sustained *in vitro* drug release but similar permeability and flux coefficient as commercial eye drops.[137] Ustundag-Okur *et al.* prepared ofloxacin microemulsions with and without 0.75% chitosan oligosaccharide lactate. Both formulations enhanced the precorneal residence time compared to the drug solution. However, the addition of chitosan resulted in no significant difference in the residence time between the two microemulsion formulations.[138] This contradicting finding may be attributed to the surprisingly nearly neutral charge of the chitosan microemulsions, resulting in less tendency of the formulation to interact electrostatically with the negatively charged ocular surface.

The drawbacks in the use of mucoadhesive agents are ocular irritation and poor solubility. Ocular irritation on the administration of mucoadhesive nanoemulsion induces blinking and rapid tear clearance, thereby reducing the amount that adheres to the ocular surface. The amine and hydroxyl groups in chitosan can be modified to improve the solubility and reduce the irritation caused by chitosan-based nanocarriers. Some approaches used to overcome this problem are the deacetylation and thiolation of polymers. Thiolation of chitosan enhances its solubility, mucoadhesion, and corneal permeation compared to its native form. Besides improving its biocompatibility, it mimic the action of mucin *via* the formation of disulfide bonds with cysteine groups to enhance its mucoadhesion. For instance, Lacrimera®, composed of chitosan with *N*-acetylcysteine thiol groups, significantly prolonged the contact time of the formulation even when administered once a day.[139]

Combining colloidal systems, such as nanoemulsions, and mucoadhesive polymers, enhances the precorneal retention time and drug penetration.

3.4.3 Nanoemulsion-based *in situ* gels. Nanoemulsion-based *in situ* gels are composite systems that combine nanoemulsions and *in situ* gels. These composite systems combine the advantages of nanoemulsions in improving the drug solubility and the prolonged retention property of gels. They are liquid preparations that undergo a phase transition to a gel when in contact with the ocular surface. This viscoelastic nature enables extended contact of the product with the conjunctiva, cornea and other adjacent ocular tissues, which is crucial for improved drug permeation through the cornea cells and tight junctions. Nanoemulsions of moxifloxacin, Acyclovir, fluconazole and other antimicrobial agents have been incorporated into *in situ* gels and shown potential in treating ocular infections.[140–142] Compared to the conventional nanoemulsions, *in situ* gels based on nanoemulsions offer sustained drug release given that the drug is gradually released from the gel over an extended period. Consequently, the frequency of administration is reduced, and patient compliance is increased. *In situ* gelling systems are formulated using polymers that respond to stimuli such as pH and temperature. Thermoresponsive *in situ* gelling systems are commonly used in ocular drug delivery systems. They are liquid below a certain temperature but change to a gel when the temperature is above the lower critical co-solute temperature. Mahboobian *et al.* formulated thermo-sensitive *in situ* gel nanoemulsions containing Acyclovir and evaluated their potential for ocular delivery.[142] Poloxamer 407 was employed as the thermoresponsive polymer to achieve a solution at room temperature and a gel at the gelation temperature of the ocular tissues. The optimised nanoemulsions exhibited a sustained release profile

compared to the standard solution used. Compared to the conventional Acyclovir solution, a 2.8-fold increase in drug permeation through the bovine corneal membrane was obtained.[142] The safety of these nanocomposite systems should be paramount and considered during development. A fluconazole nanoemulsion-based *in situ* gel composed of Cremophor RH40, polyglycerol, triacetin, poloxamer 407 and poloxamer 188 was reported to be non-toxic and non-irritating on human retinal pigment epithelial cells.[141] Likewise, the safety of a ketoconazole nanoemulsion-based *in situ* gel was confirmed through a human retinal pigment epithelial cell viability assay, hen's egg test on the chorioallantoic membrane (HET-CAM), and Draize test.[143]

Hence, the use of nanoemulsion-based *in situ* gels is an excellent and safe strategy for prolonging the drug residence time on the ocular surface and for sustained drug release. In addition, the gel matrix can act as a protective shell against environmental factors, including enzymes, thereby protecting sensitive drugs and preventing their premature degradation.

3.4.4 Nanoemulsion-based microneedles. Microneedles (MNs) are micro-technology (height ranging from 60–1000 μm) that is minimally invasive to ocular tissue. The application of microneedles on the ocular surface creates aqueous conduits on the cornea, overcoming the corneal barrier, and enhancing the intraocular bioavailability of drugs. The wound created by the insertion heals within 24 h, leaving no medical waste.[139] Initially, microneedles were developed as a highly efficient non-invasive option for transdermal delivery and local and systemic drug delivery. However, recently, they have been successfully used in topical ocular drug delivery *via* the cornea and sclera.[144–146] The benefits of microneedles include minimal invasiveness, painlessness, production of non-sharp waste, potential for self-administration, sustained release capability and enhanced bioavailability. Microneedles can be classified based on the type of material (silicon, ceramic, polymer or a combination of two or more of these materials), shape (cylinder, bullet, pyramid, and cone) and the mode of construction/action (solid, hollow, coated, dissolving and hydrogel).[147] Dissolving microneedles are preferred given that they are made from safe, biocompatible and biodegradable polymers that dissolve within a given time frame with no hazardous sharp waste. These polymers include polyvinyl alcohol, polyvinylpyrrolidone and polymethacrylate. Polymers such as polycaprolactone, polylactic acid and poly(lactic-*co*-glycolic acid) are used to form core–shell dissolving microneedles to encapsulate hydrophobic drugs that are challenging to encapsulate in the polymers mentioned earlier due to immiscibility issues.

Desirable effects were obtained from fluconazole microemulsion-based microneedles developed using polydimethylsiloxane (PDMS) for treating fungal keratitis. The dissolvable microneedles (dissolved within 3 min) consisted of outer polyvinyl alcohol polymer and inner fluconazole microemulsion layers, as shown in Fig. 4.[148] More studies on nanoemulsion-loaded microneedles in facilitating ocular drug penetration should be carried out, given that some studies have demonstrated the potential of microneedles in treating ocular infections such as keratitis.[149,150]

3.4.5 Nanoemulsion-laden contact lens. Medications can be incorporated in nanoformulations, and then loaded into contact lenses, allowing them to be gradually released directly on the cornea. These systems have been used in treating chronic eye diseases such as glaucoma.[151,152] Compared to traditional nanoemulsions, nanoemulsion-laden contact lenses

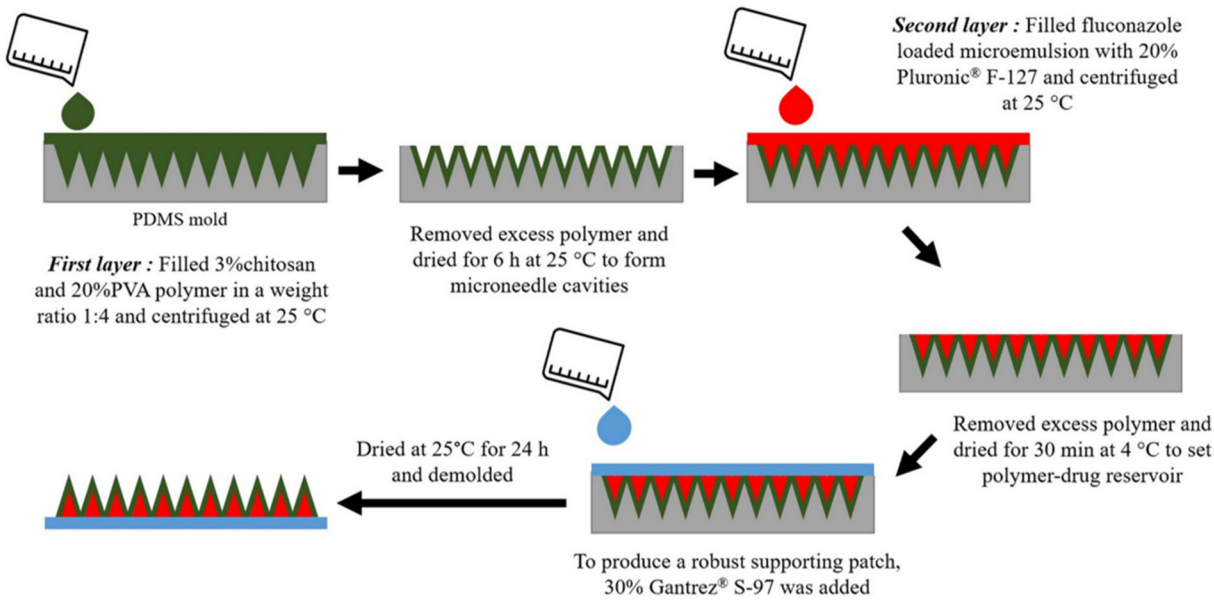

Fig. 4 Design of two-layered dissolving microneedles loaded with fluconazole microemulsion. This figure has been reproduced from ref. 148 with permission from MDPI, Copyright 2022.

offer better precorneal retention and sustained and controlled drug release, consequently reducing the frequency of administration and improving patient compliance.[153] In a recent randomised controlled trial study, drug-loaded contact lenses prolonged the contact of the drug on corneal lesions, enhancing corneal healing in patients with bacterial keratitis.[154] A less frequent regimen that will improve patient compliance may be achieved when the precorneal contact is enhanced. Also, there is potential for stimulus-responsive contact lenses to reduce drug leaching during manufacture and storage and to promote on-demand drug release.[155]

However, these systems are challenging to formulate given that factors related to the nature of the polymer, the drug and the method of drug loading into the lens need to be optimised. Most of the rigid lenses are produced from glass or polymers such as polymethyl methacrylate. In contrast, soft contact lenses are based on hydrogels such as poly(hydroxyethyl methacrylate) (HEMA) and silicone (polysiloxane). The swelling index, transmittance and oxygen permeability of the contact lens should be considered and optimised. The method of incorporating nanocarriers into the contact lens also influences the performance of the lenses. Drugs can be incorporated into contact lenses by *ex situ* or *in situ* approaches. In the *ex situ* method, the formulation is encapsulated, adsorbed or chemically conjugated to a preformed contact lens. In the *in situ* method, the drug is mixed with a base monomer solution (for the contact lens) during the polymerisation process. The soaking method, an *ex situ* approach that involves immersing the contact lens in the drug-loaded solution, is the most widely used. However, it has been reported to be more effective for loading drugs into contact lenses from emulsions than solutions. For instance, the traditional approach of soaking ofloxacin in contact lenses from the drug solution has shown limited drug uptake due to the poor solubility of ofloxacin in the contact lens matrix. However, ofloxacin was successfully incorporated into loaded contact lenses from microemulsion formulations with no significant reduction in transmittance values given that the drug was solubilised inside the microemulsion core.[156] Similarly, ofloxacin-loaded contact lenses exhibited enhanced drug release, which can be attributed to the increased and better partitioning of ofloxacin inside the contact lens matrix. Furthermore, there were no symptoms of ocular irritation in rabbit eyes on the instillation of the test extract from the ofloxacin microemulsion-loaded contact lens.[156]

The main limitation of the soaking method is the release kinetics characterised by a high initial burst release, resulting in the release of more than 90% of the drug loaded within a few hours.[153] This rapid drug release may be due to the adsorption of the drug on the lens surface and not deep inside the hydrogel matrix. To prolong drug release from contact lenses, newer loading methods (*in situ* methods) such as molecular imprinting and the supercritical fluid-assisted molecular imprinting methods are being developed. With the development of new loading methods, the utility of emulsion-laden contact lenses will be well appreciated.

4. Nanoemulsions for the treatment of ocular eye diseases

Nanoemulsions have been widely investigated as nanocarriers for the ocular delivery of antibacterial, antiviral and antifungal agents in treating eye infections, including conjunctivitis, keratitis and ocular tuberculosis. In this section, we review recent studies on the use of nanoemulsions in treating ocular eye diseases.

4.1 Nanoemulsions for the treatment of bacterial eye diseases

Based on evidence-based medicine, fluoroquinolones are commonly recommended as the drug of choice for the treatment of bacterial eye infections and perioperative prophylaxis in ophthalmic surgery. Therefore, scientists have made reasonable efforts to develop systems with improved precorneal retention time and permeability of fluoroquinolones to enhance the efficacy of drugs. For instance, ofloxacin microemulsion-loaded contact lens demonstrated potential in treating conjunctivitis in a *Staphylococcus aureus*-induced conjunctivitis rabbit model, given that there was improvement in the ocular inflammatory symptoms after a 4 day treatment.[156] Another study to investigate the prospects of besifloxacin nanoemulsions for treating bacterial keratitis showed that desirable antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* was achieved even when the dose was one-third of the dose of a commercial besifloxacin formulation.[157] Although the drug release from ofloxacin mucoadhesive microemulsions prepared with chitosan oligosaccharide lactate was delayed compared to chitosan-free microemulsions, superior antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* was obtained.[138] The superior antibacterial activity may be partly attributed to the bacteriostatic properties of chitosan oligosaccharide lactate. The broad-spectrum antibacterial activity of gatifloxacin microemulsion against *S. aureus*, *B. subtilis*, and *E. coli* was observed to be almost equipotent to that of a marketed gatifloxacin eye drop.[158] Compared with the conventional gatifloxacin eye drops, the o/w microemulsion system had greater corneal adherence and permeability in the anterior eye, increasing the drug concentration in ocular tissues. Moreover, the microemulsion was non-irritant and relatively safe for rabbit eyes.[158] Nanoemulsions have also been explored for the ocular delivery of moxifloxacin.[159,160] In one of the studies, mucoadhesive nanoemulsions developed using hydroxypropyl methylcellulose and polyvinylpyrrolidone had similar antimicrobial efficacy against *Staphylococcus aureus*-induced bacterial keratitis compared with the conventional eye drop.[160] The benefits of these systems lie in their sustained drug release, which may result in a reduced dosing frequency and increased patient compliance in treating bacterial eye diseases.

Several laboratory investigations have been performed on other classes of antibacterial agents for the treatment of eye infections. An entirely different but significant study was con-

ducted by Butt *et al.* Herein, the team explored the potential use of fatty acid-based microemulsions to treat ophthalmia neonatorum caused by *Neisseria gonorrhoeae* and *Staphylococcus aureus* infections.[161] The fatty acid α-linolenic acid, used as the lipid phase, has been reported by several studies to have activity against *N. gonorrhoeae* and *S. aureus*.[102,162] The formulations exhibited a robust inhibitory effect against *S. aureus* than *N. gonorrhoeae*.[161] In another broadened study by the same researchers, lauric acid, tridecanoic acid, myristoleic acid, palmitoleic acid, and α-linolenic acid were probed for their activity against *Staphylococcus aureus*-induced neonatal conjunctivitis.[163] The results showed that all the microemulsions prepared with the fatty acids have significant antimicrobial activity against *Staphylococcus aureus*. Rifampicin-loaded cationic nanoemulsions employing chitosan or polymyxin B as surface-modifying agents to enhance the ocular mucoadhesiveness showed antimicrobial activity against ocular infections caused by *Mycobacterium tuberculosis*.[96] However, surface modification with the cationic agents did not influence its antibacterial activity.

These findings (see Table 4) on the use of nanoemulsions and microemulsions in delivering antibacterial agents to the eye show a promising future in the treatment of bacterial eye diseases. However, there is a need for more *in vivo* studies on this to confirm the safety and efficacy of these systems.

4.2 Nanoemulsions for the treatment of fungal ocular infections

The major challenges in delivering antifungal agents are their insolubility and ocular toxicity. The use of nanoemulsions is one of the novel techniques that has been developed to overcome the difficulties of conventional ocular antifungal preparations. Ketoconazole and luliconazole nanoemulsions were non-irritant, non-toxic, and well-tolerated by rabbits.[168,171] Luliconazole nanoemulsions, which were optimised using the quality by design methodology, were transparent with monodispersed spherical droplets of size 18 nm. The transparency of the formulation is an indication of the improved solubility of the drug in the vehicle. Compared to the luliconazole suspension, the formulation exhibited better penetration into the intact cornea and conjunctiva as well as superior *in vitro* antifungal activity against *Fusarium* and *Aspergillus* isolates.[168] The *in vivo* irritancy test findings in rabbits showed no conjunctival discharge or oedema after applying the drug-loaded nanoemulsion to the eye (Fig. 5a). In addition, a slit-lamp microscope with cobalt blue light did not detect any epithelial damage (Fig. 5b), an indication that the formulation is non-irritating to the rabbit's eyes. Hence, the formulation could be a safe and effective option for treating fungal keratitis.

Voriconazole microemulsions also demonstrated improved efficacy, given that there was a significant improvement in the conjunctival redness of rabbit-induced ocular keratitis. The improved efficacy was attributed to the increase in residence time and drug concentration impacted by the microemulsion.[173] Fluconazole nanoemulsion-based *in situ* gels and microneedles were reported to be non-toxic and non-irritating on human retinal pigment epithelial cells.[141] Better antifungal activity against *C. albicans*-infected corneal tissue was achieved.[148]

The above-mentioned studies highlight the place of nanoemulsions and microemulsions in the quest to improve the therapeutic efficacy of antifungal ophthalmic preparations.

4.3 Nanoemulsions for the treatment of viral ocular infections

Herpes simplex keratitis and cytomegalovirus retinitis have become the most common viral infections diagnosed in people with non-congenital blindness. Recurrent and relapse of ocular viral infections have sparked several arguments among researchers regarding the cause of drug therapy failure in interventions. Only a few *ex vivo* studies have investigated the use of nanoemulsions as vehicles for the ocular delivery of antiviral agents. Nonetheless, the potential of delivering ganciclovir and Acyclovir in microemulsion/nanoemulsions for the treatment of viral eye diseases is encouraging. For example, chitosan-coated ganciclovir microemulsions enhanced corneal permeation through excised goat cornea compared to the conventional ganciclovir solution.[165] Similarly, optimised thermo-sensitive *in situ* gel nanoemulsions containing Acyclovir exhibited a sustained release profile compared to the standard solution used. Compared to the conventional Acyclovir solution, a 2.8-fold increase in drug permeation through the bovine corneal membrane was obtained.[142] Also, Acyclovir nanoemulsions showed no significant ocular irritation, sustained drug release profile and enhanced permeability through isolated bovine eyes.[166]

4.4 Nanoemulsions for the treatment of protozoan ocular infections

Protozoa are unicellular parasites known to cause certain ocular infections such as toxoplasmosis, toxocariasis, amoebiasis, and leishmaniasis. There is a paucity of literature on the use of nanomedicines in ocular parasitic disease; however, some researchers have reported the potential use of nanoemulsions in treating ocular *Acanthamoeba keratitis*. A study demonstrated the possible use of nanoemulsions of coumarin extracted from *Pterocaulon balansae* (Asteraceae) in treating ocular keratitis caused by *Acanthamoeba castellanii*. The formulation was reported to exhibit significant amoebicidal activity similar to chlorhexidine against *Acanthamoeba*, given that there was a 95% reduction in trophozoite viability when incubated with the coumarin nanoemulsions.[172] Furthermore, microemulsions loaded with phytosteryl glycoside significantly inhibited *Acanthamoeba* spp. and were reported to show optimal physicochemical properties.[174] More *in vivo* studies need to be done to explore the benefits of this nanocarrier in treating parasitic ocular diseases.

5. Challenges, advances and prospects

As carriers for ocular anti-infectives, nanoemulsions face some challenges that hinder their clinical translation. These challenges, including toxicity, ocular irritation, blurred vision, low

Table 4 Recent studies on the application of nanoemulsions and microemulsions for the delivery of anti-infectives to the eye

Drug/preparation	Major excipients	Formulation technique	Major outcomes	Ref.
Gatifloxacin microemulsion	Isopropyl myristate, Tween 80, Transcutol-P	Spontaneous emulsification	A stable microemulsion system with no ocular irritancy. Good corneal adherence and permeability. Enhanced drug concentration. Equipotent *in vitro* antibacterial activity was similar to that of the standard eye drop solution.	158
Ofloxacin microemulsion-laden contact lens	Glyceryl monocaprate, Pluronic F68, PEG 200,	Microemulsion or high shear homogenisation methods	No symptoms of ocular irritation. Enhanced drug release due to the slow migration of ofloxacin. Improvement in the ocular inflammatory symptoms of conjunctivitis in *S. aureus*-induced conjunctivitis rabbit model.	156
Ofloxacin chitosan-modified microemulsion	Oleic acid, Tween 80, ethanol, chitosan oligosaccharide lactate	Microemulsion or high shear homogenisation methods	Zeta potential of −0.56 mV. No significant ocular irritancy. Better antibacterial activity. Compared with the conventional eye solution, enhanced preocular residence time from both chitosan-free microemulsions and the chitosan microemulsion was obtained. No significant difference in retention time in the use of chitosan.	138
Besifloxacin microemulsion	Triacetin, Cremophor® (RH 40), Transcutol® (P)	Low-energy emulsification method	Non-irritating ophthalmic formulation. Sustained drug release and higher corneal permeability. Comparative *in vitro* antibacterial activity with a higher dose drug suspension.	157
Moxifloxacin microemulsion	Ethyl oleate, Tween 80, Soluphor P	Phase titration method	Non-irritant for ocular delivery. Steady drug release for more than an hour. High permeation rate. Preservation of the inherent bactericidal effect of the incorporated moxifloxacin in the formulation.	159
Moxifloxacin mucoadhesive nanoemulsion	Oleic acid, Tween® 80, glycerin, hydroxypropyl methylcellulose, polyvinylpyrrolidone	Hot homogenisation coupled with probe sonication technique	Zeta potential of >−30 mV. No ocular irritation. Sustained drug penetration into the intraocular tissues. Similar *in vivo* antibacterial efficacy to the conventional eye drops against *Staphylococcus aureus*-induced bacterial keratitis.	160
Azithromycin microemulsion	Oleic acid, Transcutol-P, Tween 80, propylene glycol	Phase titration	No ocular irritation. Stable for six months. Enhanced corneal absorption of azithromycin through rabbit cornea.	164
Rifampicin cationic nanoemulsion	Oleic acid, polysorbate 80, poloxamer 188, chitosan chloride and polymyxin B	High-pressure homogenisation method	No significant eye irritation. Chitosan and polymyxin nanoemulsions exhibited particle sizes of approximately 150 nm and zeta potential values of +51.3 mV and +5.5 mV, respectively. Addition of cationic agents did not influence the *in vivo* antimicrobial activity of rifampicin against ocular infection induced by *Mycobacterium tuberculosis*.	96
Fatty acid microemulsions	α-Linolenic acid, Tween 80, Cremophor EL, Transcutol-P	Phase titration by the microplate dilution method.	Stable for up to 8 weeks. Strong *in vitro* activity against *S. aureus* and *N. gonorrhoeae*. Not a strong ocular irritant.	163
Ganciclovir mucoadhesive microemulsions	Capmul MCM EP, Labrasol, Transcutol® P, chitosan	Phase titration or the spontaneous emulsification method	Stable, transparent and homogenous preparations. Tolerance to ocular tissues. Sustained *in vitro* drug release pattern. Enhanced corneal permeation through the excised goat cornea.	165

Table 4 *(Contd.)*

Drug/preparation	Major excipients	Formulation technique	Major outcomes	Ref.
Acyclovir nanoemulsions	Triacetin, Tween 20, Transcutol® P	Low energy emulsification method	Homogenous and stable formulation with droplet size less than 15 nm. Transient turbidity at low temperature. No significant ocular irritation, as shown by the results of HET-CAM and modified Draize test. Sustained drug release profile compared to the conventional eye preparation. Approximately 3-fold enhanced permeability through an excised bovine cornea.	166
Acyclovir thermosensitive *in situ* gel nanoemulsions	Triacetin, Poloxamer 407, Transcutol® P, Poloxamer188	Low-energy emulsification method	Sustained *in vitro* drug release pH, viscosity, osmolality, and refractive index were within acceptable limits for ocular administration. Enhanced permeation through excised goat cornea compared to the marketed ointment. No irritation potential from HET-CAM and modified Draize tests. No ocular swelling or erythema in the experimental rats Histopathological studies using isolated rat corneas showed no ocular tissue damage. Enhanced drug deposition on the cornea and corneal permeation in isolated rat eyes.	142
Fluconazole nanoemulsion *in situ* gel	Poloxamer 407, Poloxamer188, Cremophor RH40, propylene glycol, and Triacetin	Cold low-energy method	0.1% and 0.5% fluconazole gel was not toxic to the retinal pigment epithelium. Non-irritant and safe for ocular use based on HET-CAM and Draize rabbit tests.	141
Posaconazole nanoemulsion	Isopropyl myristate oil, Labrasol, Transcutol P	Pseudo-ternary phase diagram/water titration approach	Transparent formulation with almost 100% transmittance. Osmolality of 298–300 mOsm per kg A 2.5-fold enhancement in POZ permeability coefficient compared to the drug suspension. Superior *in vitro* activity against *Candida albicans* and *A. niger*, compared to free nanoemulsions and drug suspension.	167
Luliconazole nanoemulsion	Oleic acid, propylene glycol dicaprylate, castor oil, Cremophor®, Kolliphor® (HS15), Tween 80, Capryol 90,	—	Spherical, monodispersed with a droplet size of about 18.43 nm. Significantly improved *in vitro* antifungal activity compared to the drug suspension. Good ocular tolerability in rabbits. Increased penetration into the intact cornea and conjunctiva.	168
Clotrimazole mucoadhesive microemulsion	Oleic acid, Transcutol HP, low molecular weight chitosan, Cremophor EL		Superior *in vivo* antifungal activity on rabbits compared to uncoated formulations. Safe for ocular delivery confirmed by the *in vivo* ocular tolerance and histopathological studies.	169
Itraconazole nanoemulsion	Benzyl benzoate, Eumulgin CO40, and propylene glycol	Spontaneous emulsification method	A 7-fold increase *in vitro* drug release after 24 h compared to the aqueous suspension. Stable formulation when subjected to centrifuge test, three cycles of freeze–thaw test, and six cycles of the heating–cooling test. Stabilisation was achieved through steric repulsions, given that the zeta potential of the optimised formulations was less than −10 mV.	90
Fluconazole microemulsion	Isopropyl myristate, polyethylene glycol 400, Tween 80		Particle size ranged from 57–60 nm, while zeta potential was in the range of −20 to −32 mV. Sustained drug release up to 12 h. Significant improvement of ocular bioavailability ($p < 0.0001$) from optimized formulation composed of 45% w/w isopropyl myristate and 40% w/w of 3 : 1 Tween® 80-PEG 400 mixture.	170

Table 4 *(Contd.)*

Drug/preparation	Major excipients	Formulation technique	Major outcomes	Ref.
Ketoconazole nanoemulsion-based *in situ* gel	PEG 400, triacetin, Cremophor RH40, Poloxamer 407, Poloxamer 188.	—	Optimal physicochemical properties-globule size of 24.44 nm, PDI of 0.249, refractive index of 1.372, pH of 5, and viscosity of 186.26 mPa s. More than 80% RPE cell viability 24 h after incubation with 0.1% ketoconazole nanoemulsions *in situ* gel HET-CAM score of 0.33 against 17.33 of the positive control, and thus non-irritant. Similar irritancy score from the modified Draize test.	171
Fluconazole microemulsion-based microneedles	Eugenol, tween 80, polyethylene glycol 400, chitosan and polyvinyl alcohol.		Optimal physical properties, stability and improved fluconazole permeation (1.57% ± 0.22%) with a particle size and drug content of 121.22 ± 9.01 nm and 73.58 ± 0.54 mg mL^{-1}, respectively. Significantly higher ocular delivery of optimised formulation compared to fluconazole-loaded microneedle suspension. Improved *in vitro* fungal activity with a 61.96% ± 5.80% zone of inhibition.	148
Coumarin nanoemulsion	Egg lecithin, medium chain triglycerides and ethanol.		Particle size, zeta potential, PDI and drug content of 276 ± 54 nm, −21.5 ± 5.9 mV, 0.215 ± 0.09 and 0.705 ± 0.037 mg mL^{-1}, respectively. 95% reduction of trophozoite viability after 24 h of incubation with the nanoemulsions. Significant *in vitro* amoebicidal activity was comparable with chlorhexidine.	172

viscosity and instability, must be overcome to move more of these systems into clinical use. Preservatives and surfactants, especially cationic surfactants, are responsible for the ocular toxicity and irritation observed with most ophthalmic nanoemulsions. Benzalkonium chloride is a widely used preservative in eye drops, but has been reported to be cytotoxic to ocular tissues.[175] The cell viability of human epithelial conjunctival cells after 60 min incubation with latanoprost eye drops containing 0.025% benzalkonium chloride was reduced to 30%.[176] Hence, recent studies have investigated the use and design of preservative-free nanoemulsions. For instance, Cationorm®, manufactured by Santen, is a preservative-free emulsion designed for improved comfort and to minimise blurring in patients suffering from dry eye disease and ocular allergy symptoms. It can be used for up to three months after first opening. A similar strategy should be employed for nanoemulsions targeted for ocular infections. Surfactants have also been reported to disrupt the tear film and penetrate the epithelial cell membrane, thereby damaging the ocular tissues. Surfactants can be replaced with other classes of stabilisers, such as polymers or colloidal particles, resulting in Pickering nanoemulsions. Pickering nanoemulsions have attracted great attention since their introduction in 2012. Conversely, cyclodextrins, which are cyclic oligosaccharides derived from starch containing several (α-1,4)-glucopyranose units, have been used by some researchers to solubilise hydrophobic drugs instead of using surfactants.[177] Surfactant-free amphotericin B Pickering nanoemulsion stabilised with cyclodextrin exhibited excellent stability.[178] PFSF-LAT, a new preservative-free, surfactant-free latanoprost formulation, showed an improved tolerance profile in treating patients with glaucoma in a recent retrospective, multicentre, observational study.[179] In another study, a novel Pickering nanoemulsions stabilised with hydroxyl-functionalised diblock copolymer nanoparticles exhibited only mild toxicity in a planaria model.[180] Formulators and researchers should focus their attention on the development of preservative and surfactant-free nanoemulsions to reduce their ocular toxicity and promote their clinical use in infectious eye diseases.

Nanoemulsions also face instability issues, including creaming or sedimentation, flocculation, Ostwald ripening, and coalescence. The droplet size plays a great role in emulsion stabilisation. In addition, the nature and concentration of the oil phase, emulsifiers and preservatives influence the stability of these systems. Hence, these excipients should be optimised using a quality by design approach to achieve the critical quality attributes of the product. Adequate measures should be taken to control the particle size distribution to prevent Ostwald ripening and loss of transparency in the system. A recent study explored the effect of the number of cycles, pressure and temperature on the size and droplet distribution of a fluconazole nanoemulsion using the high-pressure homogenisation technique.[181] The stepwise process of formulation optimisation and subsequent characterisation of the nanoemulsion are illustrated in Fig. 6. The formulation and process variables usually optimised during the preparation of nanoemulsions include concentration and ratio of surfactant and oil; order, speed and time of mixing; formu-

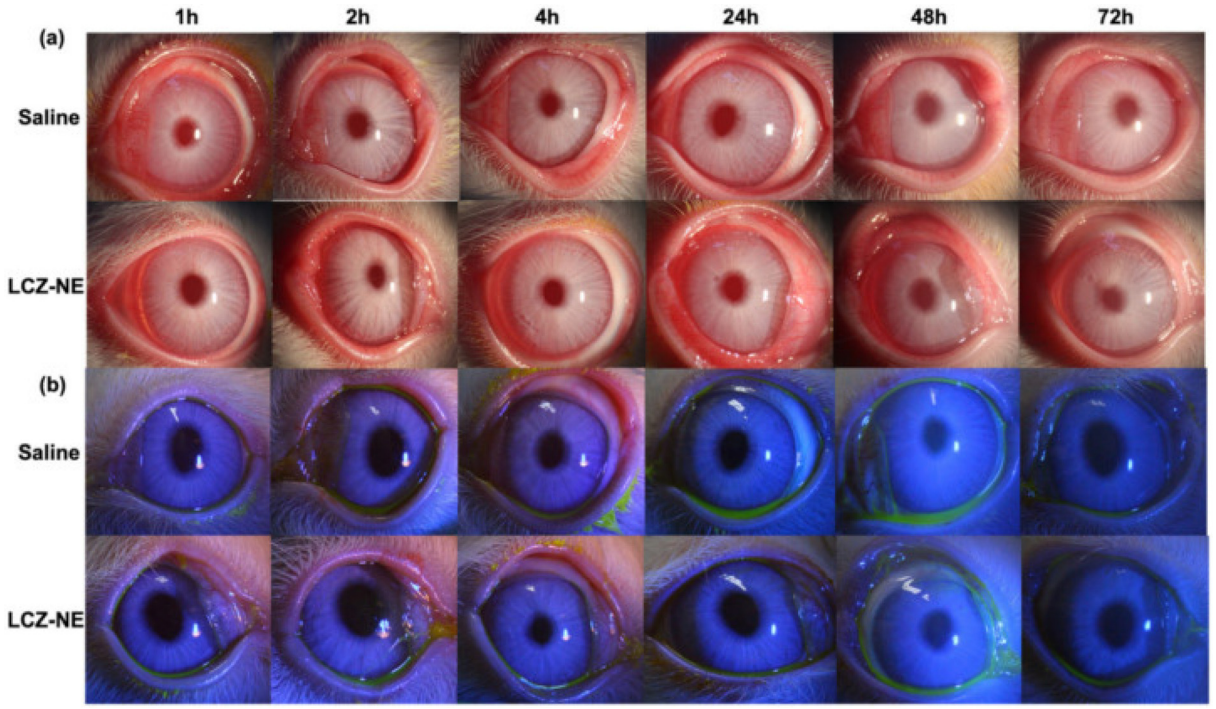

Fig. 5 Slit-lamp microscopy of rabbit eyes after one instillation of the luliconazole nanoemulsion and saline (as control). (a) Pictures with visible light and (b) cobalt blue light post dropping fluorescein-sodium into rabbit eyes. This figure has been reproduced from ref. 168 with permission from MDPI, Copyright 2022.

lation temperature, pressure and number of passes through the homogenisation chamber.[182] In addition, costly equipment such as homogenisers, ultrasonicators and microfluidisers is required to produce stable products. More efforts should be devoted to the optimisation of nanoemulsions to enhance their stability, scalability and clinical potential in treating infectious eye diseases.

New technologies such as Novasorb® are being developed to enhance the ocular retention and corneal penetration of drugs.[131] IMPACT-SVT® technology by Salvat Laboratories improves the mucoadhesion and drug permeability and was used to develop SVT-15473, a clobetasol propionate nanoemulsion, which is currently under clinical trial.[183] Ocugen's patented OcuNanoE™ technology is another technology developed to enhance the ocular retention time of drugs. It has also been employed in the development of ophthalmic nanoemulsions such as Ocu300 and Ocu310, which are under clinical trial.[184] In addition, the use of penetration-enhancing agents or technologies such as dissolvable microneedles should be given serious attention.

The issue of antimicrobial resistance is a global challenge in infectious disease therapy. Phage therapy, the use of viruses that kill bacterial cells, is a promising alternative or adjunct to conventional antibiotics in the fight against resistant organisms.[185] The findings from a recent study on the enhanced efficacy of bacteriophage-based eye drops against multidrug-resistant *Pseudomonas aeruginosa* (a major causative agent of refractory bacterial keratitis) are encouraging.[186] The incorporation of phages in nanoemulsions has also been reported to increase their structural stability and antibacterial activity.[187,188] Hence, researchers should investigate the use of nanoemulsions loaded with bacteriophages alone or in combination with conventional antibiotics for treating infectious ocular diseases, especially those caused by resistant organisms. Vaccination is an effective platform for prophylaxis of infectious diseases. Interestingly, nanoemulsions loaded with *Leishmania* peptides successfully delivered the antigens in an experimental murine model.[189] Hence, the use of nanoemulsions as antigen carriers or as vaccine adjuvants should be probed.

Despite these recent technologies and advances, there is still a paucity of clinical studies on nanoemulsions loaded with antimicrobial agents. The only approved nanoemulsions for treating infectious ocular diseases are Durezol®, approved in 2008 for the treatment of anterior uveitis; Ikervis®, approved by the European Medicines Agency in 2015 for the therapy of severe keratitis in adult patients with keratoconjunctivitis sicca; and Verkazia®, approved in 2021 by the FDA to treat Vernal keratoconjunctivitis in children and adolescents.[133,190,191] It is of great concern that no approved ophthalmic nanoemulsions are loaded with antimicrobial agents.

However, with more research, technological advancement, and collaboration among stakeholders, there are prospects for nanoemulsions to revolutionise the treatment of ocular infectious diseases and address the global issues of avoidable vision impairment and blindness.

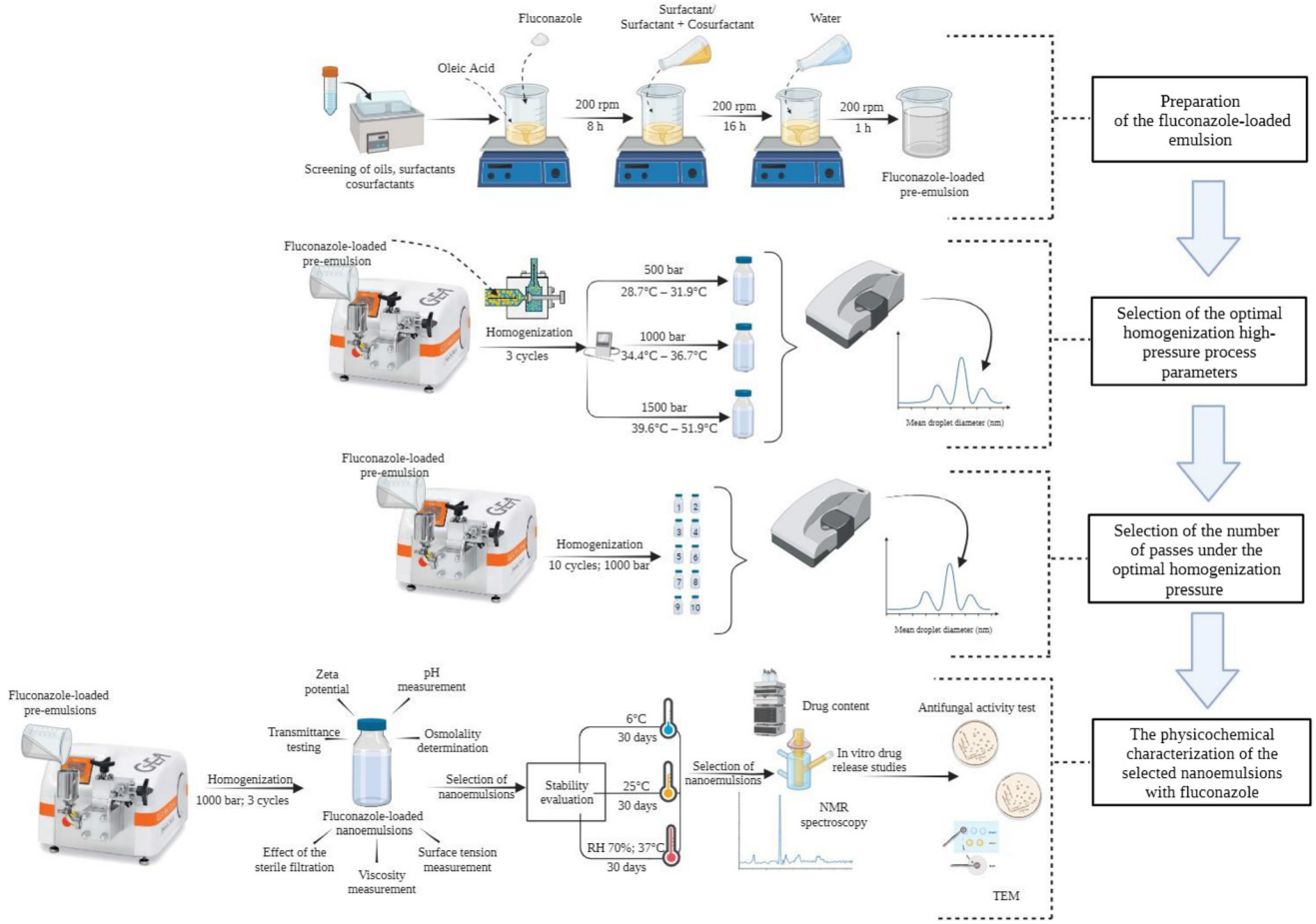

Fig. 6 Schematic of the optimisation of process parameters in high-pressure homogenization. This figure has been reproduced from ref. 181 with permission from MDPI, Copyright 2023.

6. Conclusion

Infectious eye diseases are a global health problem given that they can lead to vision impairment and blindness, compromising the quality of life. Analysis of recent studies on the use of nanoemulsions in delivering anti-infective agents to the eye shows advancement in this area. Significant progress has been made in the use of cationic and mucoadhesive nanoemulsions to enhance the precorneal retention. In addition, the development of nanoemulsion-laden contact lenses, *in situ* gels and microneedles has yielded excellent results in treating ocular infections. Consequently, there has been an improvement in the ocular bioavailability of anti-infectives, resulting in enhanced antimicrobial effects, less frequent regimens, enhanced patient compliance and therapeutic outcomes. Hence, nanoemulsions can potentially reduce the incidence of infection-induced vision impairment and blindness. However, more preclinical and clinical studies should be carried out to confirm the safety and efficacy of these nanosystems.

Conflicts of interest

The authors declare no competing interests.

Data availability

No primary research results, software or code have been included, and no new data were generated or analysed as part of this review.

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