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Preservatives in Ophthalmic Products: A Review

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Abstract: Preservatives are commonly found in many multi-dose ophthalmic products to maintain the sterility of the solution against bacteria and fungi. However, research indicates that these preservatives can have detrimental effects on the eye surface, especially with prolonged use. Benzalkonium chloride, a commonly used preservative, serves various functions, such as cleaning, disinfecting, and killing bacteria and fungi. However, its application on the eye surface can have significant consequences over time. It disrupts the tear film, promotes cell death, and triggers inflammation. Continuous use of eye drops containing preservatives can lead to damage to both superficial structures like the conjunctiva and cornea, as well as deeper structures including the trabecular meshwork and lens. Milder ocular symptoms include sensations of discomfort or irritation, such as feeling like there is a foreign object in the eye, tingling, burning, or dryness. On the other hand, more severe side effects may include inflammation, which can range from minor subclinical reactions to the development of fibrosis, increasing the risk of complications, especially during glaucoma surgery. To mitigate these risks, it is advisable to minimize the frequency of preserved eye drop use and preferably opt for preservative-free eye drops whenever possible.

Keywords: Preservatives, Multi-dose eye drops, Benzalkonium chloride, Ocular surface toxicity, Ocular inflammation, Conjunctiva, Cornea

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Introduction

Ophthalmic preparations encompass sterile liquid, semi-solid, or solid formulations intended for application onto the eyeball and/or conjunctiva or insertion into the conjunctival sac. These include categories such as eye drops, solutions for ophthalmic washing, powders for eye drops and washing solutions, semi-solid ophthalmic preparations, and ophthalmic inserts. Ophthalmic preparations are formulated using appropriate products and methods to ensure sterility and prevent contamination and microbial growth (Bonnard *et al.*, 2005). Ophthalmic medications hold a unique position due to their method of administration and formulation. Some examples of

Table 1: Some ophthalmic products available in Indian market

Drug category	Drug	Marketed product in India
Antibiotic	Moxifloxacin	Moxeza, Vigamox
Antibiotic	Tobramycin	Toba, Tobrex
Anti-inflammatory (Steroidal)	Prednisolone acetate	Predmet, Omnipred
Anti-inflammatory (Non-Steroidal)	Ketorolac tromethamine	Acular, Ketoflam
Anti-allergic	Olopatadine	Patanol, Olopat
Lubricant	Carboxymethyl cellulose	Refresh Tears, Lubricant Eye Drops IP
Anti-glaucoma (Beta-blocker)	Timolol	Timoptic, Ciplox-TZ
Anti-glaucoma (Prostaglandin analog)	Latanoprost	Xalatan, Latoprost
Mydriatic	Tropicamide	Tropicacyl, Mydrin-P
Decongestant	Naphazoline	Naphcon-A, Nap Drops

marketed ophthalmic preparations with drug category are shown in Table 1. They are designed to be instilled directly into the eye or applied to the eyelids, necessitating specific adaptations. These formulations typically include solutions, suspensions, and occasionally creams or ointments, packaged in appropriate containers like glass or plastic bottles or aluminium tubes (Baudouin *et al.*, 2010). The composition of these formulations must be compatible with the eye's mucous membrane, ensuring suitable pH and osmolarity. Additionally, the products must be free from any foreign particles that could potentially irritate or harm the eyes during administration. Regardless of the duration of treatment, the presence of water in ophthalmic preparations makes them highly susceptible to microbial contamination, including both bacteria and fungi (Ioannou *et al.*, 2007).

To address this issue, many ophthalmic preparations contain preservatives. However, even with preservatives, microbial contamination can still occur, albeit at reduced rates. To mitigate this risk, manufacturers have implemented automatic limits on the duration of use after opening, typically set at 15 or 30 days. Despite these precautions, frequent use of preserved eye drops can lead to well-documented consequences, including the development of hypersensitivity to preservatives, particularly in predisposed individuals (Labbe *et al.*, 2006). Frequent use of preservatives can weaken the ocular surface, potentially leading to the development of chronic inflammatory conditions over time. This may manifest as various clinical symptoms, including itching, burning sensations, feelings of grittiness, conjunctival redness, and pain, indicative of conjunctivitis or keratoconjunctivitis. This review consolidates and presents the current understanding regarding preservatives utilized in eye drops, with a primary focus on benzalkonium chloride (BAC). Initially, it will outline the several types of preservatives used in ophthalmic preparations and their modes of action.

Classification of preservatives and mode of action

Preservatives found in ophthalmic preparations vary based on their physicochemical properties, compatibility with other ingredients in eye drops, spectrum of activity, bacteriostatic or bactericidal efficacy, potency against pathogenic species, ocular toxicity, and allergenic potential. These preservatives encompass quaternary ammonium compounds, organomercurial derivatives, amidines, alcohols, and oxychloride complexes.

Quaternary ammoniums

Quaternary ammonium salts are chemical compounds typically characterized by a nitrogen atom surrounded by four groups, each containing between eight and thirty-five carbon atoms.

Among these compounds, benzalkonium chloride (BAC) is the most utilized in ophthalmic preparations. Other examples found in such preparations include cetrimide (cetrimonium bromide), benzododecinium bromide, and cetylpyridinium chloride. These compounds are amphiphilic, meaning they are both water-soluble and possess surfactant properties. Their primary mode of action involves acting as powerful detergents, which lead to the dissolution of bacterial cell walls and membranes. This process disrupts the semi-permeable layer of the cytoplasm, resulting in the release of intracellular contents. Their bactericidal effectiveness is rapid and becomes more potent at 37°C in an alkaline environment. Typically, benzalkonium chloride (BAC) is employed at concentrations ranging between 0.004% and 0.02%. Its primary action targets Gram-positive bacteria, such as *Staphylococcus*, even at exceptionally low concentrations. When combined with EDTA at 0.1%, its efficacy against Gram-negative bacteria like *Pseudomonas aeruginosa* is enhanced. Quaternary ammonium compounds also exhibit strong antifungal properties, particularly against *Candida albicans* and *Aspergillus fumigatus.*

Amidines

The primary agent utilized is chlorhexidine, a cationic compound belonging to the bis-diguanide family. It is typically employed in the form of water-soluble di-gluconate and demonstrates effectiveness in neutral or slightly alkaline environments (pH 8). Chlorhexidine gluconate's activity can be compromised by soaps, detergents, and anionic compounds, which nullify its action. Its mode of action involves disrupting the semi-permeable layer of cytoplasmic membranes, inhibiting transmembrane transport of cations, hydrolysis of membrane ATP, and delaying bacterial spore germination. Its antimicrobial properties are effective against Gram-positive cocci and bacilli, as well as certain Gram-negative bacteria. However, it is noteworthy that some bacteria, such as *Serratia*, *Proteus*, and *Pseudomonas*, exhibit greater resistance (Darbre *et al.*, 2004).

Alcohols

Only chlorobutanol and phenyl ethanol are employed as preservatives in ophthalmic preparations. Chlorobutanol functions by enhancing lipid solubility, allowing it to penetrate the bacterial lipid layer and exert its antimicrobial effects. Typically used at concentrations ranging from 0.2% to 0.5%, it demonstrates bacteriostatic and antifungal properties. It effectively targets both Gram-positive and Gram-negative bacteria (including *Pseudomonas aeruginosa*) as well as *Candida albicans*. On the other hand, phenyl ethanol exhibits low activity on its own but contributes synergistically when combined with other preservatives such as chlorobutanol, benzalkonium chloride (BAC), or chlorhexidine (Darbre and Harvey, 2008).

Parabens

Parabens are derivatives of para-hydroxybenzoic acid, with variants including methylparaben (E218), ethylparaben (E214), propylparaben (E216), isopropylparaben, butylparaben, and isobutylparaben. They exhibit greater efficacy against molds or fungi compared to bacteria, particularly targeting Gram-positive bacteria. Parabens are utilized in over 450 cosmetic products such as skincare creams, toothpaste, shampoos, cleansing milks, foundations, mascaras, and shaving foams.

Oxychloride complexes

More recently utilized oxidizing preservatives, such as stabilized oxychlorinated complexes, consist of small molecules that easily penetrate cell membranes and disrupt cellular functions by altering lipids, proteins, or DNA. These complexes primarily comprise chlorite ($NaClO_2$, Purite®), along with a small proportion of chlorates and traces of chlorine. Chlorite exerts its action by inducing potent oxidation of glutathione, thereby reducing cellular defenses against oxidative stress. Consequently, it exhibits effectiveness against

species with low levels of glutathione, such as *Staphylococcus aureus*. However, it demonstrates lower efficacy against *Pseudomonas aeruginosa*, *Candida albicans*, and *Alternaria alternate* (Handa *et al.*, 2006).

Sodium perborate

Sodium perborate was among the initial oxidant-type preservatives incorporated into eye drops. It disrupts bacterial protein synthesis by oxidizing their membranes, thereby inhibiting associated enzymes. In an aqueous environment, it undergoes transformation into water, oxygen, and hydrogen peroxide (H_2O_2), effectively eliminating microbes, including *Aspergillus niger* (Ingram *et al.*, 2004).

Regulation of preservatives in ophthamology

The Pharmacopoeia advises the inclusion of an antimicrobial agent (preservative) in eye drops to prevent or minimize microbial growth, which could lead to infection risk for the patient and deterioration of the product after the bottle is opened. Contamination of eye drops commonly arises from hand contact during handling or from direct contact if the dropper tip comes into contact with the eyelids, eyelashes, conjunctiva, or tears (Kaur *et al.*, 2009). Significant advancements in preservative development occurred from 1960 onwards, with the requirement for an antimicrobial agent in all multidose ophthalmic preparations being established in the 1970s. An "ideal" antimicrobial agent should possess the following characteristics: a broad spectrum of activity, either bactericidal or bacteriostatic, non-irritating or toxic to ocular tissues, compatible with both the constituents of the preparation and the container, and stable and heat resistant (Chibret, 1997).

Guidelines for this purpose are provided in the "Methods for preparing sterile products" section of the European Pharmacopoeia. When manufacturing ophthalmic preparations containing dispersed particles, precautions are taken to control particle size effectively and ensure suitability for the intended use of the product. Unless specified otherwise, ophthalmic preparations must be stored in a sterile, leak-proof container with a tamper-proof closure. The labeling of ophthalmic preparations must include the name of any antimicrobial preservative added. According to the European Pharmacopoeia, lyre collars, ophthalmic washing solutions, powders for eye drops, and powders for ophthalmic washing solutions may contain excipients intended to adjust osmotic power or viscosity, stabilize pH, increase solubility of active substances, or stabilize the preparation (Debbasch *et al.*, 2001). Multidose containers of lyre collars and ophthalmic washing solutions are equipped with an appropriate antimicrobial preservative unless the preparation itself possesses adequate antimicrobial properties. Multi-dose containers are specifically designed to dispense multiple doses of the solution they contain. When eye drops or ophthalmic washing solutions are prescribed without antimicrobial preservatives, they are preferably packaged in single-dose containers whenever feasible.

Preservation effectiveness test

The regulatory efficacy test involves deliberately contaminating the preparation, preferably in its final container, with a controlled inoculum of microorganisms. The preparation is then kept at a specified temperature, and samples are periodically taken from the container to enumerate the organisms present. Adequate preservation properties are indicated if, during the test, there is a notable decrease or absence of increase in the microbial count in the inoculated preparation (Fleurette *et al.*, 1996). Acceptance criteria regarding the reduction in microbial count over time vary depending on the category of preparation and the level of protection desired. The test microorganisms include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus niger*, each represented by unique strains. Additional strains or species that could potentially contaminate the preparation may be included, as necessary (Ferk *et al.*, 2007).

Physical properties of benzalkonium chloride

Benzalkonium chloride (BAC) can manifest in various forms, such as amorphous powder, gelatinous flakes, or a thick white to yellowish gel, displaying high hygroscopicity and emitting a mildly aromatic odor. Typically, it is supplied in a 50% aqueous solution or an 80% hydroalcoholic solution (either isopropanol/water or sometimes ethanol/water), as manufactured by the industry. BAC is highly soluble in water, alcohol, and acetone; moderately soluble in benzene; and insoluble in ether. Its aqueous solutions demonstrate low surface tension, possessing detergent and emulsifying properties, and tend to foam vigorously when agitated.

Pharmacological properties of benzalkonium chloride

Benzalkonium chloride (BAC) serves as an antiseptic, emulsifier, spermicide, and virucide. Additionally, BAC acts as a surfactant, altering the surface tension between two interfaces, which refers to the tension existing at the boundary of two media. Surfactants are amphiphilic molecules, meaning they possess two parts with different polarities: one lipophilic, capable of mixing with oil and nonpolar substances, and the other hydrophilic, capable of mixing with water and polar substances. The critical micellar concentration of BAC is 0.02%, defined as the concentration of the surfactant in solution above which some dispersed molecules aggregate to form micelles.

Mechanism of action of benzalkonium chloride

Benzalkonium chloride (BAC) exerts its antibacterial effects through multiple mechanisms. Firstly, it induces denaturation of bacterial proteins or enzymes, leading to their solubilization and depolymerization. This process results in the inactivation of enzymes involved in respiration and glycolysis, as well as dehydrogenases. Initially, this enzymatic inactivation may be reversible, but with prolonged exposure, it becomes irreversible. BAC can also bind to ribosomes, halting protein synthesis. Additionally, it can disrupt the bacterial cell membrane, leading to osmotic imbalances and cell lysis (Verin *et al.*, 1992). BAC adsorbs onto the surface of negatively charged cells, neutralizing them and altering membrane permeability, eventually causing membrane lesions. Physiologically, these effects include decreased mitotic activity and cell migration, resulting in delayed wound healing, as well as alterations in electrical potential and increased cell lysis. Benzalkonium chloride (BAC) is a bacteriostatic cationic surfactant that exhibits greater activity against Gram-positive bacteria compared to Gram-negative bacteria. It shows no activity against mycobacteria, has weak fungistatic properties, and demonstrates virucidal effects. The effectiveness of its antimicrobial activity varies depending on environmental conditions, with factors such as alkaline pH and a temperature of 37°C favoring its efficacy. However, its activity can be diminished by anionic compounds like soaps, hard water, organic matter such as blood or pus, and certain non-ionic compounds (Burstein,1980).

Pharmacokinetics

Benzalkonium chloride (BAC) is rapidly absorbed in lesser amounts by the gastrointestinal tract and, to a lesser extent, through the skin, with notable variations among individuals. It distributes to the liver, lungs, and kidneys. In rats, blood and tissue levels remain consistent for up to 24 h following oral exposure, with increasing concentrations observed in the liver, lungs, and kidneys. After intravenous or intra-arterial injection, blood concentration decreases rapidly within 30 min before stabilizing, with a half-life of 1.5 to 2 h. Pulmonary and renal concentrations are about 10 times higher than those in the blood, indicating these organs as reservoirs and targets for BAC. Elimination of BAC primarily occurs slowly through urine and feces without prior metabolism (Abelson and Schaefer, 1993).

Acute toxicity in humans

The primary effects of BAC are associated with its corrosive properties, particularly when the

concentrated substance is involved. Instances of fatal poisoning due to BAC were reported in five elderly individuals with senile dementia who accidentally ingested a 10% BAC solution. Ingestion of BAC leads to immediate sensations of burning in the mouth, throat, and abdomen, accompanied by excessive salivation. This is followed by symptoms such as agitation, anxiety, and confusion, along with muscle damage resulting in muscle twitching. In severe cases, depression of the central nervous system may occur, sometimes accompanied by convulsions. BAC chloride provokes irritation of the skin, eyes, and respiratory mucous membranes. Concentrated solutions can cause skin necrosis, with even 10% solutions already proving irritating to the skin. At ocular levels, concentrations ranging from 0.1 to 0.5% can induce serious conjunctivitis, while concentrations exceeding 10% pose a formidable threat to the cornea.

Chronic toxicity

The predominant concern associated with BAC is the development of allergic reactions. Prolonged use of topical products containing BAC can lead to skin allergies. Rare instances of occupational asthma linked to quaternary ammonium compounds, including BAC, have been reported, particularly among individuals involved in disinfection activities, such as spray disinfection in hospital settings. Bronchial provocation tests may yield positive results in these cases. Some research suggests that chronic use of nasal sprays containing BAC, with concentrations not exceeding 0.1%, may increase the risk of drug-induced rhinitis in individuals with allergic rhinitis, although the contribution of other components in these treatments cannot be completely discounted.

Adverse effects of preservatives in ophthamology

The repeated and prolonged use of preservatives on the eye can lead to sensitization and the development of allergic reactions. As awareness of preservatives increases, it becomes evident that they are not only present in ophthalmic preparations but also in various consumer products. Mercurial derivatives are known to be highly allergenic, with positive skin tests ranging from 13% to 37% depending on the series. Benzalkonium salts are considered moderately allergenic, with prevalence rates of 4% to 11% in different series. Other preservatives, such as chlorhexidine and chlorobutanol, are less commonly recognized allergens. Clinically, allergic reactions to preservatives typically manifest as conjunctivitis or blepharitis, ranging from simple conjunctival redness to papillary conjunctivitis with or without eyelid eczema (Hong and Bielory, 2009)

Toxic effects

Preservatives have the potential to be toxic to various structures of the eye, both on the surface (such as the conjunctiva and cornea) and internally (including the trabecular meshwork, lens, and retina). *In vitro* studies have demonstrated the toxicity of preservatives on diverse types of cultured cells, including epithelial cells of the cornea or conjunctiva, keratocytes, endothelial cells of the cornea, fibroblasts of the capsule of Tenon, trabecular cells, and lens epithelial cells. Patients who undergo long-term antiglaucoma treatment are more prone to developing cystoid macular edema following cataract surgery. This effect is observed across various types of eye drops containing preservatives such as epinephrine, dipivefrin, timolol, and latanoprost. The exact causes of this induction are not fully understood, although a possible association with inflammatory reactions has been suggested.

Conclusion

Preservative triggers allergic and toxic reactions in the corneal epithelium, leading to inflammation, necrosis, or edema. This results in sensations such as tingling, burning, itching, dryness, and allergic reactions for the patient. The use of antiglaucoma eye drops is of particular concern as they are required long-term, potentially impacting the functional prognosis for filtration surgery

following treatment failure. Despite this, there is still a lack of widespread awareness regarding the dangers associated with the widespread use of preservatives. Currently, preservatives are not receiving enough attention compared to other widely distributed toxic substances, despite their potential serious toxicity. However, in ophthalmology, the problem of preservatives has been taken seriously for over a decade, with clinicians and manufacturers working closely to address the cycle of pathology and iatrogenic ocular surface issues caused by their use. To mitigate ocular complications, the preferred approach is to use preservative-free eye drops whenever feasible. Alternatively, fixed combinations are favoured to minimize the impact of multiple therapies, and once-daily instillation eye drops are preferred. These strategies aim to enhance patient comfort and adherence to treatment and improve treatment efficacy, ultimately contributing to the success of future filtering surgery.

Ethical Statement

This is a review article hence no Ethical Approval needed.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

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