

Development of Ophthalmic Formulation for Dry Eye Syndrome

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Abstract

Ophthalmic formulation was prepared considering the essential requirements for the healthy eye. Acacia gum was used along with zinc sulphate as essential nutrients. The pH was adjusted to make the solution isotonic by using NaCl and other buffer solutions. The formulation was evaluated for viscosity and eye irritation test. The formulation was made in concerned with dry eye syndrome and associated disease due to old age. Formulation was observed for particle size and sterility test and finally stability test.

Keywords: Acacia; Ophthalmic drops; Dry eye syndrome; Keratoconjunctivitis sicca

Introduction

In today's scenario, working overtime on computers or watching television has been increased leading to early age dry eye syndrome. The stress can lead to adverse ocular conditions such as those associated with oxidative and/or free radical damage within the eye. These conditions can evolve a condition, disease, or disorder of the cornea, retina, lens, sclera, anterior segment, or posterior segment of the eye.

In dry eye, the eye becomes dry either because there is abnormally high rate of evaporation of tears or because there is not enough tears being produced. Individuals with dry eye experience heaviness of the eyelids or blurred or decreased vision referred to as keratoconjunctivitis sicca.

Healthy tears contain a complex mixture of proteins such as antimicrobial proteins such as lysozyme, lactoferrin, growth factors, inflammation suppressors and mucin which provides viscosity and stability of the tear and electrolytes for proper osmolarity. The contents of the tear in an eye suffering from dry eye are altered with lesser concentrations of proteins such as cytokine which promotes inflammation. Additionally, soluble mucin is greatly decreased due to loss of goblet cells which impacts viscosity of the tear film.

Mucus is a viscous, lubricating material that recruits and maintains moisture to the surfaces it coats. Mucus is actively secreted with salt and water onto surfaces that require these hydrating and lubricating properties for normal functioning. Mucus is particularly important in the normal functioning of the ocular surface [1].

The first line of treatment is usually eye drops, preferably preservative free, that act as artificial tears. Most artificial tears are hydrogels that increase the moisture content on the eye surface and give some temporary relief. These solutions and ointments give some temporary relief, but do little to arrest or reverse any damaging conditions.

Oral medicine for dry eye is also available. For example, pilocarpine, the active ingredient in Salagen TM or cevimeline, the active ingredient in Evoxac TM, is known to stimulate specific receptors in lacrimal gland and cause increased secretion of tears.

Currently, the pharmaceutical treatment of dry eye disease is mostly limited to administration of artificial tears (saline solution) to temporarily rehydrate the eyes. However, relief is short-lived and frequent dosing is necessary. In addition, artificial tears often have contra-indications and incompatibility with soft contact lenses [2].

Stimulation of tear secretion by topical application of melanocyte stimulating hormones is described [3].

Current medications that are used, including cyclosporine A, corticosteroids, tacrolimus, tetracycline derivatives and autologous serum, have been effective for management of dry eye. In addition, a topical ophthalmic formulation of cyclosporine (Restasis) has been investigated as a treatment of immune-based dry eye disease [4]. Stimulation of ocular mucin secretion has also been demonstrated with hydroxyeicosatetraenoic acid derivatives [5]. Nichols et al. [6] disclosed a method of stimulating tear secretion from lacrimal tissue by administering to the eyes an effective amount of purinergic receptor agonists such as uridine 5'-triphosphate, cytidine 5'-triphosphate, adenosine 5'-triphosphate, dinucleotides, and their analogs. Jumblatt and Jumblatt [7] demonstrated the effects of adenine analogues on secretion of high molecular weight, mucin-like glycoprotein by conjunctival goblet cells.

Acacia contains mucilage and chemical constituents such as quercetin, catechol, gallic acid, (+) catechin, (-) epicatechin, (-) epigallocatechin -5, 7-digallate and tannins [8,9]. Acacia has been used in pharmaceuticals as excipient for tablets, emulsifier and thickener. The decoction of bark yields spongy gum which is useful in sore throat, for washing ulcers, to stop bleeding from wounds, skin diseases, as an astringent for diarrhoea and leucorrhoea. Powdered gum is also given in dysentery and diabetes [10]. Gum Acacia consists principally of Arabin, a compound of Arabic acid with calcium, varying amounts of the magnesium and potassium salts of the same acid being present. Acacia is a demulcent and antioxidant [11]. It is also administered intravenously in haemolysis. Acacia is also known to have antibacterial and anti-inflammatory activities [12]. In the form of mucilage, it is used as a suspending agent. Acacia is a good emulsifying agent for fixed oils, volatile oils and also for liquid paraffin. The fried gum is considered a nutritive tonic, particularly in sexual debility. It also soothes inflamed membranes of the pharynx, alimentary canal and genito-urinary organs.

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Eye drops are sterile, aqueous or oily solutions or oil solutions or suspensions of one or more medicaments intended for instillation into the conjunctival sac. They may contain suitable auxiliary substances such as buffers, stabilizing agents, solubilizing agents and agents to adjust the tonicity or viscosity of the preparation. Unlike creams and ointments, these systems have obvious advantages such as ease of administration, ease of mixing with tear fluid and ability to spread over the corneal surface. Increased viscosity of the instilled drops improves contact time of drug with the site of application and can provide better therapeutic efficacy unlike eye solutions, which drain out rapidly from the eyes. In present study, the objective was to prepare ophthalmic drops using suitable concentration of acacia gum that enhances the contact time and also leaves a thin film of drug over the eye surface. Various concentrations of acacia were analyzed to find the suitable concentration for stability of formulation. Viscosity increases the contact time of the formulation. An ophthalmic formulation is provided for the prevention and treatment of adverse ocular conditions, including presbyopia, arcus senilis, age-related macular degeneration, and other conditions associated with aging.

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Too much viscosity produces irritation in eye, hence optimal viscosity was achieved. Viscosity measurements were done using Oswald viscometer and Brookfield viscometer. Apart from providing viscosity to the ophthalmic solutions acacia is also a natural source of calcium and magnesium. These are essential nutrients to eye.

Isotonicity was adjusted and appropriate pH was set. Formulation was observed for particle size and sterility test. Formulation was kept for standing for 3 months any conglomeration was observed. Assay of drug ZnSO₄ was performed intermittently for stability studies. Formulations were instilled in to rabbit eye and were observed for any inflammatory response.

Experimental Methods

Materials

Acacia, Whatmann filter paper, autoclave, filter, Brookfield viscometer, Ostwald's viscometer.

Hydration of acacia gum was achieved as dispersion of gum in deionised water by heating while stirring at about 80°C, autoclaving at 121°C, 15 PSI and adding 0.2% sodium citrate and stirring. Solutions of gum were prepared in concentration of 0.3%, 0.6%, 0.9% and 1.2%,

1.5%, 1.8%, 2.0% w/v. The solutions were allowed to cool to room temperature.

Zinc sulphate is the optimum source of zinc. It gives symptomatic relief to tearing, photophobia, redness, swelling, blepharospasm and itching due to allergies. Also, it may be used to control hyperemia of the palpebral and bulbar conjunctiva resulting from bacterial, allergic and vernal conjunctivitis.

Stabilizing agent

Stabilizing agent is used to reduce degradation of drug during sterilization and storage. Sodium citrate is used as stabilizing agent and it prevents the sticking together of acacia gum and zinc sulphate. Since it is an anti-coagulant and as a buffering agent, sodium citrate helps maintain pH levels. Sodium citrate also acts as a sequestering agent, attaches to calcium ions in water.

Isotonicity adjustment

Sodium chloride equivalent method [13] for isotonicity was used. The "tonic equivalent" of a drug is the amount of sodium chloride that is equivalent to 1 gram, or other weight unit, of the drug.

Formulations were filtered to remove undispersed fibers before and after sterilization. They were observed for agglomerate formation and clarity. Table 1 gives the composition of formulations F1-F7.

Evaluation of formulation

1) pH: Formulation pH was determined using a digital pH meter. pH was set to 7.4.

2) Viscosity:

i) Oswald viscometer (Ubbelohde viscometer): Basically consist of a glass tube in the shape of a U held vertically in a controlled temperature bath. In one arm of the U is a vertical section of precise narrow bore (the capillary). Above this is a bulb, there is another bulb lower down in the other arm. In use, liquid is drawn into the upper bulb by suction, then allowed to flow down through the capillary into the lower bulb. Two marks (one above and one below the upper bulb) indicate a known volume. The time taken for the level of the liquid to pass between these marks is proportional to the kinematic viscosity.

ii) Brookfield Viscometer:

The instrument measures the shearing stress on a spindle rotating at a definite, constant speed while immersed in the sample. The degree of spindle lag is indicated on a rotating dial. This reading multiplied by a conversion factor based on spindle size and rotational speed, gives a value for viscosity in centipoise. By taking measurements at different rotational speeds, an indication of the degree of thixotropy of the sample is obtained.

3) Particle size: Injections must be examined for freedom from

Formulation	F1	F2	F3	F4	F5	F6	F7
Acacia gum	0.3 g	0.6 g	0.9 g	1.2 g	1.5 g	1.8 g	2.0 g
Boric acid	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g
NaCl	0.896 g	0.896 g	0.896 g	0.896 g	0.896 g	0.896 g	0.896 g
ZnSO ₄	0.22 g	0.22 g	0.22 g	0.22 g	0.22 g	0.22 g	0.22 g
Benzethonium chloride	0.01 g	0.01 g	0.01 g	0.01 g	0.01 g	0.01 g	0.01 g
Sodium citrate	0.2 g	0.2 g	0.2 g	0.2 g	0.2 g	0.2 g	0.2 g
Water for injection (q.s.)	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml

Table 1: Composition of Formulation.

foreign particles, before and after sterilization. The earlier inspection makes possible the re-filtration of unsatisfactory solutions, which may not be permissible after sterilization.

A convenient arrangement is a box with a shielded lamp at the top. To reduce reflection it should be painted black inside, except for half the back. The container is held horizontally and rotated immediately under the lamp and then inverted once or to find heavy particles, such as glass. Movement must not be sufficiently vigorous to fill the solution with confusing air bubbles.

Human eye cannot detect particles smaller than about 50 μM . Hence samples were tested for microscopic evaluation for presence of agglomerates or particles smaller than 50 μM .

4) Assay of zinc sulphate- To 5.0 ml add 50 ml of water and 5 ml of ammonia buffer pH 10.9 and titrate with 0.1M disodium edetate using mordant black II solution as indicator each ml of 0.1M disodium edetate is equivalent to 0.002875 g of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$.

5) Sterility test: As per I.P. specifications.

6) Acute eye irritation test:

Six healthy young albino rabbits were used for the study with prior examination of both eyes of each experimental animal 24 hours before starting the experiment, to avoid any animals showing ocular defects or preexisting corneal injury. Animals should be individually housed. The temperature of the experimental animal room was kept at 20°C ($\pm 3^\circ\text{C}$) for rabbits. The relative humidity was maintained at 50-60% and not exceeding 70%. Lighting was artificial and the sequence of light hours and dark hours being was maintained at 12 hrs each alternatively. Feeding was conventional laboratory diet with an unrestricted supply of drinking water. The test is carried out by applying 0.1 mL of each formulation was instilled in the conjunctival sac of one eye of rabbits after gently pulling the lower lid away from the eyeball. The lids are then gently held together for about one second in order to prevent loss of the material. The other eye, which remains untreated, served as a control. The eyes were examined at every one hour and continued for 72 hours after application. The grades of ocular reaction, in terms of redness to the conjunctivae and cornea were recorded at each examination as per OECD TG405 [14]. The cornea is visually observed and assessed for inflammation, tear production, reaction to light, hemorrhage and gross destruction. The conjunctiva is evaluated for the degree of redness, swelling and discharge.

Results and Discussions

Ophthalmic drop evaluation

pH was set to 7.4 for all formulations. pH did not changed even after standing for 3 months.

Particle size

Microscopic examination of formulations did not show not more than 20 particles that have maximum dimension greater than 25 μM , not more than 10 particles have a maximum dimension greater than 50 μM and none has a maximum dimension greater than 100 μM . Samples of formulation were tested. No particles were found in any of the formulations on standing for three months.

Sterility test

The test showed no growth of microorganisms even after keeping for incubation at 37°C for 7 days. The ophthalmic drop was found sterile.

Conc. % w/v	Brookfield Viscometer (Shearing stress in centipoises) for spindles 3,4,5,6,7 at 2,4,10 and 20 revolutions.	Ostwald's Viscometer (viscosity in poise)
0.3 (F1)	No Shearing stress	0.076
0.6 (F2)	No Shearing stress	0.081
0.9 (F3)	No Shearing stress	0.084
1.2 (F4)	No Shearing stress	0.089
1.5 (F5)	No Shearing stress	0.094
1.8 (F6)	No Shearing stress	0.099
2.0 (F7)	No Shearing stress	0.101

Table 2: Viscosity measurements for formulations F1-F7.



Figure 1: Redness in rabbit eye due to formulations (F4, F5, F6, F7).

Rheological studies of the gum formulation [15,16]

Rheological behavior of the formulations were performed by subjecting the formulation to various rates of shear using suitable spindle of Brookfield viscometer by using spindle no.'s 3, 4, 5, 6, 7 were utilized at revolutions 2, 4, 10 and 20. Formulation is expected to show no shearing stress in this study as too much viscosity may cause uneasiness for the patient. Ostwald's viscometer shows the viscosity in poise where all the formulations show viscosity below or equal to 0.1 poise. Limits of viscosity upto 0.1 poise was considered for easy instillation of drops.

Table 2 gives the viscosity measurements for formulations F1-F7.

Biological evaluation was carried out on rabbit eye which did not showed redness or inflammation for formulations (F1) 0.3, (F2) 0.6, (F3) 0.9% w/v concentrations of acacia gum formulations. Remaining higher concentrations in formulations F4 to F7 showed redness (Figure 1) but no inflammation.

Assay was carried out as per I.P. for all the formulation and it was observed that all contain not less than 95.0% and not more than 105.0% of Zinc sulfate.

Stability studies

Formulations were kept in a desicator at 27°C for 3 months in a sealed bottle. Formulations F3, F4, F5, F6 and F7 showed agglomeration after standing for two weeks. Formulations F1 and F2 did not showed agglomeration even after standing for 3 months.

Conclusion

Our studies have found that 0.6% w/v of acacia solution produce sufficient viscosity in ophthalmic eye drop formulation to serve the purpose of maximum contact of drop. We have further seen that 0.6% w/v acacia is also better stabilized formulation. The formulation was clear and did not showed agglomeration. The drug content of formulations shows no degradation of ZnSO_4 due to acacia gum interaction.

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