FORMULATION OF ARTIFICIAL TEARS NANO EMULSION AND ASSESSMENT OF STABILITY

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ABSTRACT

Purpose:

Dry eye syndrome also known as keratoconjunctivitis sicca is a disorder of the tear film which occurs due to tear deficiency or excessive tear evaporation and may causes damage to the ocular surface. To control this disorder artificial tears or tear substitutes are used. In the present study an attempt has been made to minimize the evaporation of tear from ocular surface using castor oil and coconut oil in water nano emulsion and it's stability was assessed.

Methods:

Nanoemulsion is prepared by spontaneous emulsification. Nanoemulsion prepared by two different oil (i.e Castor oil and Coconut oil) containing various ratio of propylene glycol and PEG 400. Osmoprotectants like L-Carnitine and erythritol and humectant sodium hyaluronate were used in the formulation.

Result:

Physicochemical parameters were evaluated and optimized batch was subjected for accelerated stability study. Artificial tear fluid, the refractive index ranges from 1.340 to 1.360. Over a 90-day period, it had no significant changes in pH value, refractive index, surface tension, viscosity, osmolarity, and surface tension.

Conclusion:

From the results obtained from the accelerated stability test it was found that there are no significant changes in the physicochemical parameters and significant improvement in the stability of formulation.

KEYWORDS: Artificial tears, nanoemulsion, dry eye disease, ocular disorder

INTRODUCTION

DED is characterised by inefficiency or inability of the lacrimal glands to produce tears, and evaporative dry eye (EDE), typically attributed to excessive evaporation of the tear fluid.

Preservative-containing formulations can also cause a systemic reaction. Different symptoms are associated with dry eyes [1]. The interpalpebral ocular surface may be damaged if tears are insufficient or excessively evaporated. Keratoconjunctivitis sicca (KCS) is a condition where the lacrimal glands and ocular surface are irritated by external factors.

Keratoconjunctivitis sicca, also known as dry eye syndrome, is an abnormality of the tear film that occurs because of inadequate tears or excessive tear evaporation. As in Canada and Japan, DED is prevalent in up to 25% of countries [2.] Tear substitutes or artificial tears are used to treat this disorder. A nanoemulsion of castor oil in water has been used here to minimize tear evaporation from ocular surfaces.

L-Carnitine, erythritol, and sodium hyaluronate serve as Osmoprotectants. An accelerated stability study was conducted on the optimized batch based on physicochemical parameters [3.] The accelerated stability test results showed that physicochemical parameters did not change significantly and formulation stability improved significantly. One of the most challenging tasks facing pharmaceutical scientist is to develop a new delivery system for ophthalmic drugs.

Topical eye drops can treat a variety of ophthalmic disease; however, they are poorly bioavailable. It is expensive to scale up nanoemulsions because of expensive preparation methods, instability issues, and high costs [4.] In order to address these concerns, pharmaceutical scientists develop ophthalmic preparations. While ointments, suspensions, and emulsions help increase the bioavailability of the drug, they can cause irritation, redness, and interfere with vision, making them unsuitable for use. Moreover, chronic administration may lead to serious complications [5]

MATERIALS AND METHODS

MATERIALS USED

In the formulation batches, ingredients were listed as percentage: Calcium chloride (0.30), Sodium chloride (0.50), Polysorbate- 80 (0.05), Magnesium chloride (0.30), Zinc chloride (0.002), boric acid (0.4), glycerine (0.3), sodium perborate (0.01), and water for injection- Q.S

FORMULATION DEVELOPMENT

SYNTHETIC TEAR FORMULATION PREPARATION PROCEDURE

The formulation development process was conducted aseptically. Using previously sterilised glassware, solutions A and B contained viscosity enhancing agents and electrolytes, as well as additives. Moisture heat sterilization was used for both solutions [6.] Membrane filtering is used to add solution B to solution A. NaOH was used to adjust pH and to store in sterile containers. Since sodium perborate is itself stable, no buffering was necessary.

FORMULATION OF NANOEMULSION

Nanoemulsion for ophthalmic use are usually prepared by spontaneous emulsification. The oil and water mixture are gently stirred at room temperature with preselected surfactants and co-surfactants. The first stage of the preparation of nanoemulsion is the mixing of oil with lipophilic surfactant and mixing them with water miscible solvent. Water and hydrophilic surfactants are then mixed together in the second phase to form a homogeneous lipid phase.

When the lipid phase is added to the water phase, an o/w emulsion is instantaneously decided. First, premixing components were ultrasonicated for 10 minutes at 40% amplitude, then stirred for 5 minutes at 2000 and 16000 rpm. By evaporating under reduced pressure, the water-miscible solvent is removed in the third step. Hydrophilic surfactants and water dissolve nanodroplets of oil into an aqueous solution.

Six different nanoemulsion made by two different oil phase (i.e Castor oil - CS and Coconut oil - CO) containing various ratio of propylene glycol and PEG 400 which was shown in table:1

Table:1 Formulation Development

FORMULATION CODE	OIL (%W/V)	SURFACTANT MIX (%W/V)	1% PROPYLENE GLYCOL (%W/V)	1% PEG 400	AQUEOUS PHASE (%W/V)
CS 1	12.5	60	2.5		25
CS 2	12.5	60		2.5	25
CS 3	12.5	60	1.25	1.25	25
CO 4	12.5	60	2.5		25
CO 5	12.5	60		2.5	25
CO 6	12.5	60	1.25	1.25	25

(Oil Phase: Castor oil: Captex 8000 (1:1); Coconut oil: Captex 8000 (1:1), Surfactant Mix- 5−10 wt % surfactant concentration, Aqueous phase- water for injections, L-Carnitine; Erythritol; Sodium hyaluronate)

CHARACTERISATION OF NANOEMULSION

PHYSICOCHEMICAL EVALUATION

1. Transmittance and visual examination

Under diffused daylight over black and white backgrounds, nanoemulsions are evaluated for clarity under diffused daylight. That composed can be intelligible if the droplet diameter is 50 nm or overcast if the droplet size ranges from 50 nm to 200 nm, depending on the wavelength of light. Measurement devices, such as UV/vis spectrophotometers and colorimeters, are used to analyse nanoemulsion optical properties [7] A UV-visible spectrophotometer measures light reflection or transmission from 380-780 nm. 50, 100 and 200 times diluted nanoemulsion was observed for Transmittance.

2. Distribution of particle size

By using dispersed particles in the range of 1-500 nm, we give the Artificial tear nanoemulsion more opportunity to contact the eyeball surface, therefore increasing the stability of the emulsion after it has been prepared. Moreover, nanoemulsions can penetrate into deeper layers in the eye, counting the aqueous humor, due to the smaller droplets in the dispersed phase [8.] The droplet size and polydispersity of the nanoemulsions are determined at room temperature using the dynamic light scattering (DLS) methods using Malvern Zetasizer. Concentration (undiluted) or water (diluted) samples are used to measure nanoemulsion particle size. When analysing formulations that contain excipients that increase viscosity, dilution often becomes necessary, as it makes it impossible to measure droplet size. Analysis of examples has shown dilution by 40 to 100 and even by 500 times with deionized water. A true value of droplet diameter cannot be obtained by achieving particular viscosity values. The size of nanoemulsion droplets will not change with dilution, making particle size measurements of undiluted and diluted formulations useful in distinguishing micro and nanoemulsions. A nanoemulsion application site can be mimicked with simulated tear fluid, but the formulation ionic strength may change, resulting in colloidal droplet aggregation and, as a consequence, drastic particle size changes. DLS measures polydispersity by calculating the PDI which measures the homogeneity of particle size.

3. Zeta Potential

This value is defined as the dissimilarity between the electric potentials of two dispersion media and the stationary fluid layer, which is associated with the dispersed oil nanodroplets in an optically stimulated dispersion technique. Zeta potential measurements are performed with diluted or undiluted formulations using Zetasizer at 25°C [9] It is estimated that neutral

nanoemulsions have a zeta potential in dispersed systems between -10 mV and +10 mV. Zeta potentials greater than +30 mV indicate strongly cationic nanoparticles, whereas values less than - 30 mV indicate strongly anionic nanoparticles. Stabilized nanoemulsion formulations display zeta potential values over +30 mV or less than -30 mV.

4. pH Measurement

An electrode calibration is performed using buffers with pH 4.0, 7.0, and 10.0 and the pH computation is performed at 25°C using the potentiometric technique. Nanoemulsion pH should be similar to the physiological utility of tear fluid pH, which is between 7.0 and 7.4, so that the formulation can be applied comfortably [10] Dorzolamide hydrochloride nanoemulsions with pH values ranging from 4.34 to 5.42 were found to be non-irritating, tolerable, and intact in rabbit corneas

5. Refractive Index

In nanoemulsion formulations, refractive index (RI) refers to isotropic properties and can identify chemical interactions between excipients and medicinal substances. Nanoemulsion formulations with liquid crystals have anisotropic optical properties due to their locally ordered structure and higher viscosity. If the sample is placed between two crossed polarizers, it will shine when the light source is turned on [11] These conditions produce dark isotropic nanoemulsions. It is feasible to administer eye drops with a highest refractive index of 1.476 due to the refractive index of the tear fluid, which ranges from 1.340 to 1.360.

6. Osmotic pressure

Molecules dissolved in a solution generate osmotic pressure by interacting. At daytime, tears are physiologically osmolar between 231 and 446 mOs/kg. It's possible to get eye irritation with high osmotic pressure formulations. Assessment of osmotic pressure is built on the point at which the freezing point of the solution decreases compared with a pure solvent [12]Osmotic pressure was determined by Freezing pot depression method [13].

7. Surface tension

A tensiometer measures surface tension. Under constant temperature, Wilhelmy plates or Du Nouy rings are detached from the surface of nanoemulsions by measuring the force required. Surface tension of tear fluid is 40-50 mN/m at physiological levels. Surface tensions of formulations that are significantly lower than tear fluid (i.e., less than 35 mN/m) can cause eye infuriation, pain, and patient stiffness. Contrary to this, high surface tension formulations decrease tear film stability [14]

8. Viscosity Measurement

A viscometer (conical plate) can determine the viscosity of nanoemulsions at various preset shear rates at 25°C. Depending on the tissue texture or environment, thin emulsions may be applied to the eyes in order to promote a gas exchange between them and the environment, however, thin emulsions may also block the tear channel as high-viscosity emulsions may deposit within them [15]

8. Stability study

For stability study, the thermal and centrifugal methods are applied to assess the stability of nanoemulsion. Nano emulsions long term storage there is a chance for thermodynamic instability and changes in his characters hence to assess the stability the formulations are stored at various specific temperatures for a period of 3 months, during which the properties of the nanoemulsion, that is, viscosity, pH, refractive index, average droplet size, and the content of the drug substance, are tested at different time points. Stable formulations are characterized by the lack of phase separation, a clear appearance, and only slight changes in physicochemical parameters.100

Phase separation study

Accurately about 1 ml of Nanoemulsion was added to 100 ml of Distilled water in a beaker at 37C and vortexed for 2 min. Later the mixture is left undisturbed visually for 2 hours at the room temperature and observed visually for any phase separation. [16]

Thermodynamic stability studies

This stability studies include centrifugation, heating cooling cycle and freeze-thaw cycle.[17,18]

a) Heating Cooling Cycle

All the formulations were subjected to three heating and cooling cycles (4 to 45 °C) was recorded.

b) Centrifugation

All the formulations were subjected to centrifugation at 3500 rpm for 30 min and at each 15 min the samples were visually observed for any physical stability.

c) Freeze thaw cycles

All the formulations were subjected to Freeze thaw cycles (-18 to 25° C). The response of the formulation for freeze thaw cycle was recorded.

While storing nanoemulsions, they can flocculate, coalesce, undergo Ostwald maturation, and undergo phase inversion, resulting in phase separation. It is therefore essential that the nanoemulsion final formulation remains both physically and chemically stable throughout

production, storage, transportation, and application. The viscosity, pH, refractive index and average droplet size of the Artificial tear nanoemulsion that have been stored for 3 months at various temperature.[19]

RESULTS

EVALUATION OF NANOEMULSION

Table:2 Evaluation parameters of formulated nanoemulsion

Formulation Code	MGS (nm)	PI	Zeta potential	pH	Refractive Index	Surface tension (dynes/cm)	Viscosity (cps)	Osmolarity (mOsm/l)
CS 1	127.3± 0.6	0.318± 0.003	+32.1± 2.45	7.2 ±0.5	1.34 ±1.2	37.5 ±1.5	13.25 ±0.5	285±0.5
CS 2	123.5± 1.2	0.318± 0.003	+33.6± 5.45	7.1 ±0.3	1.35 ±0.5	38.5 ±0.5	26.5 ±1.5	276±1.5
CS 3	132.6± 0.5	0.318± 0.003	+32.5± 5.45	7.3 ±0.4	1.35 ±0.02	39.5 ±1.5	24.2 ±0.2	302±0.5
CO 1	116.4± 1.5	0.318± 0.003	+37.6± 4.25	7.3 ±0.4	1.34 ±0.02	39.6 ±1.2	9.50 ±0.2	305 ±1.2
CO 2	122.3± 0.5	0.318± 0.003	+34.3± 2.15	7.2 ±0.4	1.36 ±0.02	39.5 ±1.1	8.7 ±0.4	315±1.5
CO 3	119.5± 0.5	0.318± 0.003	+33.6± 0.35	7.4 ±0.4	1.36 ±0.02	39.2 ±0.2	9.92 ±1.2	295±0.5

PDI stands for polydispersity index, ZP for zeta potential, and RI for refractive index.

Transmission Test

Dilution	% Transmittance					
	CS 1	**CS 2**	**CS 3**	**CO1**	**CO2**	**CO 3**
50 times	76.38	82.51	79.43	90.34	90.45	91.26
100 times	86.91	90.32	84.58	92.78	91.63	91.39
200 times	93.65	91.73	90.82	97.47	96.59	97.93

Dynamic light scattering technique (DLS) determines the polydispersity index of nanoemulsion systems based on the mean particle size of the dispersed phase. By scattering laser light on particles in the solution, DLS determine the standard size and distribution of particles based on the intensity changes induced by the laser beam. In the measurement, laser

light scattering is measured as a function of time due to the Brownian motion of particles in the solution, with smaller particles moving faster. Concentration (undiluted) or water (diluted) samples are used to measure nanoemulsion particle size. From the results obtained mean globule size of the particle is observed that 119.5 nm to 132.6 nm and if the particle size is below 500 nm than it is highly stable. DLS measures polydispersity by calculating the PDI which measures the homogeneity of particle size. A polydisperse system will have a PDI of one, whereas monodisperse system will have PDI 0 to 1. Nanoemulsion are polydisperse when their PDI values are higher than 0.5, according to Kumar et al. The formulation are having less than 0.5 which indicates monodisperse system.

The zeta potential values of +30mV or below -30mV are stable. The prepared formulations are found to be within the limit. Th pH of the artificial tear is 7.1 to 7.4 which correspond to the physiological tear fluid value of pH 7 to 7.4. Refractive Index is an optical property used to identify the isotropic nature of nanoemulsion to identify the interaction between the components. The Refractive Index of tear fluid is 1.340 to 1.360 and the prepared artificial tears have the range from 1.34 to 1.36 which indicates no interaction between the components selected for the formulation.

Osmotic pressure is a colligative property and depends on the number of molecules dissolved in the solution. The physiological osmolarity of the tear film during the day ranges from 231 to 446 mOs/kg. The prepared formulations osmolarity is between 276 to 325 which will not produce any irritation in eye.

The physiological value of tear fluid surface tension is in the range of 40−50 mN/m. Nanoemulsions display low surface tension due to the presence of surfactants which enables even dispersion of the oil phase in the water-based media. Furthermore, low surface tension of the formulation may increase the wettability of the cornea. Viscosity value of coconut oil nanoemulsion is 8.7 to 9.9 mN/m however artificial tear with castor oil having higher viscosity 13.25 to 26.5 mN/m.

Based on high transmittance values of Coconut oil model, the system is transparent and clear, making it suitable for eyeball applications.

Stability

The formulations are able to sustain upto 3 cycles of heating and cooling. The formulation was able to retain its physical stability for 30 min at 3600 rpm.

By modifying the ionic strength, temperature, pH and mechanical factors, nanoemulsions may be destabilized, changes in particle size distribution and morphology may affect the release of substances from the dispersed phase [20] An emulsion aging method is available for evaluating

emulsion stability over time, while an evaluation of formula durability can also be done quickly. As a result, we determine the viscosity, pH, refractive index and average droplet size of the Artificial tear nanoemulsion that have been stored for 3 months at various temperature [20].

Figure Stability study of Artificial Tear Nanoemulsion

a) Heating and cooling cycle

Formulation code	**0**	**1st Cycle**	**2nd Cycle**	**3rd Cycle**
CS 1	Stable	Stable	Stable	Stable
CS 2	Stable	Stable	Stable	Stable
CS 3	Stable	Stable	Stable	Stable
CO 4	Stable	Stable	Stable	Stable
CO 5	Stable	Stable	Stable	Stable
CO 6	Stable	Stable	Stable	Stable

All the formulations subjected for three heating and cooling cycles (4 to 45 °C) were stable and no phase separation was observed.

b) Centrifugation

Formulation code	**0**	**15 min**	**30 min**
CS 1	Stable	Stable	Stable
CS 2	Stable	Stable	Stable
CS 3	Stable	Stable	Phase separation
CO 4	Stable	Stable	Stable
CO 5	Stable	Stable	Stable
CO 6	Stable	Stable	Stable

All the formulations except CS3 have retained its physical stability

c) Freeze thaw cycle

Formulation code	**0**	**1st Cycle**	**2nd Cycle**	**3rd Cycle**

CS 1	Stable	Stable	UnStable	UnStable
CS 2	Stable	Stable	Stable	Stable
CS 3	Stable	Stable	Stable	UnStable
CO 4	Stable	Stable	Stable	Stable
CO 5	Stable	Stable	Stable	Stable
CO 6	Stable	Stable	Stable	Stable

Majority of formulations sustain the 3 freeze thaw cycle however CS 1 & 3 could not able to sustain for 3 cycles.

Table:3 Analyses of accelerated stability

PARAMETER	**STORAGE PERIOD (DAYS) AT 40°C AND 75% RH**			
	0 Day	**30 Days**	**60 Days**	**90 Days**
APPEARANCE	Clear	Clear	Clear	Clear
pH	7.0 ±0.2	7.3 ±0.35	7.3 ±0.3	7.4 ±0.4
REFRACTIVE INDEX	1.3±0.04	1.32±0.02	1.33±0.1	1.34±0.05
SURFACE TENSION (DYNES/CM	39.7±0.3	38.4±0.5	39.3±0.6	39.7±0.5
VISCOSITY (CPS)	9.0±0.4	9.2±0.5	9.3±0.4	9.3±0.5
OSMOLARITY (MOSM/L)	305±0.7	304±0.3	303±0.4	305±0.5

DISCUSSION

Formulated nanoemulsion was accessed by means of physiochemical parameters such as visual examination, transmittance testing, particle size measurement, pH measurement, refractive index determination, surface tension, viscous nature and stability nature over accelerated condition.

To obtain transparent formulations with reduced viscosity of the dispersed phase a mixture of oils can be used (an example includes the combination of castor oil with medium chain triglycerides in 1:1 ratio, resulting in a decrease of castor oil viscosity. The refractive index for the tear fluid is 1.340 to 1.360. Eye drops must have refractive index values not higher than 1.476.

From the literature source *In vitro* studies have demonstrated that sodium hyaluronate promotes corneal epithelial cell migration and proliferation, which facilitates healing in dry eyes. Refresh®, Oasis®, and Blink® tears can provide additional relief in dry eye.

Nanoemulsion droplets differ in size according to the composition and viscosity of oil phases. In contrast to oils with lower viscosities, oils with higher viscosities produced larger droplets. Increasing the viscosity of the continuous phase can reduce droplet size in thick oils. In order to obtain nanoemulsions with fine droplets, an optimal viscosity ratio between the dispersed and continuous phases was determined. Addition of natural or synthetic polymers can enhance the viscosity of the ocular nanoemulsions and prevent the rate of evaporation of artificial tear. Over a 90-day period, it had no significant changes in pH value, refractive index, surface tension, viscosity, osmolarity, and surface tension.

CONCLUSION

Artificial tears contain Polyethylene glycol, propylene glycol which acts as demulcent as a primarily water-soluble polymer, which is applied topically to the eye to protect and lubricate mucous membrane surfaces and relieve dryness and irritation. Osmoprotectants are agents that protect cells against hyperosmolar stress-mediated injury. Since hyperosmolarity is one the characteristics of dry eye, osmoprotectants erythritol which is an emerging ingredient of artificial tears. Humectants are hygroscopic agents that facilitate the retention of water. propylene glycol retain moisture at the ocular surface.

Hyaluronic acid acts as a humectant and can bind water multiple times the amount of its weight. Sodium hyaluronate is a semisynthetic smaller molecular weight sodium salt derivative of hyaluronic acid. Like hyaluronic acid, sodium hyaluronate also acts as a humectant to provide additional hydration. Its mucoadhesive properties are proposed to increase the corneal residence time. castor oil, and mineral oil mimic the lipid layer of tear film, stabilize tear film, increase lipid layer thickness, and prevent evaporation.

Dry eye due to meibomian gland dysfunction also has significant evaporative component due to deficiency in the lipid layer. In such cases, artificial tears that contain lipid components including castor oil and mineral oil that either replenish or stabilize the lipid layer should be considered.

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