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In vitro formulation optimization of hydrogel based artificial tears for dry eyes

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Abstract
Objective: The objective of the present study was to formulate, evaluate & optimize artificial tears formulation for the treatment of dry eyes.
Methods: Carboxymethyl cellulose & Hydroxypropyl methyl cellulose hydrogel based artificial tears formulations were evaluated for clarity, pH, viscosity, surface tension, specific gravity, drop size, freezing point & osmolality.
Results & Discussion: Artificial tears formulations were prepared with different concentration of HPMC and CMC and code from B1 to B18. The solutions prepared with Guar gum & Chitosan in purified water was found turbid & subsequently rejected for the formulation of artificial tears. The formulations were evaluated for clarity, pH, Viscosity & surface tension and screened for further study. Four CMC & HPMC hydrogel based formulations B1, B7, B8 & B9 were selected for further study. The specific gravity, freezing point & osmolality determinations were done on formulations B1, B7, B8 & B9. The formulation optimization studies were done using Sigmaplot® software of Systat software Inc UK on B1, B7, B8 & B9 formulations.
Conclusion: The viscosity, surface tension, dropsize, osmolality for HPMC hydrogel based B8 & B9 formulations containing 0.5%w/v & 0.75% w/v HPMC was found optimum.

Keywords: Artificial tears, dry eyes, hydrogel, freezing point, osmolality

Introduction
The eye is an critical organ in our frame for seeing objects, colorations and different things. The human eye is one of the maximum treasured and touchy sensory organs [1]. It lets in to peer the great global and the colors round us The use of virtual generation has modern blessings in our life, however it's also related to many unfavourable outcomes on our fitness which includes stress, anxiety, depression, obesity, eye infection and eye injuries, eyes, etc [2]. Light, starting from X-rays and different ionizing radiation to infrared and longer wave bands, will have risky outcomes at the eyes if it reaches a stage inflicting photochemical reactions, photothermal harm or metabolic disturbances. Various light-induced eye diseases have been recognized, including photokeratitis, pterygium, droplet-induced climatic keratopathy, cataracts, and corneal and retinal degeneration [2].
The smartphone screen also emits blue light which negatively affects corneal epithelial cells in humans. Excessive blue light exposure caused tear film deterioration and increased levels of inflammatory markers and reactive oxygen species (ROS) [2, 3]. The cornea is the anterior part of the outer part of the eye, which protects delicate internal structures from external influences and its susceptibility to the environment, invasion of foreign bodies and physical or chemical damage [4]. Corneal wounds are responsible for blindness worldwide because most of the refractive power of the eye is controlled by the cornea. Collagen, fibrin, gelatin, alginate, chitosan etc are the different natural biopolymers that have been studied for the treatment of peripheral eye diseases [5].
The lower ocular bioavailability is the area of concern from decades due to low retention time of eye formulations in the eye [5]. The dynamic lacrimal system is an additional barrier that limits the retention time of an ophthalmic preparation on the surface of the eye resulting in low ocular bioavailability. The increased loss of ocular treatment solutions requires more frequent instillations for proper healing with better bioavailability [5]. The improvement of the bioavailability of the active substances is mainly based on an increase in the ocular residence time due to the decrease in the rate of drainage thanks to the increase in the viscosity and the mucoadhesive properties of the polymers [5, 6]. Viscosity improvers commonly include high molecular weight hydrophilic polymers such as carbomers, hyaluronic acid, polyvinyl alcohol,

poloxamers and various polysaccharides including cellulose derivatives, chitosan, guar gum, gum gellan and xanthan gum [6, 7]. Most of these agents have a lubricating effect and significantly reduce surface tension. The viscosity and mucoadhesive properties of ophthalmic solutions largely depend on the concentration, the weight of the molecules, and therefore on the nature of the viscosifying agents, these parameters must be adjusted for an optimal effect on the corneal wound and the protective potential, the solution must have a prolonged action retention time [6-8].

Dry eyes is a ailment that arise because of disturbance of lacrimal practical unit. It is a ailment of tears and corneal surface that consequences in visible discomfort, visible disturbance and tear movie instability with capacity harm to the ocular floor [9]. Dry eye is fundamental in globe. As a result, eye medical professional and different fitness care professionals, including eye specialist, popular practitioners are assist those sufferers to therapy from those symptoms. The commonly prescribed remedy for control of dry eyes is over the counter tear lubricants also called Artificial Tears [10]. A wide variety of tear lubricant formulations are to be had that modify with the aid of using their mechanism of action. In this lubricants may be decided on for sufferers with moderate to slight dry eye [10]. Side outcomes of lubricants, which include burning on instillation due to mismatches of eye drop with tear pH and osmolality [10-12]. The most important element of synthetic artificial tears is the natural hydrogel polymers, so referred to as due to their capacity to retain water & swell. The following hydrogels had been utilized in synthetic tear substitutes which include hydroxypropyl methylcellulose, carboxy methylcellulose, polyvinyl alcohol [11-15]. The present study was envisaged and performed in a view to formulate a patient acceptable non-irritant artificial tears pharmaceutical preparation with carboxymethylcellulose (CMC), Hydroxypropylmethylcellulose (HPMC), Chitosan, Guar gum.

Materials and Methods

Carboxymethyl cellulose (CMC), Hydroxypropylmethylcellulose (HPMC), Chitosan was purchased from stellar Bio-sol India Ltd. Guar gum was purchased from Shubhlaxmi Industries India. Tween 80 was purchased from Loba Chemie Mumbai India. All other chemicals used were of analytical grade and were used as received.

Preparation of Artificial Tears: Eighteen formulations of artificial tears were formulated with different percentage of HPMC, CMC, Guar gum and chitosan in purified water. The polymers were allowed to hydrate, dissolve in purified water for 12 h with slow stirring using mechanical stirrer; the hydrogel solution was clear and viscous. Prepared 10 ml hydrogel solution for each formulation was transferred in clear glass bottles that were used in the packaging of eye drops.

Clarity Test: the clarity test for prepared formulations was done manually. The prepared formulation solutions were tested visually under white tube light against black & white background for the presence of any particulate matter or turbidity in the solution [9].

pH: Adequate volume of each formulation was taken in 30ml glass beaker and pH was recorded on a systronic pH meter which was previously standardized [10].

Viscosity: Viscosity of the formulations was measured using Ostwards viscometer at laboratory temperature.

The formula used in the determination of viscosity is as follows

$$\frac{V1}{V2} = \frac{D1\ t1}{D2\ t2}$$

Where V1, D1 & t1 are the viscosity, density of the formulation at t1 time of flow for test sample

V2, D2 & t2 are the viscosity, density of water at t2 time of flow for purified water

Surface Tension: The surface tension of each prepared formulation was determined by drop method using stalagmometer. The surface tension can be determined as follows

$$\text{Surface Tension} = 72.8 \text{ X } \frac{D1}{D2} X \frac{n2}{n1}$$

Where: 72.8 is the surface tension of water dyne/cm

D1 is the density of the formulation & n1 is the number of drops

D2 is the density of purified water & n2 is the number of drops

Drop Size Determination: The drop size of the formulations was determined by adopting weight method. A commercially available flexible plastic dropper bottles were filled with 10ml of prepared formulations. The bottle was fixed in the downward position and pressed gentle to deliver a drop of formulation. The drop was weighed immediately on analytical balance. For each solution, 10 drops were dispensed and average weight was determined. The procedure was repeated in triplicate and standard deviation was calculated. The drop size of purified water was taken as reference standard [9]

1drop of purified water = 0.05ml =50mg

0.05ml = 50µl

Freezing point depression: The depression in freezing point of prepared artificial tears formulation was determined to adjust the isotonicity. The apparatus used was Beckmann freezing point apparatus having digital thermometer with resolution 0.01 ºC. The weight of Sodium Chloride (g/100ml) required to adjust tonicity was calculated as follows [7, 8]

$$\text{Weight of NaCl required (g/100ml)} = \frac{0.52 - A}{B}$$

Where A is the freezing point of prepared Unadjusted Artificial Tears Formulation

B is the freezing point of 1 %w/v Nacl solution

Osmolality: Osmolality of each formulation was calculated using data of freezing point depression using following equation [7]

$$\text{Osmolality} = \frac{\Delta T}{1.86} X\ 1000$$

The units of Osmolality is mOsm/kg

Where: ΔT is the depression of freezing point of test solution below the freezing point of pure solvent. 273.16K is the

freezing point of purified water. 1.86 is the cryoscopic constant for purified water.

The osmolality of isotonic normal saline solution (0.9%w/v NaCl) is 290.32 mosm/kg was used as reference standard. The osmotic gap between formulation and normal saline was determined for each formulation.

Results and Discussion

Eighteen artificial tears formulations were prepared with different concentration of HPMC and CMC and code from B1 to B18 (Table 1). The natural polymers Guar gum and Chitosan were insoluble in purified water. The solutions contain 0.25%w/v guar gum & 0.25%w/v chitosan was turbid & failed in clarity test for eye drops, there natural polymers guar gum & chitosan were rejected in the formulation of artificial tears. The pH of all formulations B1 to B18 was in the range of 6.6 to 7.0.

Table 1: Artificial Tears Formulations

Formulation Code	CMC (%W/V)	HPMC (%W/V)	Total polymer concentration
B1	0.25%	-----	0.25%
B2	0.5%	-----	0.5%
B3	0.75%	-----	0.75%
B4	1.0%	-----	1.0%
B5	1.5%	-----	1.5%
B6	2%	-----	2%
B7	-----	0.25%	0.25%
B8	-----	0.5%	0.5%
B9	-----	0.75%	0.75%
B10	-----	1%	1%
B11	-----	1.5%	1.5%
B12	------	2%	2%
B13	0.125%	0.125%	0.25%
B14	0.25%	0.25%	0.5%
B15	0.375%	0.375%	0.75%
B16	0.5%	0.5%	1%
B17	0.75%	0.75%	1.5%
B18	1%	1%	2%

The viscosity & surface tension of all formulations was determined (Table 2). The viscosity of all formulation was found from 1.92 to 450cp. Viscosity of B1 to B6 formulations were in the range of 4.79 to 46.4 Cp, B7 to B12 in the range of 1.92 to 26 & for formulations B13 to B18 was in the range of 16.9 to 450. The viscosity of formulation B1, B7, B8 & B9 was 4.79, 1.92, 5.97 & 8.80 respectivly. Formulation B1 contains 0.25%CMC, formulation B7, B8 &B9 was HPMC based containing HPMC 0.25%, 0.5% & 0.75% respectively. Ideally the viscosity of eye drops formulations should be in the range of 6-12cp.

Surface tension of all formulations was in range of 32.5 dynes/cm to 86.92 dynes/cm. the surface tension of formulation B1, B7, B8 & B9 was found 40.3, 32.5, 41.4 & 45.8dynes/cm. ideal surface tension of eye formulations must be in the range of 40-46 dynes/cm. The artificial tear formulation code B1, B7, B8 & B9 was selected for further investigations.

Specific gravity of the selected formulation B1, B7, B8 & B9 was determined using 20ml specific gravity bottle. The procedure was repeated in triplicate. The average value of specific gravity ± SD was recorded. Specific gravity = weight of 20ml of formulation/ weight of 20ml of purified water

The specific gravity of formulation B1, B7, B8 & B9 was found 1.04 ± 0.03, 1.05 ± 0.02, 1.09 ±0.01, 1.21 ± 0.01.

Table 2: Clarity, Viscosity and Surface tension of Artificial Tears Formulation

Formulation Code	CMC		HPMC		Total polymer concentration w/v	Clarity	Viscosity (cp)	Surface tension dynes/ cm	Drop Size (µl)
	Concentration w/v	Volume	Concentration w/v	Volume					
B1	0.25%	15 ml	_	_	0.25%	Clear	4.79	40.3	40.85±0.2
B2	0.5%	15 ml	_	_	0.5%	Clear	14.5	75.2	25±0.45
B3	0.75%	15 ml	_	_	0.75	Clear	17.5	76.6	------
B4	1.0%	15 ml	_	_	1.0%	Clear	22.5	75.1	------
B5	1.5%	15 ml	_	_	1.5%	Clear	34.2	77.6	-----
B6	2%	15 ml	_	_	2%	Clear	46.4	79.9	------
B7	_	_	0.25%	15 ml	0.25%	Clear	1.92	31.6	32.92±0.18
B8	_	_	0.5%	15 ml	0.5%	Clear	5.97	40.2	44.55±0.18
B9	_	_	0.75%	15 ml	0.75%	Clear	8.80	44.2	45.81±0.5
B10	_	_	1.0%	15 ml	1%	Clear	12.40	85.1	25.41±0.25
B11	_	_	1.5%	15 ml	1.5%	Clear	19.4	86.4	-----
B12	_	_	2%	15 ml	2%	Clear	26.0	85.9	-------
B13	0.25%	7.5 ml	0.25%	7.5 ml	0.5%	Clear	16.9	87.3	--------
B14	0.5%	7.5 ml	0.5%	7.5 ml	1.0%	Clear	24.2	84.4	-------
B15	0.75%	7.5 ml	0.75%	7.5 ml	2%	Clear	65.3	84.5	------
B16	1%	7.5 ml	1%	7.5 ml	3%	Clear	168	88.1	-----
B17	1.5%	7.5 ml	1.5%	7.5 ml	4%	Clear	247	87.9	------
B18	2%	7.5 ml	2.0%	7.5 ml	5%	Clear	450	88.4	------

Drop size for artificial tear formulations coded B1 to B18 was determined. For CMC based B1 & B2 formulation containing 0.25%w/v & 0.5%w/v CMC the drop size was found of size 40.85±0.2 & 25±0.45 µl respectively. The drop size for HPMC based formulations B7, B8, B9 & B10 was found 32.92±0.18, 44.55±0.18, 45.81±0.5 & 25.41±0.25µl. For all other CMC, HPMC and mixture of CMC-HPMC based formulations it was not possible to determine the drop size as the formulations were highly viscous & was not flow from the container on slight squeeze.

The freezing point & osmolality of formulation B1, B7, B8 &B9 was presented in Table 3 was compared with the osmolality of normal saline (293mOsm/kg). the osmolality of HPMC based B8 & B9 was near to the osmolality of normal saline hence the formulations was isotonic. The osmolality of formulations B1 & B7 was lower than the normal saline hence was hypotonic. 0.217g/100ml, 0.160g/100ml sodium chloride was added in formulation B1 & B7 and was made isotonic.

Table 3: Freezing point & Osmolality of selected Artificial Tears Formulation

Formulation Code	CMC		HPMC		Total polymer concentration w/v	Freezing Point	Osmolality mOsm/kg
	Concentration w/v	Volume	Concentration w/v	Volume			
B1	0.25%	15 ml	_	_	0.25%	- 0.23	215±1.2
B7	_	_	0.25%	15ml	0.5%	- 0.36	268 ±0.88
B8	_	_	0.5%	15ml	0.75	- 0.41	280 ±0.5
B9	_	_	0.75%	15ml	1.0%	- 0.48	288±2.1
Normal Saline	0.9%	-	-	-	-	- 0.52	293

Effect of polymer concentration on specific gravity, viscosity, surface tension & drop size was presented in Fig 1. It has been found that for HPMC based artificial tear formulations as the concentration of polymer increased the specific gravity, viscosity, surface tension & drop size increased. It was found that the specific gravity, viscosity, surface tension & drop size of CMC based B1 formulation containing 0.25%w/v CMC was nearly equivalent with B8 HPMC based formulation containing the 0.5%w/w of HPMC.

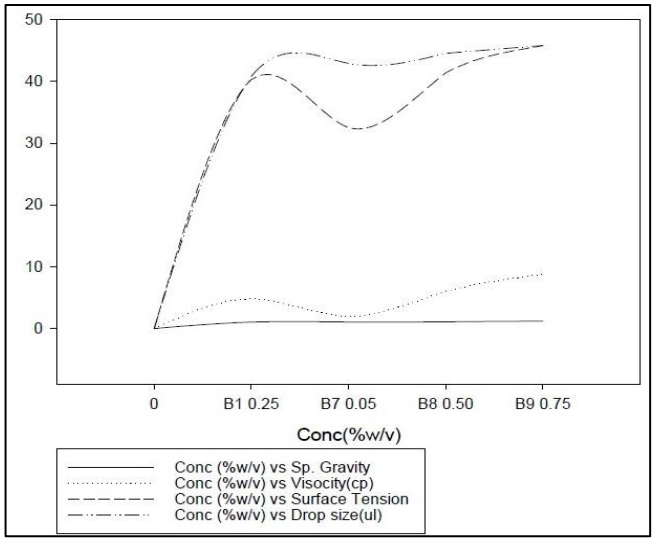

Fig 1: Effect of Polymer Conc. On specific gravity, Viscosity, Surface Tension & Drop Size

Effect of viscosity on the surface tension & dropsize was evaluated (Fig 1A) for HPMC based artificial tears formulation B7, B8, B9 containing 0.25%, 0.5%, 0.75%w/w HPMC & CMC based B1 Formulation containing 0.25% CMC. An increase in viscosity there was increase in the surface tension as well as dropsize was resulted with HPMC based formulations. It was obtained that there was no proportional increase in surface tension & drop size with respect to viscosity in case of HPMC based Formulations. An increased viscosity of eye preparations enhanced the ocular retention time in the eye thereby improves the ocular bioavailability. It was found, there was no proportional increase in the viscosity of HPMC based B7, B8 & B9 formulations with respect to the concentration of the polymer.

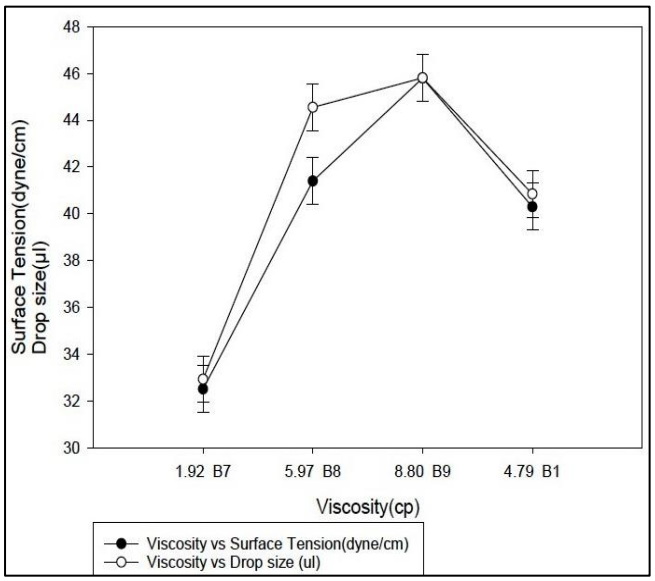

Fig 1A: Effect of Viscosity of the Surface Tension & Drop Size

The formulation optimization studies were done using Sigmaplot® software of Systat software Inc by correlation of viscosity, surface tension, drop size, osmolality & polymer concentration Fig 2 & Fig 3. The objective of optimization study was to select best formulation among B1, B7 B8 & B9 artificial tears formulations. For formulations B1, B7 B8 & B9 the viscosity, surface tension and drop size was compared on 3D line graph to evaluated the effect of viscosity, surface tension on the drop size of artificial tears formulation. It has been found that the drop size for formulation B9 was highest at 45.81 µl with highest viscosity & surface tension among all four artificial tear formulations. The drop size, viscosity, osmolality for HPMC based B8 & B9 formulations containing 0.5%w/v & 0.75%w/v was found optimum.

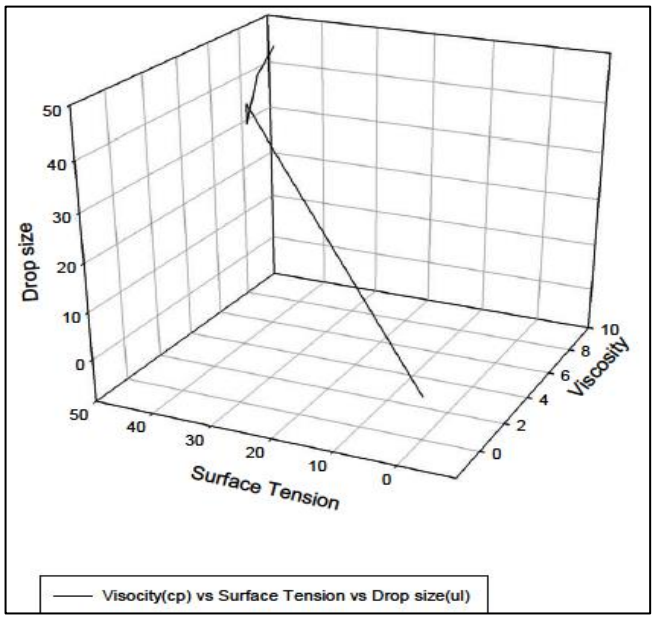

Fig 2: 3D Line Plot corelation of Viscosity, Surface Tension & Drop Size

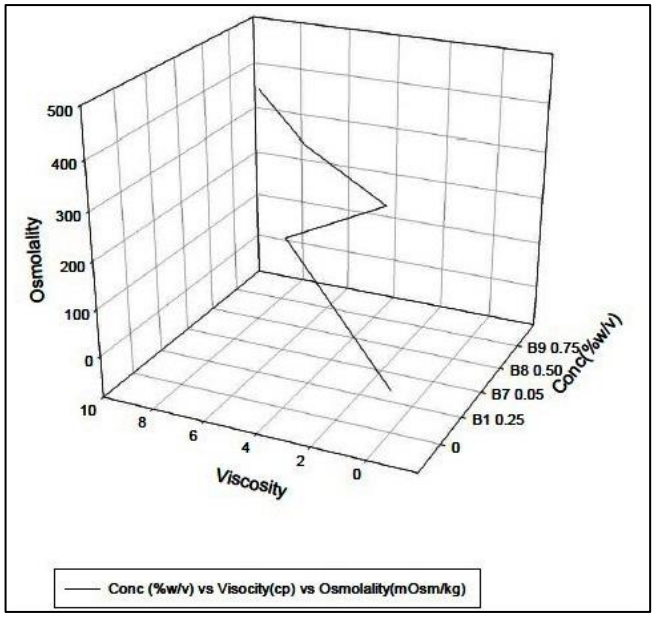

Fig 3: 3D Line Plot corelation of Polymer cons, Surface Tension & Drop Size

Conclusion

Eighteen artificial tear formulations coded B1 to B18 for the treatment of dry eyes based on hydrogels & natural polymers were formulated and evaluated. The natural polymers used were Guar gum & Chitosan. The formulations based on natural polymers chitosan & Guar gum was rejected in the preparation of artificial tears as the formulations failed in clarity test for eye drops. The hydrogel forming polymers used in the formulation of artificial tears were CMC, HPMC in different concentrations alone and in combination. The prepared formulations were evaluated for clarity, viscosity, surface tension, specific gravity, osmolality & drop size. Out of eighteen formulations only 4 formulations based on CMC & HPMC coded B1, B7, B8 & B9 was selected for further investigations after determination of clarity, viscosity, surface tension & drop size in the initial screening. The viscosity, clarity, drop size & osmolality for Formulation B8 & B9 was found optimum after performing optimization studies using sigmaplot® software. Hydrogel HPMC based B8 & B9 formulations containing 0.5%w/v & 0.75%w/v HPMC respectively were found suitable to be used as artificial tears in the treatment of dry eyes.

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