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Formulation and evaluation of *in situ* ophthalmic gels of Diclofenac sodium

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ABSTRACT

In ocular delivery the physiological constraints imposed by the protective mechanisms of the eye lead to low absorption of drugs, resulting in a short duration of the therapeutic effect. Thus with the use of these in situ gelling systems, residence time of the drug in the eye is increased. Continuous delivery of drugs in a controlled manner to the anterior chamber of the eye will eliminate the requirement for frequent drug administration, causing better patient compliance and resulting in extended duration of action. The present work describes the formulation and evaluation of an ophthalmic delivery system of an anti-inflammatory drug diclofenac sodium, based on the concept of pH triggered in situ gelation by using sodium alginate, In vitro release studies indicated Among the all formulations F1 shows better drug release when contacted with STF solution at 8 hrs study period. It shows antimicrobial, antibacterial and antifungal efficacy with selected microorganisms. These results demonstrate that the developed system is an alternative to conventional ophthalmic drops, patient compliance, industrially oriented and economical.

Keywords: Ophthalmic delivery systems, *in situ* gelling, diclofenac sodium, pH triggered.

INTRODUCTION

A new approach is to try to combine advantages of both solutions and gels, such as accuracy and facility of administration of the former and prolonged residence time of the latter. Thus in situ gels can be instilled as eye drops and undergo an immediate gelation when in contact with the eye. In situ-forming hydrogels are liquid upon instillation and undergo phase transition in the

ocular cul-de-sac to form viscoelastic gel and this provides a response to environmental changes. In situ-forming hydrogels are liquid upon instillation and undergo phase transition in the ocular cul-de-sac to form visco-elastic gel and this provides a response to environmental changes. in situ gel-forming ophthalmic drug delivery systems prepared from polymers that exhibit reversible phase transitions (sol–gel–sol) and pseudoplastic behavior to minimize interference with blinking . Such a system can be formulated as a liquid dosage form suitable to be administered by instillation into the eye which, upon exposure to physiological conditions, changes to the gel phase, thus increasing the pre-corneal residence time of the delivery system and enhancing ocular bioavailability. Gel systems are better retained in the eye than conventional eye drops and are better tolerated by patients than inserts and ointments. Like ointments, gels are also difficult to administer for some patients. In this respect in situ gels are interesting since these are conveniently dropped as a solution into the conjunctival sac, where they undergo a transition into a gel with its favorable residence. The sol-gel-sol transition occurs as a result of chemical and physical change induced by the physiological environment. Here we described the in situ ophthalmic gels were prepared by the pH triggered method. pH triggered in-situ gelling system are low viscosity polymeric dispersion in water which undergoes spontaneous coagulation and gelation after instillation in conjuctival cul-de-sac[1].

Advantages of *in situ* forming gel:

- Generally more comfortable than insoluble or soluble insertion. Less blurred vision as compared to ointment.
- Increased bioavailability due to – Increased precorneal residence time Decreased nasolacrimal drainage of the drug which cause undesirable side effects arising due to systemic absorption of the drug through naso-lacrimal duct is reduced.
- Drug effect is prolonged hence frequent instillation of drug is not required.
- The principle advantage of this formulation is the possibility of administering accurate and reproducible quantities, in contrast to already gelled formulations and moreover promoting precorneal retention

EXPERIMENTAL SECTION

Diclofenac sodium was purchased as a gift sample by Yarrow chem. Products, Mumbai, HPMC, Carbopol, sodium alginate (Ranbaxy chemicals pvt ltd). All other chemicals were of analytical grade.

Formulation of ophthalmic gel:

The diclofenac sodium was dissolved in 75ml distilled water and then sodium chloride was added and stirred continuously until dissolved. Benzyl alcohol was added as preservative after that HPMC was added and allow hydrating and swelling. The carbopol was sprinkled over this solution and allow for hydrating overnight. The desirable pH was adjusted by 0.5mol/inah and then the solution was stirred instantly until a uniform solution was obtained. Finally volume was made up 100ml by using distilled water.

Table 1: Formulation of diclofenac sodium *in situ* ophthalmic gels

Ingredients	F1	F2	F3	F4
Drug	0.1%	0.1%	0.1%	0.1%
Sodium Alginate	1.5%	0.8%	-	1.5%
HPMC	-	0.2%	1.5%	-
HPC	-	-	-	0.5%
HEC	0.75%	-	-	-
Carbopol	-	-	0.5%	-

Evaluation of ophthalmic gel

1. General appearance [2]

The general appearance of the formulation was observed which included colour and clarity of solution.

2. Drug content [2]

It is determined by taking 1ml of the formulation and diluting it to 100ml with distilled water. 5 ml was withdrawn and further diluted to 25 ml with distilled water. Concentration was determined at 200-400mm by using UV visible spectroscopy.

3. pH of the ophthalmic gels

The prepared in situ gel formulations were evaluated for pH measurement by using pH meter.

4. *In vitro diffusion study*

In vitro release of diclofenac sodium was carried out in formulations with different concentrations of sodium alginate, HPMC, HPC, HEC, Carbopol using a dialysis membrane. The diffusion medium was taken as 100ml of simulated tear fluid composition and stirred at 50rpm at 37^0C $\pm 0.5^0$C. One end of the diffusion tube was covered by a dialysis membrane. The formulation was kept in that diffusion tube and the diffusion tube was kept in the diffusion medium so as the formulation came in contact with the simulated tear fluid. The drug samples were withdrawn at the interval of 30 mins for the period of 8 hrs from diffusion medium and analyzed by a U.V spectrophotometer at 276nm using simulated tear fluid as blank.

In Vitro Diffusion studies: Parameters

Instruments : Magnetic stirrer and diffusion tube
Medium : 100 ml simulated tear fluid at pH 7.4
Temperature : 37 ± 0.5^oc
RPM : 50
Duration : 8h
Sampling time : 30 min
Amount withdrawn : 1ml
λmax : 276nm

5. Sterility testing [3]
Direct inoculation
Preparation should be examined during usage. Sterile media was pipette out by sterile pipette and with sterile syringe then aseptically transferred the specified volume of material to a vessel and inoculate the media and incubate for 7 days.

6. Anti microbial studies
Microbiological assay
Microbiological assay is a type of biological assay performed with microorganism e.g.. Bacteria, yeast, & Moulds. In typical microbial assay evaluation is performed with a culture of microorganisms. Many therapeutic agents, which either inhibit the growth of the microorganism (or) essential for their growth can be standardized by microbiological assay.

Methods:
The procedure employed in microbial assay may be divided into two broad classifications.

- Disc diffusion method.
- Turbidimetric method (or) Serial dilution method

Disc Diffusion Method:
Filter paper discs of 6mm diameter were impregnated with optimum amount of drug. The discs may be dried in incubator and stored in refrigerator. Liquid cuture of the bacteria in broth was flooded on a solid medium in a plate (muller Hinton agar (or) nutrient agar) and excess was thrown away. Alternatively, the culture plate may sub cultured by the bacterial culture by using swab. The medicated discs were then placed on the plate and incubated overnight the zone of inhibition around the disc is noted.

Turbidimetric method or serial dilution method
In the turbidimetric assay of drug, the potency of the drug is based on inhibition of microbial growth as indicated by measurement of the turbidity (transmittance) of a suspension of suitable microorganism in a fluid medium to which have been added graded amounts of the test compounds changes in transmittance produced by known concentration of reference material which were compared with results.

7. Antibacterial and antifungal studies:
In this study the initial work has been carried out by disc Diffusion method. In the presence study 4 gram positive bacteria, 4 gram Negative bacteria and 1 fungi were selected.

Gram (+) Strain	Gram(-)Strain	Fungi
Staphylococcus Aureus	*Escherichia Coli*	*Candida Albicans*

Procedure:
Accurately weighed quantity of the above ingredients were suspended in 1000ml of distilled water and boiled to dissolve completely. The pH of the medium was adjusted to 7.3 ± 0.2 at 25ºC. It was sterilized by autoclaving at 121ºC for 15 minutes.

Preparation of sterilization of inoculums:
Each bacteria and fungi prone culture was transferred into 100ml of Muller Hinton Nutrient Broth (NB) and Sabouraud's dextrose broth (SDB) respectively. The inoculated broths were incubated at 37ºC for 24 hours and 27ºC for 72 hours for bacteria and fungi. After incubation inoculums were standardized to CFU/ml of bacteria and CFU/ml for fungi for colony forming unit method.

Antimicrobial activity of synthesised compound by disk diffusion method
The synthesized compounds were dissolved in dimethyl sulfoxide to a final concentration of 100µg/ml. The sterile disk (6mm in diameter) was impregnated with 10µg of the sample and test against microbial cultures.

Antibacterial activity:
Muller Hinton Agar medium was prepared and transferred into sterile petri plates, 200µl of the standardized bacterial inoculums was spread on agar medium using sterile cotton swab. The test impregnated discs were placed on the inoculated agar medium. Ciprofloxacin 5µg/ml capacity disc were used as positive reference standard to determine the sensitivity of each microbial species tested. All petri plates were incubated at 37ºC for 24hours. After incubation diameter of inhibition was measured.

Antifungal activity:
Sabouraud dextrose agar medium was prepared and transferred into sterile petri plates. 200µl of the standardized fungal inoculums was spread agar medium using cotton swab. The test impregnated discs were placed on the inoculated agar medium. Co-trimaxazole 25µg/ml was used positive reference standard to determine sensitivity of each microbial species tested. All petri plates were incubated at 27ºc for 72 hours. After the incubation diameter of zone of inhibition was measured. Triplicate the results of antibacterial and antifungal.

Determination of minimum inhibitory concentration (MIC):
The minimum inhibitory concentration was found for all the test compounds against various strains of fungi.

Tube dilution method:
This method depends upon the inhibition of growth of microbial culture in a uniform solution of the test sample in a fluid medium that is favorable to its rapid growth.

The entire test sample were dissolved in dimethyl sulfoxide and diluted to highest concentration (5000µg/ml) to be tested, and then fold serial dilutions were made in a concentration range from 500µg/ml to 7.5µg/ml in sterile test tubes containing standardized inoculums. All the tubes were incubated at 37ºC for 24 hours, after incubation minimum inhibitory concentration values were determined.

RESULTS AND DISCUSSION

The preparation of in situ ophthalmic gelling system was carried out by using different polymeric systems like sodium alginate, hydroxyl propyl methyl cellulose, hydroxyl propyl cellulose, hydroxyl ethyl cellulose and carbopol. Then the formulations were subjected to general appearance, pH, gel strength, in vitro diffusion studies, and drug content, antimicrobial studies, anti bacterial and antifungal studies.

Table 2: Evaluation Parameters

Formulation code	pH measurement	Gelling capacity	Drug content (%)
F1	7.4	++	81.5
F2	7.4	++	75
F3	7.4	++	71
F4	7.4	++	72

++ Gelation immediate remains for few hours

Table 3: *In vitro* release data of ophthalmic gel

% CUMULATIVE RELEASE				
Time (min)	FORMULATION			
	F1	F2	F3	F4
0	0.000	0.000	0.000	0.000
30	21.809	3.793	6.462	18.473
36	26.698	8.835	13.866	20.993
90	36.637	13.928	15.673	21.868
120	38.000	17.736	20.498	28.088
150	39.372	19.912	25.370	31.365
180	50.763	21.775	28.955	35.006
210	51.255	25.656	29.236	38.014
240	54.749	28.907	30.185	40.714
270	64.279	32.855	31.140	41.769
300	68.894	33.837	31.769	42.164
330	71.548	34.492	36.070	43.894
360	75.556	39.488	38.077	51.642
390	80.932	39.859	39.432	52.791
420	83.018	41.899	41.465	60.952
450	85.785	42.954	43.849	69.858
480	87.238	44.017	47.253	73.843

Figure 1: Comparative *In vitro* release profile all formulations

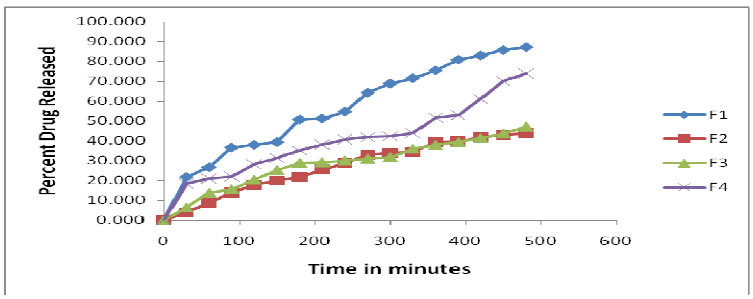

Table 4: Sterility testing data for diclofenac sodium *in situ* ophthalmic gel

Number of days	Sample number	Nutrient broth	Sabouraud broth
1	A	+	+
	B	+	+
	TEST	+	+
2	A	+	+
	B	+	+
	TEST	+	+
3	A	+	+
	B	+	+
	TEST	+	+
4	A	+	+
	B	+	+
	TEST	+	+
5	A	+	+
	B	+	+
	TEST	+	+
6	A	+	+
	B	+	+
	TEST	+	+
7	A	+	+
	B	+	+
	TEST	+	+

Clarity of all formulations was found to be satisfactory. The pH was within acceptable range and hence would not cause any irritation upon administration of the formulation. Table 2 also shows the result of drug content for all formulations. The drug content was found to be in acceptable range for all formulations. Percent drug content in all four formulations were in the range 71-82 %. The two main prerequisite of gelling system are viscosity and gelling capacity. The formulation should have an optimum viscosity which will allow its instillation into the eye as a liquid which will then undergo rapid sol-gel transition due to pH change. Moreover, to facilitate sustained release of drug to the ocular tissue the in situ formed gel should preserve its integrity without dissolving or eroding for a prolonged period of time. All the formulations gelled instantaneously on contact with STF. The in vitro release studies were carried out for all formulations using STF as the dissolution medium. The data of these studies are presented in Table. 3. results indicated that F1 showed better sustaining effect amongst all formulations. This may be due to the higher concentration of sodium alginate along with HEC. The formulation F1 passed the sterility test as there was no appearance of turbidity and hence no evidence of microbial growth when incubated for not less than 7 days. The in vitro efficacy study indicated that diclofenac sodium retained its antimicrobial efficacy when formulated as an in situ gelling system and the drug was active against the selected strains of microorganisms.

CONCLUSION

The present work was carried out to develop a in situ ophthalmic gel prepared by using diclofenac sodium as a model drug. It is a newer approach to improve easy eye instillation, residence time and bioavailability and prolong drug release. From the study conducted the

following conclusion were drawn by varying the concentration of polymers with two different gums ratio, it is to obtain the increased residence time and sustained drug release. Among the all formulations F1 shows better drug release when contacted with STF solution at 8 hrs study period. It shows antimicrobial, antibacterial and antifungal efficacy with selected microorganisms. The developed formulation is a viable alternative conventional eye solution by virtue of its ability to enhance bioavailability through its longer precorneal residence time and ability to sustain drug release.

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