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Research Article

Fabrication of Ophthalmic Insitu Gel of Diclofenac Potassium and its Evaluation

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Abstract: Ophthalmic formulations in the form of insitu gel system can be applied as solution or suspension that undergoes gelation after administration. Diclofenac Pottasium is one of the commonly used nonsteroidal antiinflamatory drug usually for the treatment of inflammation. Insitu gel formulation of diclofenal potassium ophthalmic drug delivery system used to reduce the inflammation caused by various diseases as well as to prevent postoperative inflammation in cataract surgery. Insitu gel formulation of ophthalmic delivery of diclofenac was fabricated using sodium alginate and hydroxy propyl methyl cellulose in different concentrations under aseptic conditions. Various evaluation tests as well as stability testing of formulations were carried out to identify the ideal formulation.From the parameters,the ideal formulation was identified, that having the polymer combination of Sodium alginate and Hydroxy propyl methyl cellulose in the ratio of .Thus the formulation canbe utilized for its sustained release property that may improve patient compliance.
Keywords: Diclofenac Pottasium, *Insitu* gel formulation,Ophthalmic drug delivery system.

INTRODUCTION

Ophthalmic drug delivery [1] is one of the most interesting and challenging endeavors of pharmaceutical sciences.Recently pharmaceutical industry specializing in the development of ophthalmic preparations have in treating ophthalmic disorders. New therapies may become available for preventing blindness [2] caused by degenerative diseases, including age related macular degeneration (AMD), macular edema, and diabetic retinopathy. Biotechnological products may also become available to treat the causes of multifactorial eye disorders like glaucoma [3,4].This specialized therapeutic system may release the active pharmaceutical ingredient in a controlled manner[8]. Ophthalmic drug delivery systems intended for treatment of various eye diseases [5].

Diclofenac potassium [6,7] is a non steroidal anti-inflammatory drug which acts specifically on inflammatory sites and there by decreases the inflammation. It is also used as 0.05% and 0.1% eye drops for the inhibition of intraoperative miosis (but it does not possess intrinsic mydriatic activity) and to prevent post operative inflammation in cataract surgery.

In situ hydro gel formulations [9] applied as solutions or suspensions that undergo gelation after instillation.These systems are more acceptable for the patients. Since they are administered into the eye as a solution and undergoes an immediate gelation when in contact with the eye.Studies have shown that the precorneal residence time of some *insitu* gelling for several hours. The *in situ* gelation has been the most attractive feature of these system.Various polymeric combinations [11], have been successfully used for fabrication. Hence, they are promising means for overcoming the shortcoming of conventional topical ophthalmic dosage forms like eye drops, suspensions and ointments.

MATERIALS AND METHODS

Materials

Diclofenac Potassium obtained as a gift sample from Novartis India Pvt. Ltd, Mumbai. Sodium Alginate purchased from Loba chemie Pvt Ltd Mumbai, Hydroxy propyl methyl cellulose (HPMC), and D-Mannitol were purchased from Finar chemicals,Ahammedabad and Merck Ltd ,New Delhi respectively. All other chemicals used were of AR grade.

Fabrication of Ophthalmic Solution

1. Compatibility Studies [25]

Compatibility between the drug and polymers were studied by FourierTransform Infrared spectroscopical method using KB disc method.

2. Preparation of Sodium alginate and HPMC solution.

Accurately weighed Sodium alginate and HPMC [8,11] were finely powdered in aseptic chamber with help of mortar and pestle. This mixture was added to sterile Phosphate buffer (pH 7.4) with constant stirring at a speed of 4000 rpm.Stirring was continued until a homogeneous solution was obtained (Batch- A

formulation). The same steps were repeated to get Batch-B formulations by using acetate buffer pH 5.0 instead of phosphate buffer pH 7.4.

3. Preparation of Drug polymer mixture [10,12]

A homogenous solution of drug-polymer mixture was prepared by continuous stirring in the following sequence of addition of Mannitol and finally the preservative Cetrimide. The pH was maintained at 7.4 for batch-A and pH 5.0 for batch-B. under aseptic condition (Laminar Air Flow Bench with HEPA Filter). The obtained solution was kept in sterile vial and sealed. They are stored for evaluation studies. Formulation chart is represented in Table No-1.

Evaluation of Ophthalmic Solutions [13,14,15]

1. Test for clarity

The clarity of the formulations was determined by black and white background. The vials were held horizontally and gently rotated immediately under the lamp and inverted once or twice to detect foreign particles.

2. Determination of pH

The pH of all formulations was determined immediately after preparation as well as after 24 hours of storage at Refrigerator with help of digital pH meter.

3. Sterility test

Direct inoculation technique was used for sterility testing of the ophthalmic solutions. Sterile Fluid Thioglycollate and Soya bean Casein media were used to detect bacteria and fungi growth respectively. Medias were sterilized by moist heat sterilization technique. One set of positive control (Bacillus Subtilis and Aspergillus Niger) and negative control for each medium were used for the comparative study. Few ml of ophthalmic solutions were aseptically transferred in to the sterile media. The media were incubated at 32.5°C and 22.5°C for detecting the growth of bacteria and fungi respectively.

4. Measurement of surface tension [16]

Surface tension was measured by Stalagmometer by drop count method. The number of drops were counted and calculated surface tension.

5. Determination of viscosity of ophthalmic formulations

Viscosity of the formulated solution and gel were measured by Brookfield DVE digital viscometer. Guard leg was mounted on the viscometer. Helipath spindle no.18 was used for measurement of viscosity of solution. Helipath spindle was inserted in the test material until fluid level was at the immersion groove on the spindle. The spindle was attached to the lower shaft of the viscometer. The shaft was lifted slightly; holding it firmly with one hand while screwing the spindle. The spindle code 00 was used for measurement of the viscosity of solution and spindle code S 18 was used for measurement of the viscosity of gel. The motor was turn on and spindle was rotated. The viscosity was noted from the display window and the readings were recorded.

6. Gel Characteristic studies [17]

Gelation studies were carried out in locally fabricated gelation cells. The cells were of cylindrical reservoirs capable of holding 3 ml of solution. The study was carried out using STF solution-A(sodium chloride 0.670 g, sodium bicarbonate 0.200 g, calcium chloride dihydrate 0.008 g and distilled water sufficient to make 100 g) and solution-B (bovine serum albumin 0.268g, lysozyme 0.268g, D-glucose 0.15g, sodium chloride 0.65g and distilled water qs to100g) which simulated the divalent cation content. The formulation (100 µl) was carefully placed into the cavity of the cup using a micropipette and 2 ml of gelation solution (STF solution-A and B) were added slowly in to it. Gelation was assessed by visual examination.

7. Drug content [18,19,22]

Drug content studies were done by UV spectrophotometric method. A quantity equivalent to 1.0 mg of formulation of Diclofenac potassium was dissolved in 25 ml distilled water. From this solution 2 ml samples were withdrawn and suitable dilutions were made and analyzed spectrophotometrically at 281.0 nm. The obtained data were taken to calculate drug content.

8. *In-vitro* release study [20,21,23]

The *in vitro* release of the formulation was studied using cellophane membrane. Freshly prepared STF- pH 7.4 used as the dissolution medium. One ml of formulation (equivalent to 0.5mg of Diclofenac potassium) was accurately placed into this assembly. The medium was stirred continuously at low speed. Aliquots,were withdrawn at hourly intervals and replaced with a fresh medium. The samples were suitably diluted and analyzed by UV-visible spectrophotometer at 281nm and calculated percentage drug release.

9. Release kinetics study [21,24]

To analyse the mechanism for the release kinetics of the dosage form, the data obtained was fitted to Zero order, Higuchi matrix, and Peppas model. After comparing the r-values , the best-fit model was selected.

10. Stability studies [20,21]

Stability testing of the ideal formulation was kept at 4ºC, 37ºC and 45 ºC for the period of 90 days. Three containers of each formulation type were used. The samples were analysed for their appearance, pH, viscosity, gelation nature, content of drug, *In- vitro* drug release at the end of test period and the results were recorded.

RESULTS AND DISCUSSION

Appearance and Clarity

On careful visual inspection against dark and white background, all the solutions were found clear and transparent.

pH of the Formulation

The pH of the formulations were determined and found that pH of the solutions were in the range of 4.5-7.4.This pH range is acceptable and this may minimizes the discomfort and irritation of cornea.

Sterility of the Formulation

The formulations prepared aseptically showed no turbidity after incubation at specified conditions up to 7 days. However, considerable turbidity was observed at the same time in all the media incubated as positive control with specific bacterial cultures. From the observations, the growth of microorganism was within the limit and thus the solutions proved for its sterility.

Determination of Surface tension

The surface tension of the prepared solutions were measured. The obtained values were very close to the value of normal human tears (43 dyne/cm). This will ensure good spreading of the solutions.

Viscosity of the Formulation

Viscosity of ophthalmic solutions after instillation is desired feature for sustaining therapeutic actions of diclofenac Potassium by providing its precorneal residence time. The increase in viscosity was achieved by inclusion of Sodium alginate and HPMC.These polymers may undergo phase transition in presence of divalent ions in tears. Sodium alginate has been used extensively for polymeric dispersions in buffers that typically show low viscosity up to pH 5 and coacervate in contact with tear and thus forms gel. The polymeric chains undergo hydration and swell to form a gel structure leads to its increased viscosity Among the various strengths attempted, the formulae having the composition of 2.5% w/v of Sodium alginate and 0.2% w/v HPMC was found satisfactory results.. These compositions could maintain good clarity in solution form and sufficiently high viscosity, when converted in to gel form (*In situ*).

Gel Characteristics

The *in vitro* gelation studies were performed to assess the gel characteristics, whichsubsequently would affect drug diffusion in the simulated tear fluids. Sodium Alginate at 0.5%w/v possessed weak gelation in 2-3 minutes in stimulated tear fluid, where as at 1% concentration possessed instantaneous but weak gelation. Alginates at higher concentrations (1.5%-2.5%) showed instantaneous gelation .When Sodium alginate matrices are brought in contact with tear, the polymer tend to hydrate, forming a superficial gel, which eventually erodes. Its gelling properties have been utilized for making *in situ* gelling systems for ocular delivery

Drug Content

The drug content of Diclofenac Potassium were evaluated to identify the ideal formulationDrug content of all the formulation was found in the range of 0.045 to 0.048.This was almost similar to the drug loaded.

***In-vitro* release study**

In vitro diffusion studies were conducted for the formulations to optimize the ideal formulation.The diffusion medium used was simulated tear fluid (STF). The *in vitro* drug release conditions may be very different from those likely to be encountered when instilled in to the eye. However, the results clearly show that the gel have ability to retain the drug for prolonged period. In the *cul-de-sac* of the eye, the gels will probably undergo faster dissolution. No significant difference in the *in vitro* release of formulation prepared with phosphate (pH 7.4) and acetate buffer (pH 5.0) was noted. Results indicated that the **A5** showed better sustaining effect amongst all formulations, which showed 82.75% release in 10 hours. This may be due to the higher concentration of Sodium alginate along with HPMC.

The result of physicochemical values are reported in Table No: 2.The percentage release profile readings of comparable formulations are reported in Table No: 3 and corresponding plots are represented in Figure No: 1.

Table No:1 Formulation Chart for the Ophthalmic Solution

Formulation code	Diclofenac Potassium (%w/v)	Sodium alginate(%w/v)	HPMC (%w/v)	Mannitol (%w/v)	Cetrimide (%w/v)
A1	0.05	0.5	0.2	5.0	0.1
A2	0.05	1.0	0.2	5.0	0.1
A3	0.05	1.5	0.2	5.0	0.1
A4	0.05	2.0	0.2	5.0	0.1
A5	0.05	2.5	0.2	5.0	0.1
B1	0.05	0.5	0.2	5.0	0.1
B2	0.05	1.0	0.2	5.0	0.1
B3	0.05	1.5	0.2	5.0	0.1
B4	0.05	2.0	0.2	5.0	0.1
B5	0.05	2.5	0.2	5.0	0.1

Table No:-2 Physicochemical Evaluation parameters of the formulations

Formulation Identity	pH *	Surface tension(dynes/cm)*	Viscosity(cps)*	Drug Content(gm)*
A2	7.4	43.71±0.413	15.93±0.4	0.0456±0.141
A3	7.4	44.76±0.382	17.1±0.21	0.0468±0.152
A4	7.4	44.95±0.267	19.9±0.25	0.0477±0.178
A5	7.4	43.16±0.289	21.6±0.09	0.0485±0.098
B1	5.1	42.23±0.413	11.9±0.16	0.0454±0.182
B5	5.1	44.94±0.398	20.2±0.24	0.0478±0.214

* Three observations ± SD.

Release kinetics Studies

The regression coefficients for the formulations were fitted to Zero order , First order, and Higuchi's plots.The results shows all the formulations follows Higuchi's order release Kinetcs.The comparative plots indicated that the drug release was significantly prolonged by using the *in situ* gelling system due to the addition of the polymers, Sodium alginate and HPMC.

Table No:3 Percentage Drug Release Profile of Comparable Formulations

Sl No	Time (Hrs)	Formulation Identity*					
		A2	A3	A4	A5	B1	B5
1	0	0	0	0	0	0	0
2	0.25	12.22 ±0.23	12.95 ±0.83	11.09 ±0.32	10.70 ±1.03	26.06 ±0.96	8.11 ±0.93
3	0.5	25.55 ±0.15	18.93 ±0.52	23.03 ±0.94	22.38 ±1.19	31.28 ±1.03	16.22 ±0.45
4	1	35.55 ±1.03	37.87 ±0.71	32.05 ±0.31	36.98 ±0.93	52.12 ±0.18	29.41 ±0.36
5	2	52.22 ±0.24	45.84 ±0.81	45.07 ±0.72	43.79 ±0.67	76.10 ±0.12	45.64 ±0.51
6	3	63.33 ±0.61	52.82 ±0.13	51.08 ±0.62	53.52 ±0.91	79.23 ±1.03	49.70 ±0.28
7	4	67.77 ±1.92	58.80 ±1.02	59.09 ±0.42	60.34 ±0.72	--	53.76 ±0.92
8	5	78.88 ±0.98	66.78 ±0.98	67.11 ±0.14	66.18 ±0.83	--	64.91 ±0.81
9	6	--	74.75 ±0.24	72.12 ±0.62	69.09 ±0.19	--	72.02 ±0.92
10	7	--	--	74.12 ±0.92	71.04 ±0.28	--	80.13 ±0.63
11	8	--	--	--	73.96 ±0.82	--	81.14 ±0.82
12	9	--	--	--	78.85 ±0.72	--	--
13	10	--	--	--	82.75 ±0.73	--	--

* Three observations ± SD.

Stability Studies

Various physico-chemical evaluations were undergone in selecting ideal formulation.From the results the formulation **A5** was found to be satisfactory for the following parameter, hence was selected for assessing its shelf-life at accelerated condition of temperature and humidity over a period of 90 days. The results revealed that the doesnot showed any significant changes of the ideal formulation after the stability testing period. Thus the ideal formulation have adequate shelf-life.

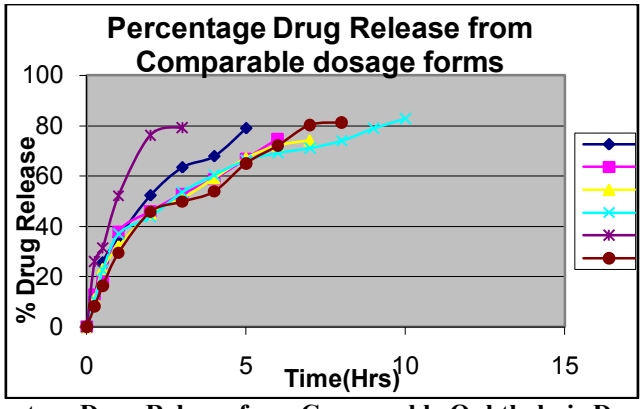

Figure No-1: Percentage Drug Release from Comparable Ophthalmic Dosage forms.

CONCLUSION

Modern research in novel approach in drug therapy is focused in maximizing the therapeutic efficacy of the drug. Many polymers have been used to fulfill the objective of sustained release of drug through ocular drug delivery to prevent the loss of drugs through tears. The present work was aimed at fabricating and evaluating ophthalmic drug delivery of Diclofenac potassium for *in situ* gel. Diclofenac potassium was successfully formulated as an *insitu* gelling system for the sustained release of drug for a prolonged time. It has been viewed for its ability to enhance pre-corneal residence time and thereby ocular bioavailability .The ease of administration along with its ability to provide sustained release could probably result in less frequent administration ,thus enhancing patient compliance. With increasing the concentration of polymers, sodium alginate & hydroxy propyl methyl cellulose, which was used as phase transition element for sustaining the diffusion of Diclofenac potassium, it was observed that the onset of gelation was faster and formed gels were more viscous, resulting in the retardation of release of diclofenac potassium. pH of ophthalmic formulation is one of the major factors for the solubility of Diclofenac potassium. The gelling systems were prepared using acetate buffer (pH 5.0) and phosphate buffer (pH 7.4) in order to examine the effect of pH on the Release profile of the drug from the corresponding batches, as sodium alginate is a well known vehicle with a low viscosity upto pH 5.0 and coacervation occurs at pH 7-7.4.The samples showed no significant changes during the period of 90 days and are stable.

Based upon the release profiles of the drug loaded polymer, the formulation (**A5)** containing polymer concentration about 2.5% showed a compatible higher sustained release with better pattern of drug release. The optimum concentration of HPMC (0.2%) for ideal gelation and release characteristics, in combination with 2.5% Sodium alginate. This combination could serve as a suitable *in situ* gelling vehicle for ophthalmic use. Therefore it can be concluded that the *in situ* gels have desirable characteristics for the localized delivery of the drug within the eye.

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REFERENCES

1. Remington, The science and Practice of pharmacy, 21st edition, Mack Publishing Co., Pennsylvania,1995: 850, 861- 862.
2. Tortora GJ; Principle of anatomy and physiology, 10th edition, 2003: 531-545.
3. Desai SD; Encyclopedia of Pharmaceutical Technology, Vol. 11, Marcel Dekker, New York, 1995: 43-75.
4. Lieberman HA and Lachmam L; Pharmaceutical Dosage Forms: Parentral Medication, 2nd edition, Vol.1 Marcel Dekker Inc., Inc., New York, 1993, 541-581.
5. Ross and Wilson, Anatomy and physiology in health education and illness, 9th edition, 2001, 192-192.
6. Guyton AC, Hall TE; Text book of medical physiology, 11th edition, 617- 635.
7. Kaur IP, Kanwar M; Drug Delv., Ind. Pharma, 2002: 28, 473-493.
8. Gupta SK; Ophthalmic drug delivery systems, The Eastern Pharmacist, 1988:71-74.
9. Chein YW; Novel Drug Delivery System, Marcel Dekker Inc., New York, 2nd edition, Vol. 50, 1992: 269-300.
10. Patel GM, Patel MM; Resent Advances and Challenges In Ocular Drug Delivery System, Pharma Times, 2007; 39(1): 21-25.
11. Sattone MF; Mucosal Drug Delivery, Ocular – Progress & Problems in Ophthalmic Drug Deli

very-Business briefing, Pharmatech, 2002; 606-613.
12. Rowe RC, Sheskey PJ and Wellev PJ; Handbook of Pharmaceutical Excipients, 4th edition, 2003; 89-92: 447-450.
13. Hong-Ru Lin and Sung KC; Journal of controlled release, 2000; 69:379-388.
14. Edsman K, Carlfos J, Peterson R; Euro. J. of Pharmaceutical Sciences, 1998: 6, 105-112., 113-119.
15. Paulson M, Hagerson H, Edsman K; European Journal of Pharmaceutical Sciences, 1999; 9: 99-105.
16. Cho KY, Chung TW, Kim BC, Kim MK, Lee JH, Wee WR, Cho CS; International Journal of Pharmaceutics, 2003; 260: 83-91.
17. Saettone MF, Chetani P, Cerbai R, Mazzanti G, Braghiroli L; International Journal of Pharmaceutics, 1996; 142,103-113.
18. Ruel-Gariepy E, Leroux JC; European Journal of Pharmaceutics and Biopharmaceutics, 2004; 58: 409-426.
19. Sankar A, Chandrasekar K, Durga S, Prasanth KG, Nilani P, Geetha G, Ravichandran V, Vijayakumar A and Raghuraman S; Indian Journal of Pharmaceutical Sciences, 2005; 67(4): 475-476.
20. Srividya B, Cardoza RM, Amin PD; Journal of Controlled release, 2001;73, 205- 211.
21. R.C.Doijad.F.V.Manvi, V.S.N.Malleswara Rao, Prajakta Alase. Indian Journal of Pharmaceutical Sciences, 2006; 64(6): 814-818.
22. Pandit JK, Bharat Di, Shrinatha A, Ridhurkar DN, Singh S; Indian Journal of Pharmaceutical Sciences, 2007; 69(1), 37-40.
23. Kumar MT, Bharathi D, Balasubramaniam J, Kant S, Pandit JK; Indian Journal of Pharmaceutical Sciences, 2007; 67(3): 327-333.
24. Shirwaikar AA and Rao PG; Indian Journal of Pharmaceutical Sciences, 1995; 57(4):143-147.
25. Silverstein RM, Wepster FX; Spectrometric Identification of organic compounds, John Wiley & Sons Inc, New York, (IR) 71-143, (1H-NMR) 144- 216.