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FORMULATION AND EVALUATION OF OPHTHALMIC IN-SITU GEL OF DICLOFENAC SODIUM USING NATURAL GELLING AGENT

[1]SONU DWIVEDI, [2]DR. JITENDRA BANWEER, [3]Mr. ABHISHEK BANKE

[1]M. Pharma Pharmaceutics, Department of Pharmacy, Sagar Institute of Research and Technology Pharmacy Bhopal (M.P.)

[2]Director, Department of Pharmacy, Sagar Institute of Research and Technology Pharmacy Bhopal (M.P.)

[3]Assistant Professor, Department of Pharmacy, Sagar Institute of Research and Technology Pharmacy Bhopal (M.P.)

Abstract: The pH-sensitive ophthalmic in situ gel of Diclofenac sodium was successfully formulated by using TSP as gelling agent and Sodium CMC as viscosity enhancer and release retardant. The formulated pH sensitive in situ gels were then evaluated for appearance, clarity, pH, gelling capacity, refractive index, drug content, viscosity, sterility, antimicrobial efficacy, ocular irritation and in vitro release in artificial tear fluid. The formulations were liquid at around pH 6 and underwent rapid gelation upon raising the pH to 7.0. The gels were stable for the period of 8 hours and provided delayed release. Response surface design, regression analysis, contour and surface plots, and desirability function obtained from response optimizer have been proven to be a useful approach for the optimization of formulations.

Index Terms - Ophthalmic delivery system, formatting, TSP, Sodium CMC and Anatomy of Eye.

INTRODUCTION: Ophthalmic delivery system is a challenging area for the formulation chemist due to unique anatomy and physiology of the eye, which consists of three layers namely: Epithelium, Stoma and Endothelial. Outer epithelium layer act as barrier for hydrophilic drug, while stroma acts as diffusion barrier for lipophilic drug. Endothelium is lipoidal in nature [1], The anatomy, physiology and biochemistry of the eye render this organ exquisitely impervious to foreign substances. The challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage. Topical drug delivery is a desirable route for drug administration. It will provide better relief, easy administration and improve patient compliance [2], Conventional dosage forms like eye drop, is commonly used but it has some disadvantages like rapid spillage of drug from eye results low therapeutic effect, repeated drug administration is needed. Repeated dose administration results damage to eye tissue. However viscous semisolid formulations like ointment and gel provide sustained release but it has some drawbacks like blurred vision, sticky sensation results eye irritation [1]. In ophthalmic drug delivery, a challenging task is normal-ocular protective mechanisms like blinking, tears drainage; that promotes rapid clearance, reduce bio-availability which results short duration of pharmaceutical response [2]. To increase corneal residence time and improve bio availability, different ophthalmic delivery systems like gels, suspension, collagen shield and inserts are developed. Because of blurred vision, variability in dose instilled, sticking of eye lids and patient-discomfort these

formulations have not been widely accepted.[4] In recent years, there have been significant research efforts for the design of ophthalmic delivery system that are provided sustained and controlled drug release. Insitu gelling system is such type of delivery system, where it is liquid upon instillation and undergoes phase transition to viscous gel in accordance with pH, temperature, electrolyte composition [5]

In-Situ Gel Ophthalmic in-situ gelling is comprising of environmentally sensitive polymers that will be altered structurally with the small changes in specific conditions like pH, temperature and ionic strength in the environment. In-situ forming gels are liq- uids during instillation into the eye and then undergoes rapid gelation in the cul-de-sac of the eye to form viscoelastic gels in response to environmental changes (Fig. 2); lastly release the drug slowly under physiological conditions . Consequently, the residence time of the gel formed in-situ will be extended and the drug is released in a sustained manner which leads to enhanced bioavailability, minimized systemic absorption and reduced frequent dosing regimen resulting to improved pa- tient compliance . Furthermore, some other potential ad- vantages such as simple manufacturing process, ease of ad- ministration, and deliverance of accurate dose have been ex- hibited by in-situ gelling system.

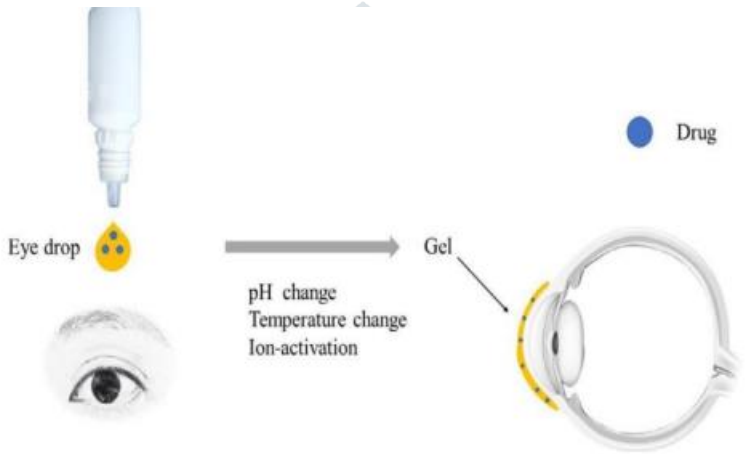


Fig. 1 – In-situ forming gels process. The formulation is liquid when instilled into the eye which undergoes gel formation rapidly in the cul-de-sac of the eye in response to environmental changes such as pH, temperature and ion; finally release the drug slowly under physiological conditions.

The prepared in-situ gel formulations were evaluated for clarity, pH measurement, gelling capacity, drug content, rheological study, in vitro diffusion study, isotonicity, antibacterial activity, in-vivo ocular testing in rabbits and accelerated stability studies. © 20XX JETIR Month 201X, Volume X, Issue X www.jetir.org (ISSN-2349-5162) Paper id Journal of Emerging Technologies and Innovative Research (JETIR) www.jetir.org 27 The formulation should have an optimum viscosity that will allow for easy instillation into the eye as a liquid (drops), which would undergo a rapid sol-to-gel transition (triggered by pH, temperature or ion exchange). Texture analysis The firmness, consistency, and cohesiveness of hydrogels are evaluate by using texture analyser which mainly indicates the syringeability of sol so can formulation can easily be administered in-vivo. Higher values of adhesiveness are needed to maintain the intimate contact with the tissues. Physical parameters:-The formulated in-situ gel solution is tested for clarity, pH, gelling capacity, and drug content estimation. Gelling capacity:-The gelling capacity of the prepared formulation is determined by placing a drop of the formulation in a vial containing 2.0 ml of freshly prepared simulated tear fluid and visually observed. The time taken for its gelling is noted. Rheological studies:-The viscosity measurements can be calculated using Brookfield viscometer, Cone and Plate viscometer. The in-situ gel formulations were placed in the sampler tube. From the literature it was evident that, the formulation before gelling should have a viscosity of 5 to 1000 mPas. And after ion gel activation by the eye, will have a viscosity of from about 50- 50,000 mPas. The samples are analyzed both at room temperature at 25°C and thermo stated at 37°C ± 0.5°C by a circulating bath connected to the viscometer adaptor prior to each measurement. The angular velocity of the spindle was increased 20, 30, 50, 60, 100, 200 and the viscosity of the formulation is measured. All the formulations exhibited Newtonian and pseudoplastic flow characteristics before and after gelling in the simulated tear fluid respectively. In vitro drug release studies:-In vitro release study of in-situ gel solution was carried out by using Franz

diffusion cell. The formulation placed in donor compartment and freshly prepared simulated tear fluid in receptor compartment. Between donor and receptor compartment dialysis membrane is placed (0.22µm pore size). The whole assembly was placed on the thermostatically controlled magnetic stirrer. The temperature of the medium was maintained at 37°C ± 0.5°C. 1ml of sample is withdrawn at predetermined time interval of 1hr for 6 hrs and same volume of fresh medium is replaced. The withdrawn samples are diluted to 10ml in a volumetric flask with respective solvent and analyzed by UV spectrophotometer at respective nm using reagent blank. The drug content calculated using the equation generated from standard calibration curve. The % cumulative drug release (%CDR) calculated. The data obtained is further subjected to curve fitting for drug release data. The best fit model is checked for Krosmeyers Peppas and Fickinian diffusion mechanism for their kinetic Isotonicity Evaluation:-Isotonicity is important characteristic of the ophthalmic preparations. Isotonicity has to be maintained to prevent tissue damage or irritation of eye. All ophthalmic preparations are subjected to isotonicity testing, since they exhibited good release characteristics and gelling capacity and the requisite viscosity. Formulations are mixed with few drops of blood and observed under microscope at 45X magnification and compared with standard marketed ophthalmic formulation. Antibacterial activity The microbiological growth of bacteria is measured by concentration of antibiotics and this has to be compared with that produced by known concentration of standard preparation of antibiotic. To carryout microbiological assay serial dilution method is employed. Ocular irritancy test The Draize irritancy test was designed for the ocular irritation potential of the ophthalmic product prior to marketing. According to the Draize test, the amount of substance applied to the eye is normally 100µl placed into the lower cul-de-sac with observation of the various criteria made at a designed required time interval of 1hr, 24hrs, 48 hrs, 72hrs, and 1week after administration. Three rabbits (male) weighing 1.5 to 2kg are used for the study. The sterile formulation is instilled twice a day for a period of 7 days, and a cross-over study is carried out (a 3 day washing period with saline was carried out before the cross-over study). Rabbits are observed periodically for redness, swelling, watering of the eye. Accelerated stability studies Formulations are placed in ambient colour vials and sealed with aluminium foil for a short term accelerated stability study at 40±2 °C and 75±5% RH as per International Conference on Harmonization (ICH) states Guidelines. Samples are analyzed every month for clarity, pH, gelling capacity, drug content, rheological evaluation, and in vitro dissolution.[18][17].

PROPOSED METHOD: The objective of the present research study was to formulate, develop and optimize in situ gelling systems for ocular and periodontal therapeutic applications. To achieve these objectives the following specific aims of this research work were set.

- To extract the natural polymer and optimize the extraction process.
- To formulate, develop and optimize mucoadhesive in situ gelling ocular drug delivery systems containing Diclofenac Sodium using natural polymer and in combination.
- To optimize the ratio of natural polymer and in combination.
- To characterize the in situ gelling systems and gels with respect to viscometric properties
- To compare the developed in situ gelling system with respective marketed preparation.

Preformulation studies Formulation studies are needed to ensure the development of a stable as well as therapeutically effective and safe dosage form. The Preformulation studies, performed in this research include melting point , orgenoleptic properties ,identification of drug, solubility analysis, partition coefficient and drug compatibility. Melting point determination: Melting point determination of DIiclofenac sodium is done by using Melting Point Apparatus. In that method the presealed capillary is filled by the small amount of drug. Then capillary and thermometer were placed in Melting Point Apparatus. Then see capillary for melting the drug. The temperature were noted when the drug start to melt and the drug till complete melt. Melting Point: Melting Point of DIiclofenac sodium is found in the range of 280 to 282 degree celcius[26]

Partition coefficient determination:- The partition coefficient of DIiclofenac sodium was determined by shaking flask method in n-octanol: water system. 10 mg of drug DIiclofenac sodium was added into 50 ml each of octanol and water. The mixture was shaken for 24 hours until equilibrium was reached. Phases were separated in a separating

funnel and the aqueous phase was filtered through 0.2µ filter, suitably diluted and amount of DIiclofenac sodium in aqueous phase was determined by measuring the absorbance at 258nm using UV spectrophotometer. The partition coefficient (Po/w) of DIiclofenac sodium was calculated from the ratio between the concentration of DIiclofenac sodium in organic (Coil) and aqueous phase (Caq.) using following equation.

Table 1: Partition Coefficient of DIiclofenac sodium

Test	Specification	Observation
Partition Coefficient	octanol:water : PBS (pH 7.4)	4.57

Table: 2 Shows Organoleptic Properties of Diclofenac Sodium

S.No.	Organoleptic Properties	
1	Color	Yellowish white.
2	Crystalinity	crystalline powder
3	Hygroscopicity	No Hygroscopicity
4	Taste	Bitter
5	Odour	odorless

Solubility properties

For quantitative solubility studies, known amount of drug (10mg) was suspended in a series of different solvents and shaken for 24 hrs. Using wrist action shaker (York India). Solubility of DIiclofenac sodium in different solvents is recorded.

Table 3: Solubility of Diclofenac sodium in different solvent

Solvent	Solubility		
Solubility in water (µmol/ml)	Slightly soluble	0.0019	
Solubility in 5% PG water (µmol/ml)			
Solubility in Hexane (µmol/ml)	soluble	21.0	
Solubility in IPM (µmol/ml)	Freelly soluble	43.0	

Identification of Drug

Determination of λmax: The λmax was found to be at 276 nm. UV Spectrophotometric analysis of drug: Ultraviolet absorption in the range 200 to 400 nm of a 25 mg/ml solution in methanol was determined.

a. Preparation of Buffers and Reagents:

Sodium hydroxide solution 0.2 M – 8.0 gm of sodium hydroxide was dissolved in distilled water and diluted to 1000 ml with distilled water.

Potassium dihydrogen phosphate solution 0.2 M – 27.218 gm of potassium dihydrogen phosphate was dissolved in distilled water and diluted to 1000 ml..

Phosphate buffer solution

PH 7.4 – 250 ml of 0.2 M potassium dihydrogen phosphate was placed in 1000 ml volumetric flask. 112 ml of 0.2 M sodium hydroxide was added and then volume was adjusted with distilled water up to 1000 ml. PH was adjusted to 7.4 with dilute sodium hydroxide.

Data and Sources of Data

For this study secondary data has been collected. From the website of KSE the monthly stock prices for the sample firms are obtained from Jan 2010 to Dec 2014. And from the website of SBP the data for the macroeconomic variables are collected for the period of five years. The time series monthly data is collected on stock prices for sample firmsand relative macroeconomic variables for the period of 5 years. The data collection period is ranging from January 2010 to Dec 2014. Monthly prices of KSE - 100 Index is taken from yahoo finance.

Theoretical framework

Variables of the study contains dependent and independent variable. The study used pre-specified method for the selection of variables. The study used the Stock returns are as dependent variable. From the share price of the firm the Stock returns are calculated. Rate of a stock salable at stock market is known as stock price. Systematic risk is the only independent variable for the CAPM and inflation, interest rate, oil prices and exchange rate are the independent variables for APT model.

Consumer Price Index (CPI) is used as a proxy in this study for inflation rate. CPI is a wide basic measure to computeusualvariation in prices of goods and services throughout a particular time period. It is assumed that arise in inflation is inversely associated to security prices because Inflation is at lastturned into nominal interest rate andchange in nominal interest rates caused change in discount rate so discount rate increase due to increase in inflation rate and increase in discount rateleads todecreasethe cash flow's present value (Jecheche, 2010). The purchasing power of money decreased due to inflation, and due to which the investors demand high rate of return, and the prices decreased with increase in required rate of return (Iqbal et al, 2010).

RESULTS OF DESCRIPTIVE STATICS OF STUDY VARIABLES

Quantitative Estimation of Drug:

Determination of absorption maxima – The standard sock solution of DIiclofenac sodium was prepared by dissolving 50 mg of drug in methanol in 100 ml volumetric flask. Stock solution of DIiclofenac sodium was further diluted in methanol to get standard solution concentration of 100 mcg/ml. The resulting solution was then scanned between 200 -400 nm. UV visible spectrophotometer (shimadzu 1601 UV Japan).

Standard curve of DIiclofenac sodium in phosphate buffer solution (PH 7.4) Accurately weighed 100 mg of DIiclofenac sodium was dissolved in 100 ml of pH 7.4 phosphate buffer to give a solution of 1 mg/ml (1000 µg/ml) concentration and this served as the first standard stock solution. From this stock solution 1 ml was taken and diluted to 100 ml using pH 7.4 phosphate buffers to get a solution of 10 µg/m concentration and this solution served as the second standard solution. Into a series of 10 ml volumetric flasks, aliquots of second standard solution (i.e.) 2 ml, 4 ml, 6 ml, 8ml, 10ml and 12 ml was added and the volume made up to 10 ml using pH 7.4 phosphate buffer. The absorbance of these solutions was measured against reagent blank at 276 nm using Shimadzu (UV-1601) UV spectrophotometer. Standard curve was plotted with concentration on x-axis and absorbance on y-axis[32][35]

Calibration curve

The calibration curve was constructed by analyzing 10 different Concentrations of standard solution, prepared on the same day The range of solutions varied from 2.0 to 20.0 µg/ml All determinations were conducted in triplicate.

Calibration Curve: Standard curve of DIiclofenac sodium in phosphate buffer solution (PH 7.4) Table 2: Absorbance value of DIiclofenac sodium in PBS pH 7.4 (λmax 292 nm).

Calibration Curve:

Standard curve of DIiclofenac sodium in phosphate buffer solution (PH7.4) Table Absorbance value of DIiclofenac sodium in PBS pH 7.4 (λmax 276 nm).

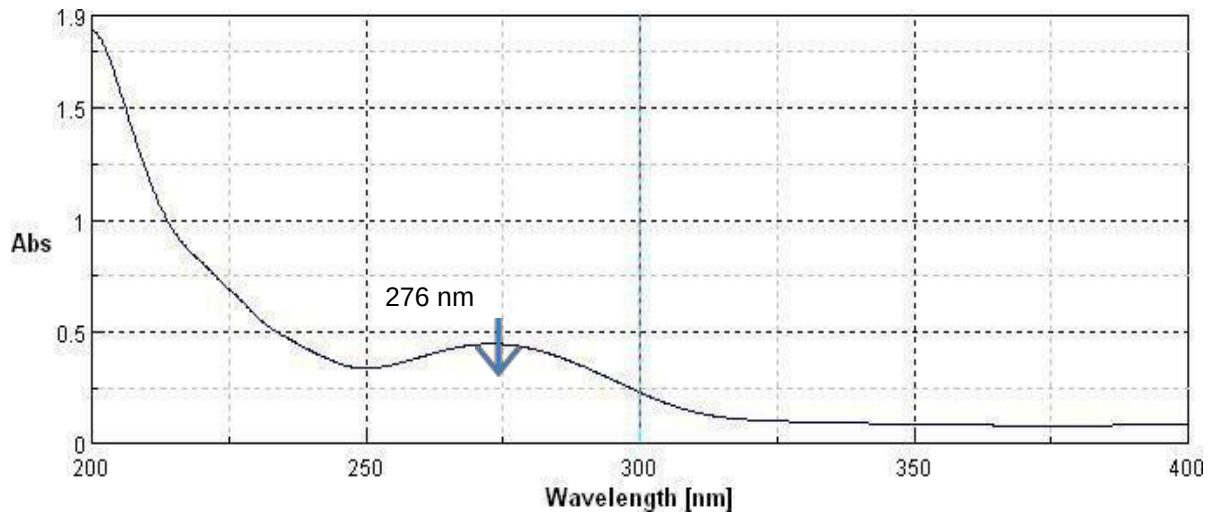


Fig. 2: UV spectra of Diclofenac sodium.

Table 4: Data of concentration and absorbance

Sr. No.	Concentration (µg/ml)	Absorbance at 276 nm
1	2	0.1012
2	4	0.1891
3	6	0.2492
4	8	0.3219
5	10	0.3991
6	12	0.4849
7	14	0.5972

The graph of absorbance versus concentration for pure Diclofenac sodium is shown in Figure 2. It was found to be linear in the concentration range of 2-14 µg/ml at 276 nm. Hence the drug obeys the Beer's- Lambert law in this range.

Table 5: Data for standard curve parameters

Sr. No.	Parameters	Values
1	Correlation coefficient (R)	0. 922
2	Slope	0.039
3	Intercept	0.016

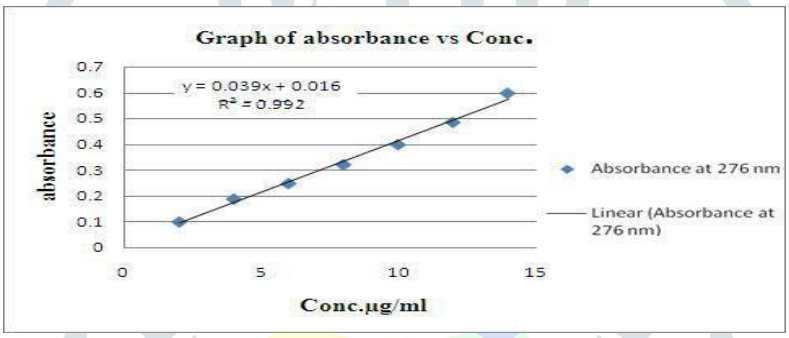


Fig. 3: Calibration curve of Diclofenac sodium in phosphate buffer (pH 6.8)

IR Spectroscopy of Diclofenac Sodium

An FT infrared (FT-IR) spectroscopy study was carried out to check the compatibility between the drug DIiclofenac sodium. The spectra obtained from FT infrared spectroscopy studies at wavelength from 4000 cm to 400-1 cm are shown Figures characteristic peaks obtained. This study was carried out to find out the possible drug DIiclofenac sodium . FT-IR of DIiclofenac sodium showed the following characteristic peaks peak at 3265-1 cm due to carboxylic group 2931-1 cm due to alkanes group stretching 1724-1 cm due to stretching of carbonyl group, 1294-1 cm due to stretching of amines, in between 1100 to 1400-1 cm due to the presence of halogen group. thus revealing compatibility of the selected drug. .Infrared spectrum of Diclofenac sodium was recorded. The observed peaks are match with the peaks given in pharmacopoeia which confirms that the supplied samples was of Diclofenac sodium.[38]

Table 6: Data for standard curve parameters.

Sr. No.	Parameters	Values
1	Correlation coefficient (R)	0. 992
2	Slope	0.016
3	Intercept	0.069

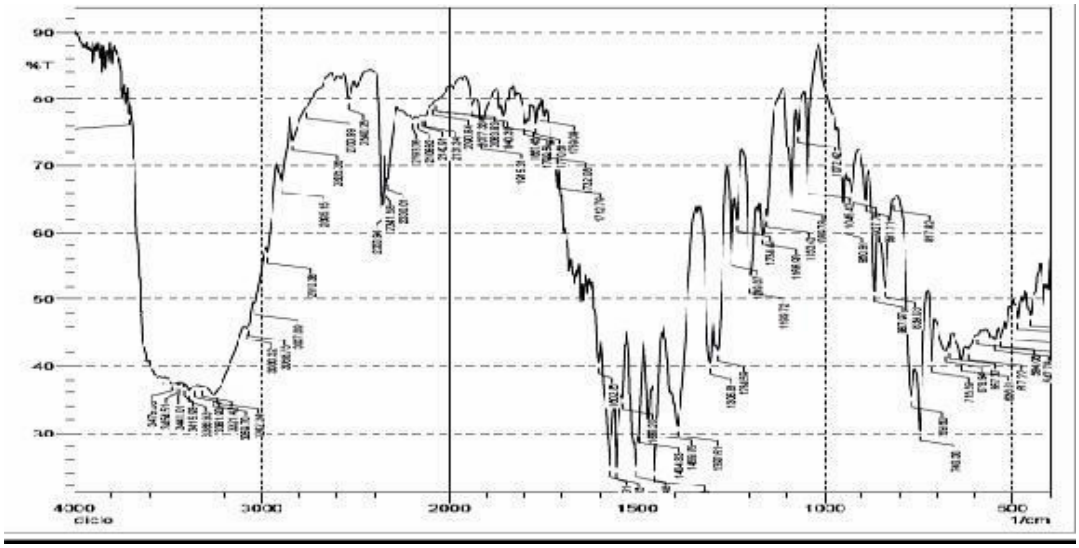


Fig. 4 FTIR interpretation of DIiclofenac sodium

Visual appearance, clarity, pH and Refractive index

The formulations were light yellow in colour. When examined against white and dark background, the solutions were devoid of any gritty particles. These formulations were clear except F5and F6 due to high concentration of polymer, which were slightly translucent. The pH values were within acceptable range of 5.0 to 7.0 and would not cause any irritation upon administration in the eyes.

Refractive index of tear fluid is 1.31 to 1.38. It is recommended that eye care solution should have refractive index values not higher than 1.48. The situ gel had refractive index values ranging from 1.34 to 1.37 which are within the recommended values. Data reflected that no visual problem may cause in patient after administration of formulation. Clarity, pH and Refractive index for all the 6 formulations:

Table 7: Clarity, pH and Refractive index of ATF, Optimized and all the formulations

S.No.	Formulation no.	Clarity	pH	Refractive index
1	F1	Slightly Translucent	5.02	1.33
2	F2	Clear	5.05	1.36
3	F3	Clear	6.01	1.36
4	F4	Clear	6.00	1.31
5	F5	Slightly Translucent	05.8	1.36
6	F6	Clear	5.03	1.38

Gelling Capacity and Drug Content

In-situ gelling capacity of developed formulation was observed in a thermostatically maintained artificial tear fluid in a vial. Out of the thirteen formulations, formulations F5, F6, have shown strong gel forming capacity. The gel formation was observed within a minute and lasted for more than 8 hours. The formulation F3, F4, F5, F6, have shown gel formation after few minutes and remained for 6-8 hours. The formulations F1 and F2 have shown low gelling capacity. The optimized formulation also have shown gel formation after few minutes and remained for 6-8 hours. The drug content in all the formulations and optimized was found within a range of 98.92% to 101.3%.

Table 8: Gelling Capacity and Drug Content

S.No.	Formulation no.	Gelling Capacity	Drug Content (%)
1	F1	+	92.89
2	F2	+	93.11
3	F3	++	99.93
4	F4	++	98.13
5	F5	+++	100.06
6	F6	+++	108.92

Rheological Study

Increasing the shear rate from 3 to 100 rpm at pH 6, F4, F5, and F6 Optimized formulations showed Newtonian behavior whereas other formulations showed Pseudo- plastic behavior.

So, F3, F5, F6 and Optimized formulations were subjected for further study at physiological conditions (pH 7.0 and Temperature 37ºC). pH of the formulations were raised to 7.0 by addition of 0.5N NaOH and temperature was maintained to 37ºC on a thermostatically controlled water bath. The formulations at physiological condition showed Pseudo plastic behaviour as solutions transformed into gels with high viscosity .F5 and F6 Optimized formulation with concentration of TSP of 1.0% and 1.2 % showed highest gelling behaviour whereas F1 with TSP concentration of 0.2 % showed lowest gelling behaviour. Thus, the obtained results also confirmed that increase in the concentration of TSP results in increased gelling behaviour of the formulation.

Kinetic study

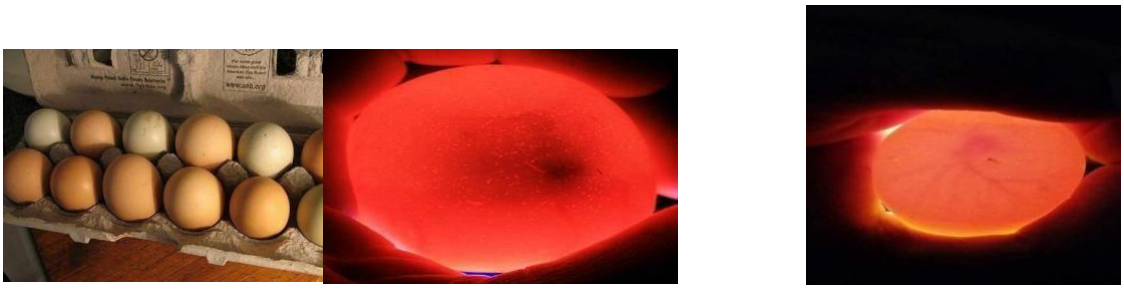
Various mathematical models were used to predict the drug release pattern from in-situ gelling system. The formulations formed opaque matrix immediately upon addition to the dissolution medium, due to increase in pH of the artificial tear fluid. The drug release pattern obtained for the gelled samples is characteristic of hydrophilic matrices. The initial fast release of Diclofenac sodium may be due to the fact that the in situ gels are formulated in water and hence the polymers (TSP) are completely hydrated. Hydrated TSP when come in contact with ATF leads to gelation. In this prehydrated matrix, water penetration is no longer limit for drug release leading to an apparent diffusion controlled release. Diclofenac sodium release from the in situ gel of the optimized formulation follows the Higuchi square root law and Krosmeyer-Peppas law with R2> 0.98.Higuchi matrix model suggests that the drug release occurs by diffusion mechanism. The polymers can absorb a significant amount of water to form an elastic gel and, at the same time, release the dissolved entrapped drug by diffusion through swollen regions of the gel. Similarly, the release index (n) of the optimized formulations was 0.508, which indicated that the formulation showed drug release by Non- Fickian diffusion mechanism.

Table 9: Drug Release Kinetics of formulated batches and optimized batch

Formulation Code	Zero Order	First Order	Higuchi	Korsmeyer-Peppas	
	R2	R2	R2	R2	N
F1	0.978	0.994	0.998	0.995	0.615
F2	0.957	0.997	0.994	0.993	0.527
F3	0.979	0.943	0.997	0.997	0.494
F4	0.936	0.995	0.986	0.982	0.531
F5	0.950	0.993	0.991	0.989	0.533

Ocular irritation test

Ocular irritation test was performed by HET-CAM test in fertilized hen egg. The scoring pattern was assigned based on degree of damaging over developed blood vessels. 0.9% Sodium chloride solution and 0.1N NaOH were used as negative and positive control respectively to compare the results obtained from optimized and marketed formulations. Test for each sample was performed in triplicates. Both optimized and marketed formulations have shown no any irritation with total of '0' score as that of negative control whereas positive control have shown all the three responses of hyperemia, hemorrhage, and coagulation within 30 seconds of application.



Collection of Fertilized egg Candling of Fertilized egg Candling of Fertilized egg on 9th day

Gative Control (0.9% Normal Saline) Positive Control (0.1 N NaOH), Optimized Formulation, Marketed Formulation

Fig.. 5: Images captured during HET- CAM experiment

Sterility test and Antimicrobial efficacy test

Sterilization by autoclaving had no effect on the physical and chemical properties of the formulation. There was no appearance of any bacterial and fungal colonies in the plates when the plates were incubated for not less than 14 days at 37ºC. Hence, there was no evidence of microbial growth. The optimized formulation therefore passed the test for sterility and sterility was achieved by this technique without affecting the nature of formulation. The zone of inhibition (ZOI) measured was compared with that of control (Standard Diclofenac sodium) for Pseudomonas aeruginosa and Staphylococcus aureus. At concentration of 100 mcg/ml and 20 mcg/ml, the optimized formulation have shown the efficacy of about 100.67% against Staphylococcus aureus and at concentration of 25 ppm and 5 ppm, the optimized formulation have shown the efficacy of about 102.72%. Thus, the study indicated that the Diclofenac sodium retained its antimicrobial efficacy even after formulated as an in situ gelling system and excipients did not interfere with the antibacterial properties of Diclofenac sodium.

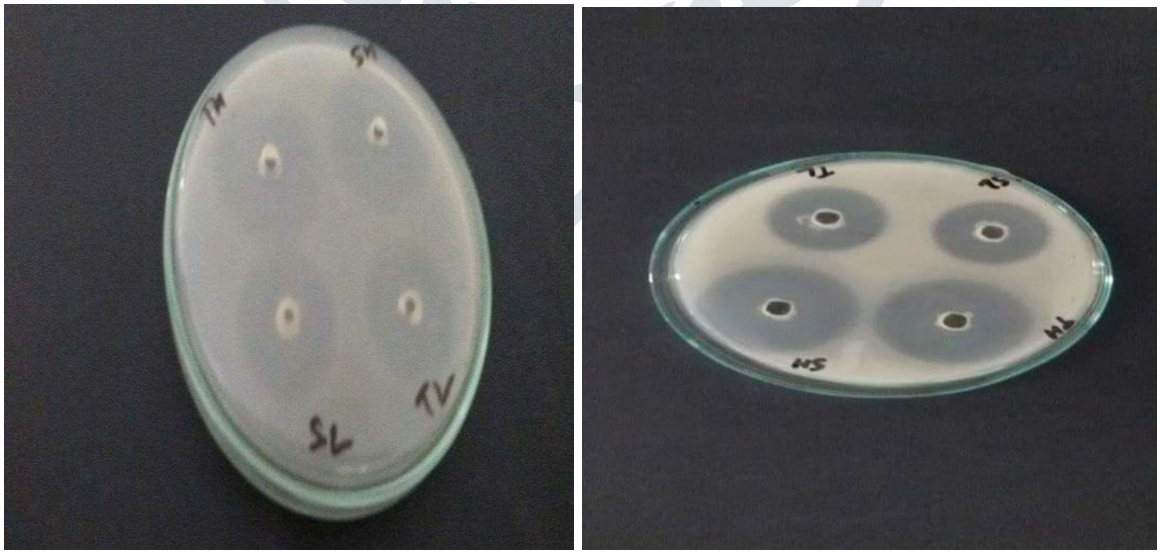


Fig: 6. ZOI against S. Aureus (Pure Diclofenac sodium as standard)

CONCLUSION

The pH-sensitive ophthalmic in situ gel of Diclofenac sodium was successfully formulated by using TSP as gelling agent and Sodium CMC as viscosity enhancer and release retardant. The formulated pH sensitive in situ gels were then evaluated for appearance, clarity, pH, gelling capacity, refractive index, drug content, viscosity, sterility, antimicrobial efficacy, ocular irritation and in vitro release in artificial tear fluid. The formulations were liquid at

around pH 6 and underwent rapid gelation upon raising the pH to 7.0. The gels were stable for the period of 8 hours and provided delayed release. Response surface design, regression analysis, contour and surface plots, and desirability function obtained from response optimizer have been proven to be a useful approach for the optimization of formulations. The optimized formulation has shown sustained drug release over the period of 8 hours. So, this formulation can be used an alternate to conventional eye drops to improve the bioavailability through its longer pre corneal residence time and ability to sustain drug release. Ease of administration and reduced frequency of administration is its another importance which results in better patient compliance. The antibiotic efficacy studies have shown that developed formulation is responsive towards Staphylococcus aureus and Pseudomonas aeruginosa and the HET- CAM test proved to show no any ocular irritancy upon application. From all the experimental results it can be concluded that the present work was satisfactory in developing pH Sensitive ophthalmic in situ gel of Diclofenac sodium.

REFERENCES

1. Ali, Macha, S., P.M. Hughes, and A.K. Mitra, Overview of ocular drug delivery, in Ophthalmic Drug Delivery Systems, Second Edition. 2003, CRC Press, 1-12.
2. Gaudana, R., et al., Recent perspectives in ocular drug delivery. Pharmaceutical research, 2009; 26(5): 1197-1216.
3. Anand, B.S., S. Dey, and A.K. Mitra, Current prodrug strategies via membrane transporters/receptors. Expert Opinion on Biological Therapy, 2002; 2(6): 607-620.
4. Tangri, P. and S. Khurana, Basics of ocular drug delivery systems. International Journal of Research in Pharmaceutical and Biomedical Sciences, 2011; 2(4): p. 1541- 1552.
5. Singh, K.P. and S.P. Verma, Novel Polymeric in Situ Gel Forming System for Ophthalmic Drug Delivery. International Journal of Drug Delivery Technology, 2013. 4(1).
6. Balpande, H.M., et al., Compatibility study of metformin with pharmaceutical excipients. Int. J. Chem. Tech. Res, 2013; 5(1684): e1693.
7. Chidambaram, M. and K. Krishnasamy, Drug-Drug/Drug-Excipient Compatibility Studies on Curcumin using Non-Thermal Methods. Advanced pharmaceutical bulletin, 2014; 4(3): 309.
8. Reddy, J. and M.G. Ahmed, Sustained ocular delivery of sparfloxacin from pH triggered in situ gelling system. MU J Pharm, 2013; 40(3): 16-25.
9. Mahesh, N. and A.A. Hajare, Ion activated in situ gel system for ophthalmic delivery of Moxifloxacin hydrochloride. Latin American Journal of Pharmacy, 2010; 29(6): 876-82.
10. Patil, S., et al., Formulation and evaluation of an in situ gel for ocular drug delivery of anticonjunctival drug. Cellulose chemistry and technology, 2015; 49(1): 35-40.
11. Ramchandra, L.U., et al., Design and Development of pH-triggered in situ gelling system of ciprofloxacin. International research journal of pharmacy, 3(5): 418-422.
12. Marques, M.R., R. Loebenberg, and M. Almukainzi, Simulated biological fluids with possible application in dissolution testing. Dissolution Technol, 2011; 18(3): 15-28.
13. Srividya, B., R.M. Cardoza, and P. Amin, Sustained ophthalmic delivery of ofloxacin from a pH triggered in situ gelling system. Journal of controlled release, 2001; 73(2): 205-211.
14. Dr.B.K.Dubey1, Mithun Bhoumick1, Vimal Kumar Shahwal1*, Abhay Upadhyay in Ophthalmic Drug Delivery System: An Overview, Ijbr2 (6).2011.391-410
15. Yumei Wu a, b, Yuanyuan Liu a, b, Xinyue Li a, , DerejeKebebe a, b, e, Bing Zhang a, b, .
16. USCH+LOMB In corporated.2019 “Discussed about eye or ophthalmic disease”
17. Jing Ren a, b, Jun Luf, Jiawei Li a, b, c, Shouying Du d ,Zhidong Liu a , b , in .Research progress of in-situ gelling ophthalmic drug delivery system, Asian Journanalof Pharmaceutical Sciences 14 (2019) 1– 15.
18. Americanoptometic Association 2019-Discussed about dry eyes diseases. Gourav Rajoria*, Arushi Gupta in In-Situ Gelling System: A Novel Approach for Ocular Drug Delivery Am. J. PharmTech Res. 2012; ISSN: 2249-3387
19. Takuzo Kamishita, "Gel preparations for topical application of diclofenac sodium." U.S. Patent US4670254, issued October, 1983.US4670254
20. CharlierC,Michaux C:Dual inhibition of cyclooxygenase-2 (COX-2) and 5- lipoxygenase (5-LOX) as a new strategy to provide safer non-steroidal anti- flammatory drugs. Eur J Med Chem. 2003- Discussed about mechanism of action.
21. Dr.B.K.Dubey1, Mithun Bhoumick1, Vimal Kumar Shahwal1*, Abhay Upadhyay in Ophthalmic Drug Delivery System: An Overview, Ijbr2 (6).2011.391-410

22. Yumei Wu a, b, Yuanyuan Liu a, b, Xinyue Li a, , DerejeKebebe a, b, e, Bing Zhang a, b, .
23. USCH+LOMB In corporated.2019 “Discussed about eye or ophthalmic disease”
24. Jing Ren a, b, Jun Luf, Jiawei Li a, b, c, Shouying Du d ,Zhidong Liu a , b , in .Research progress of in-situ gelling ophthalmic drug delivery system, Asian Journanalof Pharmaceutical Sciences 14 (2019) 1– 15.
25. Americanoptometic Association 2019-Discussed about dry eyes diseases. Gourav Rajoria*, Arushi Gupta in In-Situ Gelling System: A Novel Approach for Ocular Drug Delivery Am. J. PharmTech Res. 2012; ISSN: 2249-3387
26. Takuzo Kamishita, "Gel preparations for topical application of diclofenac sodium." U.S. Patent US4670254, issued October, 1983.US4670254
27. CharlierC,Michaux C:Dual inhibition of cyclooxygenase-2 (COX-2) and 5- lipoxygenase (5-LOX) as a new strategy to provide safer non-steroidal anti- flammatory drugs. Eur J Med Chem. 2003- Discussed about mechanism of action.