

Original Research Article**Design and Evaluation of Ion Activated *In Situ* Ophthalmic Gel of Moxifloxacin Hydrochloride and Ketorolac Tromethamine Combination using Carboxy Methylated Tamarind Kernel Powder**Shashank Nayak N^{1*}, Srinivasa U²¹Ph.D Research scholar Pacific Academy of Higher Education and Research University Udaipur, Rajasthan, INDIA and
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Abstract: *In situ* gel is a novel ophthalmic dosage form, which persists as solution form at room temperature but undergoes gel transformation when instilled into ophthalmic cavity, thus enhancing the patient compliance. In this present study ion activated *in situ* ophthalmic gel of Moxifloxacin hydrochloride and Ketorolac tromethamine combination are prepared by using gelrite (0.1-0.25% w/v) as gelling agent and carboxy methyl tamarind kernel powder (0.2-0.45% w/v) as viscosity enhancing agent and rate controlling polymer, Benzalkonium chloride in suitable proportion was used as preservative. The compatibility study between drug and polymer was done by FTIR and DSC. The formulations were sterilized by autoclaving at 121⁰ C at 15 PSI at 20 min. The formulations passed various evaluation parameters like visual appearance, clarity, pH, gelling capacity, drug content and *in vitro* diffusion studies, antimicrobial study, stability study. The drug release pattern was analysed by derivative spectroscopy using ZCP method. The release pattern of the best formulation indicated that both the drug showed sustained release pattern for a period of 12 hours thus enhancing the contact time of drug with ocular tissues and reducing the nasolacrimal drainage. From this present work it can be concluded that *In situ* ophthalmic gel is an alternative to convention drug delivery system.

Keywords: Zero crossing point (ZCP), gelrite, Carboxy methyl Tamarind kernel powder, Zone of inhibition, Differential scanning calorimetry

INTRODUCTION

Eye drops are the most conventional ophthalmic delivery systems which result in poor bioavailability and therapeutic response since the high tear fluid turnover and dynamics cause rapid precorneal elimination of the drug. A high frequency of eye drop instillation is associated with decrease patient compliance. Addition of excess drug in the formulation to solve the bioavailability problems is potentially dangerous if the drug solution drained from the eye into systemically absorbed from the nasolacrimal duct. Numerous ophthalmic vehicles such as inserts, ointments, suspensions and aqueous gels have been developed in order to lengthen the residence time of instilled dose and enhance the ophthalmic bioavailability. These ocular drug delivery systems, however, have not been used extensively because of some drawbacks such as blurred vision from ointments or low patient compliance from inserts. Above mentioned problems can be overcome by the use of *in situ* gelling systems, a liquid dosage form suitable to be administered by instillation into eye, which upon exposure to physiological conditions, changes to the gel

phase thus increasing the precorneal residence time of the delivery system and enhancing the ocular bioavailability. It comprises the ease of eye drop instillation and patient compliance as well as sustained release property that is described to intensify ocular bioavailability. Depending on the method employed to cause sol to gel phase transition on the ocular surface, the following three types of systems have been recognized:

- PH-triggered - The polymers used in this system are pseudo latexes - carbomer (carbopol), cellulose acetate phthalate latex (CAP-latex).
- Temperature-dependent - Poloxamers (Pluronic), cellulose derivatives (MC, HPMC), Xyloglucan.
- Ion-activated induced - Alginates, Gelrite (Gellan gum).

In this current work ion activated *in situ* gel of Moxifloxacin HCl and Ketorolac Tromethamine combination using gelrite as gelling agent and carboxy

methyl tamarind kernel powder as viscosity enhancing agent

MATERIAL AND METHODS

Moxifloxacin hydrochloride and Ketorolac tromethamine and gelrite were obtained as gift sample from micro labs Bangalore. Carboxy methyl tamarind kernel powder was purchased from tamarind magic Hyderabad.

Simultaneous estimation of Moxifloxacin HCL and Ketorolac tromethamine in Simulated tears fluid

Wavelength scan was done from 200-400 nm by using 2-10 µg/ml solution for Moxifloxacin HCL and 2-14 µg/ml for ketorolac tromethamine in double beam UV spectrophotometer Shimadzu (UV-1601) to get λ_{max} and absorbance of the standard solution were noted down to get zero order derivative peak of various standard solution

First order derivative spectroscopy of Moxifloxacin HCL and Ketorolac Tromethamine [1, 2]

The obtained zero order peaks was converted into first order derivative spectra to get the ZCP of both the drugs, which is further used for simultaneous estimation of drugs in the in situ formulations.

Preparation of ion activated *in situ* gelling system [3]

Required quantity of both the drugs was dissolved in 70 ml of deionised water, gelrite was added slowly with constant stirring at 20 rpm using magnetic stirrer. Carboxyl methyl tamarind kernel powder was sprinkled over the solution and left for nearly 3 hours. Stirring was continued at 20 RPM followed by addition of benzalkonium chloride and the volume was made upto 100 ml with deionised water. The formulae of the preparation are given in Table 1.

Table-1: Composition of *In situ* ophthalmic gel

Ingredients(% w/v)	F1	F2	F3	F4	F5	F6
Moxifloxacin HCL	0.5	0.5	0.5	0.5	0.5	0.5
Ketorolac tromethamine	0.5	0.5	0.5	0.5	0.5	0.5
Gelrite	0.1	0.15	0.2	0.25	0.25	0.25
Carboxy methyl Tamarind kernel powder	0.2	0.25	0.3	0.35	0.40	0.45
Benzalkonium chloride	0.01	0.01	0.01	0.01	0.01	0.01
Deionized water QS	100	100	100	100	100	100

RESULT AND DISCUSSION

Zero order and first order spectra of Moxifloxacin HCL and Ketorolac tromethamine in Simulated tear fluid

The maximum absorption of Moxifloxacin HCL was found out at 288 nm, Figure 1 and for ketorolac tromethamine was found out at 322 nm, Figure 2. The zero order derivative spectra of

Moxifloxacin HCL which had absorption maxima of 288 nm was deravated in software and was converted to first order spectra. The ZCP was found at 286nm Figure 3. The zero order spectra of Ketorolac tromethamine which had absorption maxima of 322 nm was deravated in software to first order spectra. The ZCP was found at 328nm Figure 4.

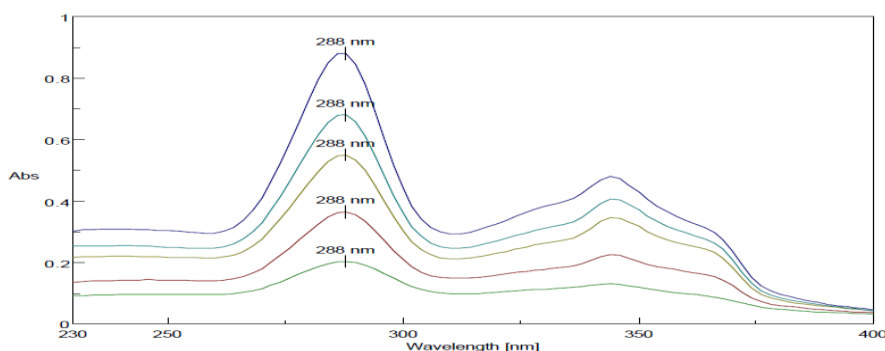


Fig-1: Overlay of λ_{max} of Moxifloxacin HCL (2-10 µg/ml)

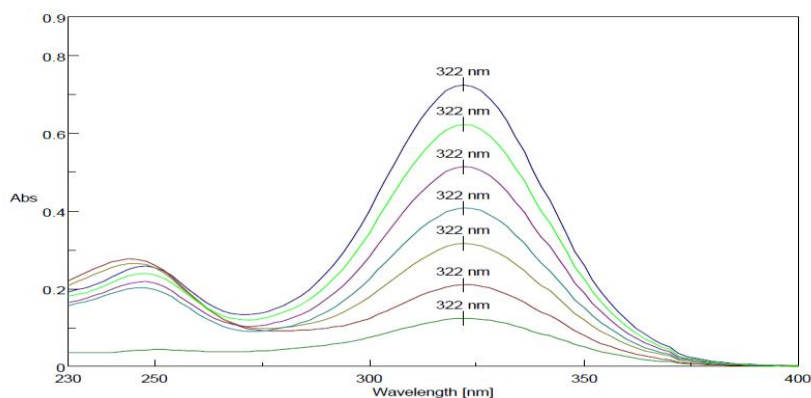


Fig-2: Overlay of λ max of ketorolac tromethamine (2-14 μ g/ml)

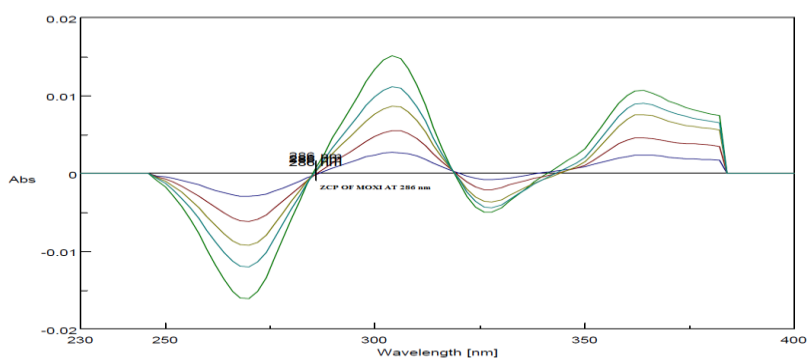


Fig-3: ZCP of Moxifloxacin HCL at 286 nm

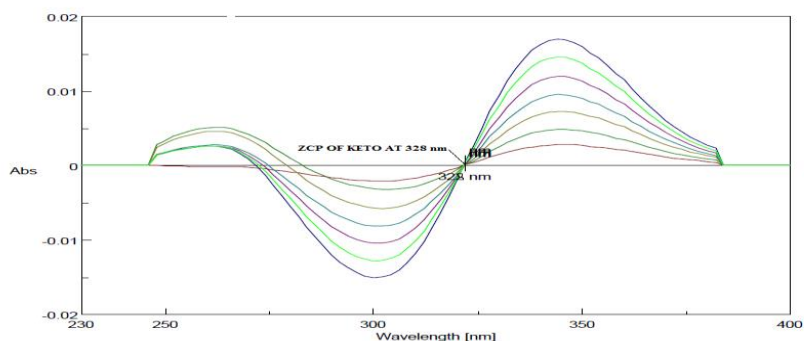


Fig-4: ZCP of Ketorolac tromethamine at 322 nm

Visual appearance, Clarity, pH, Drug content [4-10]

All the preparations were light yellow and translucent in nature. The pH was in the range of 7.3 to 7.4. The drug content was in the range of 98.5 to 99.26 % for both the drugs.

***In vitro* gellation study**

Gelling strength of formulations having different proportions of gelrite and carboxy methyl tamarind kernel powder were evaluated by placing a

drop of polymeric solution in vials containing 1 ml of freshly prepared simulated tear fluid, equilibrated at 37⁰ C. The gel formed and time taken for gellation was assessed visually. The formulation F6 showed gellation for a period of 12 hours, the composition of artificial tear fluid used was NaCl 0.670 g, sodium bicarbonate 0.200 g, calcium chloride -2 H₂O 0.008 g, purified water q.s. 100.0 g. For easy evaluation 20 ml of preparation were mixed with 20 ml of STF and maintained at 37⁰ C Figure 5,6.

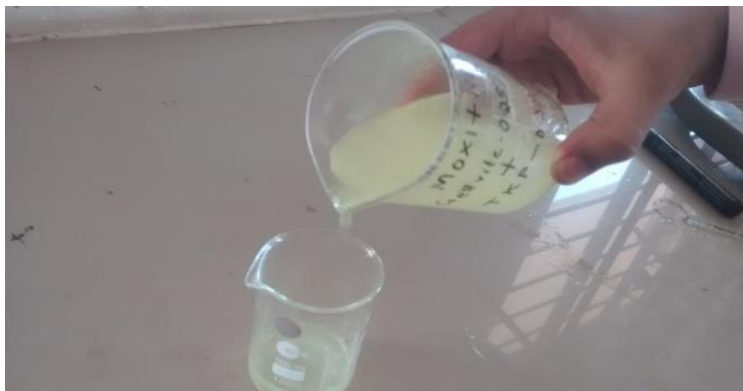


Fig-5: No gelation at room temperature-low viscosity (Non physiological condition)



Fig-6: Gellation at physiological condition- high viscosity

Rheological studies

All the formulations were within 50 cps before gellation at non physiological condition and upto 30,000 cps after gellation at physiological condition which was determined by using brook fields viscometer by using T bar spindle and spindle no 0. The formulations showed pseudo plastic flow.

In Vitro drug release studies

In-vitro release studies were carried out using bi-chambered donor receiver compartment model

(Franz diffusion cell) and this was placed on magnetic stirrer and temperature was adjusted to $37 \pm 0.5^{\circ}\text{C}$. The drug content was analyzed using UV Spectrophotometer at 286 and 322 nm against reference standard using simulated tear fluid as blank. The release studies of Moxifloxacin HCL and Ketorolac tromethamine in mentioned in figure 7, 8. The best formulae F6 showed controlled release for a period of 12 hours and showed peppas model of kinetics.

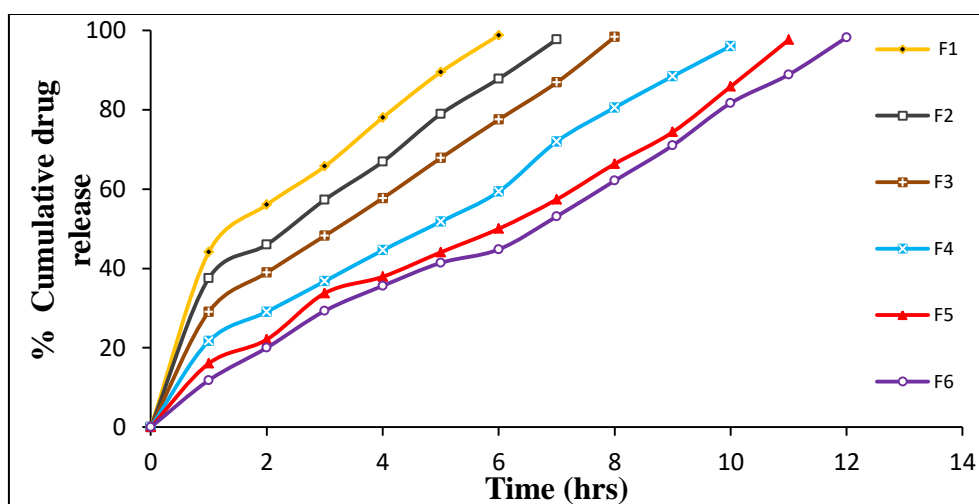


Fig-7: % CDR of Moxifloxacin HCL at 322 nm (ZCP of keto)

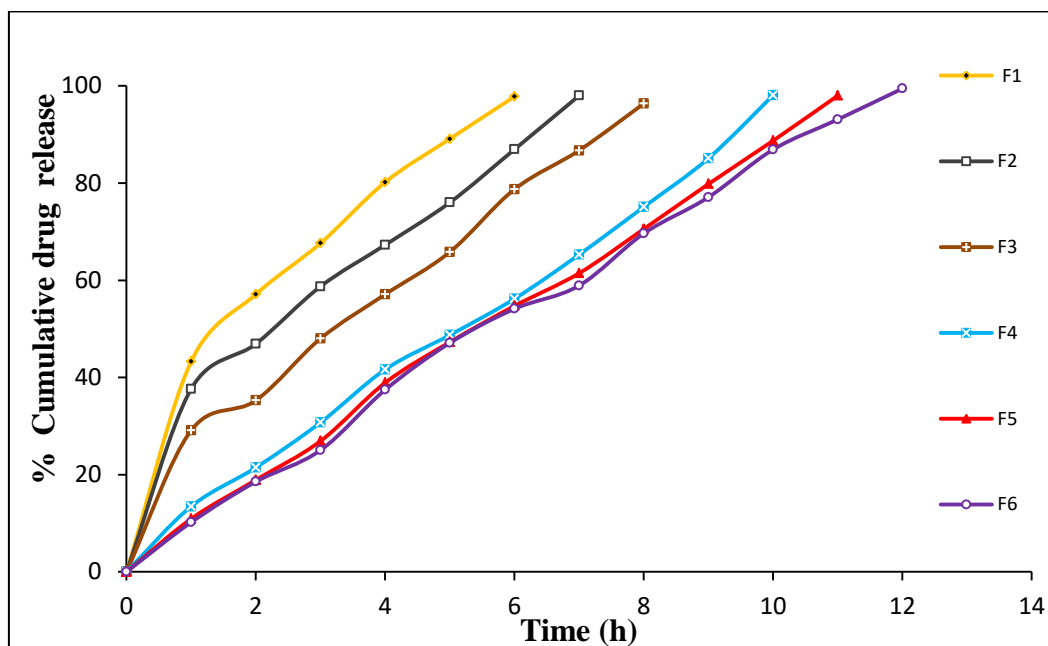


Fig-8: % CDR of Ketorolac tromethamine at 286 nm (ZCP of Moxi)

Sterility studies

The sterility testing was done by direct inoculation technique for a period of 14 days. All the formulations passed the sterility test since there was no growth of microorganisms in both fluid thioglycolate and soyabean casein digest media.

Antimicrobial activity

The Zone of Inhibition was better with E coli(41 mm with formulation and 42 mm with marketed preparations) when compared to S aureus (36 mm with formulations and 37 mm with marketed preparation) Fig-9, 10.The present study results indicate that Moxifloxacin hydrochloride retained its antimicrobial efficacy when formulated as an in situ gelling system.



Fig-9: ZOI of Moxifloxacin HCL with S aureus

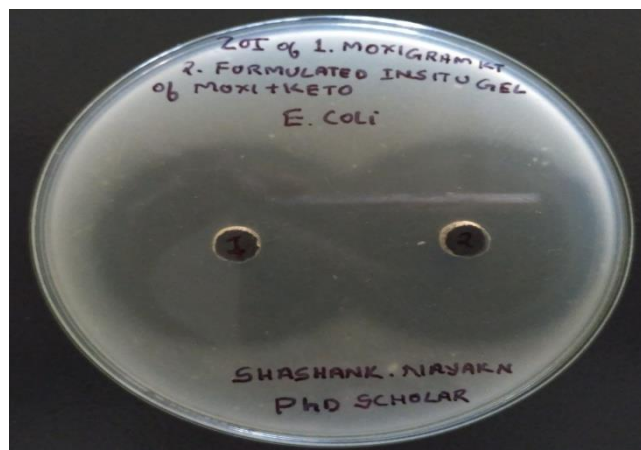


Fig-10: ZOI of Moxifloxacin HCL with E coli

Compatibility study

Compatibility study was done by FTIR, Figure 11 and DSC study which indicated that drugs are compatible with the polymers. The endothermic peak of

Moxifloxacin HCL was found out at 256.55 degree C, and Ketorolac tromethamine was found at 169.90 degree C. The optimized formulae showed nearly similar peak at 169.33 and 239.34 degree C.

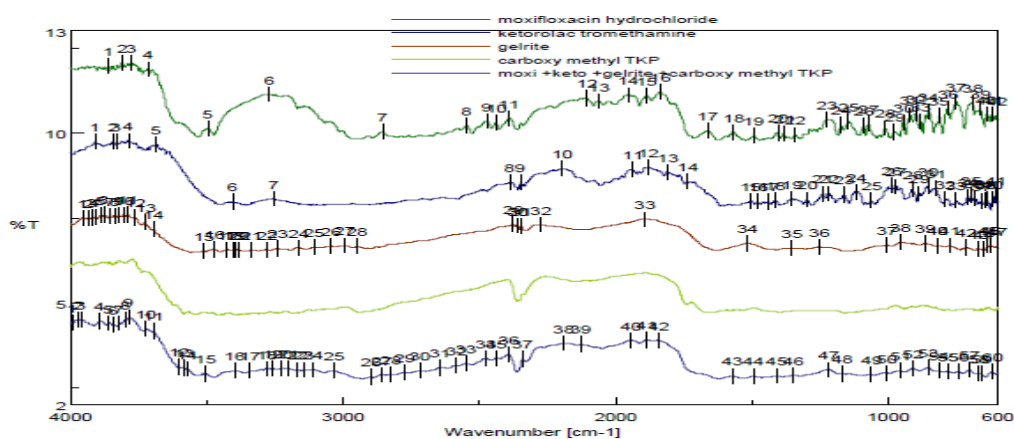


Fig-11: FTIR of Individual drugs, polymers and optimized formulation

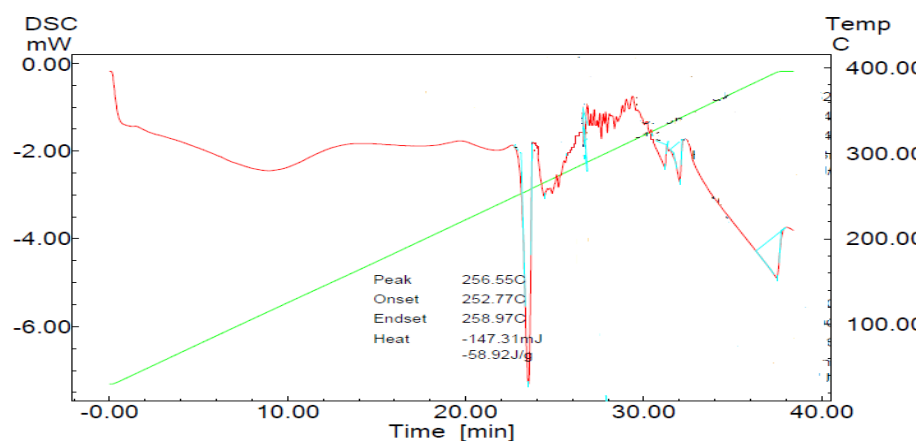


Fig-12: DSC of Moxifloxacin HCL- Peak was found at 256.55 degree C

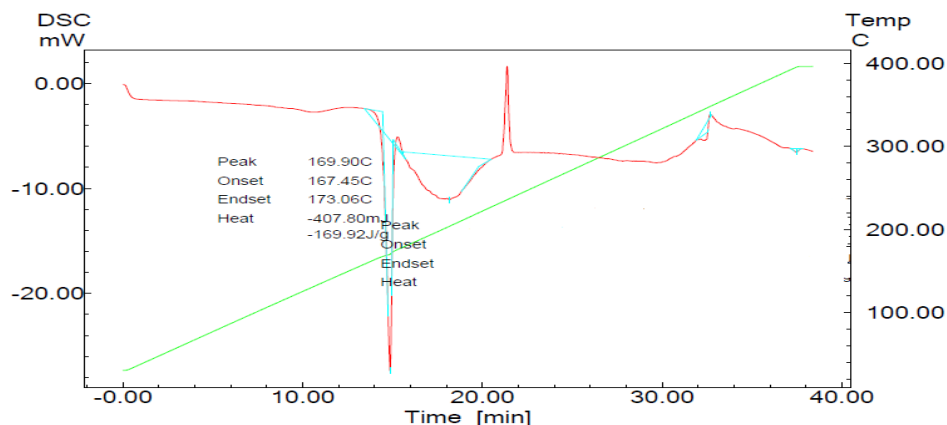


Fig-13: DSC Of Ketorolac tromethamine-Peak was found at 169.90 degree C

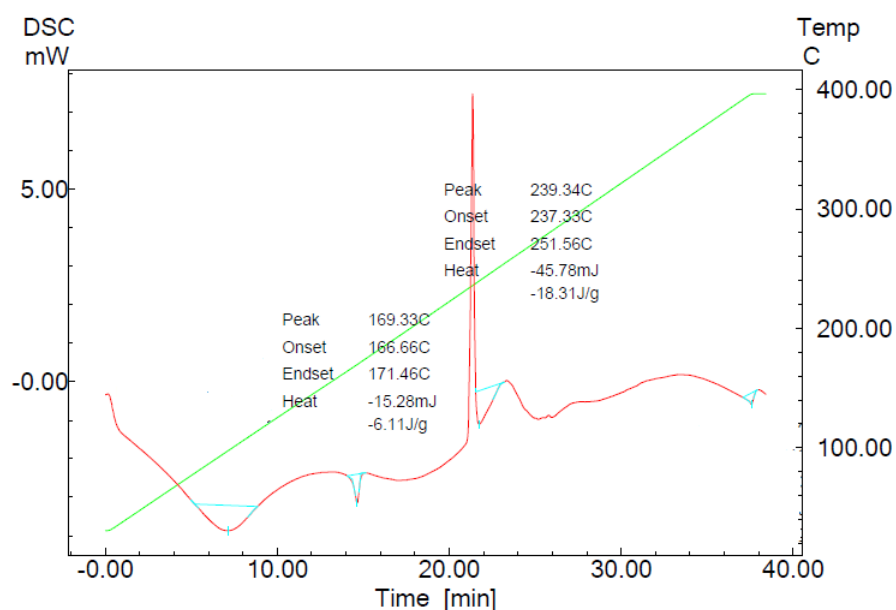


Fig-14: DSC of Moxifloxacin HCL and Ketorolac tromethamine with gellrite, carboxy methyl tamarind kernel powder and other excipients- peak at 169.33 and 239.34 degree C

Irritation study- Red blood cell lysis test

The irritation study was done by analysis of RBC of humans without formulation, Figure 15 and with formulation, Figure 16. The study demonstrated

that RBC didnot swell or shrink with formulations indicating it is isotonic.

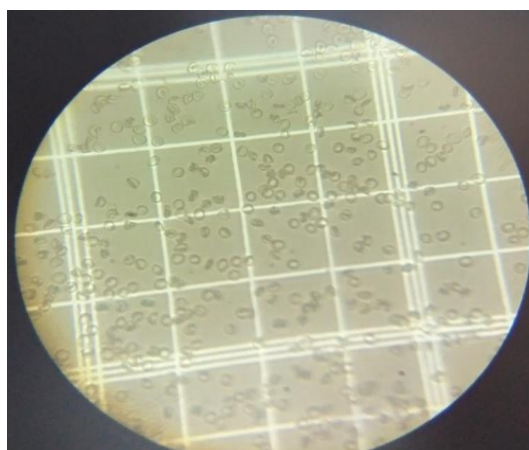


Fig-15: RBC's without formulation

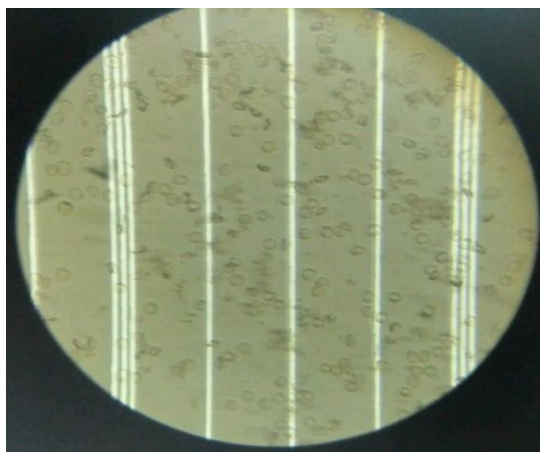


Fig-16: RBC's with formulation

Accelerated stability study

Stability study was done according to ICH guidelines for 6 months indicated that the formulations retained its various characteristics during the study

SUMMARY AND CONCLUSION

From this present work it was concluded that formulation F6 having 0.25% w/v of gelrite and 0.45 % of carboxy methyl TKP showed controlled release for a period of 12 hours and followed peppas model of kinetics. Hence it can be concluded that *in situ* ophthalmic of moxifloxacin HCL and ketorolac tromethamine combination can be an alternative to conventional eye drops.

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REFERENCES

1. Mevada, D. R., Bhalodiya, K., Maniar, B., Dadhanian, K., & Faldu, S. (2014). Development and Validation of First Order Derivative Spectrophotometric Method for Simultaneous Estimation of Bromhexine Hydrochloride and Phenylephrine Hydrochloride in their Combined Pharmaceutical Dosage Form. *Pharma Tutor*, 2(6), 132-138.
2. Kumar, N. V., Gurupadayya, B. M., Mukherjee, S., & Sreenidhi, M. K. (2015). Development and validation of zero and first order spectrophotometric method for determination of trimipramine in bulk and pharmaceutical dosage form. *Indo American Journal of Pharmaceutical Research*, 5(2), 690-697.
3. Shaikh, A., Farheen, T., Shahi, S., & Zaheer, Z. (2015). Formulation development and evaluation of in situ ophthalmic gel of epinastine hydrochloride. *Indo American Journal of Pharmaceutical Research*, 5(10), 3071-3078.
4. Nagargoje, S., Phatak, A., Bhingare, C., & Chaudhari, S. (2012). Formulation and evaluation of ophthalmic delivery of fluconazole from ion activated in situ gelling system. *Der Pharmacia Lettre*, 4(4), 1228-1235.
5. Talat, F., Sadhana, R. S., Azmat, M. S., Imran, T., & Nityanand, Z. (2013). Formulation development and evaluation of in situ ophthalmic gel of sodium cromoglycate. *Pelagia Research Library, Der Pharmacia Sinica*, 4(2), 109-118.
6. Makwana, S. B., Patel, V. A., & Parmar, S. J. (2016). Development and characterization of in-situ gel for ophthalmic formulation containing ciprofloxacin hydrochloride. *Results in pharma sciences*, 6, 1-6.
7. Kavitha, K., & Rajas, N. J. (2011). Sustained ophthalmic delivery of Levofloxacin hemihydrate from an ion activated in-situ gelling system. *Int J Pharm Tech Res*, 3, 702-706.
8. Gupta, S. K., Arora, P., & Yadav, R. (2014). Sustained Ophthalmic Delivery of Ketorolac Tromethamine from an ion activated In Situ gelling system. *International journal of pharmacy & life sciences*. 5(12), 4063-4073.
9. Rasala, T. M., Kale, V. V., Lohiya, G. K., Moharir, K. S., Itadwar, A. M., & Awari, J. G. (2011). Chemistry and pharmaceutical applications of excipients derived from tamarind. *Asian Journal of Chemistry*, 23(4), 1421.
10. Shaikh, S. S., Shivsharan, K. J., Pawar, R. K., Misal, N. S., Mene, H. R., & More, B. A (2015). Tamarind Seed Polysaccharide: A Versatile Pharmaceutical Excipient and its Modification. *Int. J. Pharm. Sci. Rev. Res.*, 33(1), 157-164