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Formulation And Evaluation Of Ophthalmic Liquid Crystal In-Situ Gel Of Natamycin

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Summary
The eye is considered as a crucial part of our body. We know that because of eye one can be able to see whole world, the nature. If someone lacking this thing or some problem associated with it they only know importance of it. In this research study natamycin an antifungal agent used for the treatment of ophthalmic fungal infections such as fungal keratitis and conjunctivitis was selected as the drug to be delivered to the eye using a sustained release ophthalmic in-situ liquid crystal gelling system. The in-situ gelling system used comprised of GRAS approved excipients with glyceryl mono-oleate (GMO) an emulsifier and stabilizer as the primary excipient. The liquid crystal structural and release mechanism of the gelling system was also studied to establish the utility and potential of the GMO based in situ gel as a drug carrier. Preformulation studies were followed by preliminary formulation and selection of suitable composite. Various compositions of sol containing natamycin in GMO, tween 40, DMSO sol system were prepared and evaluated. From the result discussion the following conclusions are made:PLM used to confirm the liquid crystal structure suggested a lyotropic liquid crystals with lamellar structures for both GMO-tween 40-sol as well as preformed in-situgel system.The pH range for formulation (F1-F5) in both sol and gel form was between pH 6.8 to 7.3, i.e around neutral to slightly alkaline.Fast gelling time was seen for all the GMO-tween 40 formulations (f1-f5) i.e. time for first detection of gelation at 37°C ± 0.5 °C was between 90 and 60 sec.The F1 to F5 formulations show high swelling index with the highest index of 102.22 observed for F3 formulation. F3 formulation contains a 1:1 ratio Tween 40 and DMSO respectively.
The viscosity of F2 gel was highest 112.32 cps as it contains no DMSO and comparatively higher surfactant tween 40. F3 formulation also showed sol to gel conversion with high viscosity of 93 cps. In the presence of STF the sol converts to gel resultingincrease in viscosity of gel by approximately tenfold.The release of the drug from the formulation F1, F2 were found to be 95.66 % and 97.63% at the end of 5 and 6 hrs respectively. The formulation F3 showed complete release in 7 hr. The release of drug from formulation F4 and F5 was found to be 98.9% and 96.32 % at the end of 9 and 7 hrs.The antimicrobial studies indicate that natamycin retained its antimicrobial activity after formulated into an in-situgel. The drug was active against the selected Candida albicans and Aspergillus fumigatus organism as indicated by zone of inhibition. F3 formulation showed better antimicrobial effects with ZOI of 16 and 15 mm for Candida albicans and Aspergillus fumigates respectively.The best selected formulation (F3) passed the test for isotonocity as the blood cells observed under the optical microscope did not show any observable change (shrinkage due to hyper-tonicity or bulging/ eventual bursting of blood cells due to hypo-tonicity) The in-situ gelling formulations (F3) was non-irritant to rabbit eyes and did not cause any damage to corneal membrane. Finally it may be concluded that F3 in-situ gelling systems comprising of 15%w/w of natamycin in GMO 55%w/w, Tween 40 15%w/w and DMSO 15%w/w showed liquid crystal (lyotropic-lamellar) microstructures with a sustained release of natamycin for 7 hours, the system had good antimicrobial effect against Candida albicans and Aspergillus fumigatus is a thus a potential ophthalmic drug delivery system.
Keywords: *Ophthalmic, In-Situ Gel, Natamycin, Liquid Crystals, Ocular Drug Delivery, GMO*

INTRODUCTION

Ophthalmic drug delivery is the most interesting and challenging drug delivery system for pharmaceutical researchers. The challenge to the investigator is to circumvent the protective barriers of the eye without causing permanent tissue damage .The conventional ophthalmic dosage forms which are widely used, are eye drops, ointment, cream, suspension, emulsion. Due to some disadvantages like rapid precorneal elimination of solution, no sustained effect, loss of drug by drainage, blurred vision and sticking of eyelids in ointments, less patient compliance for the therapeutic treatment of most of the ophthalmic problems or diseases, topical dosage forms are clearly preferred, because in case of systemic administration of drugs, only a very small fraction of dose reaches to the eye. Thus by topical administration, can achieve an optimum concentration of drug (Nanjawade et. al., 2007, Bochot et. al., 1998, Charrueau et al., 2001).

Topical application of drugs to the eye is the well established route of administration for the treatment of various eye diseases like dryness, conjunctiva, eye flu etc. For illness of the eye, topical administration is usually ideal over systemic administration, before reaching the anatomical barrier of the cornea, any drug molecule administered by the ocular route firstly crosses the precorneal barriers. These are the first barriers that slow the penetration of drug into the eye and consist of the tear film and the conjunctiva. The protective mechanisms of the eye such as blinking, baseline and reflex lacrimation, and drainage decrease the bioavailability of drug and also help to remove rapidly foreign substances like the dust particles bacteria, including drugs, from the surface of the eye. There are most commonly available ophthalmic preparations such as drops and ointments about 70% of the eye dosage formulations in market. But these preparations when instilled into eye they are rapidly drained away from the ocular surface due to blinking tear flow and lachrymal nasal drainage of the eye. With conventional ophthalmic solution normal dropper used which delivers about 50-75µl per drop and portion of these drops rapidly drain until the eye is back to normal resident volume of 7µl (Gonjari et. al. 2007, Gupta et. al. 2008, Qi et. al. 2007). Due to this drug loss in front of the eye, very small drug is available to enter the cornea and inner tissue of the eye. Actual corneal permeability of the drug is relatively low and very small corneal contact time (about 1-2 min) in humans for instilled solution usually less than 10%. Therefore only small amount of drug actually penetrates the cornea and reaches intraocular tissue. Due to these limitations, newer pharmaceutical ophthalmic formulation such as in-situ gel, nanoparticle, liposome, nanosuspension, microemulsion, intophoresis and ocular inserts have been developed in last three decades increase the bioavailability of the drug as a sustained and controlled manner. An ideal ophthalmic drug delivery must be able to release the drug in sustained manner and to remain in the area of front of the eye for prolong period of time. As a result it is necessary to optimize ophthalmic drug delivery; the best way to do so is by adding of polymers of various grades, development of colloidal suspension or using erodible or non erodible insert, development of viscous gel to prolong the precorneal drug retention Micro particle suspension or polymeric solution can be bio adhesive systems (Hady et. al. 2003, Matapady et. al. 2009, Gonjaril et. al. 2009).

Various problems faced in poor bioavailability of the eye instilled drugs are Binding by the lachrymal proteins Drainage of the instilled solutions lachrymation and tear turnover limited corneal area and poor corneal metabolism non-productive absorption/adsorption. To improve the bioavailability of drug, various approaches have been used which increase in the duration of drug action. There are mainly two different approaches are categorized. The first one is based on to provide the controlled and continuous ophthalmic drug delivery which is known as sustained drug delivery systems. The second involves maximizing corneal drug absorption and minimizing precorneal drug loss. Ideal ophthalmic drug delivery system must be able to sustain the drug release and to remain in the vicinity of front of the eye for prolonged period of time. Consequently it is imperative to optimize ophthalmic drug delivery, recently some novel ophthalmic dosage forms are available like erodible, non-erodible ophthalmic inserts, medicated lenses or in situ gelling systems to prolong the drug duration (Jagur-Grodzinskia et. al. 2010, Liu et. al. 2005, Lee et. al. 2004).

MATERIALS AND METHODS:

Natamycin was obtained as gift sample from Bimal Pharma Pvt. Ltd., Mumbai, Tween 40, GMO, DMSO were all obtained from Croda International, Mumbai, Maharashtra, India. All other chemicals and reagents used were of AR Grade.

Selection of Active Pharmaceutical Agents: Natamycinwas selected as the drug candidate as it is widely prescribed for treatment of fungal keratitis.Natamycin possesses strong antifungal properties.Natamycin is highly active against young, dividing fungal cells but it does not kill spores.Natamycin acts as a unique food preservative since it is an effective inhibitor of all target organisms without causing negative effects on product quality. It has a much broader spectrum of activity against yeasts and molds than any other fungicide allowed for use in the food industry (Majithiya et. al. 2006, Pisal et. al. 2004, Ganguly et. al. 2004).

Preformulation Study: Prior to development of dosage forms, it is essential that certain fundamental properties such as physical and chemical properties of the drug molecule and other derived properties of the drug powder are determined. This information will dictate many of the subsequent events and possible approaches in formulation development (Kubo et. al. 2003, Zhou et. al. 1996).

Identification and characterization of natamycin:

Organoleptic properties: Natamycin was evaluated for its organoleptic properties like appearance, color and odour.

Melting point: The melting point of natamycin was determined by capillary method using Melting point determination apparatus.

Solubility: The solubility of the drug was evaluated by dissolving drug in different solvents like DMSO and organic solvents at room temperature was determined.

Method: An excess amount of natamycin was added to the vials containing the 10ml of solvent and content was stirred for 24 Hrs. the mixture was then filtered through whatman filter paper no.41. The solubility of natamycin in the samples was determined spectrophotometrically at 304 nm.

Ultra Violet Spectroscopy: The stock solution (100µg/ml) was prepared by dissolving the drug (10 mg) in 100 ml of DMSO. A solution of Natamycin50 µg/ml in phosphate DMSO was prepared from the stock solution and scanned in the range of 200-800 nm to determine its λmax in the DMSO.

Fourier Transform Infrared Spectroscopy: Fourier Transform Infrared (FTIR) spectroscopy was conducted. The procedure consists of placing the drug's sample in FTIR sample holder.It was placed in the light path and scanned in the range of 4000-400 cm^{-1} on Jasco FTIR-4100. The spectrum was recorded.

Differential Scanning Calorimetry: The thermal behavior of natamycin was studied using Shimadzu DSC TA60 WS Thermal Analyzer. Accurately weighed quantity of natamycin was placed in Aluminum pan and was sealed. An empty pan was used as a reference.

RESULTS AND DISCUSSIONS:

Selection of the Active Pharmaceutical Agent Natamycin was selected as the drug candidate for ophthalmic formulation based on literature survey.

Preformulation Study

Identification and characterization of drug

Description: Natamycin is a white or almost white crystalline powder which complies with the specifications of the United State Pharmacopoeia (USP).

Melting point: Melting point of natamycin was found to be 200^0C which complies with the value mentioned in the literature.

Solubility: Natamycin is listed as practically insoluble in water, slightly soluble in methanol, and soluble in glacial acetic acid, dimethylformamide and Dimethyl sulfoxide (DMSO).The solubility of natamycinin DMSO was found to be 476.16ug/ml.

UV spectra: UV spectra of natamycin exhibited wavelength of maximum absorbance at 304 nm with the absorbance value 0.973 which complies with the value mentioned in the literature. UV spectrum of natamycin is shown in Figure 1.

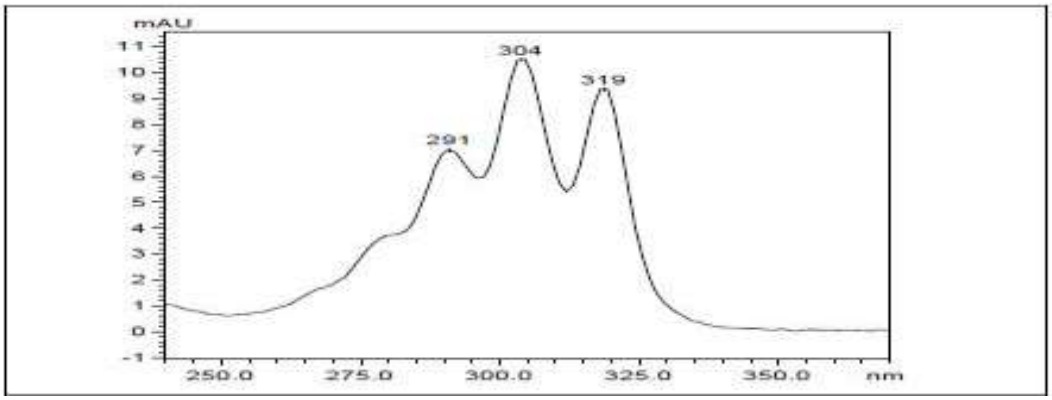
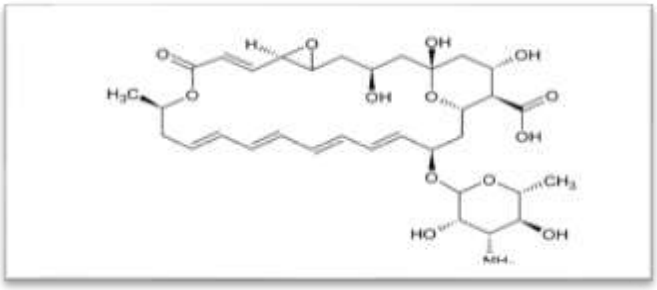


Fig.1: UV spectrum of natamycin

FTIR Spectra: The FTIR spectrum of Natamycinwas recorded which was found to be in accordance with its chemical structure22-(3-amino-3,6-dideoxy-ßD-mannopyranosyl)oxy-1,3,26-rihydroxy-12-methyl-10-oxo-6,11,28–trioxatricyclo[22.3.1.05.7]octacosa14,16,18,20-pentaene-25-carboxylic acid since the characteristic bands for the functions such as C=O, -NH, O-H were observed which are the reported peaks as per literature. It is shown in Figure 8 while the FT-IR characteristic peaks of natamycin are shown in Table 2.

Chemical Name:22-(3-amino-3,6-dideoxy-ßD-mannopyranosyl)oxy-1,3,26-rihydroxy-12-methyl-10-oxo-6,11,28–



trioxatricyclo[22.3.1.05.7]octacosa14,16,18,20-pentaene-25-carboxylic acid

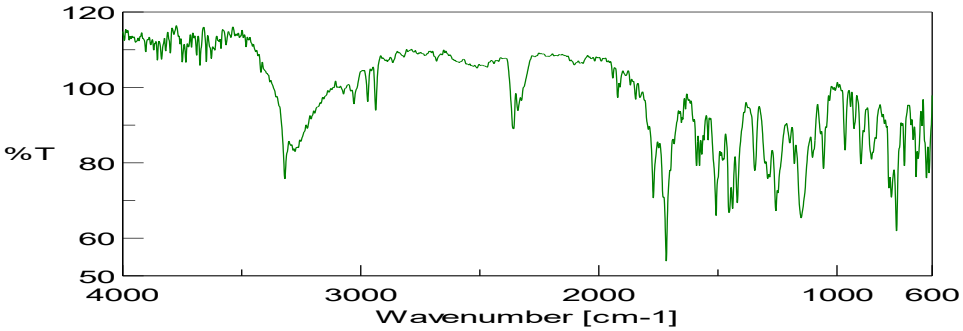


Fig.2: FT-IR spectra of natamycin

Table 2: FT-IR characteristic peaks of natamycin

Assignment	wave number cm-1 (Recorded)
C=O stretch	1636.3
O-H stretch	2970.8
N-H stretch	3419
COOH stretch	1751

Differential Scanning Calorimetry: The thermogram of natamycinshowed an endothermic peak at 157.90^{0}C with an onset at 151.85^{0}C. DSC thermogram is shown in Figure 3.On the basis of melting point, UV spectrum, Infrared spectrum and DSC thermogram it was concluded that the procured sample confirms the tests of natamycin for purity and quality.

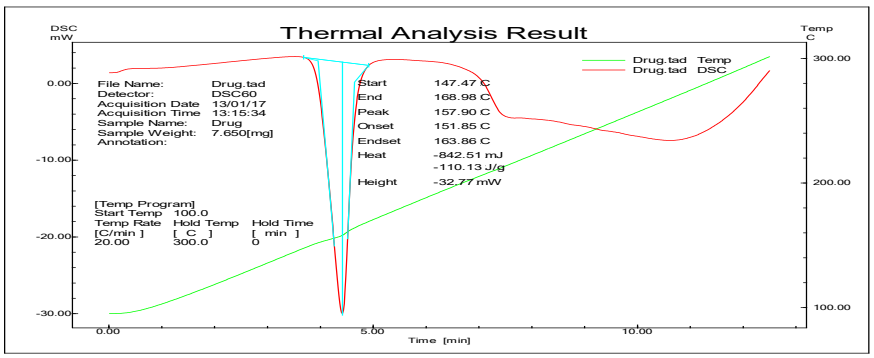


Fig.3: DSC thermogram of natamycin

UV Spectrophotometric method for estimation of natamycin: The standard solution of natamycin was prepared in the range of 2-10 µg/ml by the dilution of suitable aliquots of the stock solution. The calibration curve of natamycin was prepared by plotting the absorbance of the standard solution (y-axis) against its concentration (x-axis) measured at 304nm. The standard solution of natamycinshowed a linear curve with correlation coefficient of 0.9993. The equation of line is y = 0.06x + 0.037. These observations are shown in Table 3 and Figure 4.

Table 3: UV spectrophotometric calibration curve of natamycin

Sr. No.	Concentration(µg/ml)	Absorbance
1	2	0.163
2	4	0.274
3	6	0.393
4	8	0.523
5	10	0.638

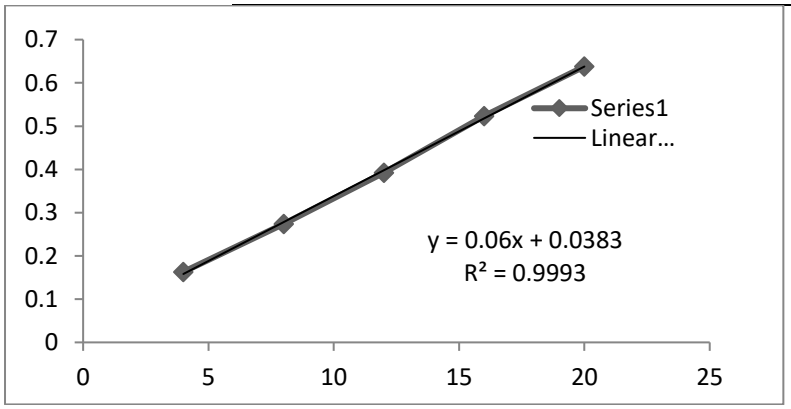


Fig. 4: Calibration curve of natamycin

Evaluation of formulation of *in-situ* gel:

Test for Clarity test / Appearance: The formulations were observed for general appearance i.e. color, odour and for the presence of suspendedparticulate matter. The clarity of the preparation was checked using against black and white background.(Mohan *et al.*, 2009; Mohamed, 2004) and the sol and preformed gel was found to be clear and translucent.

PLM Images: (Polarized Light Microscope): Various formulations were observed by polarized light microscope (PLM). PLM is used to confirm the liquid crystal structure. A lyotropic liquid crystal consists of two or more components that exhibit liquid-crystalline properties in certain concentration ranges. Lyotropic liquid crystal phases are formed by amphiphilic molecules. These often consist of a polar head group attached to one or more non-polar chains and are often known as surfactants (surface active agents).When these are dissolved in an appropriate solvent they self-assemble so the polar (hydrophilic) heads protect the non-polar (hydrophobic) tails. These structures are known as micelles. At low surfactant concentrations these are roughly spherical. As the surfactant concentration increases then other phases are formed. These include the hexagonal phase where the amphiphiles form cylinders that pack in a hexagonal array and the lamellar phase where the amphiphiles form a bilayer structure. From the PLM it is observed that the liquid crystal formed are lyotropic having lamellar structures. (Fig 5-14)

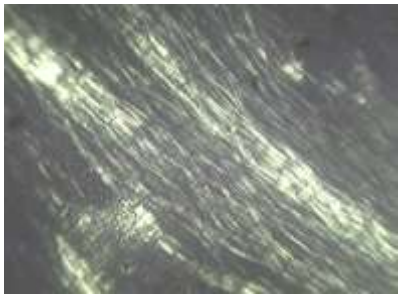


Fig. 5: Sol F1

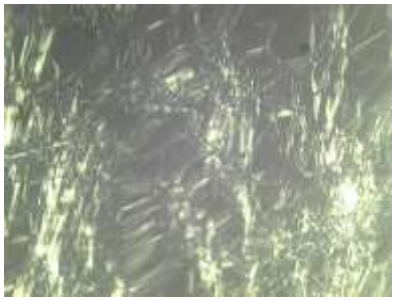


Fig.6: Sol F2

Fig.7: Sol F3

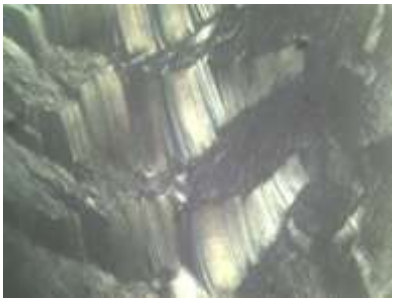


Fig.8: Sol F4

Fig.9: Sol F5

Fig.10: Gel F1

Fig.11: Gel F2

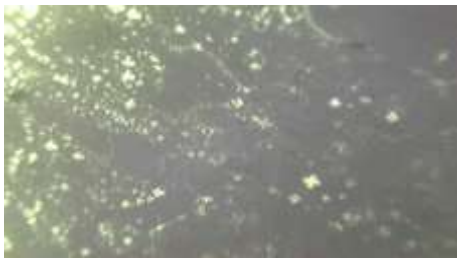


Fig.12: Gel F3

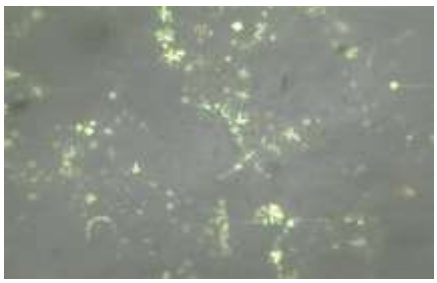


Fig.13: Gel F4

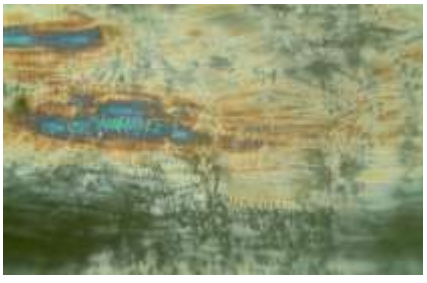


Fig.14: Gel F5

Determination of PH: The pH of all formulations was recorded using a calibrated digital pH meter immediately after preparation.

Table 4: pH of sol and perform gel formulation.

Sr. No.	Formulation Code	pH of sol	pH of gel
1	F1	6.9	7.0
2	F2	6.8	6.9
3	F3	7.2	7.3
4	F4	7.0	7.1
5	F5	7.1	7.2

The pH range for formulation in both sol and gel form is between pH 6.8 to 7.3, i.e around neutral to slightly alkaline.

Gelling capacity

The gelling capacity is determined by placing a drop of the formulation in a vial containing 1.0 ml of freshlyprepared simulated tear fluid and visually observed. The amount of simulated tear fluid (STF) required for its gelling is noted.

Table 5: Determination of gelling capacity of formulations

Sr. No.	Formulation Code	Amount of STF required (µl)
1	F1	120
2	F2	130
3	F3	140
4	F4	140
5	F5	150

Sol-gel transition temperature and gelling time: For thermo sensitive in situ gelling system the sol-gel transition temperature may be defined as that temperature at which the phase transition of sol meniscus is first noted when kept in the sample tube at specific temperature and then heated at specified rate. Gel formation is indicated by a lack of movement of sol on titling the tube. Gelling time is the time for first detection of gelation as defined above at 37°C ± 0.5°C

Table 6: Gelling time of sol formulations at 37°C ± 0.5°C

Sr. No	Formulation Code	Gelling time (sec.)
1	F1	60
2	F2	70
3	F3	80
4	F4	85
5	F5	90

Swelling studies

In this method polymer or material adsorbs water from the surrounding of eye and then expandsto converts in to gel transition.Myverol18-99 (glycerol mono-oleate)is used as swelling material.It is the substance which is polar 1400 lipid that swells in water to form lyotropic liquidcrystalline phase structures. This polymer has bioadhesive properties and can be degraded *in vivo*by enzymatic action.The sponge with the size (5x5x2 cm) was firstly soaked overnight in STF and then formed sol of 1gm was weighed accurately (W1) and placed on a sponge containing 50ml of STF pH 7.4, It was kept aside for 24 hrs. After 24 hrs gel was weighed (W2) and swelling index was calculated using the following formulae:

W2-W1/W1 *100

Where, W1 = Initial weight of sol (1gm)

W2 = Weight of sol after time period t.

Table 7: Swelling index of natamcyin *in-situ* gel formulations

Sr. No.	Formulation Code	Swelling Index (%)
1	F1	97.77
2	F2	100
3	F3	102.22

4	F4	95.55
5	F5	100

The F1 to F5 formulations show high swelling index with the highest index of 102.22 observed for F3 formulation. F3 formulation contains a 1:1 ratio Tween 40 and DMSO respectively.

Drug content: The drug content was determined by accurately placing 100µl of formulations in a test tube and suitably dilutedwith simulated tear fluid (STF) to obtain a concentration of 10µg/ml. By using UV-Visible spectrophotometerthe drug concentration was determined. The contents of natamycin in all the formulations were between 99.20 to 102.58 % of the labeled content.

Table 8: Determination of drug content

Sr. No.	Formulation Code	Drug Content (%)
1	F1	100.50±0.86
2	F2	99.80±1.08
3	F3	102.58±0.85
4	F4	99.79±0.95
5	F5	101.28±0.58

All readings taken in triplicate n±SD

Rheological studies: Viscosity and rheological properties of *in-situ*forming drug delivery systems can be assessed by usingBrookfield rheometer. The viscosity of these formulations should be such that no difficulties are envisaged during their administration to the patient,especially during parenteral and ocular administration.

Viscosity of formulation in sol and gel form :

Table 9: Viscosity of formulation in sol and gel form

Sr. No.	Formulation Code	Viscosity (cps) of sol	Viscosity (cps) of gel
1	F1	8.43	70.52
2	F2	7.00	112.32
3	F3	10.21	94.32
4	F4	10.28	105.23
5	F5	11.45	90.56

The viscosity of F2 gel was highest 112.32 cps this may be due to the fact that the formulation comprises of only GMO and Tween 40.

In the presence of STF the sol converts to gel this is due to GMO which swells rapidly forming a gel. The viscosity of gel increased approximately tenfold when compared with sol.

***In vitro*diffusion studies:** *In vitro*diffusion studies of *in-situ*gel solution was carried out by using Franz diffusion cell. The formulation placedin donor compartment and freshly prepared simulated tear fluid (STF) in receptor compartment. Between donor and receptor compartment cellulose acetate membrane was placed (0.22µm pore size). The whole assembly is placed on thethermostatically controlled magnetic stirrer. The temperature of the medium was maintained at 37°C ± 0.5°C. 1mlof sample was withdrawn at predetermined time interval of 1hr for 7 hrs and same volume of fresh medium was replaced. The withdrawn samples are diluted in a volumetric flask with respective solvent to specific volumeand analyze by UV spectrophotometer at respective nm using reagent blank. The drug content is calculatedusing the equation generated from standard calibration curve then the % cumulative drug release (%CDR) iscalculated. The data obtained is further subjected to curve fitting for drug release data.

Table 10: Cumulative percent drug release from F1-F5 formulations

Time (hrs.)	Cumulative % drug release				
	F1	F2	F3	F4	F5
1	22.34	16.58	19.33	15.31	20.33
2	41.56	35.71	31.88	22.89	32.65
3	59.99	49.74	45.74	30.99	43.62

4	80.19	65.05	57.39	45.05	54.32
5	95.66	81.37	68.87	50.73	69.02
6	-	97.63	79.45	69.13	80.32
7	-	-	95.85	77.15	96.32
8	-	-	-	81.63	-
9	-	-	-	98.9	-

The release of the drug from the formulation F1, F2 were found to be 95.66 % and 97.63% atthe end of 5 and 6 hrs respectively. The formulation F3 showed complete release in 7 hr. The release of drug from formulation F4 and F5 was found to be 98.9%and 96.32 % at the end of 9and 7hrs.

Drug release Kinetics: The in-vitro release profiles were fitted to various kinetic models in order to find out the mechanism of drug release. The rate constants were calculated from the slope of the respective plots. High correlation (R2=0.9031) was observed in the Higuchi plot for F3. Other formulation prepared F1-F5 except F3 did not show curve fitting for any release kinetic model as indicated by very low correlation coefficients. The drug release from F3 was thus proportional to square root of time, indicating that the drug release from in-situ gel was diffusion controlled. The data obtained was also fit in Korsmeyer-Peppas model in order to find out n value, which describes the drug release mechanism. The n value (0.8029) obtained from Korsmeyer-Peppas was more than 0.5, which indicated that the mechanism of the drug release was Anomalous and Non Fickian diffusion controlled.

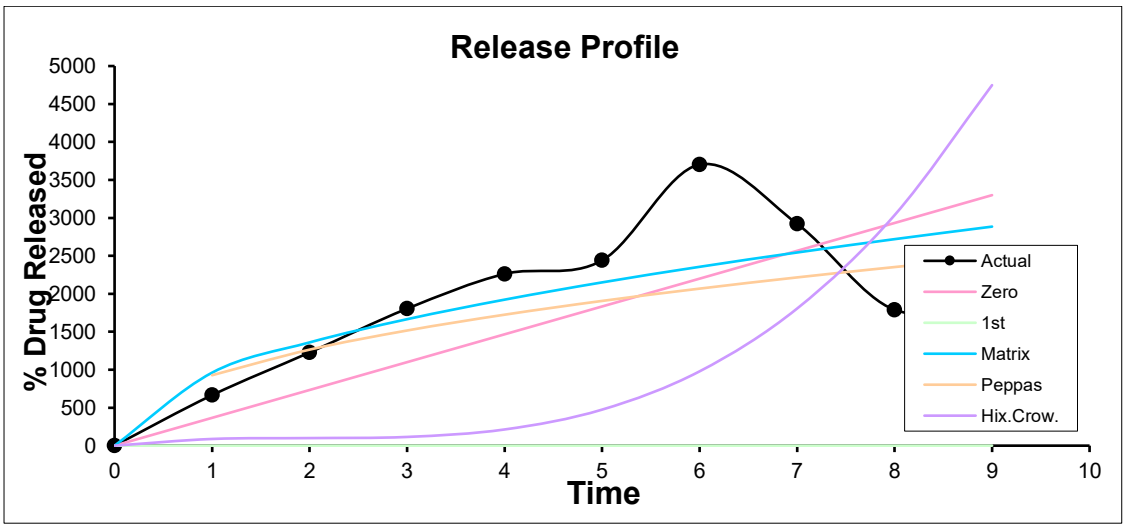


Fig 15: Release profile of various formulation of *in-situ* gel

Antimicrobial efficacy: Marketed eye drops and developed formulation each of 1 ml was added in to cups bored in to sterile nutrient agar previously seeded with *Candida albicans and Aspergillus fumigates*. After allowing diffusion of the solutions for 2 hours, the agar plates are incubated at 37°C for 24 hours. The zone of inhibition (ZOI) is measured around each cup. The entire operation except incubation is carried out in an aseptic environment using laminar air flow unit.

Fig-16:Std. C. albicance

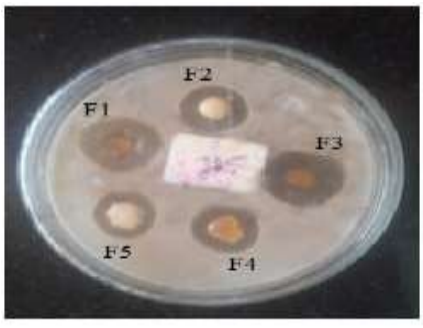


Fig-17: Sol C. albicance

Fig-18: Gel C. albicance

Fig-19: Std. A. Fumigatus

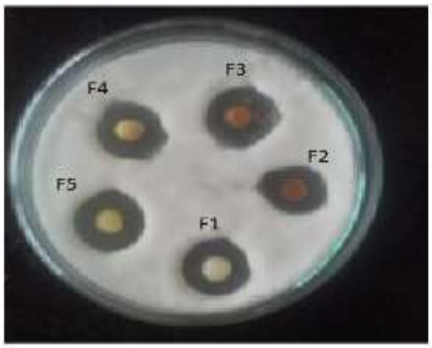


Fig-20: Sol A. Fumigatus

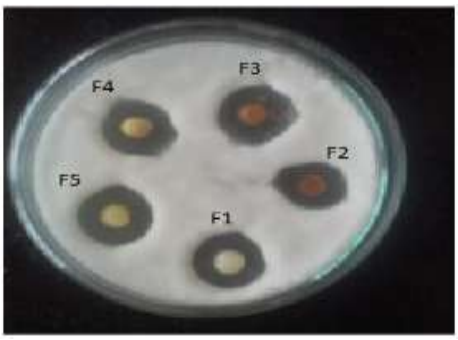


Fig-21: Gel A. Fumigatus

Table 11: Measured mean diameter of zone of Inhibition for fungi in the presence of standard, sol and gel formulations

The result of the antimicrobial efficacy tests shown in (Figure -16-21). The study indicates that natamycin retained its antimicrobial activity after formulated into an *in-situ*gel. The drug was active against the selected *Candida albicans and Aspergillus fumigates* organism as indicated by zone of inhibition.

Isotonicity evaluation: Isotonicity is important characteristic of the ophthalmic preparations. Isotonicity should be maintained toprevent tissue damage or irritation of eye. All ophthalmic preparations undergo isotonicity testing, Formulationsmixed with few drops of blood and observed under microscope at 45X magnification and compared with standard .1% NaCl aqueous solution.

Sr. No.	Formulation Code	Zone of Inhibition (mm)	
		Candida albicans (C.A.)	***Aspergillus fumigatus (A.F.)***
1	F1	13	11
2	F2	12	12
3	F3	16	15
4	F4	12	13
5	F5	13	12
6	Std.(3 µg/ml) Drug Solution	15	14
7	Std.(5 µg/ml) Drug Solution	18	17

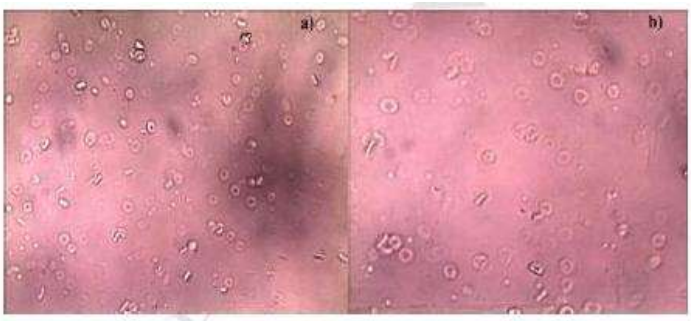


Fig.22: Test for Isotonicity a) 0.1% Nacl solution b) Optimized gel formulation F3

The formulation passed the test for isotonocity as the blood cells observed under the optical microscope did not show any observable change (shrinkage due to hyper-tonicity or bulging/ eventual bursting of blood cells due to hypo-tonicity)

Ocular irritancy test: In-vivo eye irritation testing was carried out using rabbit and as per Draize test protocol. Optimized formulation F3 were used for this test. The formulations were found to be nonirritating with no ocular damage or abnormal clinical signs to the cornea, iris or conjunctivae observed. Hence the formulation was suitable for the eye instillation.

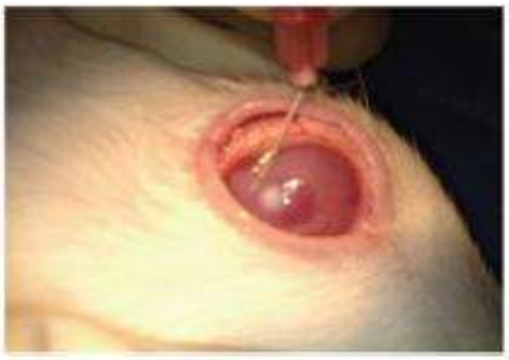


Fig.23: Injection of optimized formulation into rabbit eye

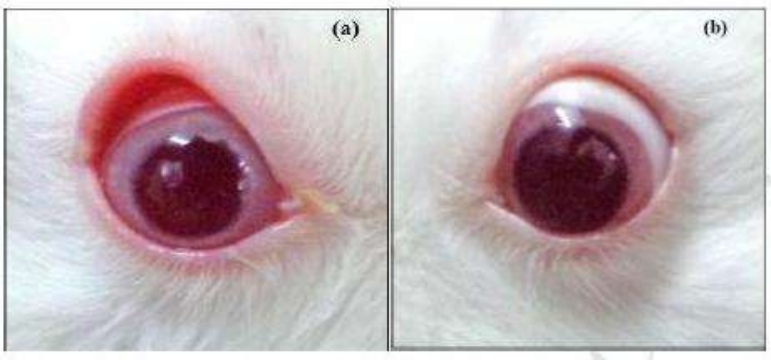


Fig.24: a) Untreated left eye b) treated with optimized formulation (F3) right eye

Table 12: Ocular irritation study for selected best formulation (F3)

Formulation code	Optimized formulation (F3)			
Time (hr)	1	24	48	72
Redness	0	0	0	0
Inflammation	0	0	0	0
Excessive tearing	0	0	0	0

Thus, the prepared *in-situ* gelling formulations (F3) were found to be non-irritant to rabbit eyes and do not cause any damage to corneal membrane.

CONCLUSION:

Finally it may be concluded that F3 in-situ gelling systems comprising of 15%w/w of natamycin in GMO 55%w/w, Tween 40 15%w/w and DMSO 15%w/w showed liquid crystal (lyotropic-lamellar) microstructures with a sustained release of natamycin for 7 hours, the system had good antimicrobial effect against *Candida albicans and Aspergillus fumigatus* is a thus a potential ophthalmic drug delivery system.

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