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RESEARCH ARTICLE

Formulation Development and Evaluation of Nepafenac Novel *In Situ* Gel

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ABSTRACT

The aim of present study was to formulate and evaluate Nepafenac liposomal *in situ* gel. Liposomes were formulated in different ratios using soya lecithin and cholesterol using Rotary Flash Evaporation method. Liposomes were evaluated for drug entrapment efficiency, %entrapment efficiency, particle size analysis, zeta potential and *in vitro* release studies. Optimized liposomes (F4) were developed into *in situ* gels. The thermo reversible liposomal *in situ* gels of Nepafenac were prepared by using poloxamer 407 and with mucoadhesive polymers like HPMC E15 and chitosan. Formulations were sterilized by autoclaving at 121^0C, at 15 lb pressure for 20 min. The formulations were evaluated for drug content, clarity, pH, gelation temperature, gelation capacity, viscosity, *in-vitro* drug release studies. Drug and Polymer incompatibilities were evaluated using FTIR spectrophotometer. The drug content of the formulations was in the range 83%-92%. pH of the formulations was in the range 6.43 to 7.42, gelation temperature was in the range 27.5^0C-40^0C. *In Vitro* drug release was in the range of 78%-99% within 12 hours, the extent of gelation and consequently the release of Nepafenac depended on the concentration of polymers used. Nepafenac was released slowly from gels, for a period of 12 hours. Formulation PC5 with poloxamer 407 (18% w/w) and chitosan (0.3% w/w) was found to be suitable as it released 78% of drug for a period of 12 hours. Poloxamer 407/chitosan (PC5) combination was found to have optimum pH and gelation temperature which is required for an *in situ* gel drug delivery system.

KEYWORDS

In Situ Gel, Liposomes, Entrapment Efficiency, Zeta Potential

INTRODUCTION

Eye drops that are conventional ophthalmic delivery systems often result in poor bioavailability and therapeutic response because high tear fluid turnover and dynamics cause rapid precorneal elimination of the drug. A high frequency of eye drop instillation is associated with patient non-compliance. Inclusion of excess drug in the formulation in an attempt to overcome bioavailability problem is potentially dangerous if the drug solution drained from the eye is systemically absorbed from the nasolacrimal duct. Various 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[1]. Several new preparations have been developed for ophthalmic use, not only to prolong the contact time of the vehicle on the ocular surface, but also to slow down drug elimination·

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Ocular Sustained Drug Delivery Systems

In the novel drug delivery system various approaches like *in situ* gelling, use of mucoadhesive polymers, polymer coated Nanoparticle and Liposomal formulations are used. These delivery systems delay the elimination of active ingredient from eye and also improve corneal penetration of drug molecule.

Liposomes

Liposomes are biocompatible and biodegradable lipid vesicles made up of natural lipids and about 25–10 000 nm in diameter. They are having an intimate contact with the corneal and conjunctival surfaces which is desirable for drugs that are poorly absorbed, the drugs with low partition coefficient, poor solubility or those with medium to high molecular weights and thus increases the probability of ocular drug absorption. The corneal epithelium is thinly coated with negatively charged mucin to which the positive charged surface of the liposomes may bind. Roonal Jain et al. formulated and evaluated soft contact lenses coated with ciprofloxacin entrapped in liposomes. Ciprofloxacin released from the liposomes coated on contact lens inhibited the Staphylococcus aureus and Pseudomonas aeruginosa. Approximately 40% of the Ciprofloxacin was retained up to three months[2].

Niosomes

Niosomes are nonionic surfactant vesicles that have potential applications in the delivery of hydrophobic or amphiphilic drugs. Niosomes are developed as they are chemically stable as compared to liposomes, non toxic and do not require special handling techniques[14]. Ghada Abdelbary et al, investigated the feasibility of using nonionic surfactant vesicles (niosomes) as carriers for the ophthalmic controlled delivery of a water soluble local antibiotic; gentamicin sulphate. They showed a substantial change in the release rate and an alteration in the % EE of gentamicin sulphate from niosomal formulations upon varying type of surfactant, cholesterol content[3].

Implants

For chronic ocular diseases like cytomegalovirus (CMV) retinitis, implants are effective drug delivery system. Earlier non biodegradable polymers were used but they needed surgical procedures for insertion and removal. Presently biodegradable polymers such as Poly Lactic Acid (PLA) are safe and effective to deliver drugs in the vitreous cavity and show no toxic signs. Intravitreal implants of Fluocinolone acetonide were developed for the treatment of posterior segment and reported to control the ocular inflammation of retina[4].

Dendrimers

Dendrimers are large and complex molecules with well defined chemical structure. Dendrimers can successfully used for different routes of drug administration and have reported to have better water-solubility, bioavailability and biocompatibility. The residence time was longer for the solutions containing dendrimers with carboxylic and hydroxyl surface groups. Vandamme et al[5].developed and evaluated poly (amid amine) dendrimers containing fluorescein for controlled ocular drug delivery and determined the influence of size, molecular weight and number of amine, carboxyl ate and hydroxyl surface groups in several series of dendrimers. The residence time was longer for the solutions containing dendrimers with carboxylic and hydroxyl surface groups.

Micro emulsion

Micro emulsion is dispersion of water and oil stabilized using surfactant and co-surfactant to reduce interfacial tension and usually characterized by small droplet size 100 nm, higher thermodynamic stability and clear appearance. Selection of aqueous phase, organic phase and surfactant/co-surfactant systems are critical parameters which can affect stability of the system[16]. Vandamme et al reported optimization of these components results in significant improvement in solubility of the drug molecule e.g. Indomethacin, Chloramphenicol for eye diseases[6].

Nanosuspensions

Nanosuspensions have emerged as a promising strategy for the efficient delivery of hydrophobic drugs because they enhanced not only the rate and extent of ophthalmic drug absorption but also the intensity of drug action with significant extended duration of drug effect. For commercial preparation of nanosuspensions, techniques like media milling and high-pressure homogenization have been used. Pingatello et al formulated nanosuspension of Flurbiprofen using Eudragit RS 100. The higher drug level in the aqueous humor was reported using Eudragit RS 100 nanosuspensions for the ophthalmic controlled delivery of ibuprofen[7].

Nepafenac is a non steroidal anti inflammatory drug (NSAID) and a potent inhibitor of COX enzyme which involves in the synthesis of Pg which mediates the inflammation reaction. It is used in the treatment of cataract surgery to reduce the pain after the surgery. At present 0.1% Nepafenac suspensions are available as eye drops with the brand name of Nevanac Alcon, manufactured by Alcon Pharmaceuticals Ltd. Nepafenac exhibits rapid precorneal elimination and poor ocular bioavailability, when given in the form of conventional eye drops. To overcome this, an attempt has been made to formulate liposomal temperature triggered *in situ* gelling system of Nepafenac to sustain the drug release and to decrease the frequency of drug administration.

MATERIAL AND METHODS

Nepafenac was obtained from Mylon Pharmaceuticals Ltd as a gift sample, Poloxamer 407, HPMC and Chitosan were purchased from Horizon Chemicals Limited, Sodium benzoate, Sodium chloride, Sodium hydroxide, Sodium bicarbonate, Calcium chloride, Hydrochloride and Di Sodium hydrogen ortho phosphate were purchased from S.D. fine chem limited.

Preparation of Nepafenac Liposomes

Procedure for Preparation of Nepafenac Liposomes

Liposomes were prepared by using conventional rotary evaporation sonication method[8]. Liposomes were prepared by taking cholesterol and lecithin in different ratios and chloroform: methanol in 2:1. After the solvent evaporation, the residue was collected and to this PBS of pH 7.4 was added and then subjected foe sonication to remove the residue which was attached to the walls of the round bottom flask. The conditions which were maintained in RFE were bath temperature at 60^0C, 100 rotations and vacuum pressure at 474 psi.

Table 1: Compositions of Liposomes

Form. code	Cholest-erol	Lecithin	Chloro -form	Methanol
F1	1	2	2	1
F2	1	3	2	1
F3	1	4	2	1
F4	1	5	2	1
F5	2	3	2	1
F6	2	5	2	1
F7	2	7	2	1
F8	3	4	2	1
F9	3	5	2	1
F10	3	7	2	1

Evaluation of Liposomes

Particle Size Analysis

The particle size of liposomes was determined by using motic digital microscope model no. (DMW201). All the prepared batches of liposomes were viewed under microscope to study their size. Size of liposomal vesicles from each batch was measured at different location on slide by taking a small drop of liposomal dispersion on it and average size of liposomal vesicles were determined[9].

In Vitro Release Studies

The *in vitro* diffusion studies were carried out by using 500 ml beaker containing 250 ml Phosphate buffer pH 7.4. The beaker was assembled on a magnetic stirrer and the medium was equilibrated at 37±5^{0}C. Dialysis membrane was taken and one end of the membrane was sealed. After separation of non-entrapped Nepafenac liposome dispersion was filled in the dialysis membrane and other end was closed.

Drug Entrapment Efficiency

Drug entrapped within the liposomes was estimated after removing the unentrapped drug, which was separated by collecting the supernatant after subjecting the dispersion to centrifugation in a cooling centrifuge at 1000rpm at a temperature of 4^{0}C for 30mts, where upon the pellets of liposomes were washed again with PBS to remove unentrapped drug. Then analyzed at 235 nm. Percentage entrapment efficiency determined by using the ratio of entrapped drug to the total drug[11].

% Drug Entrapped (PDE) = (Amount of drug in sediment / Total amount of drug) × 100.

Zeta Potential

This method is used to determine charge on empty and drug loaded vesicles surface using Zetasizer 300HSA (Malvern Instruments, Malvern, UK). Analysis time was kept for 60 seconds and average zeta potential and charge on the liposome was determined. The obtained value indicates that the surface of liposomes is dominated by the anions and proved that prepared liposome have sufficient charge to avoid aggregation of vesicles[12].

Fourier Transforms Infrared (FTIR) Spectra

Fourier transform infrared (FTIR) spectra were measured using an optical bench (Shimadzu FTIR 8400S, Japan) to determine the possible interactions between the drug and polymers. The drug, individual polymer (poloxamer 407, HPMC E15, and chitosan), 1:1 physical mixture of drug and polymer (each 10 mg) were prepared and mixed with 400 mg of potassium bromide. About 100 mg of this mixture was compressed to form a transparent pellet using a hydraulic press at 15 tonnes pressure. It was scanned from 4,000 to 400 cm in a Shimadzu FTIR 8400S spectrophotometer. The IR spectrum of the physical mixture was compared with those of pure drug and polymers that indicates incompatibility if any.

Preparation of In Situ Gel

The poloxamer 407 polymer of different concentrations were weighed accurately as per the composition table. The polymer was dissolved in one ml of cold water at temperature 4^{0}c and kept it refrigerator for 12hrs.Then weighed quantities of other ingredients like HPMC E15/Chitosan, Nepafenac, sodium benzoate and sodium chloride solutions were added to soaked polymer solution and stirred well. Finally optimized batch (F4) liposomes were incorporated into the gel and pH was adjusted to 7.4 with the help of 0.1N NaOH. All the formulations after preparation refrigerator and further used for evaluation.

Evaluation of Ophthalmic *In situ* Gel[13, 14, 15]

Drug Content

The drug content was determined for all *in situ* gel formulations using simulated tear fluid of pH 7.4 as medium. Accurately measured volume (100µl) of formulation was taken and suitably diluted with STF to get a concentration of 10µg/ml and scan at 235 nm by using UV-Visible spectrophotometer. Nepafenac concentration was determined. All experiments were performed in triplicates.

Test for Appearance/ Clarity

All formulations were checked for general appearance i.e. Color, odors, any suspended particulate matter etc. The clarity was checked using wooden board against black and white background.

Determination of pH

The pH of all formulations was recorded using a calibrated digital pH meter immediately. All the formulations were checked for pH in triplicate.

Table 2: Formulae of Poloxamer 407 and HPMC E_{15} *In Situ* Gel Formulations

Formulation Code	Drug (mg)	Poloxamer 407(mg)	HPMC E_{15} (mg)	NaCl (mg)	BZC (mg)	D.W up to (ml)
PH1	0.3	14	0.3	0.9	0.01	2
PH2	0.3	14	0.5	0.9	0.01	2
PH3	0.3	16	0.3	0.9	0.01	2
PH4	0.3	16	0.5	0.9	0.01	2
PH5	0.3	18	0.3	0.9	0.01	2
PH6	0.3	18	0.5	0.9	0.01	2

Note: Concentration is in %w/v

Table 3: Formulae of Poloxamer 407 and Chitosan *In Situ* Gel Formulations

Formulation Code	Drug (mg)	Poloxamer 407 (mg)	HPMC E_{15} (mg)	NaCl (mg)	BZC (mg)	D.W up to (ml)
PC1	0.3	14	0.3	0.9	0.01	2
PC2	0.3	14	0.5	0.9	0.01	2
PC3	0.3	16	0.3	0.9	0.01	2
PC4	0.3	16	0.5	0.9	0.01	2
PC5	0.3	18	0.3	0.9	0.01	2
PC6	0.3	18	0.5	0.9	0.01	2

Note: Concentration is in %w/v**.**

Gelation Temperature

Gelation temperature was performed according to Gilbert et al[16]. 1ml aliquots of prepared formulations were transferred in a test tube and the test tube was sealed with a parafilm and immersed in a water bath at 4^0C the temperature of the bath was increased in increments 1^0C and left to equilibrate for 15 min at each new temperature setting. The samples were examined for gelation which is considered to have occurred when meniscus would no longer move when tilted more than 90^0 that temperature was consider as gelation temperature of the formulation. All the experiments were performed in triplicates.

Rheological Studies

The prepared formulations were allowed to gel in the simulated tear fluid at 25^0C, 35^oC and the viscosity determination was carried out by using R/S PLUS Brookfield viscometer cone and plate with cone C50-1 with angular velocity run from 10 to 100 rpm. The experiment was performed in triplicate.

Autoclaving Sterilization

To study the effect of autoclaving sterilization on physicochemical properties of Nepafenac *in situ* gels, the selected formulations were subjected to the autoclaving sterilization conditions following

recommendation by the US Pharmacopeia (The United States Pharmacopeia Convention, 2007). Briefly, screw cap test tubes containing 10g of Nepafenac *in situ* gel were placed in an autoclave. Formulations were subjected to autoclave at 121^{0}C, under a pressure of about 15 psi, for 20 min. Then, the formulations were evaluated for physicochemical properties like flow ability, % labeled amount, pH, sol–gel transition temperature and in vitro drug release, and compared with the formulations before autoclaving.

In Vitro Drug Release Method

The following *in vitro* drug release methods were performed and suitable method was selected to optimize the *in vitro* drug release of ocular *in situ* gel of Nepafenac.

Modified Franz Diffusion Cell

In vitro drug release studies of samples were carried out by using Modified Franz diffusion cell. Dialysis membrane previously soaked in STF (pH 7.4) was taken and placed in between donor and receptor compartments. In the donor compartment 0.25 ml of formulation was added. Volume of the diffusion medium was maintained 25 ml in receptor compartment and temperature maintained at 34 ± 0.5°C, and rpm was maintained at 25 by using hot plate magnetic stirrer. Aliquots were withdrawn at intervals of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 hours and replaced by equal volumes of diffusion medium. Aliquots were suitably diluted with STF (pH 7.4) and analyzed by UV Spectrophotometer at 235 nm[17].

Sterility Testing

Sterility testing was performed for optimized formulation. 2ml of the optimized formulation was withdrawn with a sterile syringe then, aseptically transferred to thioglycolate medium (20ml) and soya bean - casein digest medium (20ml) in a test tube separately. The test formulation or optimized formulation mixed with the media. The inoculated media was incubated for 14 days at 30-35^{0}C in case of fluid thioglycolate medium and 20-25^{0}C in case of soya bean-casein digest medium. The test tubes were observed for turbidity and microbial growth at 14th day[18,19].

RESULTS AND DISCUSSION

Analytical Method Development for Nepafenac

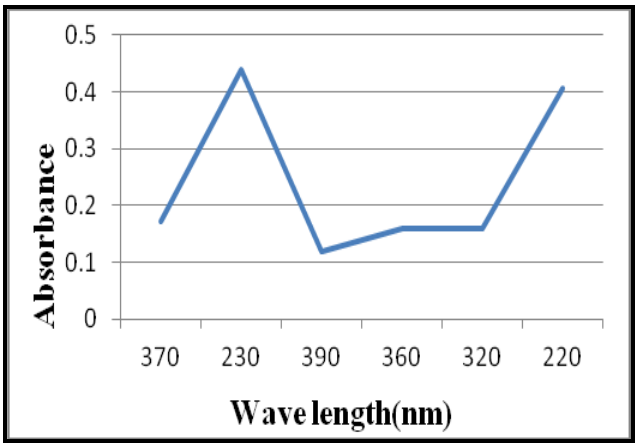

Figure 1: Absorbance maxima in D.W

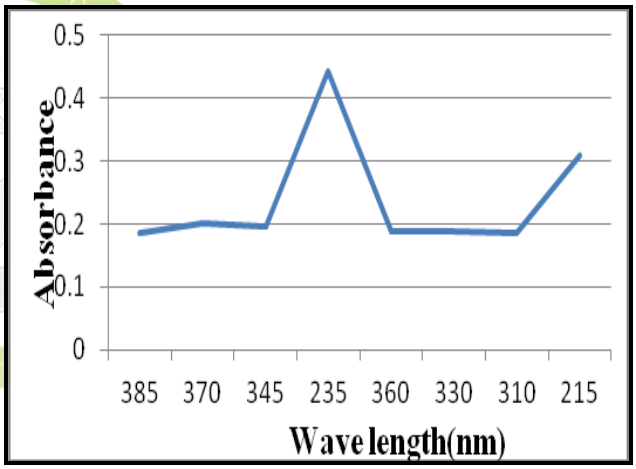

Figure 2: Absorbance maxima in PBS pH 7.4

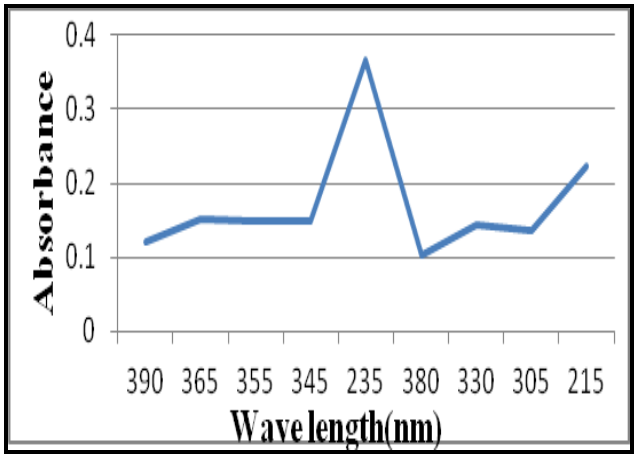

Figure 3: Absorbance maxima in STF

Standard Graph in Various Media

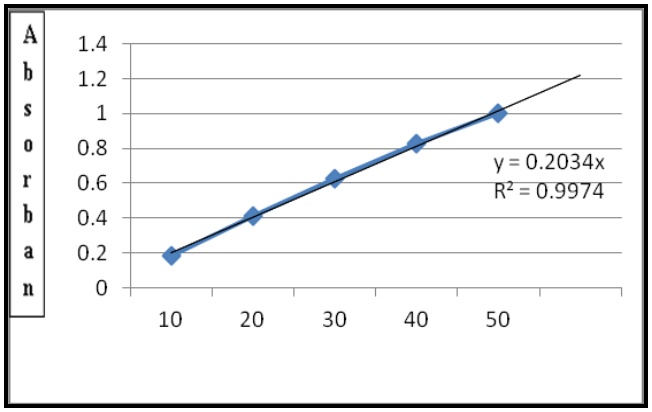

Figure 4: Standard Graph in STF

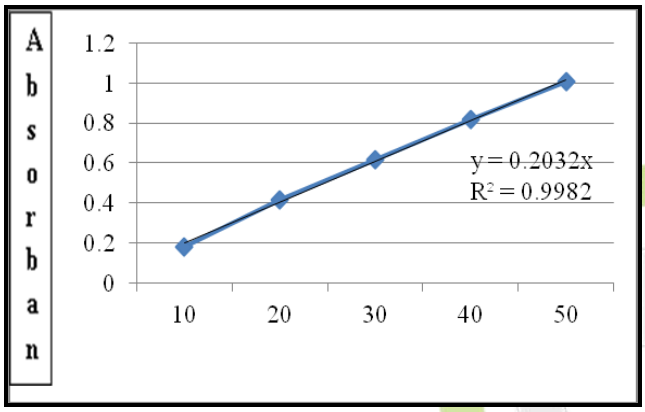

Figure 5: Standard Graph in PBS pH 7.4

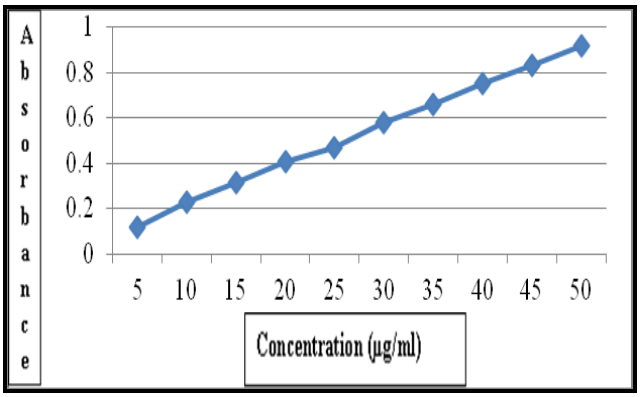

Figure 6: Standard Graph in Distilled Water

Particle Size Analysis

The particle size of liposomes was determined by using motic digital microscope model no. (DMW201). All the prepared batches of liposomes were viewed under microscope to study their size. The prepared optimized liposomes are found to have good mean particle size and it was proved that as the concentration of the cholesterol increased the particle size also increased and entrapment efficiency decreased. In formulation F4, the ratio of cholesterol and lecithin is 1:5 is selected as optimized formulation based on poly dispersity index value which is closer to 3.

Table 4: Poly Dispersity Index of All Formulations

Formulation code	Cholesterol: Lecithin	Particle size Diameter (nm)	PDI	Cumulative %drug release	%Entrapment Efficiency
F1	1:2	131.8	0.324	84.82	92
F2	1:3	114.2	0.317	73.56	91
F3	1:4	106.3	0.315	71.39	90
F4	1:5	104.8	0.252	96.25	98
F5	2:3	252.3	0.438	80.95	95
F6	2:5	150.8	0.470	83.86	97
F7	2:7	142.8	0.521	89.15	93
F8	3:4	130.8	0.364	92.68	94
F9	3:5	162.3	0.422	88.12	96
F10	3:7	175.0	0.459	91.04	93

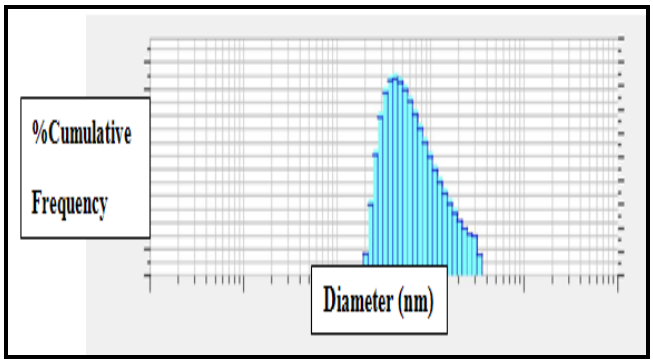

Figure 7: Particle size analysis of optimized formulation

In Vitro Release Studies

The *in vitro* diffusion studies were carried out by using 500 ml beaker containing 250 ml Phosphate buffer pH 7.4. The beaker was assembled on a magnetic stirrer and the medium was equilibrated at 37±5^{0}C. Dialysis membrane was taken and one end of the membrane was sealed. After separation of non-entrapped Nepafenac liposome dispersion was filled in the dialysis membrane and other end was closed. The drug release from the different liposomes is ranging from 13% to 96% up to 12hours. Drug release from the optimized formulation was found to be 96.25% in 12hrs.

Table 5: Drug release from liposomes

Time in hr	Cumulative % drug release
0	0.00
1	13.48±1.05
2	29.59±1.23
3	43.29±0.79
4	54.82±0.81
5	63.56±1.25
6	71.39±1.11
7	75.11±0.77
8	80.95±0.86
9	83.86±0.77
10	89.15±0.99
11	92.68±1.23
12	96.25±0.33

Estimation of Entrapped Drug

Drug entrapped within the liposomes was estimated after removing the unentrapprd drug, which was separated by collecting the supernatant after subjecting the dispersion to centrifugation in a cooling centrifuge at 1000rpm at a temperature of 4c for 30mts, where upon the pellets of liposomes were washed again with PBS to remove unentrapped drug. Then analyzed at 235 nm by UV Spectrophotometer. Entrapped drug was found to be 10mg.

Drug Entrapment Efficiency

The percentage entrapment efficiency was determined and maximum entrapment efficiency was found to be 100%.

Zeta Potential

This method is used to determine charge on empty and drug loaded vesicles surface using Zetasizer 300HSA (Malvern Instruments, Malvern, UK). Analysis time was kept for 60 seconds and average zeta potential and charge on the liposome was determined. The zeta potential of optimized formulation (F4) which is selected based on Poly Dispersity Index was -24.7 mV which indicates that the surface of liposomes is dominated by the anions and proved that prepared liposome have sufficient charge to avoid aggregation of vesicles.

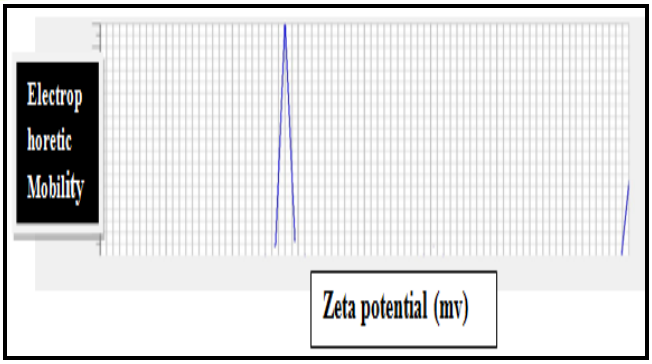

Figure 8: Zeta Potential Analysis of Optimized Formulation F4

Fourier Transforms Infrared (FTIR) Spectra

Interaction study was performed with FTIR spectrophotometer with a range from 4,000 to 400 cm^{-1}. The FTIR spectra of pure drug and excipients and physical mixture of drug and

excipients was studied. From FTIR spectra there were no disappearance characteristic peaks of the drug and polymers and no appearance of new peaks in the physical mixture. So there was no interaction between drug and polymers.

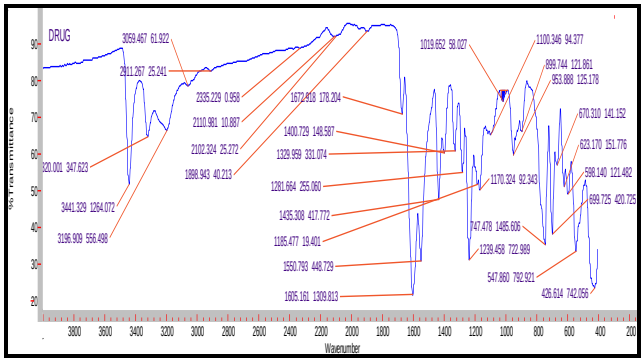

Figure 9: FTIR Study of Pure Drug

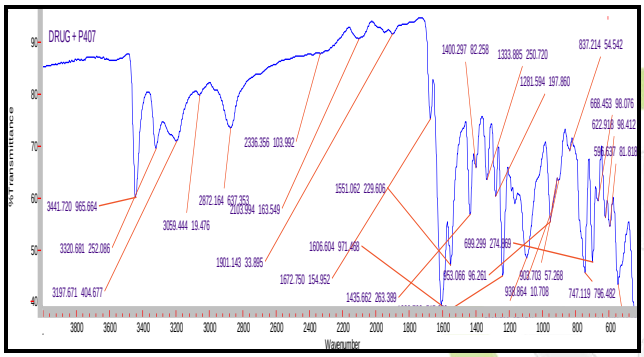

Figure 10: FTIR Study of Drug and Pol-407

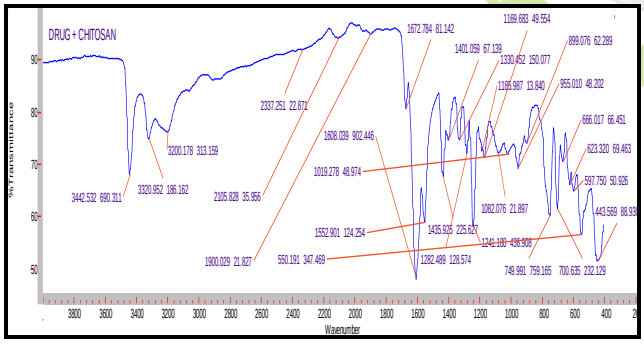

Figure 11: FTIR Study of Drug and Chitosan

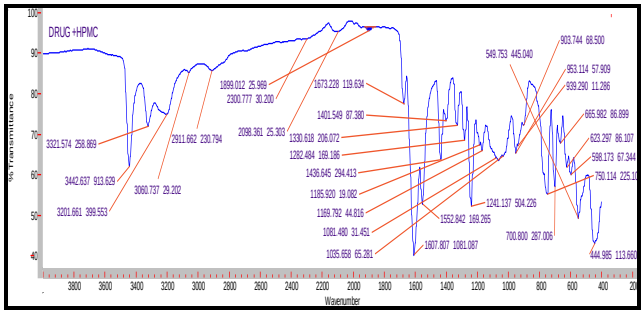

Figure 12: FTIR Study of Drug and HPMC

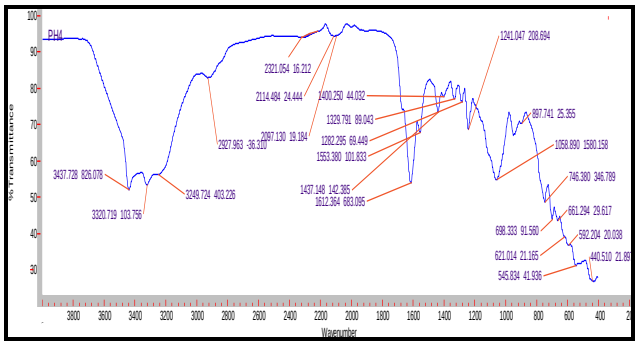

Figure 13: FTIR Study of PH4 Formulation

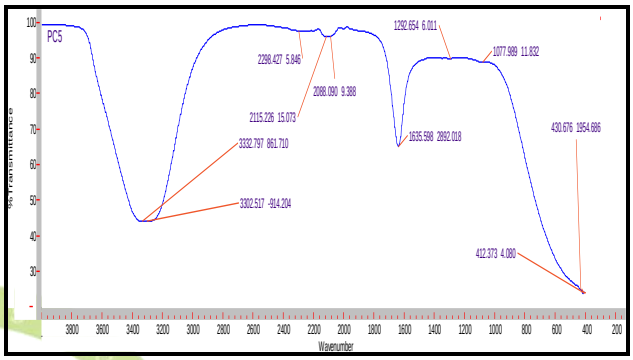

Figure 14: FTIR Study of PC5 Formulation

Drug Content

Drug content was determined for the prepared formulations by UV visible spectrophotometer at 251 nm using simulated tear fluid as a blank. The percentage of drug was calculated. The drug content of poloxamer 407 and HPMC E15 gels was in the range of 84.2%-92%, poloxamer 407 and chitosan was in the range of 83%-92%.

Test for Appearance/ Clarity

All formulations were checked for general appearance i.e. Color, any suspended particulate matter etc. The clarity was checked using wooden board against black and white background. All formulations were found to be clear by visual examination and no suspected particulate matter was found.

Determination of pH

The pH of each formulation was recorded using a calibrated digital pH meter immediately after preparation. The pH of formulations was observed in the range of 6.42-7.4.The pH of the all the formulations were in the desired range.

Table 6: Evaluation of Pol-407 and HPMC *In Situ* Gels

Formulation code	Drug content	Clarity	pH	Gelation Temperature
PH1	84	Clear	7.32±0.01	38.8ºC±0.31
PH2	85	Clear	7.37±0.02	38.5ºC±0.71
PH3	86	Clear	7.36±0.02	35.5ºC±0.17
PH4	92	Clear	7.40±0.02	34.5ºC±0.75
PH5	84	Clear	7.31±0.01	35.5ºC±0.16
PH6	86	Clear	7.42±0.05	27.5ºC±0.72

Table 7: Evaluation of Pol-407 and Chitosan in Situ Gels

Formulation name	Drug content	Clarity	pH	Gelation temp.
PC1	83.0±1.0	Clear	6.51±0.04	40.0ºC±0.71
PC2	87.6±1.5	Clear	6.48±0.01	37.5ºC±0.14
PC3	87.3±1.5	Clear	6.43±0.06	36.5ºC±0.14
PC4	87.0±1.5	Clear	6.49±0.01	36.0ºC±0.01
PC5	88.0±1.7	Clear	6.47±0.02	34.5ºC±0.13
PC6	92.0±1.0	Clear	6.51±0.01	27.5ºC±0.72

Table 8: Effect of Autoclave on *In Situ* Gels

Formulation code	pH(before)	Assay(before)	pH(after)	Assay(after)
PH4	7.4±0.02	87.4±1.7	7.37±0.03	86.5±1.0
PC5	6.46±0.02	90.2±0.6	6.43±0.03	89.7±0.5

Gelation Temperature

Gelation temperature of all formulation was determined. The temperature at which the gel no longer moved when tilted more than 90^0 was recorded as gelation temperature of formulation. The gelation temperature of the formulations was in the range of 27.5^0C-40.0^0C.

Rheological Studies

The prepared formulations were allowed to gel in the simulated tear fluid at 25^0C, 35ºC and the viscosity determination was carried out by using R/S PLUS Brookfield viscometer cone and plate with cone C50-1 with angular velocity run from 10 to 100 rpm. The experiment was performed in triplicate.

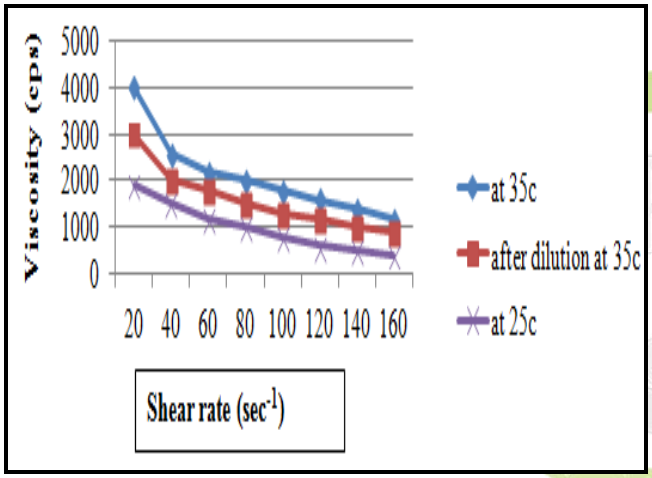

Figure 15: Rheogram of Nepafenac Novel *In Situ* Gel

Autoclaving Sterilization

The optimized formulations PH4 and PC5 were autoclaved at 121^0C and 15 lb pressure for a period of 20 min. The effect of autoclaving on the formulation was examined and the parameters observed were assay and pH of the formulation before and after autoclaving. It was found that the pH of the formulation was remained same and drug content was reduced to some extent by 2%.These studies indicated the prepared formulation were stable in terms of pH and drug content even after autoclaving at 121^0C at 15 lb pressure for 20 min.

In Vitro Drug Release

In vitro drug release studies was performed by, using different methods by maintaining simulated eye conditions like temperature, rpm and pH etc. Based on drug release and reproducibility suitable method was selected for further study. So modified USP II apparatus was selected based on drug release when simulated eye conditions were maintained. From this study 50 ml of STF pH 7.4, rpm 25 and temperature 35^0C were maintained for *in vitro* drug release studies. For all formulations the *in vitro* drug release studies were performed and the amount drug released was calculated the *in vitro* drug release was ranged 78%-99% of drug was release in 10 hours. From *in vitro* drug release studies and gelation temperature of the all for formulations PH4 and PC5 showed sustained release up to 12 hours and have gelation temperature closer to the ocular surface temperature i.e. 35^0C hence these formulations were selected for further study.

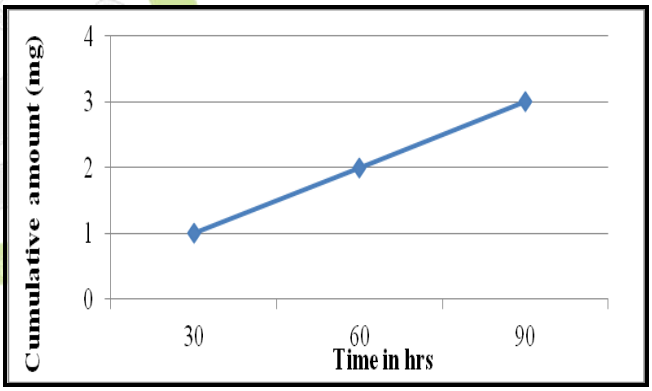

Figure 16: *In Vitro* Release of Pure Drug

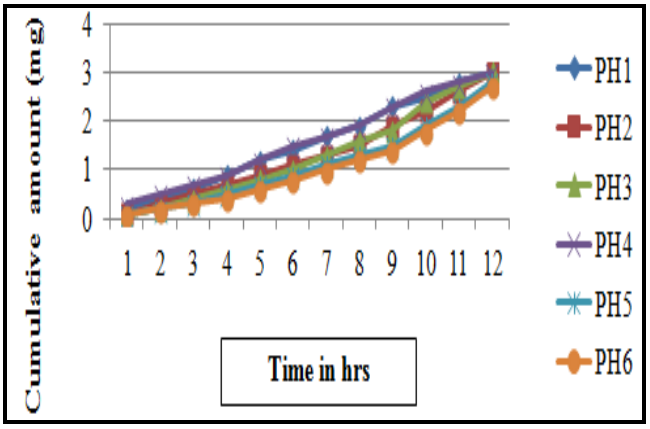

Figure 17: *In Vitro* Drug Release of P-407 and HPMC *In Situ* Gels

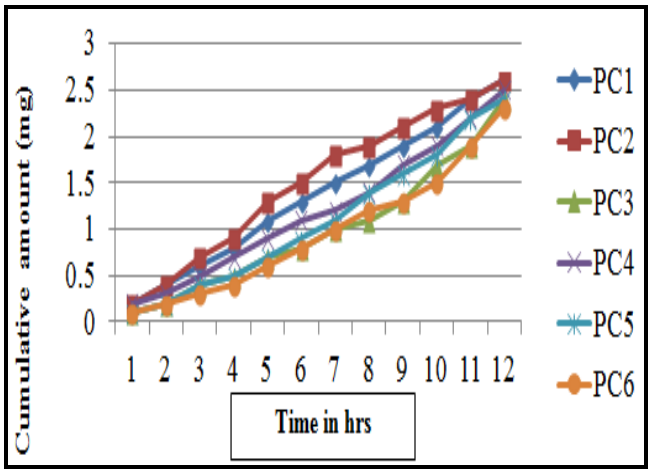

Figure 18: *In Vitro* Drug Release of P-407 and Chitosan *In Situ* Gels

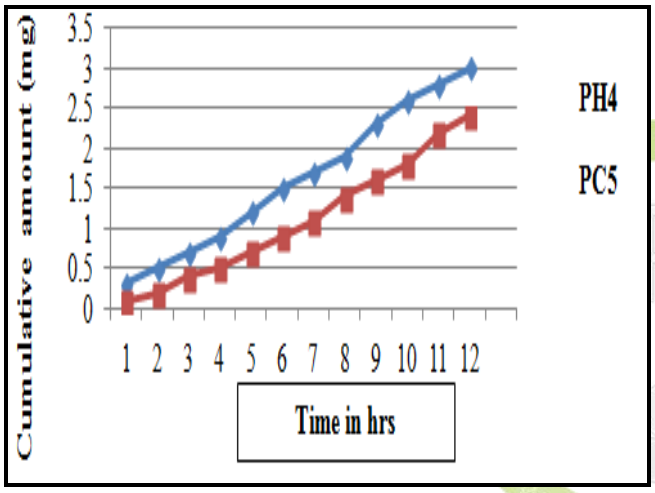

Figure 19: *In Vitro* Drug Release of Poloxamer 407 and HPMC E15 *In Situ* Gel (PH4) and Pc5 Gel

Sterility Testing

Optimized formula was aseptically transferred to thioglycolate medium (20ml) and soyabean-casein digest medium (20ml) separately. The inoculated media incubated for 14 days at 30-35^{0}C in case of fluid thioglycolate medium and 20- 25^{0}C in case of soya bean-casein digest medium.

There was no sign of microbial growth was observed in both fluid thioglycolate medium and soya bean casein digest medium. So autoclaving is the suitable method for sterilization. There was no growth of both aerobic and anaerobic bacteria and fungi.

Figure 20: Soya Bean-Casein Digest Medium and Fluid Thioglycolate Media

CONCLUSION

The different formulations of liposomes containing Nepafenac were prepared by using Rotary Flash Evaporator. The percentage entrapment efficiency was determined and maximum entrapment efficiency was found to be 98%. The prepared optimized liposomes are found to have good mean particle size and it was proved that as the concentration of the cholesterol increased the particle size also increased and entrapment efficiency decreased. In formulation F4, the ratio of cholesterol and lecithin is 1:5 is selected as optimized formulation based on poly dispersity index value which is closer to 3 and drug release as 92% for 10 hours. Optimized formulation F4 was evaluated for entrapment efficiency and drug content. In this present study Nepafenac thermo reversible mucoadhesive novel *in situ* gels were formulated with poloxamer 407, HPMC E15 and chitosan. The prepared formulations were evaluated for drug content, pH, gelation temperature, *and in vitro* drug release and sterility test. From FTIR spectra of the drug and physical mixture, it was found that there is no significant interaction.

The drug content of the formulations is ranging from 83%-92%, pH of the formulations is ranging from 6.48 to 7.4, gelation temperature is ranging from 27.5^{0}C to 40.0^{0}C, *in vitro* release is ranging from 78% to 99%. The formulation PC5 with drug content 88.0%, pH 6.47, and gelation temperature 34.5^{0}C, *in vitro* drug release is 78% within 12 hours.

Gelation temperature of the formulation PC5 is same as that of temperature of ocular surface, pH of the PC5 formulation is compatible with lachrymal fluid pH and *in vitro* release is sustained for 12 hours. Hence this combination can be used as an *In situ* gelling vehicle, to enhance ocular bioavailability and to improve patient compliance.

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