ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 15, Issue 9, 2022

Online - 2455-3891
Print - 0974-2441

Research Article

ACACIA CATACHU GUM *IN SITU* FORMING GELS WITH PROLONGED RETENTION TIME FOR OCULAR DRUG DELIVERY

MANDEEP SINGH*, DHRUV DEV

Department of Pharmaceutics, Shivalik College of Pharmacy, Nangal, Punjab, India. Email: msk286046@gmail.com

Received: 19 May 2022, Revised and Accepted: 10 July 2022

ABSTRACT

Objective: The object is to study acacia catechu gum *in situ*, forming gels with prolonged retention times for ocular drug delivery.

Methods: This study was sample collection and extraction, pre-formulation research, drug melting point and solubility preparation of standard stock solution, lambda max determination, and preparation of ciprofloxacin hydrochloride *in situ* gel.

Results: The melting point of ciprofloxacin hydrochloride was found to be 290°C. The solubility of ciprofloxacin hydrochloride in pH 2.0 and pH 6.8 media is 7.88 0.005 mg/ml and 0.080 0.05 mg/ml. The $_{max}$ of ciprofloxacin hydrochloride was found to be 276–277 nm in simulated tear fluid pH 7.4. Prepared *in situ* gelling systems were evaluated for interaction studies to ensure that no interaction occurred between drugs and polymers. The pH of the formulations was found to be 7.1–7.4, and the drug content was in the range of 92–98%. All the prepared *in situ* gelling systems were evaluated for sterility. After 7 days of incubation, the results showed no microbial growth in all formulations.

Conclusion: The developed formulation is a viable alternative to the conventional eye drops by virtue of its ability to enhanced bioavailability through its longer precorneal residence time.

Keywords: *In situ* gelling system, Natural gum, *Acacia catachu*, Ciprofloxacin hydrochloride, Ocular Drug delivery.

 DOI: http://dx.doi.org/10.22159/ajpcr.2022v15i9.45269. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

The current state of ocular medicine delivery is competitive and rapidly changing. Traditional ocular medication administration methods such as solutions, suspensions, and ointments have drawbacks such as increased precorneal excretion, high changeability, decreased vision, and a short residence period. Poor drug bioavailability from ocular dosage forms is caused by tear generation, temporary residence time, and corneal epithelial impermeability [1]. Effective eye treatment necessitates a therapeutic medication level in the eye that is stable over time [2]. At the same time, ophthalmic formulations must meet the predefined criteria of efficacy, stability, sterility, and tolerance [3]. To avoid irritation, a preparation should have a pH range of 6.6–9.0 and an appropriate viscosity to greatly increase contact duration [4]. To circumvent these constraints and increase the contact period between the medication and the eye environment, several techniques have been devised [5]. To increase residence time, researchers have used numerous techniques such as prodrugs, mucoadhesives, lyophilisates, particulates, dendrimers, vesicular systems, and *in situ* gelling systems [2]. Polymer-based viscosity increase through *in situ* gelling is the most often employed method [6]. A less viscous solution turns into a gel phase under the impact of various stimuli in an *in situ* gel system, also known as a smart gel system (pH, ionic strength, and temperature). This phase transition happens in the conjunctiva cul-de-sac in ocular *in situ* gel. Chitosan, carbopol, sodium alginate, poloxamers, pluronic, Gelrite (Merk), xanthan, and pluronic copolymers are examples of such gels [7-11]. By enhancing drug retention duration, a variety of synthetic and natural polymers have the potential to boost drug bioavailability [12]. A polymer-fabricated delivery system should have drug loading capacity, a longer residence time at target tissues, sustained release potential, as well as safety, biocompatibility, and biodegradability [13]. By modifying physiological circumstances, an *in situ* gelling system that exists as a liquid dosage can be converted into a gel phase [14]. Ocular drugs are delivered using a variety of biological macromolecules such as carbohydrates, lipids, peptides, oligonucleotides, and their derivatives. Biopolymers for ocular drug delivery should be chosen based on their stability, biodistribution, clearance of the reticuloendothelial framework by mononuclear phagocytes, and penetration into the target [15].

Due to these auxiliary qualities (extensive polydispersities, branching atomic structure, diverse receptive destinations on glycoside units, and non-toxic nature), polysaccharides are seen as fundamentally similar to other synthetic polymers [16]. Polysaccharide-based polymers, including as chitosan, alginate, heparin, and pectin, have the ability to recognize cells by focusing on moieties through explicit receptors [17,18].

Natural polymers are preferred over synthetic polymers due to biocompatibility, biodegradability, availability, and low cost. Furthermore, in an ongoing comparative random investigation of five distinct over-the-counter ophthalmic medications containing synthetic polymers: carboxymethyl cellulose, glycerin/polysorbate-80, polyethylene glycol-400, and polyvinyl alcohol/PEG-400 [19,20], a transient decrease in visual quality has been observed. On the other hand, albumin, guar gum, and hyaluronic acid have shown a high level of resemblance and patient consistency. Arabinogalactan, *Bletilla striata* polysaccharide, and tamarind seed polysaccharide are novel polymers that are similar to human lachrymal secretion and can be used to treat dry eye disease.

Due to their defensive capabilities, these unique polymers can be used as carriers to safely transport medications while reducing their toxicity and expediting the recovery of diseased tissues, resulting in a synergistic effect. Other polymers, such as gum cordia, *Sterculia foetida*, and locust bean gum, have shown the ability to deliver drugs to ocular tissues, although more research is needed. Despite the use of natural polymers in topical medicine delivery in both classic and new ocular preparations, the utility of these formulations is limited due to a lack of effective scale-up developments and sterility concerns with ophthalmic dosage structures [21].

Colossal endeavors are still required for the extraction, refinement, and verification of natural polymers at modern level.

Acacia catechu is one of the gum-producing species of the Acacia family found in India and the Indian subcontinent. It is also known as "Khair, khadir, kattha tree, cutch tree, and catechu." The plant is of special economic importance. Besides its cultivation for "kattha," the wood is used to produce strong and durable timber, poles, charcoal used in the paper industry, and fodder is a rich nitrogen source [22,23,25].

At room temperature, the effect of changing the pH (3, 5, 7, and 9) and the presence of different concentrations of solutes such as NaCl (0, 0.1, 0.5, 1, aand 2%), sucrose (0, 1, 3, 5, and 10%), glucose (0, 1, 3, 5, and 10%), and fructose (0, 1, 3, 5, and 10%) on the flow behavior of a 1% gum solution was investigated. Acacia catechu gum dissolves in cold water (to a concentration of 43–48% v/v), but not in ethanol. At low doses, acacia gum dissolves quickly in cold water, but at greater doses, shear is required for complete breakdown. Data on toxicity there is minimal or no acute, short-term, or subchronic toxicity in acacia catechu gum.

When injected intraperitoneally or orally, acacia catechu gum caused no oral toxicity in rats fed a single dose of 250–500 mg/kg. Catechu gum is negative in multiple genotoxicity assays, is not a reproductive or developmental toxin, and is not carcinogenic. Acacia catechu is not deemed an eye irritant based on the findings of ocular irritation tests. This activity was thought to be caused by the presence of tannins and flavonoids in the product.

This study represents another mechanism whereby *A. catechu* extract can control blood sugar levels. A 90-day oral safety study was conducted on a combination *S. baicalensis* and *A. catechu* product in male and female rats. A dose of 1000 mg/kg/day was identified as the no-observed-adverse-effect level and was the highest dose used in this study. No effects were observed with respect to body weight, feed consumption, clinical observations, organ weights, gross findings, spermatogenesis, estrus staging, ophthalmology, neurology, histopathology, or blood chemistries. Structure of acacia catechu gum shown in Fig. 1. These results indicate a very high level of safety [27].

According to several studies, *A. catechu* heartwood is an excellent source of catechins and epicatechins, as well as flavonoids, which have significant antioxidant activity. Both *in vitro* and *in vivo* investigations have demonstrated the antioxidant activity. Antioxidant activity is thought to be responsible for the anti-inflammatory, antineoplastic, tissue protectant, and analgesic properties that have been seen and may be linked to the antihypertensive and antidiarrheal properties. Despite A. catechu's long-term use and the general safety of catechins and epicatechins, more well-controlled safety studies in animals and humans are needed. Human efficacy studies that are well-controlled are also required. In addition, few studies have attempted to link different effects to individual elements. Asian Institution Exchange and thermoreversible gelling agent [28-31].

Ciprofloxacin is a fluoroquinolone antibiotic that has demonstrated *in vitro* activity against staphylococcus and Bacillus species. It has been suggested as a possible agent in the treatment and prevention of endophthalmitis [23,32].

Thus, with an aim of pH triggered, *in situ* gel system using ciprofloxacin HCL (0.3% w/v) and *Indian catechu* gel as gelling agents was formulated. The prepared dosage form was tested for various physicochemical and biological properties to check their possible use as future alternatives of conventional ophthalmic drugs. This work aims to formulate and evaluate a thermo-sensitive ocular *in situ* gel of ciprofloxacin acacia catechu gum as a gelling agent. Visual appearance, clarity, pH, and sol-gel transition temperature of formulas were tested. Comprehensive rheological properties of the prepared formula were also studied. In addition, irritancy test of the best formula was assessed. The release of the drug was studied using *in vitro* dissolution test. To study the retention of the gel in the eye, a pioneer work was conducted using an auto refractometer apparatus to track the contact time of ciprofloxacin gel in comparison to marketed eye drop of ciprofloxacin. In addition, sterility test and stability test were also conducted.

METHODS

Materials

Sample collection and extraction

In January 2022, gums were taken from the bark of an Indian catechu tree in Rupnagar, Punjab, India. Before washing with distilled water, dirt and pollutants were physically removed from the natural excipient (gum). The taxonomist at the University of Sargodha's Department of Botany validated the sample. It was washed, dried in the oven at 60°C, and then steeped for 24 h in 500 mL of distilled water. Acetone was used to precipitate the gel, which was then strained through a muslin cloth and dried. An electrical grinder (Anex, Germany) was used to grind the dry gel, which was subsequently sieved through # 80 and dried in a desiccator. Polysaccharides contents of dried gel powder were confirmed by molisch and iodine test [24,25]. Samples were preserved in sealed vial and stored in a dry place at room temperature.

Ciprofloxacin HCL active salt, as a gift sample, was received from Glenmark Pharmaceutical Ltd., Baddi. Indian catechu gum was collected locally. Propylene glycol benzalkonium chloride was received from S.D fine-Chem Ltd. Sodium chloride calcium chloride dehydrates were purchased from Fischer scientific. Sodium bicarbonate was received from rankem haryana. Instruments use for preparation of *in situ* gel UV visible, FTIR, Magnetic Stirrer, Mechanical Stirrer, Digital melting points apparatus, Franz Diffusion cell. Acacia catechu raw gum and Acacia Catechu gum powder form shown in Figs. 2 and 3.

Methods

Preformulation studies

Preformulation testing is the first step in the rationale development of dosage forms of a drug substance. It can be defined as an investigation of the physical and chemical properties of a drug substance alone or when combined with excipients. The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms that can be mass produced.

Identification of ciprofloxacin hydrochloride

Identification of Ciprofloxacin hydrochloride was carried out by UV spectrophotometer.

Melting point determination

Melting point of Ciprofloxacin hydrochloride was determined by open capillary method.

Solubility

Solubility of ciprofloxacin hydrochloride was determined based on co solvency method using propylene glycol, glycerin, and water.

Determination of λ_{max}

A solution of ciprofloxacin hydrochloride containing the concentration 10 µg/ml was prepared in simulated tear fluid (STF) pH 7.4 and UV spectrum was taken using Shimadzu (UV-1800) double beam spectrophotometer. The solution was scanned in the range of 200–400 nm.

Preparation of calibration curve in STF and distilled water

- Estimation of ciprofloxacin hydrochloride by spectrophotometric method

  A simple and rapid method for estimation of ciprofloxacin hydrochloride by UV spectrophotometric method was developed in STF. Ciprofloxacin hydrochloride in STF of pH 7.4 shows λ_{max} at

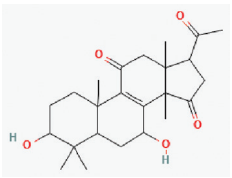

Fig. 1: Structure of *Acacia catechu* Gum [26]

Fig. 2: *Acacia catechu* raw gum

Fig. 3: *Acacia catechu* gum powder form

Fig. 4: Final product *in situ* gel of ciprofloxacin HCL

276 nm.

- Preparation of STF
  Dissolve 0.670 g of sodium chloride, 0.2 g of sodium bicarbonate, and 0.008 g calcium chloride di hydrate in 100 ml of de ionized water and adjust the pH to 7.4 using 0.5 M sodium hydroxide and 0.5 M hydrochloric acid.
- Preparation of Standard Stock Solution
  The standard stock solution was prepared by dissolving 100 mg ciprofloxacin hydrochloride of in 100 ml of STF, to get the 1mg/ml concentration of solution
- Working Standard Solution
  From above stock solution, 2 ml was pipetted out in to a 10 ml volumetric flask and made up to 10 ml with STF to give a concentration of 20 µ/ml, respectively.
- Procedure for calibration of ciprofloxacin hydrochloride using STF at λ_{max} 276 nm

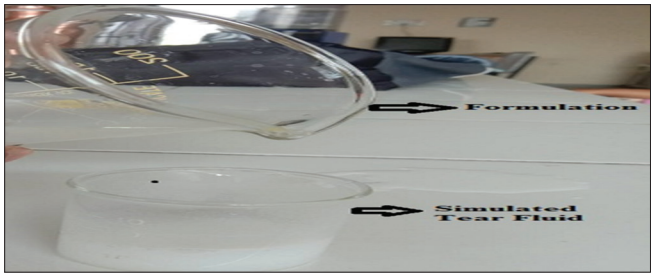

Fig. 5: Gelling capacity of formulations

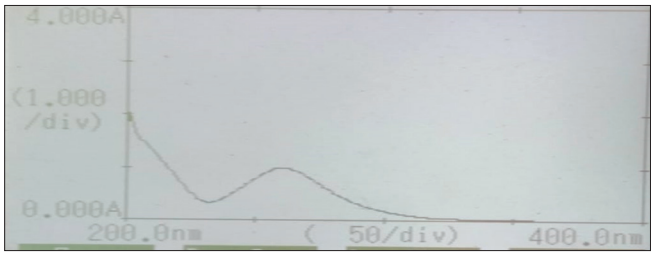

Fig. 6: UV spectrophotometric of ciprofloxacin hydrochloride

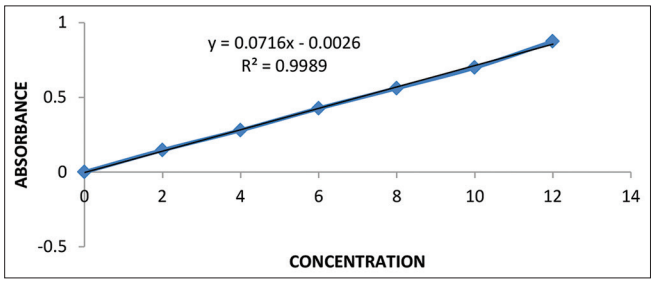

Fig. 7: Calibration curve of ciprofloxacin hydrochloride in STF

Above working standard solution 1–6 ml was taken and was diluted to 10 ml to get 2–12 µg/ml and absorbance was taken at λ_{max} 276 nm. The obtained data are given in Table 1 and standard plot of absorbance versus concentration was plotted which is given under.

Preparation of *in situ* gels of ciprofloxacin hydrochloride

Procedure for preparation of *in situ* gels of ciprofloxacin hydrochloride polymer solution was prepared by dispersing Gelrite in de ionized water by heating up to 90°c for 20 min followed by cooling to room temperature, drug solution was prepared by dissolving ciprofloxacin hydrochloride in mixture of propylene glycol and water (100:8), drug solution was mixed with polymer solution using a magnetic stirrer, and Benzalkonium chloride in concentration of 0.01% was added which acts as preservative. The prepared *in situ* gels were filled in glass vials closed with rubber closures and sealed with aluminium caps and sterilized by autoclave at 121°C 15 psi for 20 min. Final product *in situ* gel of ciprofloxacin Hcl shown in Fig. 4.

Evaluation of *in situ* gels of ciprofloxacin hydrochloride

Interaction studies

IR spectra were taken using Fourier transform infrared spectrophotometer (840, Shimadzu, Japan). The pellets of drug and potassium bromide were prepared by compressing the powders at 20 psi on KBr-press and the spectra were scanned in the wave number range of 4000–600 cm^{-1}. FTIR study was carried on pure drug, physical mixture of drug, and polymers, formulations to confirm the compatibility of drug with other excipients used in the preparation of *in situ* gels. IR spectra of drug and excipients [33].

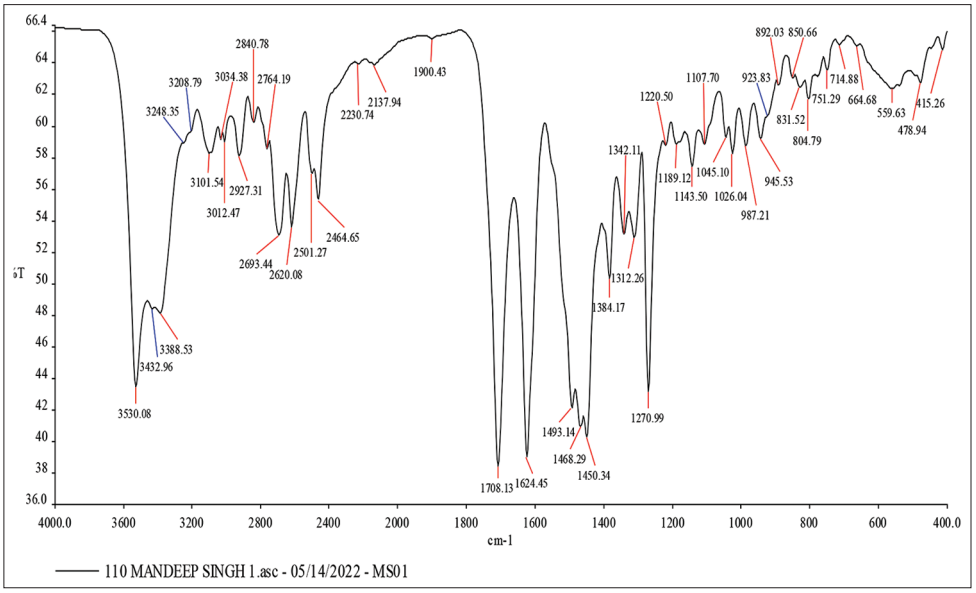

Fig. 8: IR spectra of ciprofloxacin hydrochloride

Table 1: Standard calibration data of ciprofloxacin HCL in STF

S. No.	Concentration (µg/ml)	Absorbance
1.	0	0.000
2.	2	0.147
3.	4	0.280
4.	6	0.486
5.	8	0.561
6.	10	0.699
7.	12	0.874

Visual appearance and clarity

Visual appearance and clarity were checked under fluorescent light against a white and black back ground for presence of any particulate matter.

pH

The pH of the prepared *in situ* gelling system after addition of all the ingredients was measured using pH meter.

In vitro gelation

Gelling capacity of formulations was evaluated to identify the formulations suitable for the use as *in situ* gelling systems. Gelling capacity was determined by mixing the formulation with STF in the proportion 25:7 and examined visually. The composition of STF was sodium chloride (0.670 g), sodium bi carbonate (0.2g), calcium chloride dehydrate, and bi-distilled water quantity sufficient up to100 g. Physiological pH (7.4±0.2) was adjusted by adding the required amount of 0.1 N HCL [34]. Gelling capacity of formulations shown in Fig. 5.

Rheological studies

Viscosity of the instilled formulation is an important factor in determining residence time of drug in the eye. The prepared solutions were allowed to gel in the STF, and then, the viscosity determination was carried out using Brooke field viscometer RVT model in spindle no S-34, angular velocity ran from 10 to 100 rpm. Viscosity of the formulations increased with increase in polymer concentration. The hierarchy of shear rate was reversed and average of two readings was used to calculate viscosity [35].

Sterility testing

Sterility testing is intended for detecting the presence of viable form of microorganisms and was performed for aerobic and anaerobic bacteria and fungi using fluid thioglycolate medium and soyabean casein digest medium, respectively, as per the Indian pharmacopoeia [36].

Preparation of media

Fluid thioglycolate medium and soyabean casein digest medium were prepared by suspending all ingredients in 1000 ml of distilled water, separately boiled until it dissolves completely. Then, it was sterilized by autoclaving at 15 lbs pressure, 121°C for 15 min and cooled. After cooling 25 ml of both the medium were transferred to the test tubes.

Preparation of samples

The sterile formulations were taken into laminar airflow. Sterile formulation was removed from the vials by help of syringe. This solution was passed through the membrane filter of 0.45 µm size with the help of vacuum pump. After filtration, the filter paper was removed from funnel and it was cut into two half. One half was dropped in bacterial media (Fluid thioglycolate) and the other half was dropped in the fungal media (Soyabean casein digest). The media were kept for incubation for 7 days at 37°C. Both the media were observed every day for any microbial contamination and compared with a positive and negative control.

Drug content analysis

The aliquots from the calibration curve method were taken (2–12 µg/ml) that were stored in vials. The vials containing formulation were properly shaken for 2–3 min. One millileter of the formulation was transferred into 100 ml volumetric flask with 1 ml calibrated graduated pipette, 50 ml of STF with pH 7.4 were added that gel was completely crushed with the help of glass rod followed by vigorous shaking until the formed gel gets completely dispersed to give clear solution. Final volume was adjusted to 100 ml with STF, aliquot of 1 ml was taken and further diluted to 10 ml with STF, obtained solution was filtered through 0.45 µm filter membrane and the drug concentration was determined by UV Visible spectrophotometer at 276 nm [37].

In vitro release studies

In vitro drug release from the formulations was studied by the diffusion cell. Here, the pH of the Lacrimal fluid and the blinking rate of the eye was taken into consideration and was simulated. The procedure for standard calibration is same as mentioned under drug content determination.

Table 2: Formulation of *in situ* gels of ciprofloxacin Hydrochloride

S. No.	Ingredients	Concentration (%W/v)					
		F1	F2	F3	F4	F5	F6
1.	Ciprofloxacin hydrochloride	0.3	0.3	0.3	0.3	0.3	0.3
2.	Acacia catechu gum	50	100	150	200	250	300
3.	Propylene glycol	8	8	8	8	8	8
4.	Benzalkonium chloride	0.01	0.01	0.01	0.01	0.01	0.01
5.	De ionized water	100	100	100	100	100	100

Table 3: Preliminary evaluation of visual appearance, clarity, pH, and drug content

Formulation code	Visual appearance	Clarity	pH	Drug content
F1	Transparent	Clear	7.11	96.65
F2	Transparent	Clear	7.22	97.01
F3	Transparent	Clear	7.24	96.08
F4	Transparent	Clear	7.26	95.29
F5	Transparent	Clear	7.30	93.43
F6	Transparent	Clear	7.35	91.97

Table 4: Evaluation of gelling capacity

Formulations	Gelling capacity
F1	++
F2	+++
F3	+++
F4	+++
F5	+++
F6	+++

++ gelation immediate and remains for few hours, +++ shows gelation immediate and remains for extended period

Table 5: Rheological studies of *in situ* gels before gelation

Share rate (RPM)	Viscosity of the formulation (Pa.s)					
	F1	F2	F3	F4	F5	F6
10	550	825	1120	1380	1635	1855
20	380	640	790	860	1190	1270
50	260	420	440	485	655	850
100	190	240	260	280	320	485

Table 6: Rheological studies of *in situ* gels after gelation

Share rate (RPM)	Viscosity of the formulation (pa. s)					
	F1	F2	F3	F4	F5	F6
10	1322	1407	2600	2020	3282	4180
20	712	1110	1320	1545	2192	2270
50	460	680	735	890	1082	1530
100	320	405	440	500	562	620

Procedure

In vitro release studies were carried out using bichambered donor receiver compartment model (Franz diffusion cell) using cellophane membrane soaked overnight in the receptor medium (STF, pH 7.4). The diffusion medium was 100ml of STF stirred at 50 rpm at 37°C±0.5°C. One end of the diffusion tube was covered by a cellophane membrane. The 1 ml formulation was spread on the cellophane membrane and membrane was placed such that it just touches the diffusion medium (STF) present in receptor compartment. The drug samples were withdrawn at the interval of 1 h for the period of 8 h from diffusion medium and analyzed by a UV spectrophotometer at 276nm using STF as blank.

Comparative evaluation of marketed products with prepared in situ gels

In vitro release studies of marketed formulation were carried out using bichambered donor receiver compartment model (Franz diffusion cell) using cellophane membrane soaked overnight in the receptor medium (STF, pH 7.4). The diffusion medium was 100 ml of STF stirred at 50 rpm at 37°C±0.5°C (shown in Fig. 9). One end of the diffusion tube was covered by a cellophane membrane. The 1 ml formulation was spread on the cellophane membrane and membrane was placed such that it just touches the diffusion medium (STF) present in receptor compartment. The drug samples were withdrawn at the interval of 1 h for the period of 8 h from diffusion medium and analyzed by a UV spectrophotometer at 276nm using STF as blank.

Pharmacokinetic release studies

All the optimized formulations were subjected to study the release kinetics and the best fit kinetic model was determined for the optimized formulations using analysis software PCP Disso V2.

Antimicrobial efficacy studies

The antimicrobial efficacy studies were carried out to ascertain the biological activity of the optimized formulations. *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and *Escherichia coli* were used as the test organisms. Antimicrobial efficiency was determined by agar diffusion test employing Cup-Plate method. Sterile solutions of Ciprofloxacin Hydrochloride (standard solution) and the developed formulations were diluted at different concentration (test solutions); these solutions were poured in to cups bored into sterile nutrient agar previously seeded with test organisms (*P. aeruginosa*, *E. coli* and *S. aureus*), after allowing diffusion of the solutions for 2 h, the agar plates were incubated at 37°C for 24 h. The zone of inhibition measured around each cup and was compared with that of control. The entire operation except the incubation was carried out in a laminar airflow unit. Both positive and negative controls were maintained during the study [39].

Accelerated stability studies

Stability is defined as the extent, to which a product retains with in specified limits and throughout its period of storage and use, that is, shelf life. Stability studies were carried out on optimized formulations according to international conference on harmonization guidelines A sufficient quantity of formulations in previously sterilized vials was stored in desiccators containing a saturated solution of sodium chloride, which gives a relative humidity of 75±5%. The desiccators were placed in a hot air oven maintained at a temperature 40°C±0.5°C and at room temperature. Samples were withdrawn at 7 days interval for 42 Days. Percent drug remaining was calculated and plotted against time in days [40].

RESULTS AND DISCUSSIONS

Melting point determination

The melting point of ciprofloxacin hydrochloride was found to be 290°C.

Solubility study

Solubility of ciprofloxacin hydrochloride in pH 2.0 and pH 6.8 media is 7.88±0.005 mg/ml and 0.080±0.05 mg/ml. Ciprofloxacin hydrochloride

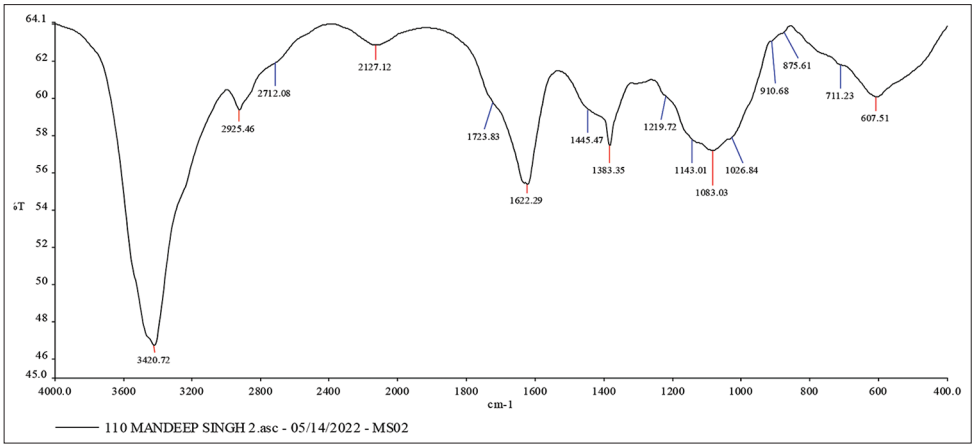

Fig. 9: IR spectra of *Acacia catechu* gum

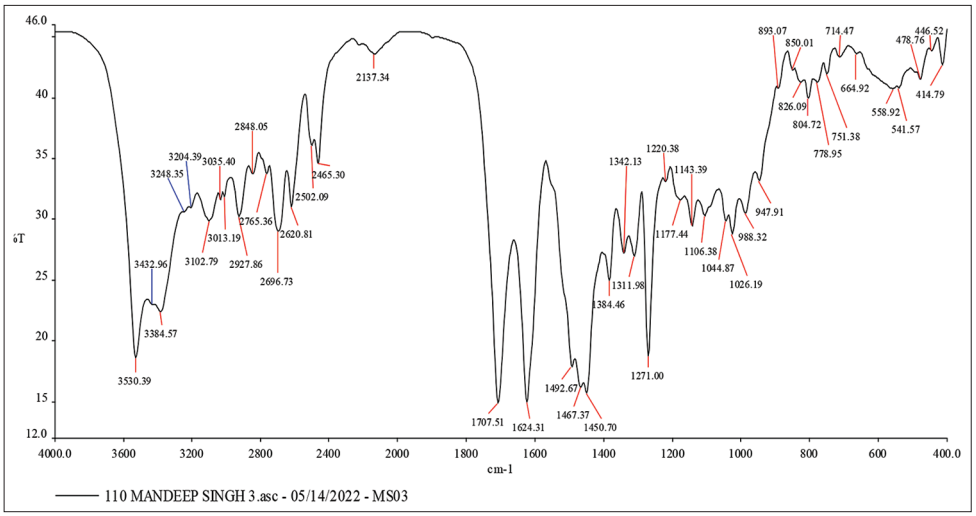

Fig. 10: IR spectra of drug and *Acacia catechu* gum

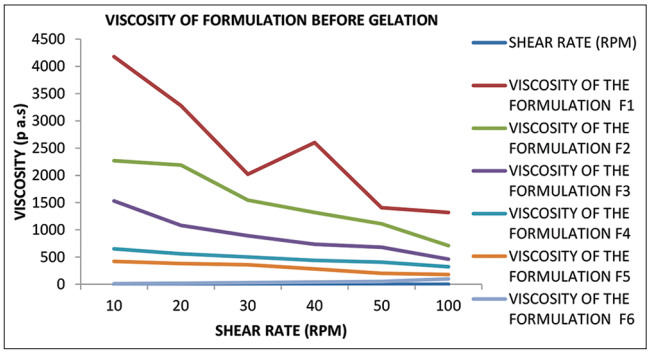

Fig. 11: Rheological studies of *in situ* gels before gelation

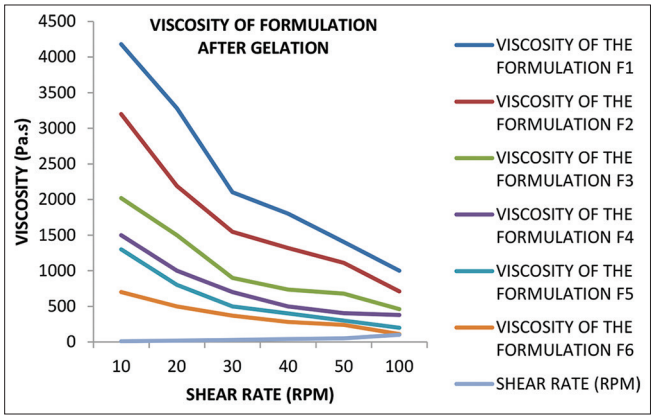

Fig. 12: Rheological studies of *in situ* gels after gelation

was soluble in co solvent mixture of propylene glycol and water, glycerin, and water, it was also found sparingly soluble in organic solvents like DMSO.

Determination of λ_{max}

λ_{max} of ciprofloxacin hydrochloride was found to be 276–277 nm in STF pH 7.4.

Evaluation of prepared *in situ* gelling system

Estimation of ciprofloxacin hydrochloride by Spectrophotometric method

A simple spectrophotometric method for estimation of ciprofloxacin hydrochloride was developed in STF and in distilled water, which exhibited λ_{max} at 276 nm in Beer's range of 2–12 µg/ml for STF. UV spectrophotometric of Ciprofloxacin hydrochloride and Calliberation curve of ciprofloxacin Hydrochloride in STF shown in Figs. 6 and 7.

Interaction studies

The prepared *in situ* gelling systems were evaluated for interaction studies to ensure that there is no interaction occurred in between drug

and polymers. For confirmation of stability of drug in the prepared formulations, the IR spectra were taken and compared with that of pure drug. The result of these studies revealed that there were no definite changes obtained in the bands of drug with respect to pure drug.

Evaluation of visual appearance, clarity, pH, and drug content

All the prepared *in situ* gelling systems were evaluated for preliminary steps such as visual appearance, clarity, pH, and drug content. These formulations were transparent and clear. The pH of the formulations was found to be 7.1–7.4, and drug content was in between 92 and 98%. IR Spectra of Ciprofloxacin Hydrochloride, IR Spectra of Acacia Catechu gum and IR Spectra of drug and Acacia Catechu gum shown in Figs. 8-10.

In vitro gelation

Prepared *in situ* gelling systems were evaluated for the *in vitro* gelation capacity. All the formulations gave satisfactory results.

Rheological studies

For the development of optimum *in situ* gelling system, two major prerequisites viscosity and gelling capacity should be taken in consideration since the ocular shear rate is very high ranging from 0.03 S^{-1} during inter-blinking periods to 4250–28500 S^{-1} during blinking, viscoelastic fluid with a viscosity that is high under low shear rate condition and low under high shear rate condition, which is called Pseudo plastic fluid, is often preferred, so dynamic viscosity of formulations was measured as the change of shear rate before and after gelation. Rheological studies of in situ gels before gelation and Rheological studies of in situ gels after gelation shown in Figs. 11 and 12.

Sterility testing

All the prepared *in situ* gelling systems were evaluated for the sterility. After 7 days of incubation, the results showed no microbial growth in all formulations.

Where "–" sign indicate the no growth

In vitro release studies

The *in vitro* release of Ciprofloxacin hydrochloride from the prepared formulations was studied through cellophane membrane using diffusion cell. The release studies of prepared *in situ* gelling systems were carried out up to 8 h.

In vitro release studies of marketed eye drops (CIPLOX) were done through cellophane membrane using diffusion cell and the release marketed product was up to 3 h.

CONCLUSION

The formulations were therapeutically efficacious. The developed formulation is a viable alternative to the conventional eye drops by virtue of its ability to enhanced bioavailability through its longer precorneal residence time, greater permeability through the tear film and corneal layers and ability to sustain drug release. Furthermore, important is the ease of administration and decreased frequency of administration resulting in better patient acceptance.

ACKNOWLEDGMENT

I am thankful to Prof. Dhruv Dev shivalik college of pharmacy, Punjab, India, for his unstinted help in conducting this study.

AUTHORS' CONTRIBUTIONS

All authors have contributed to study design, manuscript writing, and review, data analysis, and article finalization.

CONFLICTS OF INTEREST

None.

AUTHORS' FUNDING OR AUTHORS' SPONSORSHIP

None.

REFERENCES

1. Rajeshwari NP, Rachana SK. *In situ* gelling system: A novel approach for ophthalmic drug delivery. World J Pharm Pharm Sci 2014;3:423-40.
2. Sultana Y, Jain R, Aqil M, Ali A. Review of ocular drug delivery. Curr Drug Deliv 2006;3:207-17. doi: 10.2174/156720106776359186, PMID 16611007.
3. Furrer P, Delie F, Plazonnet B. Ophthalmic Drug Delivery, Modified-Release Drug Delivery Technology. Raleigh, NC: Pharmaceu Tech, Inc.; 2008. p. 59-84.
4. Remington JP. Remington: The Science and Practice of Pharmacy. Philadelphia, PA: Lippincott Williams and Wilkins; 2006.
5. Le Bourlais CA, Treupel-Acar L, Rhodes CT, Sado PA, Leverge R. New ophthalmic drug delivery systems. Drug Dev Ind Pharm 1995;21:19-59. doi: 10.3109/03639049509048095.
6. Gao Y, Sun Y, Ren F, Gao S. PLGA-PEG-PLGA hydrogel for ocular drug delivery of dexamethasone acetate. Drug Dev Ind Pharm 2010;36:1131-8. doi: 10.3109/03639041003680826, PMID 20334543.
7. Srividya B, Cardoza RM, Amin PD. Sustained ophthalmic delivery of ofloxacin from a pH triggered *in situ* gelling system. J Control Release 2001;73:205-11. doi: 10.1016/s0168-3659(01)00279-6, PMID 11516498.
8. Gupta H, Velpandian T, Jain S. Ion-and pH-activated novel *in-situ* gel system for sustained ocular drug delivery. J Drug Target 2010;18:499-505. doi: 10.3109/10611860903508788, PMID 20055752.
9. Balasubramaniam J, Pandit JK. Ion-activated *in situ* gelling systems for sustained ophthalmic delivery of ciprofloxacin hydrochloride. Drug Deliv 2003;10:185-91. doi: 10.1080/713840402, PMID 12944139.
10. Ma WD, Xu H, Nie SF, Pan WS. Temperature-responsive, pluronic-g-poly(acrylic acid)copolymers *in situ* gels for ophthalmic drug delivery: Rheology, *in vitro* drug release, and *in vivo* resident property. Drug Dev Ind Pharm 2008;34:258-66. doi: 10.1080/03639040701580622, PMID 18363141.
11. Lin HR, Sung KC. Carbopol/pluronic phase change solutions for ophthalmic drug delivery. J Control Release 2000;69:379-88. doi: 10.1016/s0168-3659(00)00329-1, PMID 11102678.
12. Ludwig A. The use of mucoadhesive polymers in ocular drug delivery. Adv Drug Deliv Rev 2005;57:1595-639. doi: 10.1016/j.addr.2005.07.005, PMID 16198021.
13. Barbu E, Verestiuc L, Nevell TG, Tsibouklis J. Polymeric materials for ophthalmic drug delivery: Trends and perspectives. J Mater Chem 2006;16:3439-43. doi: 10.1039/b605640g.
14. Pandit J, Bharathi D, Srinatha A, Ridhurkar D, Singh S. Long acting ophthalmic formulation of indomethacin: evaluation of alginate gel systems. Indian J Pharm Sci 2007;69:37. doi: 10.4103/0250-474X.32105.
15. Juliano R. Challenges to Macromolecular Drug Delivery. London, United Kingdom: Portland Press Limited; 2007.
16. Lin K, Kasko AM. Carbohydrate-based Polymers for Immune Modulation. Washington, DC: ACS Publications; 2014.
17. Pahuja P, Arora S, Pawar P. Ocular drug delivery system: A reference to natural polymers. Expert Opin Drug Deliv 2012;9:837-61. doi: 10.1517/17425247.2012.690733, PMID 22703523.
18. Majeed A, Khan NA. Ocular *in situ* gel: An overview. J Drug Deliv Ther 2019;9:337-47. doi: 10.22270/jddt.v9i1.2231.
19. Mandal S, Thimmasetty MK, Prabhushankar G, Geetha M. Formulation and evaluation of an *in situ* gel-forming ophthalmic formulation of moxifloxacin hydrochloride. Int J Pharm Investig 2012;2:78-82. doi: 10.4103/2230-973X.100042, PMID 23119236.
20. Tung CI, Kottaiyan R, Koh S, Wang Q, Yoon G, Zavislan JM, *et al*. Noninvasive, objective, multimodal tear dynamics evaluation of 5 over-the-counter tear drops in a randomized controlled trial. Cornea 2012;31:108-14. doi: 10.1097/ICO.0b013e31821ea667, PMID 22138585.
21. Krishnaswami V, Kandasamy R, Alagarsamy S, Palanisamy R, Natesan S. Biological acromolecules for ophthalmic drug delivery to treat ocular diseases. Int J Biol Macromol 2018;110:7-16. doi: 10.1016/j.ijbiomac.2018.01.120, PMID 29378276.
22. Sharma A, Bhushette PR, Annapure US. Physicochemical and rheological properties of *Acacia catechu* exudate gum. Carbohydr Polym Technol Appl 2021;2:100-27. doi: 10.1016/j.carpta.2021.100127.
23. Oztürk F, Kurt E, Inan UU, Kortunay S, Ilker SS, Başci NE, *et al*. The effects of prolonged acute use and inflammation on the ocular

penetration of topical ciprofloxacin. Int J Pharm 2000;204:97-100. doi: 10.1016/s0378-5173(00)00483-x, PMID 11011991.
24. De S, Dey Y, Ghosh A. Phytochemical investigation and chromatographic evaluation of the different extracts of tuber of *Amorphaphallus paeoniifolius* (Araceae). Int J Pharm Biol Res 2010;1:150-7.
25. Yadav R, Agarwala M. Phytochemical analysis of some medicinal plants. J Phytol 2011;3:10-4.
26. Available from: https://wwww.goldbio.com
27. Hosseini-Parvar SH, Osano JP, Matia-Merino L. Emulsifying properties of basil seed gum: Effect of pH and ionic strength. Food Hydrocoll 2016;52:838-47. doi: 10.1016/j.foodhyd.2015.09.002.
28. Bhushette PR, Annapure US. Characterization of *Acacia nilotica* exudate gum and its film. J Food Meas Char 2020;14:3058-66. doi: 10.1007/s11694-020-00541-x.
29. Bhushette PR, Annapure US. Physicochemical, functional and rheological investigation of *Soymida febrifuga* exudate gum. Int J Biol Macromol. 2018;111:1116-23. doi: 10.1016/j.ijbiomac.2018.01.117, PMID 29366895.
30. Fathi M, Mohebbi M, Koocheki A. Introducing *Prunus cerasus* gum exudates: Chemical structure, molecular weight, and rheological properties. Food Hydrocoll. 2016;61:946-55. doi: 10.1016/J.FOODHYD.2016.07.004.
31. BP British Pharmacopoeia. Stationary Office 2009. BP British Pharmacopoeia; 2010. p. 5000.
32. Al-Bazzaz FY, Al-Kotaji M. Ophthalmic *in-situ* sustained gel of ciprofloxacin, preparation and evaluation study. Int J Appl Pharm 2018;10:153-61. doi: 10.22159/ijap.2018v10i4.26885.
33. Bachhav HD, Savkare A, Karmarkar R, Derle D. Development of poloxamer-based thermosensitive *in situ* ocular gel of betaxolol hydrochloride. Int J Pharm Pharm Sci 2015;7:4-8.
34. Paradkar MU, Parmar M. Formulation development and evaluation of natamycin niosomal *in-situ* gel for ophthalmic drug delivery. J Drug Deliv Sci Technol 2017;39:113-22. doi: 10.1016/j.jddst.2017.03.005.
35. Varshosaz J, Tabbakhian M, Salmani Z. Designing of a thermosensitive chitosan/poloxamer *in situ* gel for ocular delivery of ciprofloxacin. Open Drug Deliv J 2008;2:61-70. doi: 10.2174/1874126600802010061.
36. Dewan M, Sarkar G, Bhowmik M, Das B, Chattoapadhyay AK, Rana D, *et al*. Effect of gellan gum on the thermogelation property and drug release profile of poloxamer 407 based ophthalmic formulation. Int J Biol Macromol 2017;102:258-65. doi: 10.1016/j.ijbiomac.2017.03.194, PMID 28390828.
37. Bhowmik M, Kumari P, Sarkar G, Bain MK, Bhowmick B, Mollick MM, *et al*. Effect of xanthan gum and guar gum on *in situ* gelling ophthalmic drug delivery system based on poloxamer-407. Int J Biol Macromol 2013;62:117-23. doi: 10.1016/j.ijbiomac.2013.08.024, PMID 23988556.
38. Alexandridis P, Hatton TA. Poly(ethylene oxide) poly (propylene oxide)-poly (ethylene oxide) block copolymer surfactants in aqueous solutions and at interfaces: Thermodynamics, structure, dynamics, and modeling. Colloids Surf 1995;77:1-46.
39. Asasutjarit R, Thanasanchokpibull S, Fuongfuchat A, Veeranondha S. Optimization and evaluation of thermoresponsive diclofenac sodium ophthalmic *in situ* gels. Int J Pharm 2011;411:128-35. doi: 10.1016/j.ijpharm.2011.03.054, PMID 21459137.
40. Song J, Bi H, Xie X, Guo J, Wang X, Liu D. Preparation and evaluation of sinomenine hydrochloride *in situ* gel for uveitis treatment. Int Immunopharmacol 2013;17:99-107. doi: 10.1016/j.intimp.2013.05.020, PMID 23747586.