ijlpr 2023; doi 10.22376/ijlpr.2023.13.4.P14-P28

International Journal of Life science and Pharma Research

ISSN 2250-0480

Research Article

Formulation, Evaluation, And Optimization of *Coscinium Fenestratum*-Based Niosomal *In-Situ* Gel for Anti-Cataract

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Abstract: *Coscinium fenestrat*um is a sturdy, creeping, woody plant that grows with a cylindrical, yellowish stem that belongs to the family Menispermaceae and is commonly known as tree turmeric. The research aims to develop a niosomal *in-situ* gel by incorporating a novel drug carrier, such as a niosome containing herbal extract for treating cataracts. The niosomal carrier was prepared by varying concentrations of two surfactants, while the herbal drug and cholesterol concentration was kept constant. The optimization of the niosome was done based on the size and shape of the vesicle obtained from SEM analysis. The optimized niosomal dispersion was then converted to a niosomal *in-situ* gel, and evaluations were carried out, like pH, viscosity, gelling capacity, gel strength, and isotonicity. From the preliminary phytochemical screenings of *Coscinium fenestrat*um, the ethanolic extract showed the intense presence of flavonoids, alkaloids, steroids, and saponins. The drug-loaded niosomes were stable in HPMC, HPMC K15M, and Carbopol 940 ocular *in-situ* Gel, and the optimum HLB value of the surfactant span 60 made this formulation more stable. The Brookfield viscometer determined the viscosity, which is an important factor in determining the residence time of the drug in the eye. It possessed good viscosity of about 982.66 ± 1.52 cps. From a gelling capacity study conducted, it was found that the niosomal *in-situ* Gel possesses immediate gelation and remains for an extended period. The gel strength of niosomal *in-situ* Gel was 1.30 ± 0.006 sec, and when subjected to isotonicity testing, the results were acceptable. Stability studies were conducted at two different temperatures where no color changes occurred, and there was not much difference in parameters like pH, viscosity, gelling capacity, and gel strength. So it was concluded that the niosomal *in-situ* Gel of *Coscinium fenestratum* can be an alternative method to reduce the drawbacks of conventional ophthalmic dosage forms.

Keywords: Cataract; Niosomes; In-situ gel; *Coscinium fenestratum;* Gel

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Received On 24 January, 2023

Revised On 21 February, 2023

Accepted On 3 March, 2023

Published On 1 July, 2023

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Gowtham Menon, Ravindra C Sutar, Sachin CP, Shendge R S and Girish A Kashid , Formulation, Evaluation, And Optimization of Coscinium Fenestratum-Based Niosomal In-Situ Gel for Anti-Cataract.(2023).Int. J. Life Sci. Pharma Res.13(4), P14-P28 http://dx.doi.org/10.22376/ijlpr.2023.13.4.P14-P28

1. INTRODUCTION

Herbal medicines play a serious role in curing various diseases. Most people in the world use herbal medicine for their health protection. There are many Indians who use herbal drugs regularly, like spices, home remedies, healthy foods, etc. Herbal drugs are the main portion of ayurvedic, naturopathic, homeopathic, and other systems of medicine. They are used to cure different diseases due to their fewer side effects and increased efficacy. Currently, its demand and popularity are increasing day by day. Herbal drugs use whole or parts of plants to treat diseases or wounds [1]. A novel drug delivery system gives better patient compliance and delivery at a predetermined rate. It also reduces the toxic effect with an increase in the bioavailability of drugs [1]. Traditional drug administration consists of preparing the drug in an appropriate dosage form, such as an oral tablet or an IV solution. They have been found to have certain disadvantages. NDDS are developed to defeat the drawbacks of conventional drug delivery. So many novel drug carriers are available such as niosomes, phytosomes, liposomes, nanoparticles, and ethosomes. Inside the patient's body, novel drug carriers can easily target the drug in the affected area and give a better cure. Combining herbal drugs and novel carriers can also increase their solubility and stability, protection from toxicity, increased pharmacological activity, improved tissue macrophage distribution, sustained delivery, and protection from physical and chemical degradation. Among these carriers, niosomes are a well-documented drug delivery system [2]. A cataract is a multifactorial eye disease and is the major cause of blindness and visual impairment worldwide. It is defined as any opacity (or dullness) within the lens that reduces the incoming light and causes a decrease in vision. It is the loss of lens transparency due to the opacification of lens [3]. The risk factors of cataracts include diabetes, ultraviolet radiation, aging, smoking, infectious disorder, nutritional deficiency, genetic abnormality, environmental factors, chemicals, and certain medications such as steroids [4,5]. The lens is a biconvex, flexible crystal-like structure that brings rays of light into focus and produces an image on the retina. The lens contains mainly water and protein fibers. Protein is arranged in a specific manner that helps to retain the lens clear and allow light to pass through it. But some proteins merge and blur a small lens area as we age. As a result of blurring, light is blocked from reaching the retina, and vision is impaired. The lens's opacification occurs through three biochemical changes: hydration, lens protein denaturation, and slow sclerosis [6]. When light enters through the blurred lens, it becomes scattered, and blurry images are formed. The lens is a flexible crystal-like structure that helps to focus light on the retina. The human lens is divided into the nucleus, cortex, and capsule. The transparency of the lens is important for its normal function. The optical capsule is a thin and transparent collagen membrane. It wraps around the lens. The human lens is free of elastic tissue. It is posteriorly secreted by basal cells of elongating fibers and anteriorly by lens epithelium [7]. The cortex contains the youngest lens fibers and intracellular substances and comprises cortical substances. The nucleus of the lens contains the oldest fibers. The various areas of the nucleus are the embryonic nucleus, the fetal nucleus, the infantile nucleus, and the adult nucleus. In the normal eye, the light enters through a non-vascular, colorless, and transparent lens to the retina. At the retina, light rays are changed into a nerve signal. Finally, these signals are transmitted to the brain. In cataract conditions, light is blocked by a foggy lens, and blurred images are formed [8]. When the glucose level increases, the GSH level decreases correspondingly, and the red arrows indicate an increase in reactive oxygen species. Osmotic and oxidative stress work together to reduce fibrous cells' volume regulation. As a result, the cells inflate, depolarize, and have an inflow of sodium and calcium ions. The accumulation of calcium ions activates calcium-dependent proteases. Calcium-dependent proteases target cytoskeletal and crystalline proteins [9]. At the other end, proteins are further altered by the formation of advanced glycation final products (AGEs) that alter the structure and function of the crystallin proteins. The result is an increase in insoluble proteins, the formation of high molecular mass aggregates, and cataracts [10, 11]. Niosomes are non-ionic surfactant vesicles formed by non-ionic surfactants, with or without cholesterol and charge inducers. The Size of niosomes ranges from 10 to 1000 nm. They are amphiphilic, with a central core cavity as an aqueous phase and a surfactant bi-layer as a non-polar phase. It enables the capture of hydrophilic drugs in the central cavity and hydrophobic drugs in the non-polar region present in the bilayer. As a result, hydrophilic and hydrophobic drugs may be incorporated into niosomes. Niosomes are the better drug delivery system due to their vehicle drug delivery potential, high chemical stability, and economy[12]. In-situ gels are the solution or suspensions which undergo gelation after reaching a particular site to form a gel due to physicochemical changes. The word in-situ is derived from Latin, translated literally as 'in position .'In-situ gels provide convenience in administration compared to eye drops, suspensions, or ointments [13]. The administration routes for in situ gel are oral, ocular, rectal, vaginal, injectable, and intraperitoneal. It is the most widely accepted new drug administration system that improves the viscosity of a formulation in the anterior region. It will lead to increased bio-availability due to slower drainage of the cornea [14]. The role of *Coscinium fenestratum* as a herbal medicine for cataract treatment has not been widely reported. This research work aimed to understand the role of *Coscinium fenestratum* in cataract treatment by developing a niosomal in-situ gel that improves its efficiency in treating eye diseases like cataracts.

2. MATERIALS AND METHODS

2.1. Collection of plants

The fresh stems of *Coscinium fenestratum* (Gaertn) were collected from an authorized vendor in the Kannur district, Kerala, India, in October 2019. The plant material was identified and authenticated by Dr. Ratheesh Narayanan MK, Assistant Professor, Payyanur College, Payyanur, Kerala [Voucher number-PNRCLG/DOB/2020-21/035-011].

2.2. Determination of physicochemical parameters

After collecting plant materials, they were shade dried and powdered coarsely in an electronic blender and stored in air-tight containers until further use. The physicochemical parameters of plants were determined, such as total ash, insoluble acid ash, alcohol soluble ash, moisture content by loss on drying method, water, and alcohol soluble extractive values [15].

2.3. Preparation of plant extract for phytochemical analysis

The collected stems were washed thoroughly twice under running tap water followed by double distilled water removes all debris adhering to it and avoids other contaminated organic contents. They have been kept away from direct light from the

sun and were shade dried for about 20 days to protect all the active compounds. These dried plant materials were then homogenized into a fine, coarse powder with an electronic mixer and stored in air-tight containers until further use. Various organic solvents, viz. petroleum ether, chloroform, ethanol, and water, were used for extraction. 10g of homogenized coarse stem powders of *Coscinium fenestratum* was soaked in conical flasks containing 100 ml of petroleum ether, chloroform, ethanol, and water. Then it was left to rest for 30 minutes in a water bath with occasional shaking; finally, each sample extract from the different solvents was filtered through sterilized Whatman no.1 filter paper and concentrated to dryness [16]. Thus, the resulting dried extracts were stored in sterile labeled containers until further usage. Standard methods were followed to detect various biologically active components present in various solvent extracts.

2.4. Preliminary phytochemical analysis

A preliminary qualitative analysis of all ethanolic, petroleum ether, water, and chloroform extracts was carried out using standard methods [17].

2.5. Soxhlet extraction of plant materials

The collected stems of *Coscinium fenestratum* were shade dried at room temperature and coarsely powdered. The 50gm of stem powder was loaded in a thimble and wrapped with filter paper. Then the thimble was placed inside the extractor, and the required solvent (300ml) was taken in a round bottom flask and placed in the heating element. The drug was then extracted by continuous hot extraction using 95% ethanol in the Soxhlet apparatus for about 72 hours until the solvent in the siphon tube became colorless [18]. The distillation process can recover ethanol retained within the extract, then air-dried and concentrate.

2.6. Drug excipient compatibility study

The FT-IR spectrum of drug extracts with other ingredients was analyzed for compatibility study [19].

2.7. Formulation of drug-loaded niosomes

Niosomes, namely F1 to F8, have been prepared using lipid film hydration technology with two different concentrations of surfactant (2:1, 3:1) while cholesterol and drug levels keep constant at [20]. Surfactant (Span 20,60, Tween 20,80), Cholesterol, and ethanolic drug extracts were accurately weighed and dissolved in a 15ml mixture of chloroform: methanol (2:1 v/v ratio) [21]. The above mixture was sonicated for 1 min. Then it was vortexed in the round bottom flask at 58-64°C to remove the solvent for about 30 min [22]. The thin lipid layer inside the flask was then moistened with 10ml of 7.4 pH phosphate buffer at 60°C for 1 hr. The resultant dispersion was cooled in an ice bath, left for four hr. at room temperature for complete hydration, and stored at 4°C overnight before use [23, 24]. The composition of the niosome is shown in Table 1.

Table 1: Composition of developed niosome.

Formulation code	Surfactant used	Weight taken (in mg)			Surfactant: Cholesterol ratio
		Drug (Ethanolic extract of *Coscinium fenestratum*)	Surfactant	Cholesterol	
F1	Span 20	350	200	100	2:1
F2		350	300	100	3:1
F3	Span 60	350	200	100	2:1
F4		350	300	100	3:1
F5	Tween 20	350	200	100	2:1
F6		350	300	100	3:1
F7	Tween 80	350	200	100	2:1
F8		350	300	100	3:1

2.8. Evaluation of niosomes

2.8.1. Vesicle size of niosomes

An optical microscope determined the vesicle size of each formulation. Then, each formulation was spread uniformly on a glass slide and observed under the 45X magnification optical lens[25].

2.8.2. Vesicle shape of niosomes

SEM photographs of the optimized niosomes have achieved the shape, and morphological characteristics were placed in circular aluminum tips with double adhesive carbon tape and coated with gold in a HITACHI ION SPUTTER E-1010 vacuum evaporator. It has been observed that HITACHI SU6600 FE-SEM (Field Emission Scanning Electron Microscope) has an acceleration voltage of 10.0kv and magnification of 60.0k-100.0 k [26].

2.8.3. Zeta potential

Zeta potential was determined using Malvern zeta sizer nano essential. The Zeta potential of niosomal formulation is related to the stability of the niosomal vesicle. Therefore, the high zeta potential value indicates the high degree of repulsion between the vesicle and excellent stability, i.e., the dispersion will resist aggregation[27,28].

2.9. Preparation of niosomal in-situ gell

The required quantity of HPMC was dispersed in 50ml of purified water; HPMC K15M was added to the above solution and stirred slowly with a magnetic stirrer; care was taken that no lumps of HPMC were formed during stirring. Then Carbopol 940 was added to this solution, allowing hydration for one night [29]. After 24 hrs, the solution was again stirred with a magnetic stirrer, and buffer salts (Citric acid, disodium hydrogen phosphate) were dissolved. Next, benzalkonium chloride was dissolved in niosomal dispersion (BKC). The above dispersion was added to the polymer solution under constant stirring until a uniform solution was obtained. Then the distilled water was added to make up the 100 ml [30]. The prepared formulations have been autoclaved at 121°C for 20 min. The composition of niosomal in-situ Gel is listed in Table 2.

Table 2: Composition of niosomal in-situ gel.		
Sr. No.	**Ingredients**	**Quantity**
1	Niosomal dispersion	0.35%
2	HPMC	0.1g
3	HPMC K15M	0.3g
4	Carbopol 940	0.4g
5	Benzalkonium chloride	0.02g
6	Citric acid	0.407g
7	Disodium hydrogen phosphate	1.125g
8	Purified water	100 ml

2.10. Evaluation of niosomal in-situ Gel

2.10.1. Physico-chemical evaluation

The herbal niosomal in-situ gel was prepared and evaluated for color, odor, clarity, and appearance by visual observation [31].

2.10.2. Homogeneity

The developed niosomal *in-situ* gel was placed in a container and tested for homogeneousness by visual inspection. In addition, the appearance and presence of aggregate material were also checked [32].

2.10.3. pH

The accurately weighed amount of in-situ gel (0.3g) was dissolved in 100 ml of distilled water. The pH was determined using a digital pH meter at room temperature [33].

2.10.4. Viscosity

The viscosity of the in-situ gel was determined using a Brookfield viscometer (Model-RVT) with spindle number 3 and angular velocity run from 10-100 rpm/min [34].

2.10.5. Gelling capacity

The *visual method determined the in-vitro* gelling capacity of the in-situ gel. It was determined by placing 1ml of in-situ gel formulation in a glass tube containing freshly prepared simulated tear fluid (7.4) and equilibrated at 37°C [34]. The composition to prepare artificial tear fluid is listed in Table 3. The gel formation was visually inspected [34]. The time needed for gelation and the time needed to dissolve the gel was also noted.

Table 3: Composition of artificial tear fluid.		
Sr.no.	**Ingredients**	**Quantity**
1	Sodium bicarbonate	0.20 g
2	Sodium chloride	0.67 g
3	Calcium chloride dehydrate	0.08 g
4	Deionized water	100 ml
Physiological pH (7.4 ± 0.2) adjusted by adding the required amount of 0.1 N HCL		

2.10.6. Gel strength

The measurement of gel strength was performed in a 50ml graduated cylinder. 25ml of the in-situ gel formulation was put in a graduated cylinder, and on the surface of the in-situ Gel, a weight of 14.3g was placed. The time in the second required to penetrate the weight 5cm into the in-situ gel was noted [35]. The measurements were carried out in triplicate (n=3).

2.10.7. Isotonicity evaluation

The *In-situ* formulations were mixed with a few drops of diluted blood on a slide. The grower's solution was used to make diluted blood, and the slide was observed under a microscope at 45x magnification. Then it was compared with standard marketed ophthalmic formulation [35].

2.10.8. Gel Erosion Time

Briefly, niosomal gel solution (5 mL) was gently transferred into 200 mL of phosphate buffer solution (pH 6.5) at 37 °C in a temperature-controlled water bath without shaking. After that, the time needed for the complete dissolution of gel was measured at [36]. The measurements were conducted in triplicate.

2.10.9. Evaluation of Gelation Temperature

The gelation temperature of the prepared gels was evaluated by the test tube inversion method, where 2 mL of tested formulations were placed in a vial (5 mL), then immersed in a thermostatically controlled water bath. The temperature was gradually increased by 0.5 °C/min, starting from 20 to 40 °C, and at each set point, the samples were tempered for one minute, and then the test tube was inverted at 90°C [37]. The temperature at which no flow upon inversion was seen was set as a gelation temperature.

2.11. Stability study

The stability study was performed as per ICH guidelines. The formulated niosomal in-situ gel was placed in ambient colored vials and sealed with aluminum foil at different temperatures and humidity conditions like room temperature (25±2°C) and refrigerator temperature (5±3°C) for 3 months[38]. The samples were evaluated every month for appearance, clarity, and pH.

2.12. Statistical Methods

The experimental data were processed using the Graph Pad Prism® software program.

3. RESULTS AND DISCUSSION

3.1. Plant collection and authentication

The plant *Coscinium fenestrate*d (Gaertn.) was collected from Kannur district, Kerala (India) and was authenticated by Dr. Ratheesh Narayanan MK, Assistant Professor, Payyanur College, Payyanur, Kerala [Voucher number-PNRCLG/DOB/2020-21/035-011].

3.2. Physico-chemical parameters

After collecting plant materials, they were shade dried and powdered coarsely in an electronic blender and stored in air-tight containers until further use. Physico-chemical parameters were analyzed per WHO guidelines, and results were tabulated in Table 4.

Table 4: Physico-chemical parameters of *Coscinium fenestratum*.

Sr.no.	Test	*Coscinium fenestratum*	Limits
1	Total ash (% w/w)	2.60 ± 0.03	NMT 3%
2	Acid insoluble ash (% w/w)	0.60 ± 0.01	NMT 2%
3	Alcohol soluble ash (% w/w)	1.96 ± 0.03	NMT 9%
4	Water-soluble extractive value (% w/w)	12.86 ± 0.02	NLT 8%
5	Alcohol soluble extractive value (% w/w)	6.84 ± 0.01	NLT 3%
6	Moisture content (% w/w)	4.95 ± 0.02	NMT 6%
7	pH - 1% solution	6.51 ± 0.01	NA
8	pH – 10% solution	6.71 ± 0.01	NA

Values are expressed in mean ± SD (n=3)
NMT - Not more than; NLT – Not less than; NA – Not available

As per the above table, satisfactory results were obtained concerning ash values, extractive values, and moisture content. The total ash value of *Coscinium fenestratum* was 2.60 ± 0.03. The acid insoluble (0.60 ± 0.01) and alcohol soluble ash values (1.96 ± 0.03) were determined and within an acceptable range. The moisture content by loss on the drying method was 4.95 ± 0.02. The 1% and 10% solution pH was 6.51 ± 0.01 and 6.71 ± 0.01, respectively.

3.3. Preliminary phytochemical screening

The phytochemical test on ethanol, chloroform, petroleum ether, and aqueous extracts of *Coscinium fenestratum* stem powder revealed the presence of various phytoconstituents such as alkaloids, flavonoids, saponins, cardiac glycosides, steroids, terpenoids, and phenols as given in Table 5.

Table 5: Phytochemical test on various extracts of *Coscinium fenestratum* powder.

Compounds	Ethanol	Aqueous	Chloroform	Petroleum ether
Alkaloids	+++	++	+	-
Tannins	-	-	-	-
Saponins	++	+	-	-
Flavonoids	++	+++	-	+
Steroids	++	-	-	+
Terpenoids	++	-	+	-
Phenolics	++	+	-	++
Cardiac glycosides	++	+++	+	-
Carbohydrates	-	-	-	+

(+++) Abundant, (++) Moderate, (+) Present, (-) Absent
Since most of the constituents were seen in ethanolic extract, ethanol was used for further study.

3.4. Soxhlet extraction of plant material

The extraction of dried stems of the plant *Coscinium fenestratum* was carried out by the Soxhlet extraction process using ethanol as solvent. The extract obtained from the plant was then collected and concentrated. It was then weighed and kept in a desiccator filled with fused calcium chloride until it was used to prepare a niosomal in-situ Gel. A total of 9.1 g of the extract was obtained from 50 g of crude drug powder. The percentage yield of *Coscinium fenestratum* in the ethanolic extract was 18.20 %.

3.5. Preformulation study

3.5.1. Drug-excipient compatibility studies by FTIR

The drug-polymer interaction studies were carried out to ascertain any chemical interaction of the drug with excipients used to prepare in-situ gel formulation. The FT-IR spectra were obtained using JASCO FT-IR 4700 L spectrophotometer for samples A (*Coscinium fenestratum*) and B to E (*Coscinium fenestratum*+ excipients). The FT-IR results obtained are shown below in Figures 1 to 5.

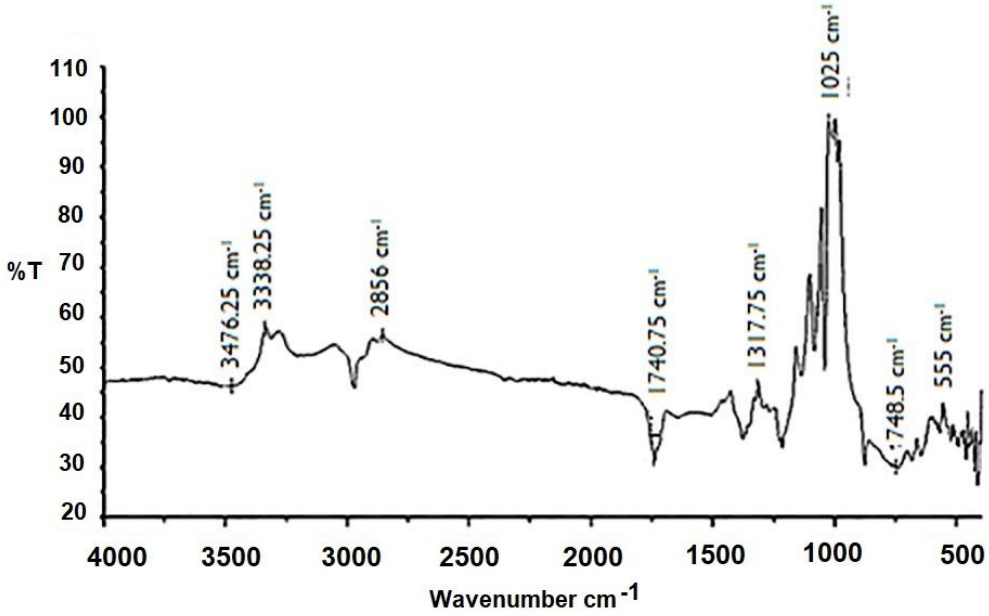

Fig 1. FT-IR spectrum of *Coscinium fenestratum* (Sample A).

Sample A: 3476.25 cm^{-1}, 3338.25 cm^{-1} (N-H stretching), 2856 cm^{-1}(C-H stretching), 1740.75 cm^{-1} (C=O stretching), 1317.75 cm^{-1} (CH3 bending), 1025 cm^{-1} (C-O stretching).

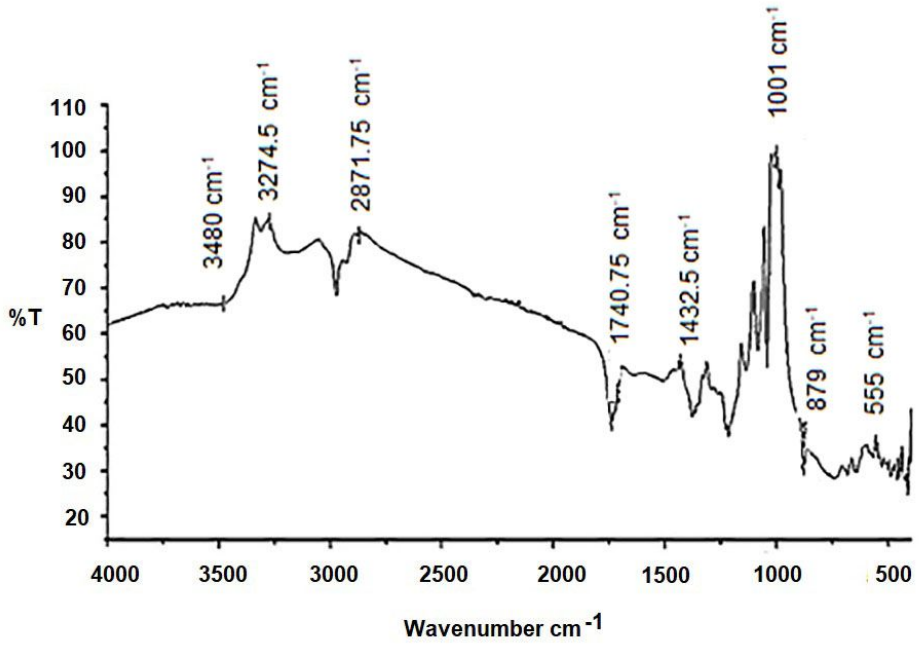

Fig 2. FT-IR spectrum of *Coscinium fenestratum* + Span 20 + Carbopol 940 + HPMC K15M (Sample B).

Sample B: 3480 cm^{-1}, 3274.5cm^{-1} (N-H stretching), 2871.75 cm^{-1}(C-H stretching), 1740.75 cm^{-1} (C=O stretching), 1432.5 cm^{-1} (CH3 bending), 1001 cm^{-1}(C-O stretching).

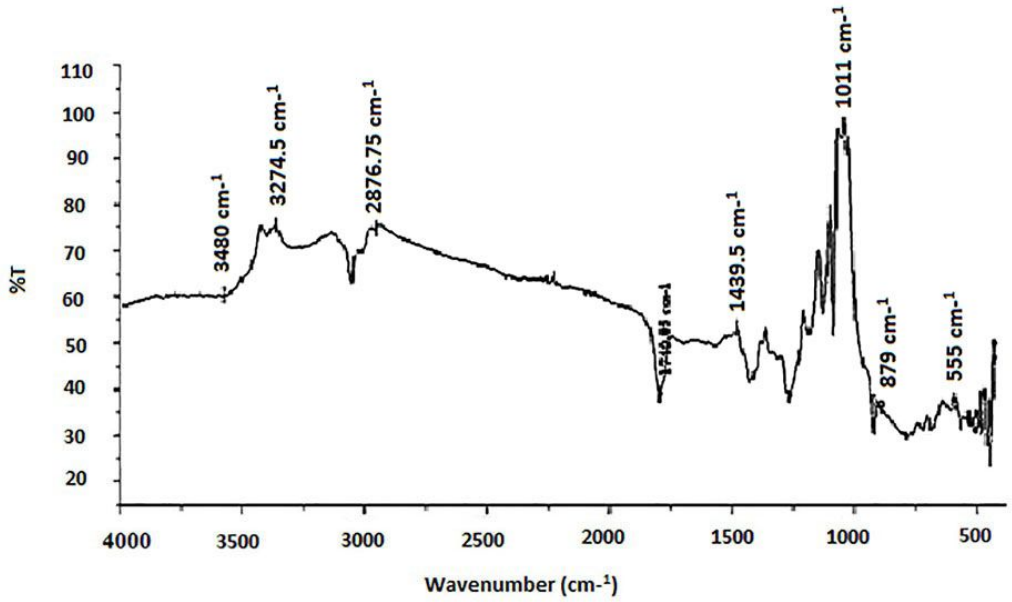

Fig 3. FT-IR spectrum of *Coscinium fenestratum* + Span 60 + Carbopol 940 + HPMC K15M (Sample C).

Sample C: 3274.5cm^{-1} (N-H stretching), 2876.75 cm^{-1}(C-H stretching), 1740.85 cm^{-1} (C=O stretching), 1439.5 cm^{-1} (CH3 bending), 1011 cm^{-1} (C-O stretching).

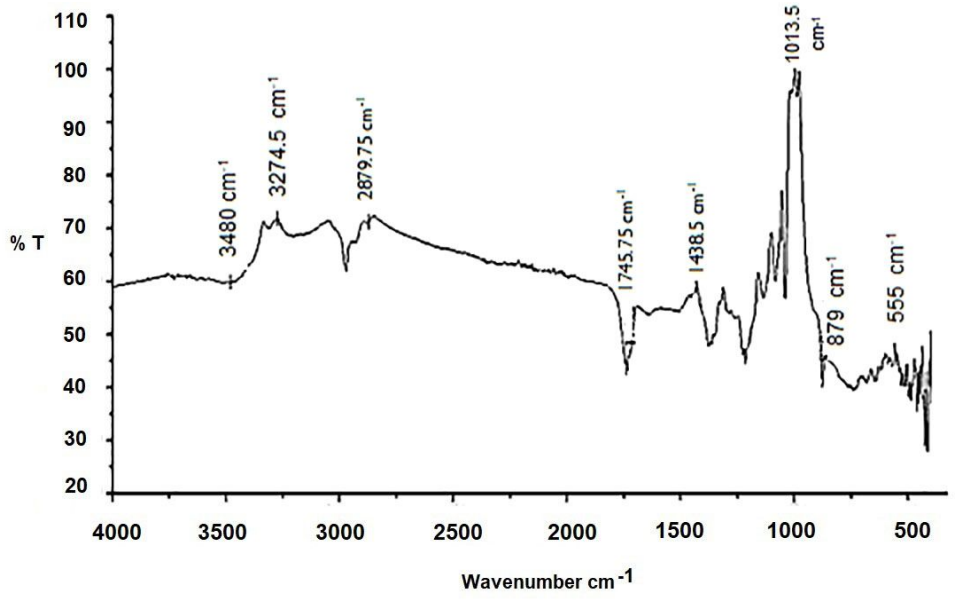

Fig 4. FT-IR spectrum of *Coscinium fenestratum* + Tween 20 + Carbopol 940 + HPMC K15M (Sample D).

Sample D: 3480 cm^{-1} (N-H stretching), 2879.75 cm^{-1}(C-H stretching), 1745.75 cm^{-1} (C=O stretching), 1438.5 cm^{-1} (CH3 bending), 1013.5 cm^{-1} (C-O stretching).

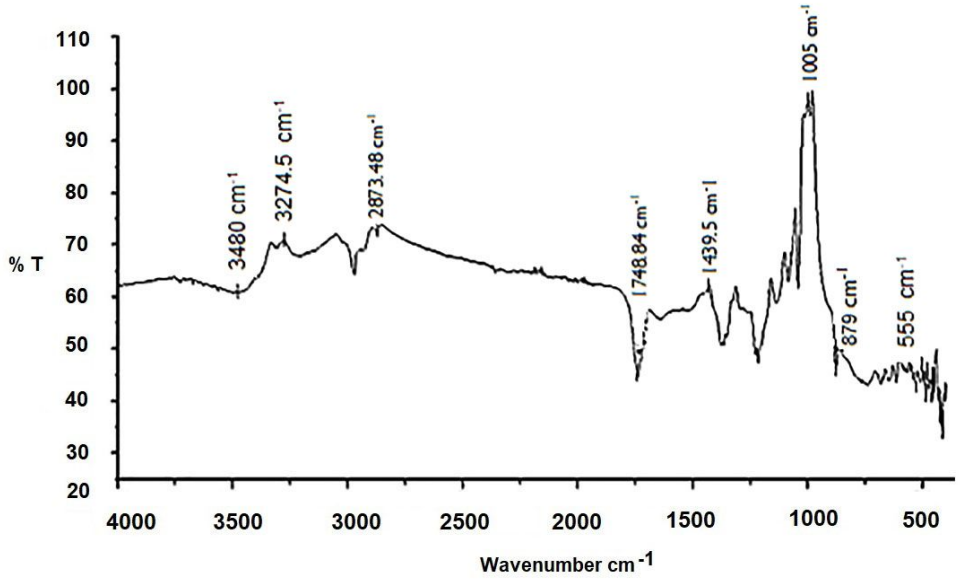

Fig 5. FT-IR spectrum of *Coscinium fenestratum* + Tween 80 + Carbopol 940 + HPMC K15M + Cholesterol (Sample E).

Sample E: 3274.5cm^{-1} (N-H stretching), 2873.48 cm^{-1}(C-H stretching), 1748.84 cm^{-1} (C=O stretching), 1439.5 cm^{-1} (CH3 bending), 1005 cm^{-1} (C-O stretching).

3.6. Formulation of drug-loaded niosome

Eight formulations named F1-F8 were prepared by different kinds of spans and tweens with varying ratios. The niosomal dispersion formed a pale brown color. Formulated niosomal dispersion and optical microscopic image are shown in Figure 6 and Figure 7, respectively.

Fig 6. Formulated niosomal dispersion of drug-loaded niosome.

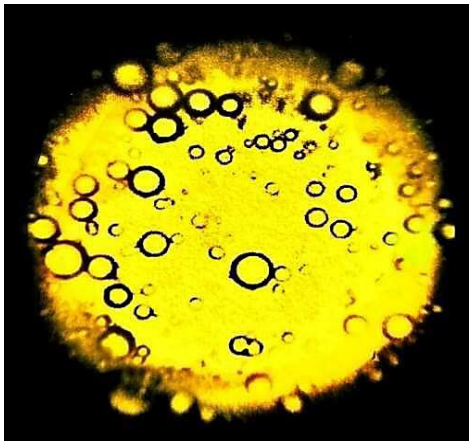

Fig 7. Optical microscopic image.

3.7. Evaluation of niosome

3.7.1. Vesicle size of niosome

The vesicle size analysis of niosomes was carried out and listed in Table 6. The F3 showed the least vesicle size of 1.45 µm compared to other formulations.

Table 6: Vesicle size of niosome.

S. No.	Formulation code	Mean particle size ± SD (µm)
1	F1	2.23 ± 0.01
2	F2	3.20 ± 0.03
3	F3	1.45 ± 0.04
4	F4	2.21 ± 0.08
5	F5	4.05 ± 0.06
6	F6	4.45 ± 0.15
7	F7	5.24 ± 0.01
8	F8	6.25 ± 0.02

Values are expressed in mean ± SD (n=3)

3.7.2. Vesicle shape of niosome

The shape and surface characteristics of formulated niosome with the least vesicle size (F3) were determined by scanning electron microscopy. SEM photographs are shown in Figure 8.

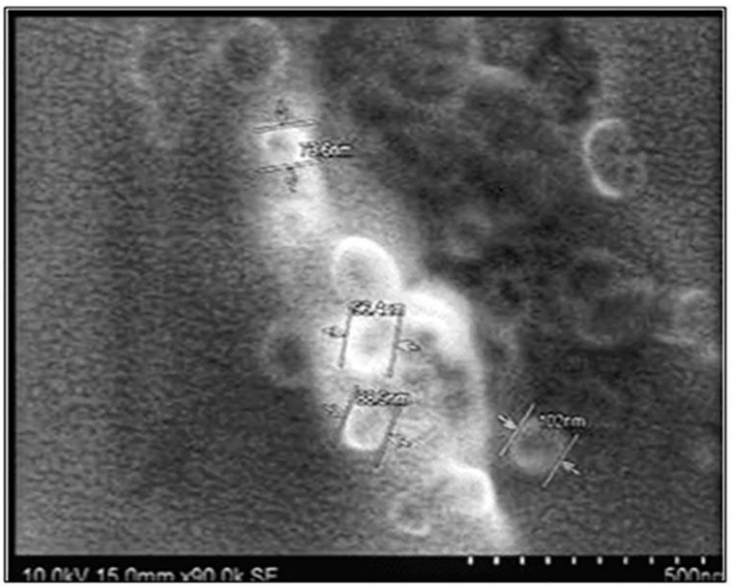

Fig 8. SEM photograph of formulated niosomes (F3).

3.7.3. Zeta potential of niosome

The zeta potential value of optimized niosomal formulation (F3) was calculated by Malvern zeta sizer nano essential, and the zeta potential was found to be -39.9. It indicates that it possesses good stability. Higher zeta potential is indicative of a stable colloidal system [53]. Zeta potential photograph is shown in Figure 9.

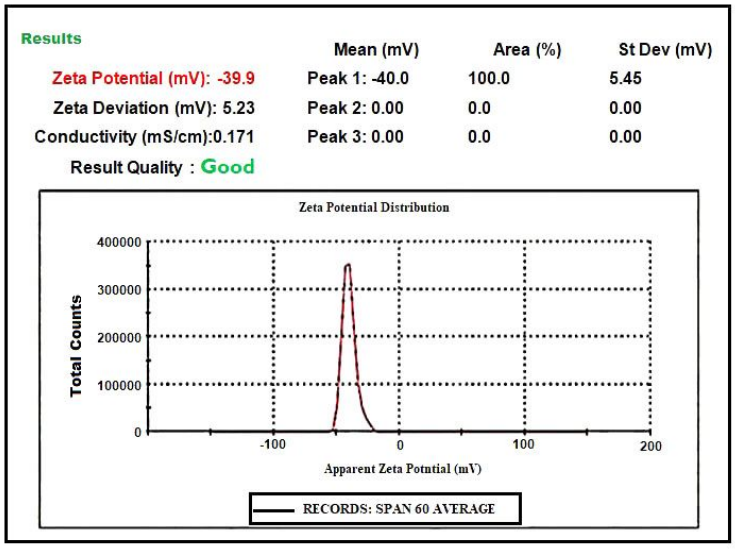

Fig 9. Zeta potential of niosome (F3).

3.8. Formulation of niosomal in-situ Gel

Based on the above-analyzed parameter F3 was considered the optimized formulation and was selected for further studies. The optimized niosomal dispersion F3 was used for the preparation of in-situ Gel. The niosomal *in-situ* Gel was prepared, which is shown in Figure 10.

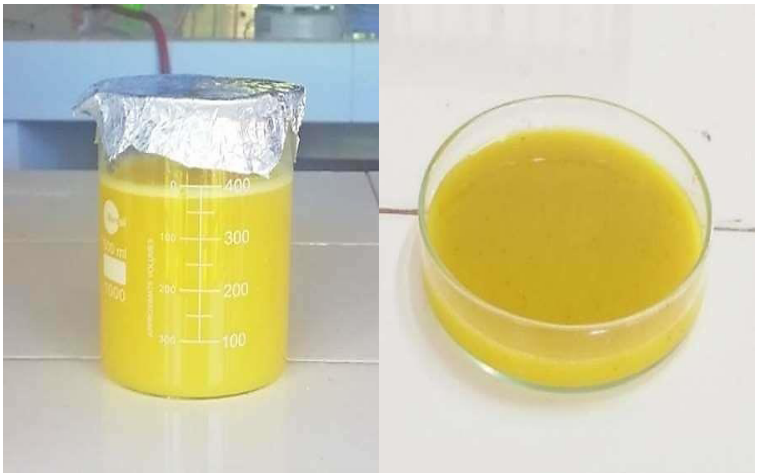

Fig 10. Formulated niosomal *in-situ* Gel.

3.9. Evaluation of niosomal in-situ Gel

3.9.1. Physicochemical evaluation

The physical parameters such as color, odor, clarity, and appearance were checked, and the results are given in Table 8, which were found to be acceptable.

Table 8: Physicochemical evaluation of in-situ Gel.

Parameters	Observation
Color	Yellow
Odor	Characteristic
Clarity	Clear
Appearance	Transparent

3.9.2. Homogeneity

The optimized niosomal in-situ (F3) was tested for homogeneity by visual inspection and checked for any aggregates. Results indicated that in-situ gel possesses uniform distribution.

3.9.3. pH

The niosomal in-situ gel formulation prepared using F3 showed favorable pH for successful ocular drug delivery. The average pH was found to be 6.62 ± 0.009.

3.9.4. Viscosity

The viscosity of in-situ gel formulation is an important factor in determining the residence time of the drug in the eye. Viscosity was determined by Brookfield viscometer in triplicate and was found to be 982.66 ± 1.52

3.9.5. Gelling capacity

Gelling capacity is important to determine whether the formulation is suitable for use in in-situ gelling systems. The gelation time and the time taken for it to dissolve were noted. Gelling capacity was determined by visual method, and it was observed that the gel was formed immediately and remained for an extended period. The F3 batch was observed and found to be 1.10 ± 0.01 min and required to form a gel.

3.9.6. Gel strength

The factors that affect gel strength are the concentration of the gelling agent, bio-adhesive polymers, and pH. Optimal niosomal in-situ gel must have suitable gel strength to be administered easily and can be retained in the ocular region without leakage after administration. The gel strength time was measured in triplicate. The gel strength of the formulation was found to be 1.30 ± 0.006 sec.

3.9.7. Isotonicity evaluation

Isotonicity testing indicates that formulation F3 exhibited no change in the shape of blood cells. However, the size of blood cells was found in the 6-7µm range, which reveals the isotonic nature of the formulation compared to standard ophthalmic marketed preparation. In addition, it indicates the maintenance of tonicity in prepared formulations.

3.9.8. Gel erosion time

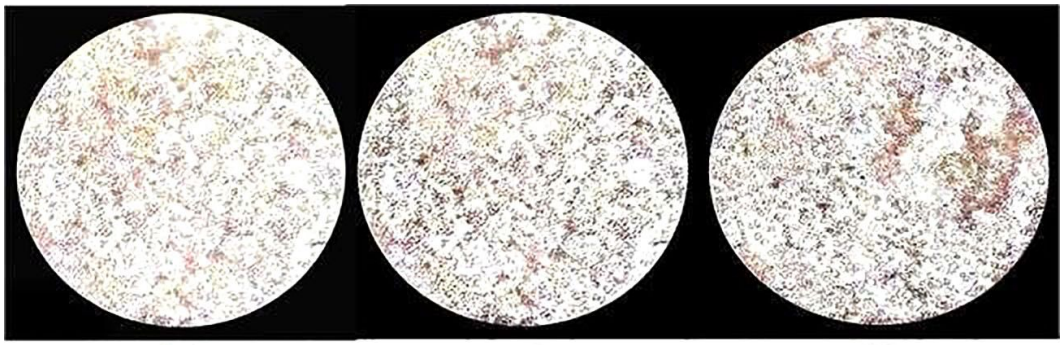

Fig 11. the shape of blood cells, (a) Blood cells, (b) Blood cells with Marketed formulation, (c) Blood cells with *Coscinium fenestratum*.

The F3 formulated in situ gel was characterized by approximately a good gel erosion time—of 4 hours which was within acceptable limits.

3.9.9. Gelation temperature

The formulated in situ gel of *Coscinium fenestratum* showed a gelation temperature of 34^0 C which was within acceptable limits.

3.9.10. Stability studies of optimized formulation

The stability studies for niosomal *in-situ* gel (F3) were performed at controlled room temperature (25 ± 2°C) and refrigerator temperature (5 ± 3 ° C) for three months. The result obtained is given in Table 11 and Table 12, respectively.

Table 11: Stability studies of niosomal in-situ gel at 25±2°C.

Sr. No.	Evaluation parameter	After one-month observation	After two-month observation
1	Color	Yellow	Yellow
2	Appearance	Clear and transparent	Clear and transparent
3	Homogeneity	Homogeneous	Homogeneous
4	pH	6.57 ± 0.03	6.55 ± 0.02
5	Viscosity	986.55 ± 0.02	987.23 ± 0.03
6	Gelling capacity	+++	+++
7	Gel strength	1.4 ± 0.06	1.3 ± 0.01

Table 12. Stability studies of niosomal in-situ gel at 5±3°C.

Sr. No.	Evaluation parameter	After one-month observation	After two-month observation
1	Color	Yellow	Yellow
2	Appearance	Clear and transparent	Clear and transparent
3	Homogeneity	Homogeneous	Homogeneous

4	pH	6.59 ± 0.001	6.62 ± 0.009
5	Viscosity	982.33 ± 0.01	985.45 ± 0.04
6	Gelling capacity	+++	+++
7	Gel strength	1.5 ± 0.03	1.6 ± 0.02

4. DISCUSSION

In this work, an attempt was made to formulate a niosomal gel of *Coscinium fenestratum*. Herbal drugs are used globally to cure different diseases due to their higher safety margins and cost-effectiveness[1]. A cataract is an opacity within the lens that reduces the incoming light. It causes a decrease in vision or the loss of lens transparency due to the opacification of the lens[2]. The lens's opacification occurs through three biochemical changes: hydration, lens protein denaturation, and slow sclerosis. When the light enters through the clouded lens, it becomes scattered, and blurry images are formed. Novel drug delivery systems are developed to defeat the drawbacks of conventional drug delivery [3]. Novel drug carriers can easily target the drug into the affected area inside the patient's body and can give a better cure. They can be used to deliver the herbal drug at a predetermined rate. Physicochemical parameters such as total ash, insoluble acid ash, alcohol soluble ash, moisture content, water, and alcohol soluble extractive values were determined and were within an acceptable range. The total ash value indicates the number of minerals and earthy materials attached to the plant [39]. In the normal, acceptable range, no inorganic impurity is present in the drugs. The water-soluble extractive value indicates the presence of sugar, acids, and inorganic compounds. The alcohol-soluble extractive value indicates the presence of polar constituents like steroids, phenols, glycosides, flavonoids, etc. The higher alcohol (ethanol) soluble extractive value recommended that ethanolic extract can be used for further study [40]. The low moisture content value could prevent the growth of microorganisms like bacteria, fungi, and yeast. The collected stems of *Coscinium fenestratum* were washed and dried. Then the extract of *Coscinium fenestratum* was made by maceration process and was subjected to preliminary phytochemical screening. The phytochemical test on ethanol, petroleum ether, chloroform, and aqueous extracts of *Coscinium fenestratum* stem powder revealed alkaloids and flavonoids, saponins, cardiac glycosides, steroids, terpenoids, and phenols [41]. Among them, the ethanolic extract contains the major constituents believed to be responsible for the anti-cataract effect of flavonoids, alkaloids (berberine), steroids, and saponins [42, 43]. Due to this fact, ethanol was selected for the Soxhlet extraction process. The drug-excipient compatibility studies by FT-IR were performed to determine whether there is any chemical interaction between the drug and excipients in the niosomal *in-situ* gel formulation. They did not show the presence of any additional peaks, and the main peaks remained unchanged in the mixtures [44,45]. The drug-loaded niosomes are prepared by varying concentrations of surfactants, while the drug and cholesterol concentration remains unchanged. The cholesterol concentration was kept unchanged because above a certain amount will disrupt the bilayer formation and may increase the vesicle size [46]. Eight different niosomal formulations were prepared by the lipid-film hydration method [47]. The niosomal dispersions were evaluated for vesicle size, shape, zeta potential, and *in-vitro* antioxidant activity. The vesicle size was determined for 50 niosomes in each formulation, where niosomes prepared by span 60 showed the lowest Size. The particle size distribution was found to be in the order tween 20 > tween 80 > span 20 > span 60 [48] Niosomal dispersion was also evaluated for its shape and surface morphology using scanning electron microscopy. The niosomes were found to be spherical and formed as good surfactant bi-layered spheres. Surface morphology confirmed the formation of niosomes [49]. The zeta potential value of optimized niosomal formulation was determined using Malvern zeta sizer nano essential. The zeta potential was found to be -39.9, indicating good stability [50]. Higher zeta potential is indicative of a stable colloidal system. The vesicle size and shape were taken as a criterion to optimize the formulation. Among the eight niosomal dispersions, the optimized formula (F3) showed a comparatively smaller particle size (1.45 ± 0.04). For plausible intravesical application, an optimal in situ Gel should be characterized with a gelling temperature range of 30-35 °C [51,52] and prolonged gel erosion time, which was 4 hours and 340C for the *in situ* gel containing niosomes of *Coscinium fenestratum*. The physical examination found that *the in-situ* Gel was free from air entrapment and foreign matters. The color of the *in-situ* Gel was found to be yellow. The formulation was clear and transparent. The pH value of the *in-situ* Gel was 6.62 ± 0.009, which lay in the normal pH of the eye and showed that the prepared *in-situ* Gel was compatible with the pH of an eye. The Brookfield viscometer determined the viscosity, which is an important factor in determining the residence time of the drug in the eye. It possesses good viscosity of about 982.66 ± 1.52 cps [52]. From a gelling capacity study conducted, it was found that the niosomal *in-situ* Gel possesses immediate gelation and remains for an extended period. The gel strength of niosomal *in-situ* Gel was 1.30 ± 0.006 sec. The prepared *in-situ* Gel was subjected to isotonicity testing, and the Size of blood cells was found in the 6-7µm range [53]. Stability studies were conducted at two different temperatures where no color changes occurred, and there was not much difference in parameters like pH, viscosity, gelling capacity, and gel strength.

5. CONCLUSION

To conclude, the findings of the present investigation showed that the herbal niosomal *in-situ* Gel was an excellent method to improve drug delivery in the case of cataracts. The drug-loaded niosomes were stable in HPMC, HPMC K15M, and Carbopol 940 ocular *in-situ* Gel, and the optimum HLB value of the surfactant span 60 made this formulation more stable. Only a few investigations were done on the herbal treatment of cataracts. So that combining a novel drug delivery system with herbal drugs provides an excellent and efficient method to treat cataract disease. The formulation provided good permeation through the cornea, while the adhesion property of *in-situ* Gel improved the contact time within the eye. Furthermore, the sustained release characteristics of this formulation were favorable to reducing the frequency of administration. Therefore, the herbal drug-loaded niosomal *in-situ* Gel can be introduced for better therapeutic ailments. Finally, it can be concluded that the niosomal *in-situ* Gel of *Coscinium fenestratum* can be an alternative method to reduce the drawbacks of conventional ophthalmic dosage forms.

6. ACKNOWLEDGMENTS

The author sincerely thanks the Management and Principal of

Sanjivani College of Pharmaceutical Education and Research, Kopargaon, Maharashtra, for encouraging and supporting this work.

7. AUTHORS CONTRIBUTION STATEMENT

Sachin. C.P. and Gowtham Menon designed and performed the experiments, derived the models, and analyzed the data. Shendge R S and Girish Kashid helped to supervise the project.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

9. ABBREVIATIONS

IV	Intravenous
NDDS	Novel Drug Delivery System
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
GSH	Glutathione
AGEs	Advanced Glycation End products
AR	Aldose Reductase
SDH	Sorbitol Dehydrogenase
HLB	Hydrophilic Lipophilic Balance
PBS	Phosphate Buffered Saline
HPMC	Hydroxy Propyl Methyl Cellulose
FTIR	Fourier-Transform Infrared Spectroscopy
HET-CAM test	Hen's Egg Test on the Chorioallantoic membrane
H_2O_2	Hydrogen peroxide
SEM	Scanning Electron Microscopy
BKC	Benzalkonium Chloride
IAEC	Institutional Animal Ethics Committee
STZ	Streptozotocin
BSL	Blood Sugar level
ANOVA	Analysis of variance
CFE	Ethanolic extract of *Coscinium fenestratum* stems
CFIG	*Coscinium fenestratum* based niosomal in-situ gel
API	Active Pharmaceutical Ingredient
ICH	*International Council for Harmonisation*

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