

Transfersosomal Gel of Chloramphenicol for Ocular Delivery

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Abstract—Ocular bacterial infections are common eye disorders that may cause serious complications when not properly treated. Conjunctivitis is one of the most prevalent ocular infections and is frequently associated with bacterial pathogens. Conventional ophthalmic dosage forms such as eye drops and ointments have limitations including rapid tear turnover, precorneal drug elimination, poor corneal permeability, and short ocular residence time. Chloramphenicol is a broad-spectrum antibacterial agent used in the treatment of bacterial ocular infections. However, conventional chloramphenicol preparations may show poor ocular bioavailability and require frequent administration. Therefore, the present study was undertaken to develop a chloramphenicol-loaded transfersomal hydrogel for ocular drug delivery. Transfersomes were selected because of their deformable vesicular characteristics and potential to improve drug delivery across biological barriers. Chloramphenicol-loaded transfersomes were prepared by the thin-film hydration method using soya lecithin and Tween 80 as formulation variables. A Central Composite Design was employed to study the effect of these variables on particle size and entrapment efficiency. Nine formulations were prepared and evaluated for particle size, entrapment efficiency, zeta potential, and vesicular morphology. The prepared transfersomes showed particle sizes ranging from 188 to 700 nm and entrapment efficiencies ranging from 73.91% to 89.87%. The optimized formulation showed a particle size of 188 nm, entrapment efficiency of 89.87%, and zeta potential of -24.28 mV. Microscopic examination revealed spherical to nearly spherical vesicular structures with relatively uniform distribution. The optimized transfersomal suspension was

incorporated into Carbopol 934 and HPC LF hydrogel. The optimized hydrogel exhibited a pH of 7.4, viscosity of 6412 cP, and spreadability of 14.84 ± 0.0213 mm². In-vitro drug release was performed using a dialysis membrane diffusion method. The transfersome, gel containing drug, optimized transfersomal gel, and marketed product showed maximum drug release of 71.41%, 75.79%, 90.63%, and 69.97%, respectively. The optimized transfersomal gel exhibited the highest drug release of 90.63% at 12 h, indicating prolonged and sustained drug-release behaviour. Overall, the developed chloramphenicol-loaded transfersomal hydrogel demonstrated satisfactory formulation characteristics and prolonged drug release, indicating its potential as an improved ocular drug delivery system.

Index Terms—Chloramphenicol; Transfersomes; Soya lecithin; Tween 80; topical gel; central composite design; Ex vivo; controlled release

I. INTRODUCTION

In Between 50% and 70% of cases of infectious conjunctivitis are caused by bacteria. If not properly treated, ocular infections can lead to permanent structural damage to the eye, potentially resulting in visual impairment or even blindness. Globally, bacterial pathogens account for 22% to 86% of all ocular infections.

Bacteria play a leading role in causing ocular infections across the globe. Contact lens use, eye traumas, surgical operations, aging, dry eyes, persistent nasolacrimal duct obstructions, and prior

infections are risk factors for these infections, which can be caused by a single organism or include numerous bacteria. Bacterial involvement is common in various ocular conditions such as conjunctivitis, keratitis, endophthalmitis, blepharitis, orbital cellulitis, and dacryocystitis.

Conjunctivitis, an inflammation of the conjunctival mucosa, is the most prevalent eye infection and poses significant healthcare and societal impacts. In chronic cases, it may extend beyond the conjunctiva, affecting nearby areas such as the eyelids, and rising the risk of both intraocular and extraocular complications. This form of conjunctivitis is frequently observed in children and older adults but may also occur in newborns and people of all ages.¹

Gram-positive bacteria are the predominant culprits, with *Staphylococcus aureus*, coagulase-negative staphylococci (CoNS), *Streptococcus pneumoniae*, and *Streptococcus pyogenes* being the most commonly identified species. On the other hand, frequent Gram-negative isolates include *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Haemophilus influenzae*. Among these, *Streptococcus pneumoniae* is recognized as one of the most virulent and fastidious organisms, frequently found in conjunctivitis and dacryocystitis cases.

Topical antibiotics help manage ocular infections by speeding up recovery, reducing discomfort, preventing transmission, and lowering reinfection risk. The main treatment for infection is eye drops or ointments using antibiotics like fluoroquinolones, aminoglycosides, and chloramphenicol. Available dosage forms include eye drops, ointments, ophthalmic solutions, microemulsions, in situ gels, contact lenses, ocular inserts, soluble ophthalmic drug inserts (SODIs), and minidiscs, offering targeted, sustained drug delivery and improved therapeutic outcomes.²

The challenge is achieving drug concentrations at the active site at the right time. The solution for ophthalmic infection with drug delivery system is necessary. The corneas anatomy, physiology, and barrier function work together to prevent rapid drug absorption. The drug is maintained at therapeutic levels in the tear film or at the site of action for eye instillation. The spread and uptake of medications applied to the surface in the precorneal and corneal areas. Nevertheless, this may lead to adverse effects and harm to the eye's surface cells. Traditional ophthalmic dosage forms exhibit low ocular

bioavailability. These dosage forms result from precorneal limitations. The main challenges include solution drainage, tear production, tear behavior, dilution, turnover rate, conjunctival uptake, poor absorption, short residence time, and the relative impermeability of the corneal epithelial membrane. The medication can solely be taken in minimal quantities through the eyes because of these anatomical and physiological limitations. For a topical formulation to be clinically effective, a balance between lipophilicity and hydrophilicity is required along with extended contact durations. The cornea and conjunctiva both absorb the active ingredient. It enters the lacrimal sac via the upper and lower canaliculus.³ It is a fundamental principle that any ophthalmic dosage form should ensure adequate biopharmaceutical properties alongside suitable ocular tolerability, especially when used for treating intraocular conditions. Nonetheless, the topical use of ophthalmic solutions (almost 90% as eye drops) does not satisfy these standards. Indeed, a remarkable tightness of the corneal epithelium regulates the entry of drug molecules into the eye's anterior segment, and the absorbed molecules are largely removed by the capillary network of the iris/ciliary body and/or tear production. Consequently, a minimal amount of the topically applied drug molecules can access the posterior segment of the eye. To address these limitations, a systemic approach for administering ophthalmic medications like anti-glaucoma drugs, corticosteroids, and some antibiotics is necessary; however, utilizing the systemic route encounters the restrictive barrier functions of the BRB and BAB. We need to develop intravitreal drug delivery systems or devices that can effectively deliver the intended doses of specific medications into the eye's posterior segment and sustain the therapeutic concentration over an extended duration.

Hydrogels, made of water-absorbing polymer networks, are perfect for delivering medications because of their swelling capacity, biocompatibility, and mechanical strength. They allow controlled release, mimic tissue, and adapt to various drugs. Their bioadhesive nature, responsiveness to stimuli, and design flexibility suit targeted delivery. Despite benefits like low toxicity and easy shaping, limitations include low strength, poor hydrophobic drug loading, and fast release, which must be addressed for broader

applications. Transfersomes on the other hand, are elastic lipid vesicles developed primarily for transdermal and topical delivery, although their use in ocular applications is still under investigation. These vesicles, also called “carrying bodies,” are similar to liposomes but include a detergent known as an “edge activator” in their bilayer, which gives them remarkable flexibility. This deformability allows them to squeeze through microscopic pores in the corneum stratum—much smaller than their own size—by adapting their shape. For example, a 500 nm transfersome can pass through a 100 nm skin pore. This enables deeper skin penetration and even potential systemic absorption. Notably, transfersomes carrying suitable levels of edge activators do not damage red blood cells, ensuring their safety. They are intriguing candidates for sophisticated drug delivery systems, including skin-based immunization, due to their excellent efficacy in delivering medications across skin barriers.^{4,5}

Chloramphenicol has been judiciously selected for ocular drug delivery research owing to its favorable pharmacokinetic properties, broad therapeutic relevance, and established safety profile. As a broad-spectrum antibiotic, it is widely employed in treating ocular infections such as conjunctivitis, keratitis, and blepharitis, effectively targeting both Gram-positive and Gram-negative bacteria. Its small molecular size and lipophilicity facilitate excellent corneal and intraocular penetration, making it ideal for topical ophthalmic formulations. Despite its clinical utility, conventional eye drops and ointments of chloramphenicol suffer from poor bioavailability (less than 5%), rapid precorneal elimination, and the need for frequent dosing. These limitations justify the exploration of novel drug delivery systems such as hydrogels, transfersomes, that can prolong drug residence time and enhance ocular absorption. Moreover, chloramphenicol’s physicochemical characteristics—such as moderate molecular weight (323.13 g/mol), good solubility, and stability—render it a suitable candidate for incorporation into advanced nanocarriers. Its favorable safety profile, with low risk of systemic toxicity when used topically, further strengthens its suitability for ophthalmic use. Additionally, its inclusion in the WHO’s List of Essential Medicines and approval by major regulatory authorities highlight its global acceptance. Thus,

chloramphenicol stands as a rational choice for research into improved ocular drug delivery strategies.⁶

In order to improve medication penetration, extend ocular residence time, and increase therapeutic efficiency against bacterial eye infections, the current research project aims to develop a transfersome-loaded hydrogel formulation of chloramphenicol for ocular drug administration. This strategy is suggested to improve patient compliance by lowering the frequency of doses, minimizing systemic adverse effects, and overcoming the drawbacks of traditional oral and ocular administration.⁷

II. MATERIALS AND METHODOLOGY

2.1 Materials

Chloramphenicol was used as the active pharmaceutical ingredient (API) and was obtained from Cipla Ltd., Bangalore. Soya lecithin was used as an unsaturated fatty acid and was procured from Sisco Research Laboratories Pvt. Ltd., Maharashtra. Tween 80 was used as a non-ionic surfactant and was obtained from Ultra-Pure-Lab Chem Industries LLP, India. Chloroform was used as an organic solvent and was procured from Sisco Research Laboratories Pvt. Ltd., Mumbai. Ethanol was used as a cosolvent and was obtained from Nice Chemicals Pvt. Ltd. Carbopol 934 was used as a gelling agent and was procured from Central Drug House (P) Ltd., New Delhi. Glycerine was used as an isotonicity agent and was obtained from Nice Chemicals Pvt. Ltd., Kochi, Kerala. Triethanolamine, used as a neutralizing agent, was obtained from S.D. Fine Chemicals Ltd., Mumbai. All other chemicals and reagents used in the study were of analytical grade.

2.2 Equipment

An electronic weighing balance (Essae-Teraoke Ltd., Chennai) was used for accurate weighing of drugs and excipients. A UV-1800 UV spectrophotometer (Shimadzu, Japan) was used for spectrophotometric analysis, while an FTIR spectrophotometer (1700S) (Shimadzu, Japan) was used for identification and compatibility studies. The melting point apparatus (Setco Ltd., Bangalore) was used for determination of melting point. A magnetic stirrer (Remi Equipment, Mumbai) was used for mixing and preparation of formulations. The viscosity of the prepared gel was

determined using a Brookfield viscometer (Brookfield Engineering Technologies, USA). Particle size and zeta potential measurements were performed using a Nano Particle Analyzer SZ-100 (HORIBA Scientific, China). A digital pH meter (Fisher Scientific, India) was used for determination of formulation pH. A bath sonicator (PCI Analytics, Thane, Maharashtra) was used for sonication. An Eppendorf Centrifuge 5430R (China) was used for centrifugation studies. A rotary vacuum evaporator (Digisun Electronics, Hyderabad) was used for solvent evaporation. Finally, a Franz diffusion cell (Sci.work, Peenya, Bangalore) was used for in-vitro drug diffusion studies.

2.3 Drug Profile:

Chloramphenicol, chemically designated as 2,2-dichloro-N-[(1R,2R)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl] acetamide, has the molecular formula $C_{11}H_{12}Cl_2N_2O_5$ with a molecular weight of 323.13 g/mol. Its chemical structure is presented in Fig. 1. Chloramphenicol is described as a white to greyish-white crystalline powder and is a synthetic, broad-spectrum antibacterial agent belonging to the amphenicol class of antibiotics. It has a reported melting point of approximately 150–153°C. The drug is slightly soluble in water but is soluble in several organic solvents, including ethanol, methanol, and acetone. Chloramphenicol is classified as an antibacterial/antibiotic drug and exhibits activity against a broad range of Gram-positive and Gram-negative microorganisms. The plasma elimination half-life of chloramphenicol is approximately 1.5–4 hours in adults, although it may vary depending on age and physiological conditions. The drug undergoes extensive hepatic metabolism, mainly through glucuronidation, and its metabolites are predominantly eliminated through the urine.⁸

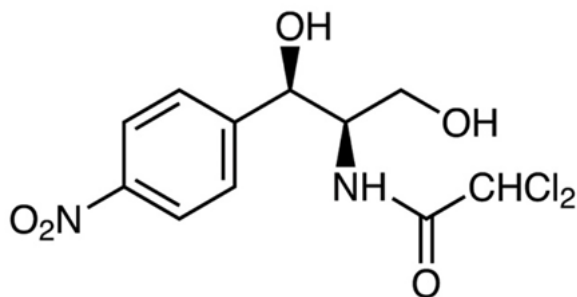


Fig no 1: Chemical structure of CHLORAMPHENICOL

2.4 Preparation of Calibration Curve.

Chloramphenicol (100 µg/mL) primary stock solution was made by precisely weighing 10 mg of the medication, dissolving it in an appropriate solvent, and then increasing the volume to 100 mL. Using a UV-visible spectrophotometer, the maximum absorption wavelength (λ_{max}) of chloramphenicol was found to be 278 nm. Phosphate buffer (pH 7.4) was used to dilute 1 mL of the original stock solution into a 10 mL volumetric flask, resulting in a secondary stock solution with a concentration of 10 µg/mL. To get working concentrations between 5 and 25 µg/mL, further dilutions were made. Each solution's absorbance was measured at 278 nm, and the absorbance was plotted against the relevant chloramphenicol concentration to create a calibration curve.⁹

2.5 Characterization of pure drug

The supplied chloramphenicol sample was characterized by melting point determination, UV-Visible spectroscopy, and FTIR analysis. The outcomes of the experiment were compared with the reported characteristics of chloramphenicol to assess its conformity with the expected properties of the drug.⁸

2.6 Determination of melting point

The melting point was determined by the capillary tube method. Finely powdered chloramphenicol was introduced into a sealed-end glass capillary and placed in the melting point apparatus. The temperature at which complete melting of the sample was observed was recorded.²¹

2.7 Preparation of chloramphenicol loaded transferosomes.

For TFH (thin film hydration), Soya lecithin, Tween 80, were dissolved in chloroform: methanol (1:2; 15ml:30 mL). The organic phase was transferred to a 500 mL round-bottom flask and evaporated under reduced pressure at 60°C until a thin dry film formed. The film was hydrated with 20 mL pH 7.4 phosphate buffer then sonicate the resulting vesicular suspension for 30 min.¹⁰

2.8 Design of experiments and optimization

Design-Expert ®Stat-Ease 360 software was used. A Central Composite Design (CCD) was employed,

which involved two quantitative variables- the concentration of Soya lecithin and tween 80 were selected. In 2 numeric factors mentioned has Soya lecithin maximum (270mg) and minimum (210mg) and Tween 80 maximum (90mg) and minimum (30mg). Particle size was selected as a response to minimize, and entrapment efficiency as a response to maximize.¹¹

Table 1: Factors and levels used in formulation of Transfersomes.

Factors	Name	unit	Low level (-1)	Middle level (0)	High level (+1)
A	Soya lecithin	mg	270	240	210
B	Tween 80	mg	90	60	30

Table 2: Central composite design (3²) of Chloramphenicol loaded Transfersomes using thin film hydration method (FT1- FT9)

Formulation Code	Chloramphenicol (mg)	Soya Lecithin (mg)	Tween 80 (mg)	Chloroform (ml)	Ethanol (ml)
F1	50	270	30	15	35
F2	50	270	60	15	35
F3	50	270	90	15	35
F4	50	240	30	15	35
F5	50	240	60	15	35
F6	50	240	90	15	35
F7	50	210	30	15	35
F8	50	210	60	15	35
F9	50	210	90	15	35

Based on the formulation chart generated by the Design of Experiments (DoE), a total of 9 formulations were prepared by using the thin-film hydration (TFH) method

2.9 Evaluation of Transfersomes

2.9.1 Particle Size and Polydispersity Index (PDI)

Dynamic light scattering (DLS) and Horiba Scientific Nanoparticle SZ-100 were used to measure the particle size and PDI of the generated transfersomal formulations at a fixed angle of 90° and a temperature of 25°C.

The mean vesicle size and size distribution were determined by diluting 0.1 mL of the suspension to 10 mL with buffer.¹²

2.9.2 Zeta Potential

Using a zeta potential analyzer, the prepared transfersomal formulations' zeta potential was ascertained. Before analysis, a 0.1 mL solution was diluted with 10 mL buffer, and the vesicles' surface charge was measured to evaluate their physical stability.¹²

2.9.3 Entrapment Efficiency (by indirect method)

Centrifugation was used to measure the entrapment efficiency of transfersomes loaded with chloramphenicol. To extract the untrapped medication from the vesicles, 2 milliliters of the transfersomal suspension were centrifuged for 30 minutes at a high speed (10,000–15,000 rpm). Spectrophotometry was used to measure the amount of free medication in the supernatant at 278 nm.¹³ The following formula was used to determine the entrapment efficiency:

$$\% \text{Entrapment efficiency} = \frac{\text{Total drug} - \text{unentrapped drug}}{\text{Total drug}} \times 100$$

2.9.4 Microscopic Examination of Optimized Ufasomes.

The optimized transfersomal preparation was examined using a bright-field microscope equipped with an image analyzer to observe the morphology and vesicular characteristics.

A small quantity of the optimized transfersomal dispersion was placed on a clean glass slide and covered with a coverslip. The preparation was examined under 40X magnification, and the vesicular structures were observed and recorded using the image analyzer.¹⁴

2.10 Preparation of transfersomal gel

The optimized transfersomal suspension was incorporated into Carbopol 934 and HPC LF gel. Preliminary trials compared 1.5% and 2% of Carbopol 934 and HPC LF, and 2% was selected in the source study based on the resulting consistency/viscosity. Triethanolamine was added dropwise for pH adjustment.²²

2.11 Evaluation of Transfersomal Gel

2.11.1 Physical Appearance:

The prepared gel was examined for clarity, color, homogeneity and presence of foreign particles.

2.11.2 Measurement of pH:

The pH of gel formulations was determined by digital pH meter.

2.11.3 Rheological Study by Viscosity Measurement:

Viscosity Of the gel was determined by using Brookfield viscometer. Accurately weighed 20gm Chloramphenicol loaded gel was transferred to 50 ml glass beaker. Different Spindle numbers were used to check the viscosity and Spindle no 63 was selected and it is immersed into the gel. The viscometer was operated at 20 rpm until the reading gets stabilized and reading was noted in centipoises.^{5, 24}

2.11.4 Spreadability:

The spreadability of transferosome gel was determined by applying weight over the gel. In brief, one gram of gel was weighed and placed over the transparent Petri plate and the initial area occupied by the gel was marked Over this was placed another weighed petri plate and incremental weights (100g) were added until maximum spreading of gel. The diameter of the formed circle was measured to express the spreadability. In these cases, spreadability is determined by the formula.^{5, 24}

$$A = \pi r^2$$

$$Si = (d^2 \times \pi / 4)$$

Where,

Si- spreading area (mm²) depending on mass,

d- spreading area diameter(mm)

2.11.5 In-vitro drug release

A Franz diffusion cell with a high media dialysis membrane that is partially permeable and has a molecular weight cutoff between 12000 and 14000 Da was used to conduct an in vitro drug diffusion investigation. The 2.4-micron pores are ideal for filtering and diffusion work after being submerged in pH 7.4 phosphate buffer for the whole nite. The membrane is positioned between the donor compartment and the Franz diffusion cell's reservoir compartment, which holds 135 milliliters of pH 7.4 phosphate buffer containing gel drug, transferosomal gel, and marketed product. The membrane is positioned over the dialysis membrane in the donor compartment. The entire assembly was fixed so that the gel-containing cell's donor compartment touched the dialysis membrane and the magnetic stirrer stirred the medium inside. Throughout the investigation, care

should be made to prevent air bubbles from entering behind the dialysis membrane. Periodically, 5 ml aliquots were removed from the receptor compartment and replaced with an equivalent volume of brand-new pH 7.4 buffer. The sample was analysed by using UV-Visible spectrophotometer at 278nm. The tests were carried out in triplicate (n=3).¹⁵

Release data were fitted to zero-order, first-order, Higuchi and Korsmeyer–Peppas models. The release exponent n from the Korsmeyer–Peppas model was used to describe the release mechanism.¹⁶

2.12 Stability Studies

Stability of the Transferosomal Suspension and Chloramphenicol loaded transferosomal gel were carried out by storing at room temperatures for one month. The formulation's alterations were tracked by measuring particle size, zeta potential for suspension, pH, spreadability for gel after 1 day, 5 days and 30 days of storage.²⁶

2.13 Ex-vivo study

2.13.1 Ex-vivo Transcorneal Permeation Study Using Porcine Cornea

Fresh porcine eyes were collected from a suitable source and transported to the laboratory under chilled conditions. The eyes were gently washed with phosphate-buffered saline (PBS, pH 7.4) to remove adhering contaminants. The cornea was carefully excised from the eyeball along with a small surrounding scleral margin and washed with PBS. The isolated cornea was kept hydrated until mounting in the diffusion apparatus. The cornea was mounted between the donor and receptor compartments of a Franz diffusion cell, with the epithelial surface facing the donor compartment. The receptor compartment was filled with PBS pH 7.4, ensuring that no air bubbles were present beneath the cornea. The receptor medium was maintained at $35 \pm 0.5^\circ\text{C}$ with continuous magnetic stirring. An accurately weighed quantity of chloramphenicol-loaded transfersomal hydrogel was applied uniformly onto the epithelial surface of the cornea. Samples were withdrawn from the receptor compartment at predetermined time intervals and immediately replaced with an equal volume of fresh, pre-warmed PBS. The collected samples were suitably diluted and analyzed for chloramphenicol content using the validated analytical method. The cumulative amount of chloramphenicol permeated through the

cornea was calculated after correcting for the withdrawn sample volume. The cumulative drug permeation was expressed per unit surface area of the cornea. The steady-state flux and apparent permeability coefficient were calculated from the permeation data. The cornea was taken out of the diffusion cell at the conclusion of the investigation, carefully cleaned, and processed to ascertain how much chloramphenicol was still present in the corneal tissue.¹⁷

The transcorneal penetration and ocular retention of the optimized transfersomal hydrogel loaded with chloramphenicol were assessed in this ex vivo investigation

III. RESULTS AND DISCUSSION

3.1 Preparation of Standard Curve

The standard curve of chloramphenicol was prepared in phosphate buffer pH 7.4 over the concentration range of 5-25 µg/mL.

The absorbance increased progressively with an increase in drug concentration, indicating good linearity.⁹ The regression equation was $y = 0.0391x$, with an R^2 value of 0.997 (Table 4). The standard curve is shown in Figure 1.

Table 3: Standard curve of Chloramphenicol

SL. No	Concentration (µg/ml)	Absorbance (Mean±SD) (n=3)
1	0	0
2	5	0.278±0.05
3	10	0.370±0.02
4	15	0.562±0.04
5	20	0.789±0.02
6	25	0.987±0.02

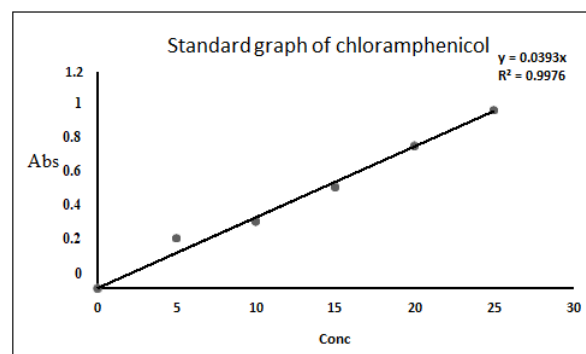


Fig No 2: Standard graph of chloramphenicol

3.2 Characterization of pure drug

The FTIR spectrum of chloramphenicol exhibited characteristic absorption bands corresponding to its principal functional groups (Fig. 5.2). A broad absorption band around 3270 cm^{-1} was observed, which was attributed to O–H and N–H stretching vibrations.

The aromatic C–H stretching vibrations were observed in the region of approximately $3000\text{--}3100\text{ cm}^{-1}$, while the aliphatic C–H stretching vibrations appeared around $2960\text{--}2870\text{ cm}^{-1}$. A prominent absorption band at 1693.56 cm^{-1} corresponded to the amide C=O stretching vibration.

The band observed in the region of $1516\text{--}1602\text{ cm}^{-1}$ was associated with amide II/N–H bending vibrations, while the characteristic NO_2 asymmetric and symmetric stretching vibrations were observed in the region of approximately $1516\text{--}1353\text{ cm}^{-1}$. The absorption band at 1068 cm^{-1} was attributed to C–O/hydroxyl-related vibrations.

In the lower wavenumber region, bands between $656\text{--}676\text{ cm}^{-1}$ were associated with C–Cl-related vibrations.⁸

Table No 4: Characteristic peaks of FT-IR Spectra of Pure drug of Chloramphenicol

Functional groups	Characteristic peaks (cm^{-1})	Peak obtained in pure drug (cm^{-1})
O–H stretching	$\sim 3400\text{--}3380\text{ cm}^{-1}$	3270 cm^{-1}
N–H stretching	$\sim 3300\text{ cm}^{-1}$	3270 cm^{-1}
Aromatic C–H stretching	$\sim 3090\text{--}3020\text{ cm}^{-1}$	$3000\text{--}3100\text{ cm}^{-1}$
Aliphatic C–H stretching	$\sim 2950\text{--}2850\text{ cm}^{-1}$	$2960\text{--}2870\text{ cm}^{-1}$
Amide C=O	1697 cm^{-1}	1693.56 cm^{-1}
Amide II / N–H bending	1568 cm^{-1}	$1516\text{--}1602\text{ cm}^{-1}$
NO_2 asymmetric/symmetric vibrations	1530 and $\sim 1358\text{ cm}^{-1}$	$1516\text{--}1353\text{ cm}^{-1}$
C–O /hydroxyl-related vibration	1068 cm^{-1}	1068 cm^{-1}
C–Cl-related region	$\sim 650\text{--}700\text{ cm}^{-1}$	$656\text{--}676\text{ cm}^{-1}$

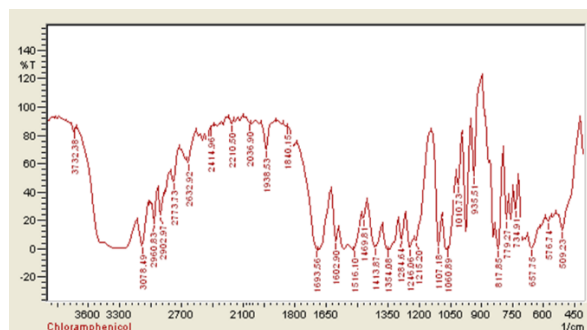


Fig No 3: FTIR Spectra of Chloramphenicol

3.3 Determination of Melting Point:

The melting point of Chloramphenicol was found to be 151°C, which complied with USP standards (149-153°C) this indicating purity of obtained drug sample.⁸

3.4 Preparation of Chloramphenicol loaded transferosomes.

Chloramphenicol loaded transferosomes were prepared by thin film hydration (TFH) using chloramphenicol, soya lecithin, Tween 80. The effect of soya lecithin and Tween 80 concentrations on particle size and entrapment efficiency was investigated using a Central Composite Design.¹⁸

3.5 Evaluation of transferosomes Prepared by Thin Film Hydration

The formulations prepared by the thin film hydration method showed particle sizes ranging from 188 to 700 nm, while entrapment efficiency ranged from 73.91 to 89.87%. The results demonstrated that changes in the concentrations of soya lecithin and Tween 80 influenced the characteristics of the prepared transferosomes.¹⁹

Table no 5: Evaluated parameters for formulation of Transferosome suspension as per CCD design using DOE by using thin film hydration.

Formulation code	Soya Lecithin (mg)	Tween 80 (mg)	Particle size (nm)	Entrapment Efficiency (%)	Zeta potential (mV)
FT1	210	30	276	73.91	-21.79
FT2	210	60	209	76.21	-19.18
FT3	210	90	198	79.64	-23.04
FT4	240	30	365	83.06	-.23.48
FT5	240	60	303	85.21	-20.64
FT6	240	90	188	89.87	-24.28
FT7	270	30	700	88.69	-24.74
FT8	270	60	668	87.77	-21.68
FT9	270	90	598	86.85	-25.09

3.6 Evaluation of transferosome by DOE by thin film hydration method:

a) Particle Size:

The particle size of obtained Transferosome prepared by thin film hydration was ranged from 188 to 700 nm as shown in table

The Model F-value of 16.83 implies the model is significant. There is only a 0.35% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant

Fit statistics:

Std. Dev.	93.70	R ²	0.8488
Mean	389.44	Adjusted R ²	0.7983
C.V. %	24.06	Predicted R ²	0.6758
		Adeq Precision	10.1055

The Predicted R² of 0.6758 is in reasonable agreement with the Adjusted R² of 0.7983; i.e., the difference is

less than 0.2.

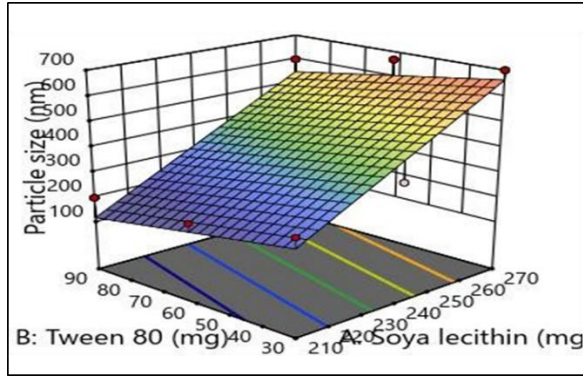


Fig no 4: 3-D graph of particle size of thin film hydration

Final Equation in Terms of Coded Factors
 Particle Size = +389.44+213.83*A-59.50*B
 Where A represents soya lecithin and B represents Tween 80.

Fit statistics:

Std. Dev.	3.76	R ²	0.7947
Mean	79.87	Adjusted R ²	0.7262
C.V. %	4.71	Predicted R ²	0.5909
		Adeq Precision	8.5631

The Predicted R² of 0.5909 is in reasonable agreement with the Adjusted R² of 0.7262; i.e., the difference is less than 0.2.

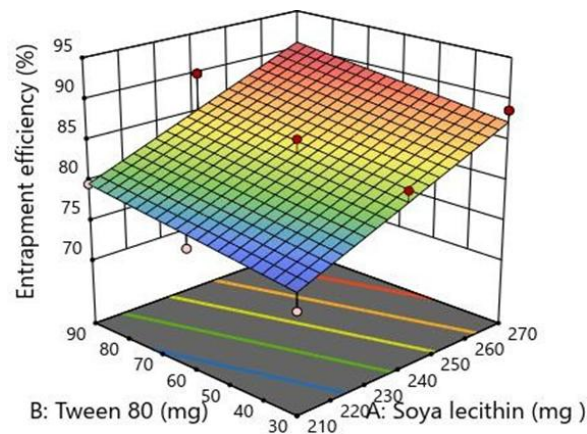


Fig no 5: 3-D graph of entrapment efficiency

The positive coefficient of soya lecithin indicates an increase in particle size with increasing concentration, while the negative coefficient of Tween 80 suggests a reduction in particle size due to improved interfacial stabilization and formation of smaller vesicles. The response surface plot (Figure 2) illustrates the combined effect of soya lecithin and Tween 80 on particle size.¹³

b. Entrapment efficiency:

The entrapment efficiency of obtained transferosome prepared by thin film hydration was ranged from 73.91 to 89.87% as shown in table 5.

The Model F-value of 11.61 implies the model is significant. There is only a 0.87% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant.

Final Equation in Terms of Coded Factors

Entrapment efficiency=+83.47+5.59*A-1.78*B

Where A represents soya lecithin and B represents Tween 80.

The positive coefficient of soya lecithin indicates that increasing its concentration improved drug entrapment, likely by providing a larger hydrophobic domain for chloramphenicol incorporation. In contrast, the negative coefficient of Tween 80 suggests that higher concentrations may reduce entrapment efficiency, possibly due to increased drug partitioning into the external phase. The combined effect of soya lecithin and Tween 80 on entrapment efficiency is illustrated in Figure 5.¹³

Table no 6: Comparison of DOE predictions of optimum formulation evaluation versus actual results

	Soya lecithin (mg)	Tween 80 (mg)	Particle size (nm)	Entrapment efficiency (%)	Desirability
DOE Prediction	235.56	90	298.34	84.42	0.819
Actual results	240	90	188	89.69	

3.6.1 Particle size of FT6 formulation

Calculation Results

Peak No.	S.P Area Ratio	Mean	S. D.	Mode
1	0.76	14.7 nm	10.2 nm	9.7 nm
2	0.24	1231.1 nm	1452.4 nm	378.4 nm
3	---	---	---	---
Total	1.00	300.8 nm	873.1 nm	9.7 nm

Cumulant Operations

Z-Average : 188 nm

PI : 0.492

Molecular Weight Measurement

Molecular Weight : ---

Parameters for Molecular Weight Calculation : ---

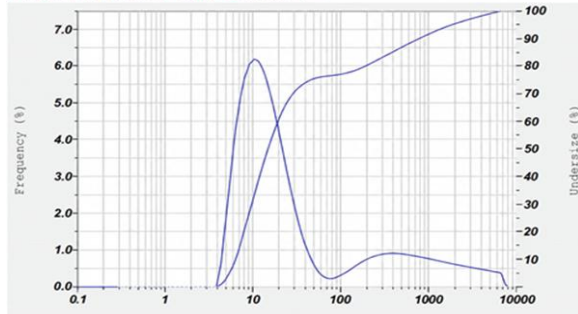


Fig no 6: Particle size of FT6

The particle size of the chloramphenicol-loaded transferosomes ranged from 188 nm to 700 nm among the thin-film hydration formulations. The variation in particle size was mainly influenced by the amounts of soya lecithin and Tween 80, which affect vesicle formation, membrane flexibility, and aggregation during formulation. An increase in soya lecithin concentration resulted in larger vesicles in some formulations, whereas the optimized combination of 240 mg soya lecithin and 90 mg Tween 80 (FT6) produced a smaller vesicle size. The optimized formulation showed a particle size of 188 nm, indicating the formation of nanosized vesicles with a suitable size for ocular drug delivery.

3.6.2 Zeta potential of FT6 formulation

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-24.28 mV	-0.000110 cm ² /Vs
2	---	---
3	---	---

Zeta Potential (Mean) : -24.28 mV

Electrophoretic Mobility Mean : -0.000110 cm²/Vs

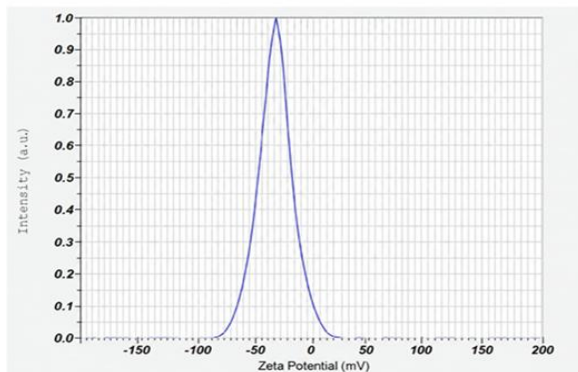


Fig no 7: Zeta potential of FT6

The zeta potential of the Chloramphenicol loaded transferosome ranged from -19.18mV to -24.74 mV for the thin film hydration formulations. The negative charge was attributed to soya lecithin, which promotes electrostatic repulsion between vesicles and helps reduce aggregation. The optimized formulation showed a zeta potential of -24.28mV, indicating satisfactory surface-charge characteristics and physical stability of the vesicular system.²⁰

3.6.3 Microscopic Examination of Optimized Transferosome

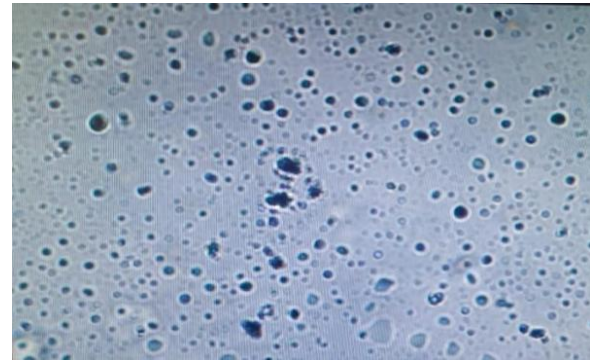


Fig no 8: Bright-field microscopic image of the optimized transferosome preparation.

Microscopic examination of the optimized transferosome formulation revealed the presence of distinct, spherical to nearly spherical vesicular structures with relatively uniform distribution, as shown in Figure 6.

The absence of major aggregation indicated good physical stability of the vesicles. The observed vesicular morphology supported the successful formation of ufasomes and was consistent with the particle-size results obtained by instrumental analysis.¹⁴

3.7 Preparation and Evaluation of Optimized Transferosomal Gel

The optimized Chloramphenicol loaded transferosomal suspension was incorporated into a Carbopol 934 and HPC LF gel to obtain a suitable topical dosage form.

The optimized gel showed a pH of 7.4, viscosity of 6412 cP at 20 rpm, and entrapment efficiency is 89.87% (Table 7). The pH was considered suitable for topical application, while the observed viscosity indicated adequate consistency of the gel.^{23, 25}

Table no 7: Evaluation of optimized Chloramphenicol loaded transferosomal gel

Formulation code	pH	Viscosity (cps)	%Entrapment efficiency	Spreadability
TG	7.4	6412	89.87	14.84±0.0213mm ²

3.7.1 In-vitro Drug Release Study

The in-vitro drug release study was performed using dialysis membrane diffusion with phosphate buffer pH 7.4 and ethanol in the ration of 70:30 as the receptor medium at $37 \pm 0.5^\circ\text{C}$. The release profiles of the optimized Transferosomal formulations, corresponding suspensions and marketed gel are presented in (table 8)¹⁶

Table no 8: In-vitro drug release studies profile of optimised formulation, formulation gel and gel+drug and marketed product.

Time	% DR T	% DR DG	% DR TG	%DR MP
0	0	0	0	0
0.5	17.3913	15.53708	15.45077	24.65473
1	28.03069	18.81714	23.82353	35.29412
2	38.36317	34.69949	35.73529	50.33248
4	49.61637	43.84917	56.53772	69.9744
6	62.19949	75.78645	65.25575	53.29923
8	67.72379	59.99245	71.03945	51.2532
10	71.40665	54.82477	75.18223	48.28645
12	64.85934	53.12328	90.63299	43.78517
24	62.19949	53.98634	86.31714	40.59798

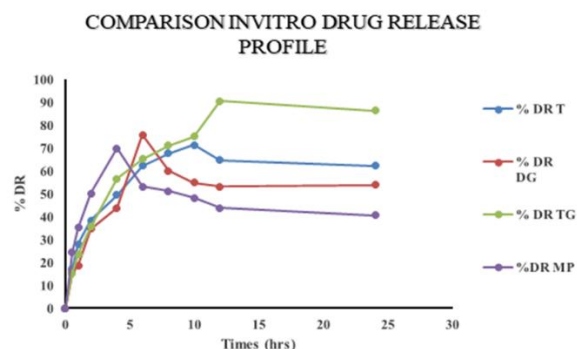


Fig no 9: Comparison graph of In-vitro drug release of formulation, gel +drug, transferosomal gel and Marketed product

The in-vitro drug release study showed that the maximum drug release was observed at different time points for the tested formulations. The highest drug release for transferosome was 71.41% at 10 h, for gel + drug was 75.79% at 6 h, for transferosomal gel was 90.63% at 12 h, and for marketed product was 69.97% at 4 h. Among all formulations, Transferosomal gel showed the highest drug release (90.63%) at 12 h, indicating a more prolonged and sustained drug-release behaviour compared with the other formulations.¹⁶

Table no 9: Kinetic values obtained from in-vitro released data

Formulation code	Kinetic Model					Mechanism of drug release
	Zero- order plot	First- order Plot	Higuchi plot	Korsmeyer- Peppas		
	R ²	R ²	R ²	n	R ²	
Formulation Suspension	0.9633	0.8501	0.8647	0.6632	0.7194	Zero order kinetics and Non-fiction diffusion
Transferosomal gel	0.8636	0.7255	0.8998	0.7743	0.5156	

“The drug-release kinetics showed that the transferosomal suspension followed zero-order kinetics with an R² value of 0.9633, while the transferosomal gel showed the highest correlation with the Higuchi model (R² = 0.8998), indicating diffusion-controlled drug release.

3.8 EX- VIVO STUDIES

3.8.1 Ex Vivo Corneal Permeation Study of Chloramphenicol Transferosomal Gel

The ex vivo corneal permeation study was carried out to evaluate the permeation characteristics of the optimized chloramphenicol transferosomal gel formulation.

The formulation exhibited a permeation flux of 12.71 µg/cm²/h across the cornea, indicating the rate of chloramphenicol permeation through the corneal membrane. The corresponding permeability coefficient (Kp) was found to be 4.67 cm/h,

demonstrating the ability of the optimized transferosomal gel to facilitate drug permeation across the corneal tissue.

Table no 10: Ex-vivo study

FORMULATION	Flux ($\mu\text{g}/\text{cm}^2/\text{h}$)	Kp(cm/h)
T-Gel	12.71	4.67

3.9 Stability study

3.9.1 Transferosomal Suspension

Optimized Formulation	Particle Size	Zeta Potential
Temp	Room Temp (25°C)	Room Temp (25°C)
1st Day	188 $\pm$ 0.566	-24.28 $\pm$ 0.54
15th Day	187 $\pm$ 0.433	-23.87 $\pm$ 0.79
30th Day	187 $\pm$ 0.598	-24.45 $\pm$ 0.65

3.9.2 Chloramphenicol Transferosomal gel

Optimized Formulation	pH
Temp	Room Temp (25°C)
1st Day	7.4 $\pm$ 0.1
15th Day	7.48 $\pm$ 0.3
30th Day	7.4 $\pm$ 0.2

IV. CONCLUSION

The present study successfully developed and evaluated a chloramphenicol-loaded transferosomal hydrogel for ocular drug delivery using the thin-film hydration method. The formulation variables, namely soya lecithin and Tween 80, significantly influenced the particle size and entrapment efficiency of the transfersomes. Among the prepared formulations, the optimized formulation showed a particle size of 188 nm, entrapment efficiency of 89.87%, and zeta potential of -24.28 mV, indicating satisfactory vesicular characteristics and physical stability. Microscopic examination confirmed the formation of spherical to nearly spherical vesicles. The optimized transferosomal suspension was successfully incorporated into Carbopol 934 and HPC LF hydrogel, which exhibited a pH of 7.4, viscosity of 6412 cP, and spreadability of 14.84 $\pm$ 0.0213 mm². The in-vitro drug release study demonstrated that the optimized transferosomal gel achieved the highest drug release of 90.63% at 12 h, compared with 71.41% for the

transferosome, 75.79% for gel containing drug, and 69.97% for the marketed product. The release data were further evaluated using zero-order, first-order, Higuchi, and Korsmeyer–Peppas kinetic models to characterize the drug-release behaviour. The transferosomal suspension showed the highest correlation with the zero-order kinetic model, with an R² value of 0.9633, while the transferosomal gel showed the highest correlation with the Higuchi model, with an R² value of 0.8998. The Korsmeyer–Peppas model showed an n value of 0.6632 for the transferosomal suspension, as reported in the study. The ex-vivo corneal permeation study demonstrated a permeation flux of 12.71 $\mu\text{g}/\text{cm}^2/\text{h}$ and a permeability coefficient (Kp) of 4.67 cm/h, indicating the ability of the optimized transferosomal hydrogel to facilitate chloramphenicol permeation across the corneal membrane. The 30-day short-term stability study at room temperature (25°C) showed that the optimized transferosomal suspension maintained its particle size and zeta potential with only minor variations. The particle size changed from 188 $\pm$ 0.566 nm on day 1 to 187 $\pm$ 0.598 nm on day 30, while the zeta potential remained within the range of -23.87 $\pm$ 0.79 to -24.45 $\pm$ 0.65 mV. Similarly, the optimized chloramphenicol transferosomal gel maintained a relatively stable pH, ranging from 7.4 $\pm$ 0.1 to 7.48 $\pm$ 0.3 during the 30-day study period. These findings indicated satisfactory physical stability of the optimized formulation during storage. Thus, the developed chloramphenicol-loaded transferosomal hydrogel demonstrated prolonged and sustained drug-release behaviour along with satisfactory ex-vivo corneal permeation and may serve as a promising approach for improving ocular drug delivery and potentially prolonging ocular residence

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