RESEARCH PAPER

Curcumin-Loaded Chitosan Nanoparticles Incorporated Into a Thermosensitive In Situ Gel For Ocular Drug Delivery In Aniridia-Associated Keratopathy

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Abstract

Background: Aniridia - associated keratopathy (AAK) is a progressive, vision - hanging corneal complaint affecting 70-85 of individualities with natural aniridia, primarily due to PAX- 6 haploinsufficiency. Being treatments including lubricants, limbal stem cell transplantation, and keratoprosthesis give limited long- term benefit, as they don't adequately address limbal stem cell insufficiency and habitual optical inflammation. Curcumin (CUR), a lipophilic polyphenol, exhibits potent anti-inflammatory (via NF- κB inhibition), antioxidant and anti-angiogenic goods applicable to AAK. still, its optical use is confined by poor waterless solubility and rapid-fire precorneal elimination.

Objective: To develop and characterize dastard- loaded chitosan nanoparticles (dastard- CHNPs) incorporated into a Poloxamer - grounded thermosensitive in situ gel for sustained optical delivery in AAK.

Methods: CHNPs were prepared via ionic gelation using sodium tripolyphosphate (TPP) across six phrasings (CNP- 1 to CNP- 6). Optimized nanoparticles were incorporated into three in situ gels (G1 – G3) containing Poloxamer 407, Poloxamer 188, and chitosan. Characterization included flyspeck size, PDI, zeta eventuality, SEM, FTIR, ruse effectiveness (EE), pH, density, and in vitro medicine prolixity and release in simulated gash fluid (pH 7.4, 34.5 °C).

Results: Optimized CNP- 4(0.2 chitosan, 0.2 TPP) showed flyspeck size 409 ± 15 nm, PDI 0.271 ± 0.02, zeta implicit 31.97 ± 1.5 mV, and EE 87. FTIR verified comity, and SEM showed flake- suchlike morphology. Optimized gel G3(18 Poloxamer 407 4 Poloxamer 188) demonstrated sol- to- gel transition (18 ± 2 cP to 2850 ± 120 cP), physiological pH, and sustained release (57 ± 4.1 over 24 h).

Conclusion: CHNP thermosensitive in situ gel is a promising non-invasive platform for sustained optical delivery in AAK operation.

Keywords: Curcumin; chitosan nanoparticles; ionic gelation; in situ gel; aniridia-associated keratopathy; PAX-6; ocular drug delivery; thermosensitive

How to cite this article: Arun Kumar MS, Naveen V, Farisha P, Ajith Kumar, Gowthamraj S, Vijayasanthanalakshmi Selvam, Gunasekaran P, Anitha A, Anil Kumar K. Curcumin-Loaded Chitosan Nanoparticles Incorporated Into a Thermosensitive In Situ Gel For Ocular Drug Delivery In Aniridia-Associated Keratopathy. Int J Drug Deliv Technol. 2026;16(65s):989-999. DOI: 10.25258/ijddt.16.65s.101

1. Introduction

Aniridia- associated keratopathy (AAK) is a severe, progressive, and vision- hanging corneal complaint that develops in roughly 70 – 85 of individualities with natural aniridia, a rare pan ocular condition caused by

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haploinsufficiency of the PAX- 6 recap factor gene (1). PAX- 6 plays a critical part in optical development, regulating the isolation and conservation of corneal epithelial cells and the limbal stem cell (LSC) niche. Loss- of- function mutations vitiate LSC renewal and corneal epithelial rejuvenescence, leading to progressive optical face failure. Clinically, AAK is characterized by dislocation of the epithelial basement membrane, habitual inflammation, conjunctivalization, stromal fibrosis, and pathological neovascularization, eventually performing in unrecoverable vision loss (2).

The pathogenesis of AAK extends beyond stem cell insufficiency and involves a patient seditious medium and oxidative stress. Elevated situations of pro-inflammatory cytokines, reactive oxygen species, and matrix metalloproteinases further aggravate corneal damage and detention epithelial mending. Despite its significant clinical burden, current treatment strategies remain largely characteristic. Conventional approaches including preservative-free lubricants, amniotic membrane transplantation, limbal stem cell transplantation, and keratoprosthesis primarily address secondary instantiations rather than the beginning molecular pathology. Long- term issues are frequently limited due to graft failure, vulnerable rejection, and the patient absence of a functional stem cell force (3). These limitations punctuate the need for pharmacological interventions that directly target inflammation, oxidative stress, and angiogenesis in AAK.

Curcumin (1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), an active compound obtained from the roots of the plant species Curcuma longa, has gained widespread interest owing to its wide-ranging medicinal effects. Curcumin displays profound anti-inflammatory effects via the blockage of the NF-κB signaling pathway, resulting in the suppression of essential inflammatory mediators like TNF-α, IL-1β, and IL-6. Further, curcumin stimulates the Nrf2 signaling pathway, thereby improving the cellular defense mechanism against oxidative stress. Additionally, curcumin possesses anti-angiogenic activity by inhibiting VEGF signaling, which aids in regulating AAK-related corneal neovascularization [4].

Despite its therapeutic potential, the clinical use of curcumin is highly constrained by its unfavorable physicochemical and pharmacokinetic behavior. The poor water solubility (<1 μg/mL) of curcumin, coupled with its unstable nature under light exposure and poor bioavailability, poses significant challenges for the development of clinically effective formulations. Ocular administration of curcumin is further complicated by its rapid pre-corneal clearance as a consequence of tear exchange, blinking, and nasolacrimal fluid drainage [5].

Nanotechnological approaches appear to be quite successful in addressing these limitations. Among several nanotechnological approaches, nanoparticles based on chitosan possess numerous advantages because of their biocompatibility, biodegradability, and natural mucoadhesive characteristics. Due to its positive charge, chitosan nanoparticles can easily interact with the negatively charged mucin layer, thus extending drug residence in the precorneal area and increasing absorption rates [6].

To increase retention of therapeutic agents in the eyes and provide for a sustained drug release mechanism, researchers have shown significant interest in developing nanoparticle-included thermosensitive in situ gels. Poloxamer-based systems (Poloxamer 407 + Poloxamer 188) demonstrate a sol-gel phase transition at physiological temperatures in the eye (34 °C). This system remains a solution at room temperature, which allows easy introduction of the drug. At physiological temperatures, this mixture converts to a gel that increases the drug residence time in the cornea [7].

In spite of advances made in the field of curcumin-based nanomedicines for the eye, there is still a need for a delivery method that addresses the pathophysiology associated with AAK disease. At the moment, there is no published research on the development of a thermosensitive in situ gel containing a CUR-CHNP formulation for this particular purpose. Therefore, this study will focus on developing an efficient system through optimization and characterization of CUR-CHNP formulation.

2. Materials and Methods

2.1 Materials

Curcumin (≥95% purity) was purchased from a pharmaceutical company. Chitosan (low molecular weight, degree of deacetylation 75–85%), sodium tripolyphosphate (TPP), Poloxamer 407, and Poloxamer 188 were pharmaceutical-grade materials. Acetone and acetic acid (glacial) were analytical reagent grades. Throughout the experiments, double distilled water was used. All chemicals were used as supplied without any further purification.

2.2 Preparation of Curcumin Loaded Chitosan Nanoparticles (CUR-CHNPs)

The CUR-CHNPs were synthesized using the ionic gelation method, as described earlier [4, 5]. Specifically, chitosan was dissolved in 1% (v/v) acetic acid solutions having chitosan concentrations ranging from 0.1–0.4% (w/v) with constant magnetic stirring. CUR was dissolved in acetone solution (concentration 2–5 mg) and was slowly added drop-wise into the chitosan solution stirred at 1000 rpm. A TPP solution (0.1–0.2% (w/v) in distilled water) was slowly added drop-wise to the reaction mixture at a rate of 1 drop/second at 1000 rpm for an hour, causing self-assembly of the nanoparticles through ionic cross-linking. The nanoparticle dispersion was subjected to centrifugation at 15,000 rpm for 30 min, followed by three washings with distilled water and lyophilization. Six formulations (CNP-1 to CNP-6) were prepared by varying concentrations of chitosan and TPP (Table 1).

Table 1. Formulation composition of curcumin-loaded chitosan nanoparticles (CNP-1 to CNP-6)

Formulation	Curcumin (mg)	Chitosan (% w/v)	Acetic Acid (% v/v)	TPP (% w/v)	Distilled Water
CNP-1	5	0.1	1.0	0.1	Q.s to 10 mL
CNP-2	5	0.2	1.0	0.1	Q.s to 10 mL
CNP-3	5	0.3	1.0	0.1	Q.s to 10 mL
CNP-4	5	0.2	1.0	0.2	Q.s to 10 mL
CNP-5	5	0.3	1.0	0.2	Q.s to 10 mL
CNP-6	5	0.4	1.0	0.2	Q.s to 10 mL

TPP: sodium tripolyphosphate; Q.s: quantity sufficient

2.3 Preparation of In-Situ Gel Using CUR-CHNP

Thermosensitive in-situ gels were prepared using the cold dispersion technique [7, 8]. A dispersion of poloxamer 407 (18-22% w/v) along with poloxamer 188 (2-5% w/v) was made in chilled distilled water (at 4°C) and kept overnight under refrigeration till a clear homogeneous solution was attained. Chitosan (0.2% w/v) was added as mucoadhesive polymer. Optimized CUR-CHNP (CNP-4) were added in the gel formulation base by dispersing them at 4°C with moderate agitation. Three formulations of in-situ gel (G1, G2, G3) having different concentrations of poloxamer were prepared (Table 2).

Table 2. Composition of CUR-CHNP-based thermosensitive in situ gel formulations (G1–G3)

Component	G1	G2	G3
CUR-CHNPs (% w/w)	1.0	1.0	1.0
Chitosan (% w/v)	1.0	1.5	2.0
Poloxamer 407 (% w/v)	0.5	1.0	1.5
Poloxamer 188 (% w/v)	0.5	0.5	0.5
Acetic acid	Q.s	Q.s	Q.s

CUR-CHNPs: curcumin-loaded chitosan nanoparticles; Q.s: quantity sufficient

2.4 Physicochemical Characterization of CUR-CHNPs

2.4.1 Particle Size, PDI, and Zeta Potential

The hydrodynamic diameter, PDI, and zeta potential were measured by dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS instrument (Malvern Instruments Ltd., UK) at an angle of backscattering of 173° at 25°C. Three replicates (n = 3) of each sample were measured, and data are presented as mean ± SD.

2.4.2 Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of curcumin, chitosan, TPP, physical mixture, and CUR-CHNPs were obtained with a KBr pellet method using an FTIR spectrometer at the wavenumber region of 400-4000 cm^{-1}.

2.4.3 Drug Entrapment Efficiency (% EE) and Drug Loading (% DL)

Drug entrapment efficiency was evaluated by isolating the drug from nanoparticles through centrifugation at 1000 rpm for 15 minutes. The nanoparticle pellet was dissolved in methanol, and the amount of curcumin was estimated using UV-Visible spectrophotometry at λmax 425 nm. The % EE and % DL were calculated according to the following formulae:

% EE = (Curcumin content in nanoparticles/Calculated theoretical content of curcumin) x 100
% DL = (Curcumin content in nanoparticles/Total weight of nanoparticles) x 100

2.4.4 Scanning Electron Microscopy (SEM)

The structure of lyophilized CUR-CHNPs was observed using scanning electron microscopy under an accelerating voltage of 15.00 kV and magnification of 500× (field size 8.5 mm, scale bar 20 µm).

2.5 Characterization of In Situ Gel Formulations

2.5.1 pH Measurement
The pH value of each in situ gel formulation was determined using a digital pH meter (25°C sol; 34°C gel) to confirm its suitability for ocular administration (pH 6.5–8.0).

2.5.2 Viscosity
Viscosity was evaluated using Brookfield viscometer. Sol phase (25°C) and gel phase (34°C) viscosity measurements were done to study the rheology of the gel and rate of gelation required for in situ delivery.

2.5.3 In Vitro Drug Diffusion
In vitro drug release through cellulose membrane was carried out using Franz diffusion cells. The donor chamber was filled with CUR-CHNP suspensions (CNP-1 to CNP-6), whereas the acceptor chamber contained STF (pH 7.4). Specimens (4 mL) were withdrawn from the receiver at fixed time intervals (0.25, 0.5, 1, 2, 4, 8 h), replenished with the receiving medium, and analyzed spectrophotometrically at 425 nm.

2.5.4 In Vitro Drug Release
Sustained- release biographies of the in situ gel phrasings (G1 – G3) were determined using dialysis membrane bags (MWCO 8 – 14 kDa) in dissembled gash fluid (STF, pH 7.4; composition NaCl 0.67, $NaHCO_3$ 0.20, $CaCl_2 \cdot 2H_2O$ 0.008) at 34.5 °C under Gomorrah conditions (n = 3). Samples (4 mL) were withdrawn at 1, 2, 4, 8, 12, 16, and 24 hours and replenished with fresh STF. Curcumin attention was quantified spectrophotometrically at λmax 425 nm (10, 11).

3. Results

3.1 Nanoparticles' Characterization

3.1.1 Nanoparticles' Size and PDI
DLS studies of optimized nanoparticles' formulation CNP-4 have shown a mean hydrodynamic diameter of 409 ± 15 nm and a PDI of 0.271 ± 0.02. This suggests a monomodal distribution pattern and monodispersity of nanoparticles, as indicated by the presence of one sharp peak (intensity of 98.5 ± 1.2%) (Table 3). These values are within an acceptable range for nanoparticles used as eye drop carriers, since particles smaller than 500 nm are generally well-tolerated in eyes (Figure 1).

Table 3. Particle size characteristics of optimized CUR-CHNPs (CNP-4)

Parameter	Value (Mean ± SD)
Particle size (nm)	409 ± 15
Polydispersity index (PDI)	0.271 ± 0.02
Peak intensity (%)	98.5 ± 1.2
Peak position (nm)	409
Size distribution	Monomodal, single peak

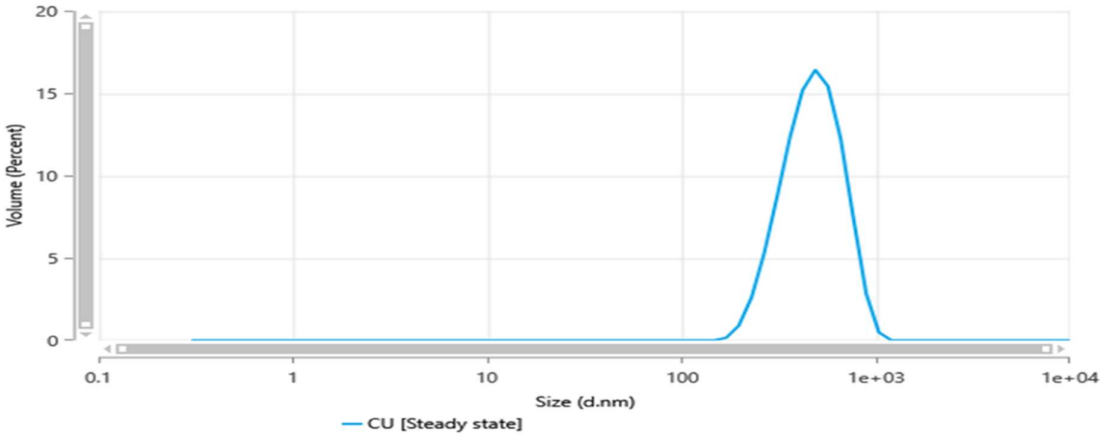

Figure 2. Zeta potential distribution curve of CUR-CHNPs (CNP-4) showing a single positive peak at +31.97 mV.

3.1.3 FTIR Spectroscopy
FTIR gamuts of pure curcumin displayed characteristic immersion bands at 3480 cm^{-1} (phenolic O – H stretch), 2940 cm^{-1} (C – H stretch), 1630 cm^{-1} (C =O enone), 1510 cm^{-1} (sweet C = C), and 1428 cm^{-1} (sweet C – H). Chitosan displayed a broad O – H/ N – H stretch at 3375 – 3400 cm^{-1} and an amide C =O band at 1650 cm^{-1}. In the dastard-

CHNP diapason, the O – H/ N – H stretch shifted to 3319.94 cm $^{-1}$, indicating hydrogen bond conformation between chitosan and curcumin (Table 5). Retention of the amide I C = C band at 1635.29 cm $^{-1}$ and cadaverous climate (469 – 408 cm $^{-1}$) verified molecular integrity of both factors, while the absence of new peaks or major peak discoveries established physicochemical comity and physical encapsulation rather than chemical revision (Figure 3A-C).

Table 5. Comparative FTIR absorption bands of curcumin, chitosan, and CUR-CHNPs

Wavenumber (cm^{-1})	Assignment	Observed in
3480	Phenolic O–H stretch	Curcumin
3375–3400	O–H / N–H stretch	Chitosan
3319.94	O–H / N–H stretch (H-bonded)	CUR-CHNPs
2940 / 2870–2920	C–H stretch	Curcumin / Chitosan
1650 / 1635.29	Amide C=O / C=C stretch	Chitosan / CUR-CHNPs
1630	C=O enone stretch	Curcumin
469–408	Skeletal / bending vibrations	CUR-CHNPs

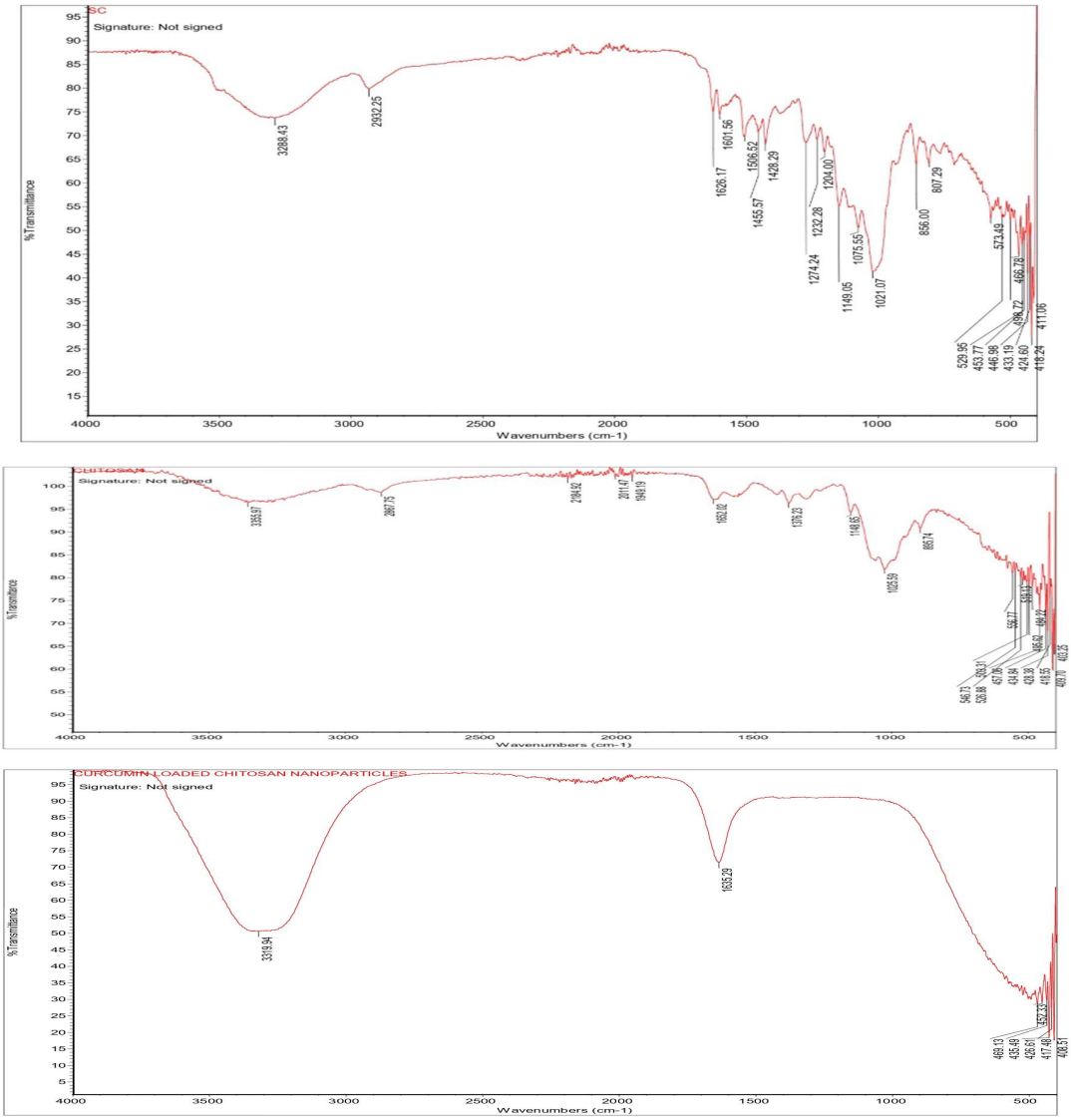

Figure 3A–C. FTIR spectra of (A) pure curcumin, (B) chitosan, and (C) CUR-CHNPs, showing characteristic peak shifts confirming encapsulation and hydrogen-bonding interactions.

3.1.4 Efficiency of Drug Entrapment

The efficiency of drug entrapment differed widely between the six preparations as shown in (Table 6). CNP-4 showed the highest efficiency of drug entrapment of 87%, CNP-5 (75%), CNP-6 (73%), CNP-3 (67%), and CNP-1 and CNP-2 had an efficiency of drug entrapment of 63%. The efficiency of drug entrapment of CNP-4 is due to the optimum ratio of chitosan and TPP (0.2% w/v : 0.2% w/v) where a tight ionically crosslinked network is formed that efficiently traps the hydrophobic curcumin inside (Figure 4).

Table 6. Drug entrapment efficiency of CUR-CHNP formulations (CNP-1 to CNP-6)

Formulation	EE (%)
CNP-1	63
CNP-2	63
CNP-3	67
CNP-4	87 (Optimized)
CNP-5	75
CNP-6	73

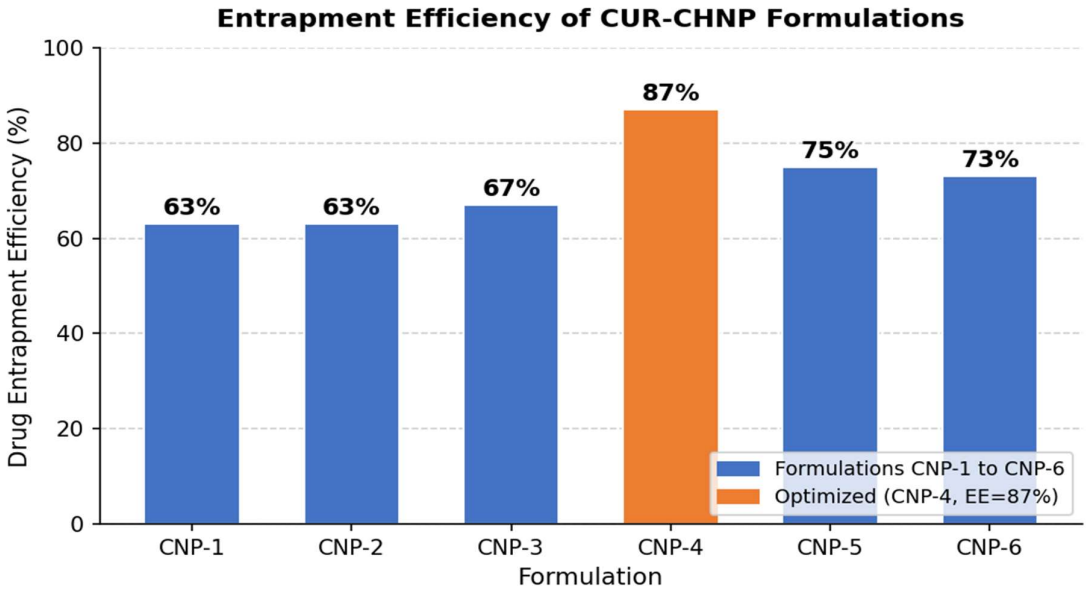

Figure 4. Bar graph depicting drug entrapment efficiency (%) of CUR-CHNP formulations CNP-1 to CNP-6. CNP-4 shows maximum EE% of 87%.

3.1.5 Scanning Electron Microscope

Observation of lyophilized CUR-CHNPs (CNP-4) using SEM analysis at magnification 500x (EHT 15.00 kV, WD 8.5 mm, scale bar 20 µm) exhibited a well-defined flake-like structure with smooth sheet-like surfaces. This particular morphology is indicative of lyophilized chitosan nanoparticles because the dehydration effect due to freeze-drying results in the transformation of hydrated spherical particles to flattened anisotropic particle structure. Uniform flakes without any crystal deposits imply homogeneity in the curcumin distribution in the chitosan framework (Figure 5).

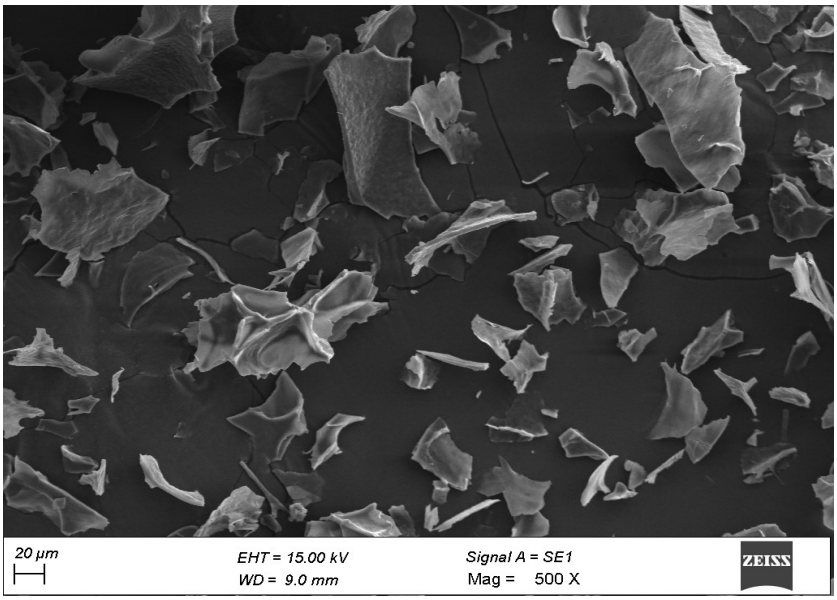

Figure 5. Scanning electron micrograph of lyophilized CUR-CHNPs (CNP-4) at 500× magnification (EHT 15.00 kV, WD 8.5 mm, scale bar 20 µm) showing characteristic flake-like morphology post-lyophilization.

3.2 Characterization of In Situ Gels

3.2.1 pH and Viscosity

All in situ gels had physiological pH during the transition from sol state to gel state, which was 7.2 at sol state (25°C) and 6.9 at gel state (34°C); both were within the ocular-compatible pH range of 6.5 to 8.0. The viscosity tests showed that there was an ideal thermogelling property of the formulated G3. It had viscosity at the sol state of 18 ± 2 cP at 25°C, which is appropriate for instillation via eye drops, and 2850 ± 120 cP at gel state (34°C), ensuring its retention on the cornea (Table 7).

Table 7. pH and viscosity of CUR-CHNP in situ gel (optimized G3) across sol–gel transition

Phase	Temperature (°C)	pH	Viscosity (cP)
Sol	25	7.2	18 ± 2
Gel	34	6.9	2850 ± 120

Values reported as mean ± SD (n = 3); cP: centipoise

3.2.2 In Vitro Diffusion of the Drug

The cumulative drug diffusion profiles for CNP-1 through CNP-6 showed variations in release behavior within 8 hours (Table 8). CNP-4 released the drug in a sustained manner with values of 28 ± 2.4% after 0.25 hours to 72 ± 5.5% in 8 hours compared to CNP-5 with higher values of 98 ± 6.6% in 8 hours, indicating that the more cross-linked CNP-4 provided sustained release, not burst release, which is desired for AAK (Figure 6).

Table 8. Cumulative drug diffusion (%) from CUR-CHNP formulations (mean ± SD, n = 3)

Time (h)	CNP-1	CNP-2	CNP-3	CNP-4	CNP-5	CNP-6
0	0	0	0	0	0	0
0.25	42 ± 3.2	38 ± 3.0	35 ± 2.8	28 ± 2.4	45 ± 3.5	32 ± 2.6
0.5	58 ± 4.1	52 ± 3.9	48 ± 3.6	38 ± 3.1	62 ± 4.4	42 ± 3.3
1	68 ± 4.8	62 ± 4.5	55 ± 4.2	45 ± 3.8	75 ± 5.2	50 ± 4.0
2	78 ± 5.3	70 ± 5.0	65 ± 4.7	52 ± 4.1	85 ± 5.9	58 ± 4.5

Time (h)	CNP-1	CNP-2	CNP-3	CNP-4	CNP-5	CNP-6
4	85 ± 5.8	78 ± 5.4	72 ± 5.1	62 ± 4.9	92 ± 6.2	65 ± 5.0
8	92 ± 6.3	86 ± 6.0	80 ± 5.7	72 ± 5.5	98 ± 6.6	75 ± 5.8

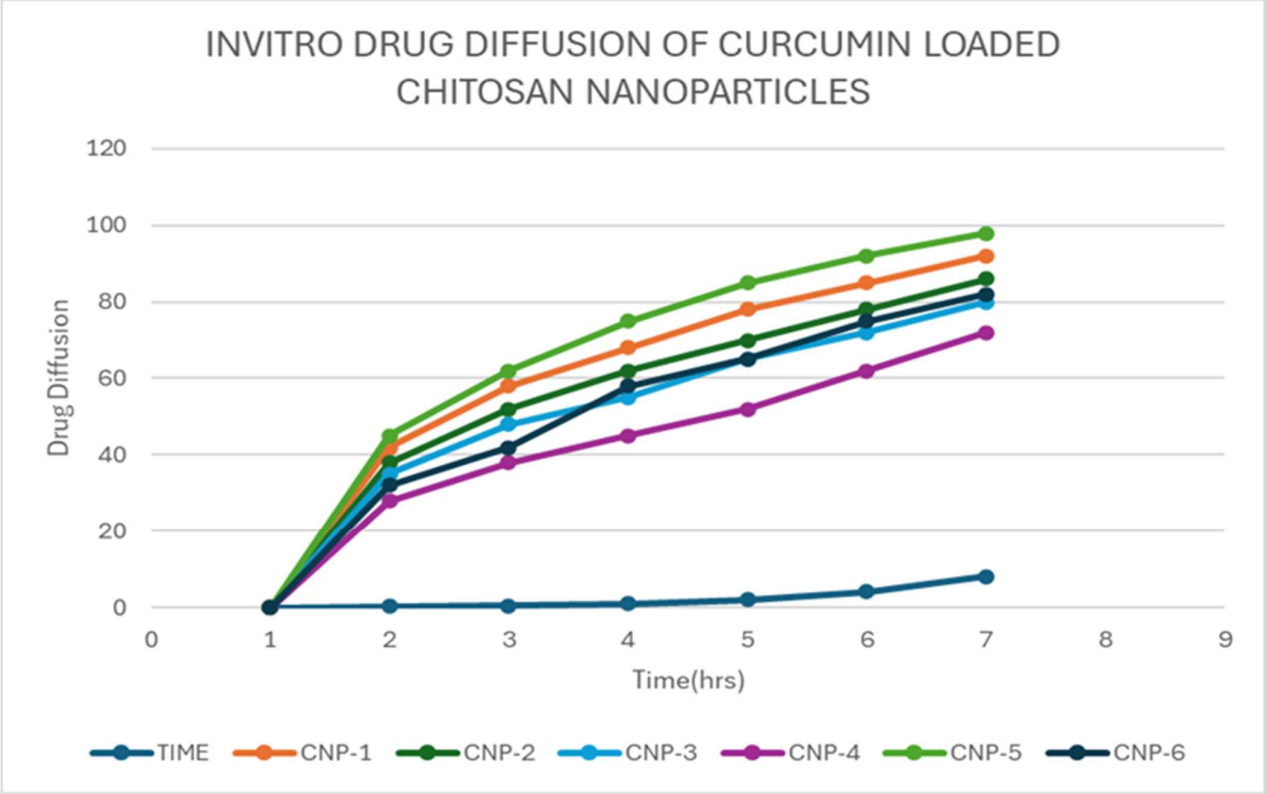

Figure 6. Cumulative drug diffusion (%) profiles of CUR-CHNP formulations (CNP-1 to CNP-6) over 8 hours, demonstrating sustained and controlled diffusion for CNP-4.

3.2.3 In Vitro Drug Release from In Situ Gel

Out of all three in situ gels, the one with the highest sustained release was G3 (18% Poloxamer 407 + 4% Poloxamer 188), having 40 ± 3.2% and 57 ± 4.1% release of CUR after 8 and 24 hours, respectively (Table 9). In comparison, viscosity-matched control, G1, showed a 50 ± 4.0% and 69 ± 5.5% release of CUR after 8 and 24 hours, respectively. It can be attributed to the lower stiffness of the gel and quicker drug release rate. However, the increased gelation power of G3 due to high Poloxamer 407 concentration creates a compact network structure, hindering the diffusion of curcumin through the nanoparticle-gel hybrid formulation, forming a biphasic release pattern (Figure 7).

Table 9. Cumulative curcumin release (%) from in situ gel formulations in STF, pH 7.4 at 34.5°C (mean ± SD, n = 3)

Time (h)	G1 (%)	G2 (%)	G3 (%)
0	0	0	0
1	30 ± 2.4	20 ± 1.8	15 ± 1.4
2	35 ± 2.8	27 ± 2.2	21 ± 1.9
4	38 ± 3.1	34 ± 2.7	28 ± 2.3
8	50 ± 4.0	46 ± 3.7	40 ± 3.2
12	59 ± 4.7	46 ± 3.7	48 ± 3.8

Time (h)	G1 (%)	G2 (%)	G3 (%)
16	64 ± 5.1	54 ± 4.3	52 ± 4.1
24	69 ± 5.5	58 ± 4.6	57 ± 4.1

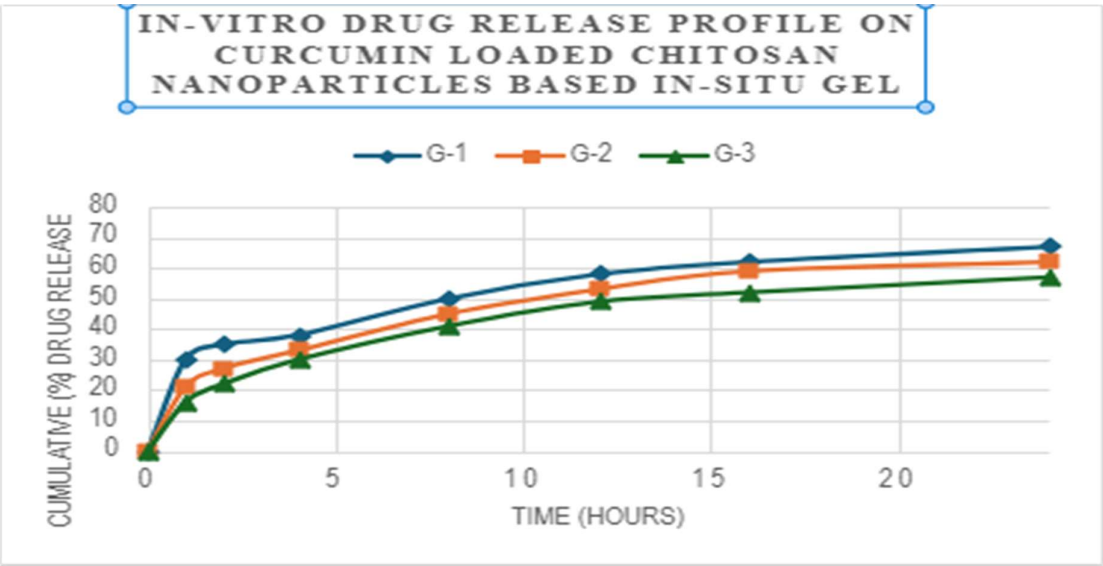

Figure 7. STF: simulated tear fluid; G1: 0.5% Poloxamer 407; G2: 1.0% Poloxamer 407; G3: 1.5% Poloxamer 407 (all with 0.5% Poloxamer 188 and 1% w/w CUR-CHNPs). Figure 7. Cumulative drug release profiles from G1, G2, and G3 in situ gel formulations over 24 hours, demonstrating superior sustained release for G3.

Discussion

The proposed study represents the first attempt to design a novel CUR-CHNP-based thermosensitive in situ gelling system suitable for AAK treatment. The design approach relies on two levels of the drug delivery control mechanism, i.e., encapsulation of curcumin within the chitosan matrix to ensure protection from degradation and improve corneal penetration, and thermosensitive polymer gelation, transforming the instilled solution to a viscoelastic depot at body temperature in an ophthalmologic application.

The CNP-4 particle diameter of 409 ± 15 nm belongs to the group of submicron particles reported to increase corneal epithelial permeation via the endocytosis pathway without causing irritation of ocular tissue [6]. The monomodal distribution with PDI equal to 0.271 indicates successful development of stable formulations. Another key characteristic of the nanoparticles, which is extremely important in AAK treatment, is the significant positive value of the zeta potential, namely +31.97 ± 1.5 mV. As a reminder, the ocular surface has a negative charge. Chitosan is a cationic substance, promoting mucoadhesion to the cornea. In turn, the precorneal retention time increases to hours instead of minutes achieved by traditional eye drops [12].

The FTIR result indicates that the curcumin was physically loaded without chemical reaction between chitosan and TPP due to the shifting O-H/N-H peak as a consequence of hydrogen bond formation and no presence of any functional groups. This supports the previously published work of non-covalent interaction mechanism for chitosan-polyphenol systems, maintaining curcumin's bioactivity [4]. The high EE% value of 87% in CNP-4 compared to other curcumin-chitosan nanoparticle formulations (usually around 60-80%) is made possible with the precise control of chitosan:TPP ionic cross-linking density [5]. The observed flake-like SEM images after lyophilization process is an acknowledged effect from freeze drying spherical chitosan nanoparticles without any cryo-protection agents, and it is suggested to enhance the mucoadhesive properties of chitosan due to its higher surface area [13].

The thermoresponsive in situ gel relies on the highly researched and studied concentration-dependant gelling property of Poloxamer 407. In concentrations greater than 16% w/v, Poloxamer 407 micelles form into a cubic lattice through an ordered packing mechanism at body temperature [8]. The inclusion of Poloxamer 188 affects the gelling temperature and enhances gel strength, while the addition of chitosan as a mucoadhesive agent enhances gel adhesion to the corneal mucin layer. The sol phase viscosity of G3 (18 ± 2 cP at 25°C) is similar to ophthalmic solutions and facilitates easy application; while the gel phase viscosity (2850 ± 120 cP at 34°C) lies in the desired range [7, 14].

The biphasic pattern of drug delivery in G3 where there is an initial moderate drug delivery and then sustained drug delivery over a period of 24 hours is scientifically plausible owing to the curcumin molecules' movement out of the nanoparticle's core into the poloxamer gel matrix, passing through the two layers of diffusion barriers, namely the chitosan crosslinking network and the poloxamer gel lattice. The release profile over a period of 24 hours is highly important in the treatment of AAK since the 24-hour release profile can provide the basis for twice-a-day drug administration, which will greatly enhance patient compliance, as opposed to the usual hourly use of lubricating eye drops [15, 16].

From the perspective of pharmacology, the prolonged ocular drug delivery system targeting AAK acts on several points in the disease mechanism: NF-kB inhibition suppresses the production of pro-inflammatory cytokines such as TNF-α and IL-1β, which causes progressive inflammatory infiltration in the stroma; the Nrf2 pathway mitigates oxidative stress resulting from the malfunctioning PAX-6 pathway that regulates antioxidant genes; and anti-angiogenesis via VEGF inhibition prevents pathological corneal angiogenesis [3, 4]. Therefore, the unique mucoadhesive nanoparticle-in-situ gel formulation presents an alternative pharmacologically multi-targeted method for treating AAK without surgical intervention that is currently not available through existing mono-therapy approaches.

Conclusion

Incorporating curcumin-loaded chitosan nanoparticles into a thermosensitive in situ gel shows promise as a multimodal drug delivery system for treating aniridia-associated keratopathy. The optimized CNP-4 formulation produced particles with a mean size of 409 ± 15 nm, a zeta potential value of +31.97 ± 1.5 mV, and 87% entrapment efficiency, while FTIR demonstrated physiochemical compatibility. The prepared G3 in situ gel exhibited sustained curcumin release for 24 hours, with a perfect sol-to-gel transition at ocular temperature, as well as a physiologically acceptable pH and viscosity profile. Considering chitosan's mucoadhesive nature, its cationic property, nanoencapsulation-based curcumin protection from degradation, and thermosensitive prolonged corneal retention, this system could overcome major obstacles in ocular drug delivery.

Abbreviations

Abbreviation	Full Form
AAK	Aniridia-associated keratopathy
CUR	Curcumin
CHNPs	Chitosan nanoparticles
CUR-CHNPs	Curcumin-loaded chitosan nanoparticles
DLS	Dynamic light scattering
EE%	Drug entrapment efficiency percentage
FTIR	Fourier-transform infrared spectroscopy
LSC	Limbal stem cell
NF-κB	Nuclear factor kappa B
PDI	Polydispersity index
PAX-6	Paired box gene 6
SEM	Scanning electron microscopy
STF	Simulated tear fluid
TPP	Sodium tripolyphosphate
VEGF	Vascular endothelial growth factor

References

1. Yazdanpanah G, Bohm KJ, Hassan OM, et al. Management of Congenital Aniridia-Associated Keratopathy: Long-Term Outcomes from a Tertiary Referral Center. Am J Ophthalmol. 2020 Feb;210:8-18. doi: 10.1016/j.ajo.2019.11.003. Epub 2019 Nov 12. PMID: 31730836; PMCID: PMC6964251.
2. Liu XF, Hao JL, Xie T, et al. Curcumin, A Potential Therapeutic Candidate for Anterior Segment Eye Diseases: A Review. Front Pharmacol. 2017 Feb 14;8:66. doi: 10.3389/fphar.2017.00066. PMID: 28261099; PMCID: PMC5306202.
3. Irimia T, Dinu-Pîrvu CE, Ghica MV, et al. Chitosan-Based In Situ Gels for Ocular Delivery of Therapeutics: A State-of-the-Art Review. Mar Drugs. 2018 Oct 9;16(10):373. doi: 10.3390/md16100373. PMID: 30304825; PMCID: PMC6212818.
4. Arozal W, Louisa M, Rahmat D, et al.Development, Characterization and Pharmacokinetic Profile of Chitosan-Sodium Tripolyphosphate Nanoparticles Based Drug Delivery Systems for Curcumin. Adv Pharm Bull. 2021 Jan;11(1):77-85. doi: 10.34172/apb.2021.008. Epub 2020 Nov 7. PMID: 33747854; PMCID: PMC7961214.
5. Mishra B, Yadav AS, Malhotra D, et al. Chitosan Nanoparticle-Mediated Delivery of Curcumin Suppresses Tumor Growth in Breast Cancer. Nanomaterials. 2024; 14(15):1294. https://doi.org/10.3390/nano14151294
6. Zamboulis A, Nanaki S, Michailidou G, et al.Chitosan and its Derivatives for Ocular Delivery Formulations: Recent Advances and Developments. Polymers (Basel). 2020 Jul 8;12(7):1519. doi: 10.3390/polym12071519. PMID: 32650536; PMCID: PMC7407599.
7. Makwana SB, Patel VA, Parmar SJ. Development and characterization of in-situ gel for ophthalmic formulation containing ciprofloxacin hydrochloride. Results Pharma Sci. 2016;6:1–6. doi:10.1016/j.rinphs.2015.06.001.
8. Longo E, Giuliano E, Gagliardi A, et al. In Situ Forming Poloxamer-Based Thermo-Sensitive Hydrogels for Ocular Application: A Focus on the Derivatives 407 and 188. Gels. 2025; 11(9):752. https://doi.org/10.3390/gels11090752
9. Gugleva V, Michailova V, Mihaylova R, et al. Formulation and Evaluation of Hybrid Niosomal In Situ Gel for Intravesical Co-Delivery of Curcumin and Gentamicin Sulfate. Pharmaceutics. 2022 Mar 30;14(4):747. doi: 10.3390/pharmaceutics14040747. PMID: 35456581; PMCID: PMC9028379.
10. Lou J, Hu W, Tian R, et al. Optimization and evaluation of a thermoresponsive ophthalmic in situ gel containing curcumin-loaded albumin nanoparticles. Int J Nanomedicine. 2014 May 21;9:2517-25. doi: 10.2147/IJN.S60270. PMID: 24904211; PMCID: PMC4039420.
11. Shen T, Yang Z. In vivo and in vitro Evaluation of in situ Gel Formulation of Pemirolast Potassium in Allergic Conjunctivitis. Drug Des Devel Ther. 2021 May 18;15:2099-2107. doi: 10.2147/DDDT.S308448. PMID: 34040348; PMCID: PMC8140898.12. Kumar D, Shafiq S. Chitosan nanoparticles for ocular drug delivery: formulation optimization and ex vivo evaluation. Int J Pharm. 2023;645:123321. doi:10.1016/j.ijpharm.2023.123321
12. Arya G, Das M, Sahoo S. Evaluation of curcumin loaded chitosan/PEG blended PLGA nanoparticles for effective treatment of pancreatic cancer. Biomed Pharmacother. 2018;102:555–566. doi:10.1016/j.biopha.2018.03.101.
13. Fathalla Z, Mustafa WW, Abdelkader H, et al. Hybrid thermosensitive-mucoadhesive in situ forming gels for enhanced corneal wound healing effect of L-carnosine. Drug Deliv. 2022 Dec;29(1):374-385. doi: 10.1080/10717544.2021.2023236. PMID: 35068268; PMCID: PMC8788381.
14. Eleraky NE, El-Badry M, Omar MM, et al. Curcumin Transferosome-Loaded Thermosensitive Intranasal in situ Gel as Prospective Antiviral Therapy for SARS-Cov-2. Int J Nanomedicine. 2023 Oct 17;18:5831-5869. doi: 10.2147/IJN.S423251. PMID: 37869062; PMCID: PMC10590117.
15. Lin X, Zhou Y, Lv K, et al. Nanomedicine-Based Ophthalmic Drug Delivery Systems for the Treatment of Ocular Diseases. Int J Nanomedicine. 2025 Jul 21;20:9221-9249. doi: 10.2147/IJN.S532074. PMID: 40718639; PMCID: PMC12292370.