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ORIGINAL RESEARCH

RETRACTED ARTICLE: Stimulus Responsive Ocular Gentamycin-Ferrying Chitosan Nanoparticles Hydrogel: Formulation Optimization, Ocular Safety and Antibacterial Assessment

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Purpose: The present study was designed to study the gentamycin (GTM)-loaded stimulus-responsive chitosan nanoparticles to treat bacterial conjunctivitis.

Methods: GTM-loaded chitosan nanoparticles (GTM-CHNPs) were prepared by ionotropic gelation method and further optimized by 3 factor and 3-level Box–Behnken design. Chitosan (A), sodium tripolyphosphate (B), and stirring speed (C) were selected as independent variables. Their effects were observed on particle size (PS as Y1), entrapment efficiency (EE as Y2), and loading capacity (LC as Y3).

Results: The optimized formulation showed the particle size, entrapment efficiency, and loading capacity of 152±3.24 nm, 60.18±1.65%, and 34.19±1.17%, respectively. The optimized gentamycin-loaded chitosan nanoparticle (GTM-CHNPopt) was further converted to the stimulus-responsive sol-gel system (using pH-sensitive carbopol 974P). GTM-CHNPopt sol-gel (NG5) exhibited good gelling strength and sustained release (58.99±2.28% in 24 h). The corneal hydration and histopathology of excised goat cornea revealed safe to the cornea. It also exhibited significant ($p<0.05$) higher ZOI than the marketed eye drop.

Conclusion: The finding suggests that GTM-CHNP-based sol-gel is suitable for ocular delivery to enhance the corneal contact time and improved patient compliance.

Keywords: chitosan, nanoparticles, gentamycin, histopathology, antimicrobial assessment, HET CAM test

Introduction

Gentamycin (GTM) is an aminoglycoside antibiotic used to treat bacterial infections.[1] Its important potential use against a wide spectrum of Gram-negative and Gram-positive bacteria.[2] It acts by inhibiting the bacterial protein synthesis mainly through binding with the 30S ribosomal subunit, interfere with the correct amino acid polymerization and elongation.[3] It is used to treat the bacterial infection like conjunctivitis and blepharitis as well as skin infection around the eye.

The eye is the most sensitive organ of our body and eye drops are the most commonly used delivery system to treat ocular diseases. The eye drops cannot attain the effective drug concentration to the ocular tissue due to poor bioavailability (≤5 %) and less corneal residence time (15–30 sec).[4,5] Therefore, frequent dosing required to achieve the effective drug concentration. There are various novel

ocular formulations have been reported to enhance the ocular bioavailability by increasing the corneal contact time. The different works of literature reported the use of chitosan in ocular polymeric nanoparticles to enhanced permeation and antibacterial activity. Levofloxacin-loaded chitosan nanoparticles were prepared and reported the sustained in vitro release and higher antibacterial sensitivity than a marketed eyed drop. The gamma scintigraphy study also reported the enhanced corneal retention due to the presence of mucoadhesive polymer chitosan.[6] Silva and its associates prepared ceftazidime-loaded chitosan nanoparticles in situ gel and reported the prolonged drug release, enhanced corneal permeation, and strong adhesion property. The higher mucoadhesive was achieved due to its ability to interact with the ocular surface, and lead to increased drug residence time in the eye. The cell line result revealed the prepared formulations were not cytotoxic on ARPE-19 and HEK293T cell lines.[7] Timolol-loaded chitosan nanoparticles were prepared and optimized using Box–Behnken design for ocular delivery. The prepared formulations exhibited enhanced permeation confirmed by the ex-vivo corneal permeation and confocal microscopy. The formulation was further evaluated for pharmacodynamic study and exhibited significant ($P < 0.05$) reduction of intraocular pressure and prolonged time activity compared to commercial TM eye drops.[8] Li et al prepared Betaxolol entrapped chitosan nanoparticle and exhibited a well-tolerated result confirmed by the human immortalized corneal epithelial cell. Further, it showed 1.99-fold and 1.75-fold higher AUC_{0-t} and MRT_{0-t} than betaxolol solution.[9] Erythropoietin entrapped topical chitosan nanoparticles were developed and depicted strong mucoadhesion over procine cornea and conjunctiva.[10] Yu and associates developed dexamethasone chitosan nanoparticles for enhancement of bioavailability through ocular delivery. exhibited prolong in vitro release and precorneal residence time than an aqueous solution. It also showed good ocular tolerance and provided a relatively longer precorneal duration.[11]

There are different natural and synthetic polymers are used to prepare ocular nanoparticles (NPs). The polymers like chitosan, flaxseed gum, galactomannans, eudragit RL 100 have been evaluated for enhancement of ocular bioavailability.[12–15] Among these, natural polymer chitosan was found to be efficient, cost-effective, and eco-friendly sources for nano-carriers.[16] Chitosan (CH) is a well-defined macromolecular type cationic polymer obtained from chitin.[17,18] It is non-toxic, biodegradable, strong bioadhesive, and penetration enhancer. It also has shown antimicrobial and antifungal property,[19] and also possesses hemostatic properties that enhance the blood clotting.[20] It acts as an antibacterial by acting on the cell wall of the bacteria. The gram-positive and gram-negative bacteria have a different cell wall. The gram-negative bacteria have thin peptidoglycan than gram-positive bacteria. CH with NH_3^+ group in the structure can adsorb on a cell wall by electrostatic interaction.[21] The presence of lipopolysaccharide and teichoic acid in the cell wall as anionic parts for Gram-negative and Gram-positive bacteria. The binding of CH with these parts can lead to damage of cell wall integrity and leakage of macromolecules from bacteria.[22] The interaction with the outer membrane of Gram-negative bacteria with chitosan may contribute to enhancing antibacterial activities.[23] It is soluble in an acidic environment (glacial acetic acid) to make the protonated form (NH_3^+) to maintain the bioadhesive property as well as permeation enhancing property.[24] This protonated form binds with the negative charge of corneal mucin and showed prolonged corneal contact time.

The nanoparticulate laden sol-gel system is used to ease the drug administration and also enhances the residence time. Carbopol 974P is a macromolecular, cross-linked polymer, and chemically belong to polyacrylic acid (PAA). It exhibited in the gel system at raised pH of the solution. It is bioadhesive in nature and its property is due to the interaction with corneal mucin by hydrogen bonding, hydrophobic interaction as well as by inter-diffusion mechanism.[25]

The objective of the present study was to prepare GTM-CHNPs by ionotropic gelation method. The formulation was further optimized by quality by design (QbD) software. The optimized formulation (GTM-CHNPopt) was transformed into the sol-gel system by using carbopol 974P polymer. The GTM CHNPopt sol-gel formulation was evaluated for clarity, pH, gelling strength, rheological study, in-vitro release, mucoadhesive strength, ex-vivo permeation, ocular tolerance, and antimicrobial assessment.

Materials and Methods

Materials

The gift sample of Gentamycin (GTM) was received from the Uni-Cure pharmaceutical Pvt. Ltd (Noida, India). The low molecular weight chitosan (CH) was procured from Sigma Aldrich (St Luis USA). Sodium tripolyphosphate (STP) was procured from the Honeywell (Fluka,

Wunstorfer Strasse, Germany). Carbopol was obtained from the SD-fine chemical (Mumbai, India). HPLC grade methanol, acetonitrile, and water were purchased from Sigma Aldrich (St Luis USA). All other chemical reagents obtained from the laboratory are used for study in analytical grade.

Methods

Formulation of GTM Nanoparticles

GTM-loaded CH nanoparticles (GTM-CHNPs) were prepared by ionotropic gelation method.[12] The different CH concentration solution was prepared by dissolving in aqueous acetic acid solution (1% v/v) and pH-5 was maintained. GTM (0.3%) was added to the different concentrations of STP in water. STP solution was added drop-wise in CH solution by using a needle in 1:2.5 ratio (STP: CH). GTM (0.3%) with continuous stirring at 2500 rpm. The suspension was separated by centrifugation at 18000 rpm for 15min (Remi-24, Cooling centrifuge, Mumbai, India) and lyophilized at 100 mbar, −120ºC using lyophilizer (Hetolyophilizer, Thermo Fisher Scientific, USA). Mannitol was used as a cryoprotectant.

Optimization

Box Behnken design (BBD) is the best tool for optimization because it gives the lesser number of formulations in an appropriate composition.[26] The selection of variables done based on preliminary study and selected variables were fitted into BBD statistical design software. The independent formulation variables CH concentration, STP concentration, and stirring speed at three levels (low, medium, and high) are shown in Table 1. Their effects were observed on the dependent responses like particle size (PS, nm), entrapment efficiency (EE %), and drug loading (DL). The experimental runs were applied into different kinetic models like linear, second-order, quadratic, and cubic to determine the best fit model.[27] The polynomial equation and three-dimensional plots (3D) were generated from the software to check the effect of independent variables on each response.[28] The general polynomial mathematical equation was given below

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_1{}^2 AB + \beta_1{}^3 AC + \beta_2{}^3 BC + \beta 11A^2 + \beta 22B^2 + \beta 33C^2 + \quad (1)$$

where Y is responses, A, B, and C are the coded value of process variables, β is coefficients (linear and interaction). AB and A^2 are interactions of coded variables for models.

Table 1 Levels of Independent and Dependent Variables Used in Experiments

Factors	Units	Level and Coded Value		
Independent Variables		**Low (−1)**	**Medium (0)**	**High (+1)**
A = Chitosan (CH)	%	0.1	0.2	0.3
B = Sodium tripolyphosphate (STP)	%	0.15	0.25	0.35
C = Stirring speed	rpm	1000	1750	2500
Dependent variables		Aim		
Y_1= Particle size (PS)	nm	Mi[illegible]200nm)		
Y_2= Entrapment efficiency (EE)	%	Maximize		
Y_3= Loading capacity (LC)		Ma[illegible]ize		

Characterization

Particle Size and Surface characterization

The particle size (PS), poly-dispersibility index (PDI), and zeta potential (ZP) of GTM-CHNPs were evaluated by zeta sizer (Malvern, zeta sizer, Malvern, USA). The appropriately diluted sample was filled in a cuvette and measured at scattering angle at room temperature.[29]

Entrapment Efficiency and Drug Load

The prepared GTM-CHNPs were transferred in the centrifugation tube and centrifuged at 18,000 rpm in the cooling centrifuge (4 ºC). The supernatant was separated and NPs pellet washed with doubled distilled water. The concentration of GTM in the supernatant was analyzed by a UV-spectrophotometer at 250 nm. The encapsulation efficiency and drug load were calculated by the given formula.[29]

$$\mathrm{EE}(\%) = \frac{Total\ GTM - unentraaped\ GTM}{Total\ GTM} \times 100$$

$$\mathrm{DL}(\%) = \frac{Total\ GTM - unentraaped\ GTM}{weight\ of\ NPs} \times 100$$

Microscopic Examination

The morphology of GTM-CHNPopt was examined by transmission electron microscopy (JEM1011, JEOL, Inc., Peabody, MA, USA). One drop of GTM-CHNPopt was placed on the carbon-coated copper grid and stained with phosphotungstic acid (2% v/v).[30] The sample kept aside for staining and air-dried. The sample grid was placed in

an electronic microscope, the image was captured and viewed by si-Viewer software.

Fourier Transform Infrared (FTIR)

FTIR instrument (ATR-FTIR, Bruker Alpha, Germany) was used to evaluate the interaction of GTM with the used carrier. The appropriate quantity of GTM and lyophilized GTM-CHNPopt was taken and kept in a sample holder for analysis. The sample was scanned at 400–4000 cm^{-1} wavenumber to check the variation in characteristic spectral peaks.[6]

Thermal Behavior Study

Thermal behavior study was performed through differential scanning calorimeter (Perkin Elmer 8000; Shelton, CT, USA). The appropriate quantity (~4 mg) of GTM and lyophilized GTM-CHNPopt were placed in the DSC pan and sealed. The pans were placed in the instrument and scanned between 0–300 ºC with a scanning speed of 5ºC/min under continuous nitrogen supply.[31]

X-Ray Diffraction Study (XRD)

The XRD study was performed by using the XRD instrument (Ultima IV diffractometer, Rigaku., Japan) to check the nature of the sample. The sample ie, GTM and lyophilized GTM-CHNPopt were placed into the XRD sample holder. The instrument was operated at 35kV tube voltage and 20mA tube current using Cu-anode as the radiation source. The sample was scanned between 5º-70º (2θ) with a scanning rate of 1º at room temperature. Each spectrum was recorded and compared to evaluate the change in diffraction angle.[31]

Formulation of GTM-CHNPopt into Sol-Gel

The optimized GTM-CHNPopt was converted into a sol-gel system by using PLGA category polymer for enhanced ocular retention corneal region. The lyophilized GTM-CHNPopt (containing 0.3% GTM) was dispersed in different concentration of the polymeric solution of carbopol and evaluated for various physiochemical characterizations.

Characterization

Clarity and Optical Transmittance

The clarity is very important criteria for the ophthalmic preparation. The presence of any visible particle produced the irritation to the ocular tissue. It was examined visually under light against the dark with the white background before and after gelation. The optical transmittance was analyzed by using a UV spectrophotometer (Genesys, 10S, UV-Vis, Thermo scientific, MA, USA) at 480 nm against STF as blank. The experiment was performed in triplicate.

pH and Drug Content

The pH of prepared formulations (NSG1-NSG6) was analyzed by Digital pH meter in triplicate. The drug content was estimated by extracting GTM into acetic acid solution (1% v/v). The formulation was vortexed, filtered by membrane filter and the extract was diluted with STF to analyze by using UV-Spectrophotometer at 250 nm in triplicate.

Gelling Strength

The gelling ability was evaluated by changing the response of formulation by doing alteration in pH. It was measured by mixing of formulation with STF (4:1) into a test tube and maintained the ocular condition. The gelling ability was inspected visually and graded according to gel strength like no gelation, gelation but dissolve quickly, and gelation but dissolve at an extended period.[32] The optimized formulation was selected and subjected to further study.

Viscosity

The rheological behavior of GTM-CHNPopt sol-gel (NSG6) in sol as well as gel state was evaluated by brook field viscometer (Fungi lab premium, SMART-H, Barcelona, Spain) at 37.5 ± 2 ºC.[33] The sample was placed in a beaker (10 mL) and spindle was dipped without touching the bottom as well as the side of the beaker. The spindle was rotated at a different speed (15, 30, 45, 60, and 100 rpm) and viscosity recorded. Similarly, the viscosity in the gel state was evaluated, and the pH of formulation was increased up to physiological pH (7.4) by using 0.1M NaOH.

Isotonicity Study

Isotonicity study was performed using the rat blood sample. A drop of blood and GTM-CHNPopt sol-gel (NSG 5) was mixed properly and placed on a glass slide under aseptic condition. The mixture was spread and smear (thin film) was prepared. Then few drops of Leishman's (neutral) stain was added over the smear and stained for two minutes. The excess amount of dye was washed with sterile water. The red blood cell (RBC) was observed by using a light microscope under 40x magnification. A sterile sodium chloride (0.9%) solution was used as control.

Drug Release

The comparative drug release study between GTM-CHNPopt, GTM-CHNPopt sol-gel (NSG 5) and marketed eye drop (0.3% Gentacin, Riyadh Pharma, Riyadh, KSA) was performed by using the dialysis membrane (MW ~ 12,000 Da). The study was performed with the diffusion cell, at 37±0.5ºC with continuous stirring of 50 rpm.[34] The membrane was placed between the donor and acceptor compartment of the diffusion cell. The sample (1 mL) was placed into the donor compartment and release media simulated tear fluid (STF) filled in the acceptor compartment. The aliquot (1 mL) was withdrawn at a definite time interval from the receptor compartment and simultaneously replaced with the same volume of STF. The released sample was further diluted and GTM concentration was analyzed by UV-spectrophotometer (Genesys 10S UV-Vis, Thermo scientific, USA) at 250 nm. The release data were fitted to different mathematical models like zero order, first order, Higuchi model, Korsmeyer-Peppas, and Hixon–Crowell model. The release graph was plotted and the regression coefficient (r^2) was calculated. Based on the maximum regression coefficient (r^2) value, the best-fit release kinetic model was selected.

Mucoadhesive Study

The mucoadhesive strength was evaluated using the physical balance method (Supplementary Figure 1). The cornea was collected from the goat eye and washed with normal saline. The cornea was placed at the opposite of the physical balance pan and equilibrated by placing it on the sample holder. The sample GTM-CHNPopt sol-gel (NSG5) was added into the sample holder, pH 7.4 was adjusted with 0.1M NaOH and allowed to stand for a few minutes with contact to the cornea. The weight (5 mg) was kept on the second pan of balance to assure the formulation was attached to the cornea and removed it. Then slowly more weight was placed onto the second pan until cornea was detached from the gel and the total weight noted. The mucoadhesive strength was calculated by the below formula and expressed in dyne/cm^2.

$$\textit{Mucoadhesive force} = \frac{\textit{Weight in gram Accerelation due to gravity}}{\textit{Surface area of mucosal surface}}$$

Corneal Permeation Study

The corneal permeation study was performed using excised goat cornea.[32] The goat whole eyeball was obtained from the local slaughterhouse and placed in normal saline (0.9% NaCl) at 4 ºC. The cornea was carefully removed and washed with simulated tear fluid. The fresh cornea was placed between the donor and acceptor compartment of a diffusion cell. The samples (2 mL) of GTM-CHNPopt, GTM-CHNPopt sol-gel (NSG5), and marketed eye drop (0.3% Gentacin, Riyadh Pharma, Riyadh, KSA) were placed into donor compartment. STF was placed into acceptor compartment as release media. The released sample (1 mL) was withdrawn at different time points from the acceptor compartment at a definite time interval and simultaneously replaced with the same volume of fresh STF. The permeated GTM sample at each time point was analyzed by using reported HPLC method[35] and drug concentration at each time point was calculated.

Ocular Tolerance Study

Corneal Hydration Test

Corneal hydration test used for the determination of ocular tolerance. After finishing the corneal permeation study, the cornea was removed and wet weight was noted by using a digital balance. Then it was kept for drying in a hot air oven at 60ºC for three days and dry weight was calculated. The corneal hydration (H) was calculated by the given formula and compared to the standard value for evaluation.

$$\text{Corneal hydration} = 1 - \left(\frac{\textit{dry weight}}{\textit{wet weight}}\right)\text{X100}$$

Histopathological Examination

The histopathological study was performed on the treated cornea with the tested samples in the permeation study.[10] After completion of the permeation study, the cornea was removed and stored into a formalin solution (8% v/v). The cornea was dehydrated with alcohol and the solid block was prepared with paraffin wax. The cross-section was cut by using microtome cutter and stained with hematoxylin and eosin.[36] The stained cross-section was examined with Motic digital optical microscope (Motic digital Microscope, B3 DMWB, Pal system, Japan) at 40x magnification lens and evaluated with the controlled treated cornea (0.9% NaCl).

Ocular Irritation

HET-CAM is the alternative method to check the irritation of formulation with the eye. It gives a similar and well-defined result as Draize test.[37] The study was performed in

freshly fertilized Hen eggs. The eggs (50–60g) were procured from the poultry form and incubated in a humidified incubator at 37.8±0.5ºC/55±2% RH for 9 days. On the 9th day of incubation, the eggs were removed, and eggshell were carefully removed from the air chamber side to avoid the break down of blood capillary. The blood capillary was developed which is similar to human eye capillary. The test samples (0.1 mL) GTM-CHNPopt sol-gel, 0.9% sodium chloride (positive control), 0.1N sodium hydroxide (negative control) were installed over the CAM of egg and damaged blood capillaries at the definite time were noted. The irritation score was given as per the standard data (ICCVAM, 2010),[38] and the mean score was calculated for every sample.

Antimicrobial Assessment

The antimicrobial susceptibility study of GTM-CHNPopt sol-gel, GTM-CHNPopt, and marketed eye drops (0.3% Gentacin) formulations were performed by cup-plate method against *S. aureus* and *E. coli* as microorganism.[39,40] The appropriate quantity of nutrient agar media was prepared and sterilized by autoclave (Astell Scientific, UK) at 121 ºC and 15 psi pressure for 15 min. The nutrient agar (9 mL) was transferred into disposable sterile petri-plates under aseptic condition. *S. aureus* and *E. coli* culture (0.1 mL) was inoculated and shaken for uniform distribution of culture and stand for solidification. The cup was prepared by using sterile borer (6 mm diameter) and test samples (100 µL) were filled in each cup. The petri-plates were kept to stand for 4h to complete dissemination of test samples. The petri-plates were incubated at 37 ºC for 48 h and the zone of inhibition was measured using the graduated scale. Normal saline solution (0.9% NaCl) used as control.

Statistical Analysis

Data were expressed as mean ± SD. One-way ANOVA was used for statistical analysis. The P<0.05 was used for statistically significant analysis.

Results and Discussion

Optimization

GTM-NPs were optimized by Box–Behnken design software and the used variables with the concentration ranges are expressed in Table 1. The design showed seventeen formulation runs in different compositions with their responses, ie, PS, EE, and DL depicted in Table 2. The experimental runs were fitted in different statistical models ie, linear, second-order, quadratic, and cubic and their results are shown in Table 3. The design showed quadratic model for all the responses and also there was no significant variation in actual and predicted regression coefficients (R^2) were observed (Figure 1). The values were found very close to each other as compared to other models as indicated the model was desirable (Table 3). The p-value of the fitted quadratic model was found to be <0.05 indicates that the model significantly fit. The value of R^2 found in the range of 0.9994 to 0.9999 (P<0.05) with high PRESS value and assured the integrity of the fit data. The lack of fit of each response for the quadratic model was evaluated and found insignificant (P>0.05), indicating the model was desirable. The polynomial equation was generated and it gives the effect of each factor on each response individually, as well as combinedly. Analysis of variance (ANOVA) of each response was applied by the software and the data indicated the model was well fitted (Table 4). The three-dimensional plot (3D-plot) was generated and showed a well-defined effect of each factor on response (Figure 2A–C).

Effect of Formulation Variables on Responses

Effect on Particle Size (PS)

The PS of GTM-CHNPs was in the range of 95.68 (F7) to 251.84 nm (F2) as shown in Table 2. The 3D-plot was used to evaluate the effect of independent variables on PS and expressed in Figure 2A. As the CH concentration increases as compare to STP, the viscosity of CH solution increases. It leads to decrease in the conductivity and more binding sites (NH_2) present for cross-linking. STP not completely cross-linked to CH and the PS increases.[10] The decrease in STP concentration lead to increase in the particle size due to aggregation of NPs. This result agreed with previously published research.[41] The third variable ie, stirring speed showed a significant effect on PS. It has shown an antagonistic effect on PS, as the stirring speed increases from 1000 to 2500 rpm the PS decreases. It increases the shear force and leads to breakdown of particle which agreed with previously published work.[42] The computer-generated second order quadratic polynomial equation of PS was given below

$$\begin{aligned} \textit{Particle size}\ (nm,\ Y1) = {} & +135.20 + 45.29 * A - 13.55 \\ & * B - 30.56 * C - 30.30 * A \\ & * B - 0.54 * A * C - 1.55 \\ & * B * C + 3\,1.60 * A^2 \\ & - 4.84 * B^2 + 4.15 * C^2 \end{aligned} \quad (1)$$

Table 2 Formulation Design-Based Composition with Actual and Predicted Results of Particle Size (Nm), Entrapment Efficiency (%) and Loading Capacity (%)

Formulation Code	Independent Variables			Dependent Variables					
	A (%)	B (%)	C (rpm)	Y_1 (nm)		Y_2 (%)		Y_3 (%)	
				Actual Value	Predicted Value	Actual Value	Predicted Value	Actual Value	Predicted Value
F1	0.1	0.15	1750	100.69	99.92	49.44	49.35	26.24	26.12
F2	0.3	0.15	1750	251.84	251.11	56.51	56.52	23.26	23.11
F3	0.1	0.35	1750	132.68	133.42	79.23	79.22	42.43	42.58
F4	0.3	0.35	1750	162.64	163.41	70.34	70.43	42	42.63
F5	0.1	0.25	1000	155.61	155.69	68.31	68.45	35.91	35.97
F6	0.3	0.25	1000	247.31	247.35	76.39	76.43	37.24	37.33
F7	0.1	0.25	2500	95.68	95.64	56.18	56.14	25.78	25.69
F8	0.3	0.25	2500	185.21	185.14	46.67	46.52	21.	21.35
F9	0.2	0.15	1000	176.38	177.08	63.8	6 5	36.32	36.38
F10	0.2	0.35	1000	153.87	153.07	82.11	81.	52.	52.32
F11	0.2	0.15	2500	118.25	119.05	38.85	38.98	.99	21.21
F12	0.2	0.35	2500	89.56	88.86	64.49	64.54	41.31	41.25
F13*	0.2	0.25	1750	136.64	135.44	60.	60.15	34.04	34.19
F14*	0.2	0.25	1750	135.64	135.44	60.19	6 5	34.14	34.19
F15*	0.2	0.25	1750	132.64	135.44	60.07	60.	34.54	34.19
F16*	0.2	0.25	1750	134.64	135.44	60.32	60.15	34.11	34.19
F17*	0.2	0.25	1750	137.64	135.44	60.16	60.15	34.13	34.19

Note: *Centre point.

The quadratic polynomial equation of PS represents CH (A) has a positive effect, STP (B) and stirring speed ( ) showed a negative effect on particle size. In the equation A, B, C, AB, A^2, B^2, C^2 are significant terms because the p-value <0.05 and significantly affect the particle size and factors AC and BC are found non-significant (P>0.05). The F-value is high due to noise (1475.86), indicates the model is significant (P<0.0001). The F-value and P-value of the lack of fit are 0.46, 0.7235 (P>0.05), represent the lack of fit are not significant, and it is good for a model (Table 4). The Predicted-R^2 (0.9972) is in reasonable agreement with the Adjusted-R^2 (0.9988). The adequate

Table 3 Statistical Model Summary for Different Kinetic Models Obtained from Design Expert Software

Response: Particle Si (Y_1)						
Source (Model)	R-Squared	Adjusted R^2	Predicted R-Squared	Std. Dev.	CV (%)	Remark
Linear	0.7582	0.7024	0.5248	24.93	—	—
2FI	0.8684	0.7894	0.4524	20.97	—	—
Quadratic	0.9994	0.9987	0.9972	1.58	1.06	Suggested
Entrapment Efficiency (Y_2)						
Linear	0.9087	0.8877	0.8165	373.55	—	—
2FI	0.9846	0.9754	0.9522	97.34	—	—
Quadratic	0.9999	0.9998	0.9992	1.61	0.22	Suggested
Drug Loading (Y_3)						
Linear	0.8811	0.8536	0.7515	280.96	—	—
2FI	0.8941	0.8305	0.4576	613.35	—	—
Quadratic	0.9996	0.9992	0.9970	3.35	0.66	Suggested

Figure 1 Actual and predicted response of independent variables on dependent variables.

precision is >4 (133.37) indicating adequate signal for the fitted model.

Effect on Encapsulation Efficiency (EE)

The %EE of GTM-CH-NPs was found in the range of 38.85% (F11) to 82.11% (F9) in Table 2. The 3D-plot was generated and represented the effect of independent variables on EE (Figure 2B). The increase in CH viscosity leads to electrostatic interaction between the NH_3^+ group of CH and PO_4^- of STP. It gives a decrease in the entrapment of GTM into NPs as well as less diffusion of GTM into the polymer matrix. But CH gave a less prominent effect than STP and stirring speed. STP concentration gives more prominent positive effect on EE, which means increasing the concentration increases the EE. It is due to more PO_4^- group available for cross-linking with NH_3^+ of CH. The more drug entrapped or diffused into the polymer matrix during cross-linking.[43] The stirring speed has a negative effect on EE but less prominent effect than STP. As the stirring speed increases, the break down of NPs takes place due to high shear force and leads to leaching of the drug from the matrix, resulted in less EE. The second order quadratic computer-generated polynomial equation of EE was given below.

$$EE\ (Y2) = +60.18 - 0.41 * A + 10.95 * B - 10.55 * C - 3.99 * A * B - 4.40 * A * C + 1.83 * B * C + 1.64 * A^2 + 2.06 * B^2 + 0.071 * C^2 \quad (2)$$

The positive and negative signs in the polynomial equation (Equation 2) represent the positive and negative effects of variables on EE. CH concentration (A) showed a negative effect on EE ie, increased the CH concentration decreases the EE. The quadratic model was found to be best fit. The F-value of 12136.03 suggested that model is significant.

Table 4 Analysis of Variance of Calculated Best Fit Quadratic Model for Responses

Model	Source	Particle Size (nm)	EE (%)	DL (%)
Quadratic	Sum of Squares	33415.25	2036.33	1130.62
	df	9	9	9
	Mean Square	3712.80	226.25	125.62
	F-Value	1475.86	12136.03	2498.55
	P-value, Prob> F	<0.0001	<0.0001	<0.0001
	Remark	Suggested, significant		
Lack of it				
Quadratic	Sum of Squares	4.5378	0.0974	0.1944
	df	3	3	3
	Mean Square	1.5126	0.0[illegible]4	0.0648
	F-Value	0.4628	3.92[illegible]	1.6465
	P-value, Prob> F	0.7235	0.1098	0.3136
	Remark	Suggested, not significant		
Residual				
Quadratic	Sum of Squares	17.609	[illegible]	0.3519
	df	7	7	7
	Mean Square	2.5156	0.0186	0.0502

The F and P-value of lack of fit of the quadratic model are 0.46 and 0.7225, indicates that the lack of fit was not significant which is good for a model. The Predicted R^2 (0.9992) is in reasonable agreement with Adjusted R^2 (0.9999) and adequate precision is >4 (1[illegible] indicated adequate signal.

Effect on Drug Load (DL)

The DL of GTM-CHNPs was found in the range of 20.99 (F11) to 52.53% (F10) as depicted in Table 2. The 3D-plot was generated and represented the effect of independent variables on DL (Figure 2C). In the case of CH (A), as the concentration increases the viscosity of the CH solution also increases. It gives a negative impact on crosslinking (gelling) between the NH_3^+ group and PO_4^- lead to decrease DL. STP (B) has a positive effect and stirring speed has a negative effect on DL. DL increases with increasing the STP concentration due to the presence of more binding sites (PO_4^-), which cross-linked with the NH_3^+ group of CH. The more drug diffused in the polymer matrix during cross-linking and resulted in increased DL, which agreed with the previously published research work.[42] The stirring speed (C) has a negative effect on DL, as the stirring speed increases the DL decrease. It is due to the breakdown of NPs with the high shear force and leaching of GTM from NPs. The positive and negative signs in the polynomial equation (Equation 3) represent the synergistic and antagonistic effects of variables on DL. The second order computer-generated quadratic polynomial equation for DL was given below-

$$\begin{aligned} \textit{Drug loading}\ (Y3) \ = & +34.19 - 0.74 * A + 9.00 * B \\ & - 6.56 * C + 0.76 * A * B \\ & - 1.43 * A * C + 1.03 * B \\ & * C - 4.14 * A^2 + 3.56 * B^2 \\ & + 0.035 * C^2 \end{aligned} \tag{3}$$

In this polynomial equation, the model terms A, B, C, AB, AC, BC, A^2, and B^2 are significant because its P-value was <0.05. The quadratic model was found to be the best fit model, and the F-value of 2498.55 suggested that the model is significant. The F-value and P-value of lack of fit were found to be 1.65 and 0.3136 (P>0.05) indicates not significant. The Predicted R^2 of 0.9970 is in reasonable agreement with the Adjusted R^2 of 0.9993 and adequate precision >4 (180.95) indicated model is desirable. It observed that the polynomial equation showed that CH has a direct negative effect.

Optimized Composition

The PS and PDI of GTM-CHNPs were found to be <200 nm and <0.5 indicates uniform size distribution. The particle size was found to be within acceptable size range, ie,

10 µm which is tolerable particle size for ophthalmic instillation.[35] The PS and PDI of GTM-CHNPopt (composition- CH 0.2%, STP 0.25%, and stirring speed 1750 rpm) were found to be 143.3 nm (Figure 3A) and 0.113±0.014. The zeta potential of GTM-CHNPopt was found positive and high, ie, 25.1 mV (Figure 3B), indicates that NPs dispersion is stable as well as non-aggregated. The EE and DL of GMT-CHNPopt were found to be 60.18 ±2.65% and 34.19±1.87%, respectively. The morphology of GMT-CHNPopt was further confirmed by TEM study and it showed spherical and smooth surface particles without aggregation (Figure 4).

Fourier Transform Infrared (FTIR)

The FTIR spectra of pure GTM and optimized GMT-CHNPopt were done for determined of compatibility between drug and excipients and spectra is depicted in Figure 5A. The IR spectra of GTM showed intense characteristic peak at 605.23 cm^{-1} (SO_2 band) and 2925.44 cm^{-1} due to alkyl groups (CH_2 and CH_3) asymmetric stretching. The most prominent peak at 1036.90 cm^{-1} due to amide group stretching confirmed the chemical structure GTM. The characteristic peak of alkyl group of CH in the spectra of GTM-CHNPopt overlaps to the alkyl group peak (CH_2 and CH_3 asymmetric stretching) of GTM. The same characteristic peaks of GTM present in the spectra of GTM-CHNPopt indicates that there is no interaction takes place between drug and polymer.

Thermal Behavior Study (DSC)

The thermal behavior of GTM and lyophilized GTM-CHNPopt was analyzed by DSC instrument (Figure 5B).

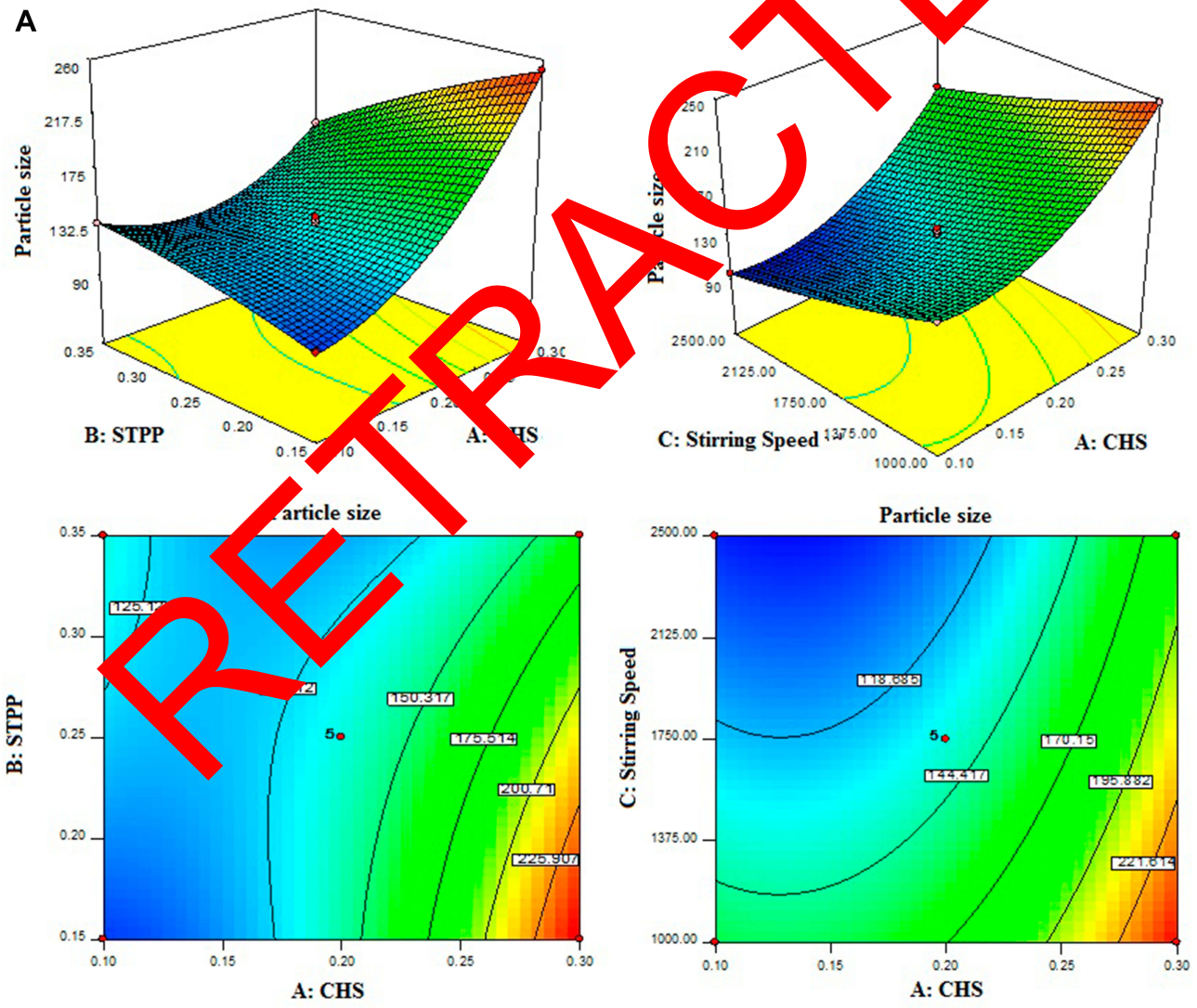

Figure 2 Continued.

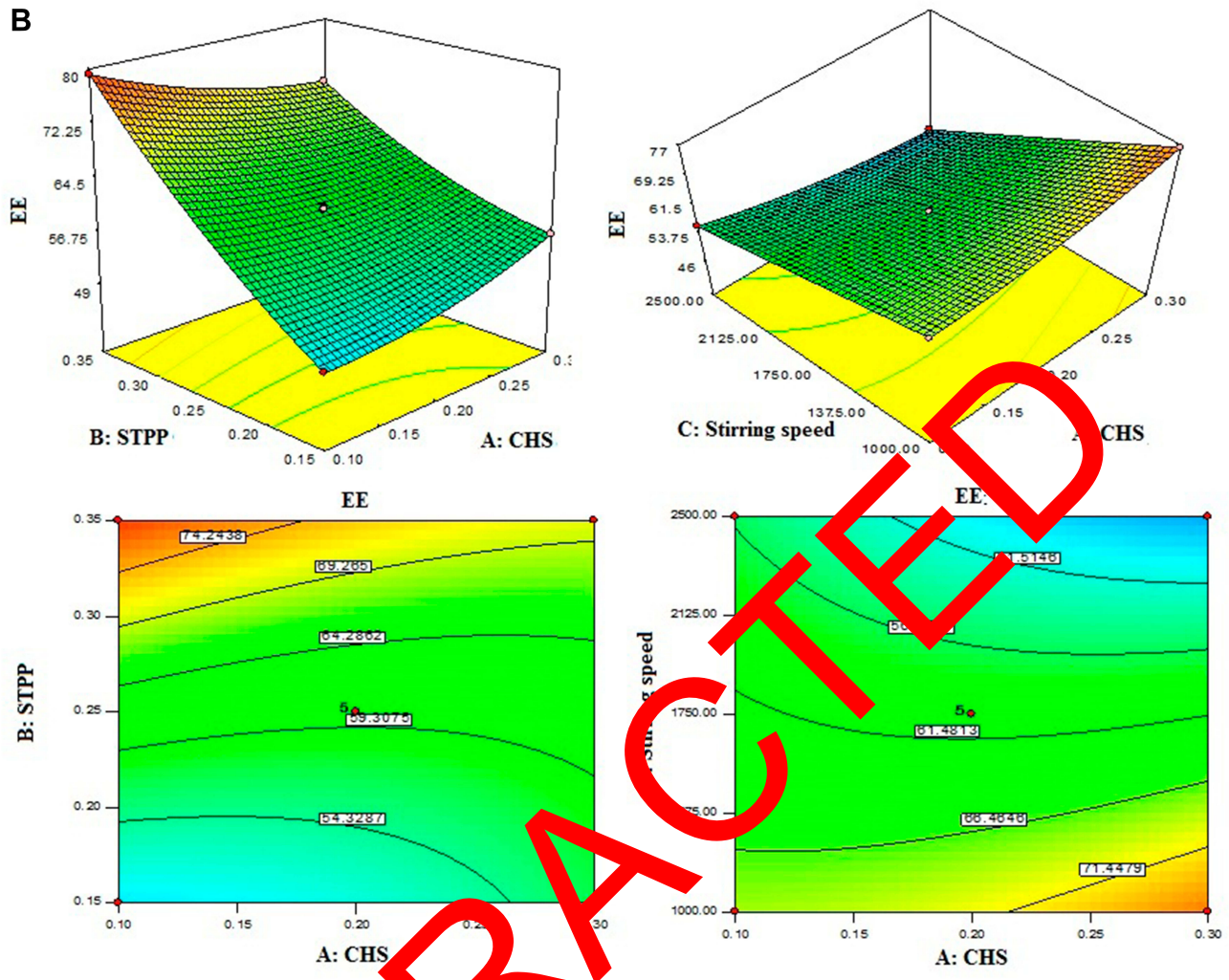

Figure 2 Continued.

GTM showed the characteristic endothermic thermal peak at its melting point of 244 °C. However, the lyophilized GTM-CHNPopt exhibited only a broad peak with a slight shift in the melting point. It indicates that GTM was encapsulated into the polymer matrix and it was further confirmed by XRD analysis.

X-Ray Diffraction Study (XRD)

The spectral analysis of pure GTM and lyophilized GTM-CHNPopt was performed to evaluate the crystallinity. The XRD spectra of GTM showed the intense characteristic peak at 2 theta value 38.0º (d-2.3660) and 44.2º (d-2.0474) indicates its crystallinity (Figure 6A). Moreover, lyophilized GTM-CHNPopt showed only the characteristic CH peak at 2 theta value 19.2º (d- 4.6189), which means GTM crystallinity has been reduced (Figure 6B). It indicates GTM was completely dissolved or encapsulated in chitosan and distributed in disordered form.[44]

Evaluation of GTM-CHNPopt Sol-Gel

Clarity and Optical Transmittance

The clarity is a very important parameter for ocular preparation because if any visible particle present it produced the irritation (inflammation). All the prepared sol-gel systems (NSG1 – NSG6) were found clear on visual observation as well as further confirmed by optical transmittance. The optical transmittance was found in the range of 93.34±0.34 to 97.76±0.28% (>90%) (Table 5). The clarity (optical transmittance) increased with an increase in carbopol concentration due to an increase in crosslinking density in the gel state.[45]

pH and Drug Content

The pH is an important parameter for ocular tolerability and quantification of gelling capacity. It was measured by digital pH meter and depicted in Table 5. The pH range was found in the range of 5.92±0.36 to 6.54±0.34 which is

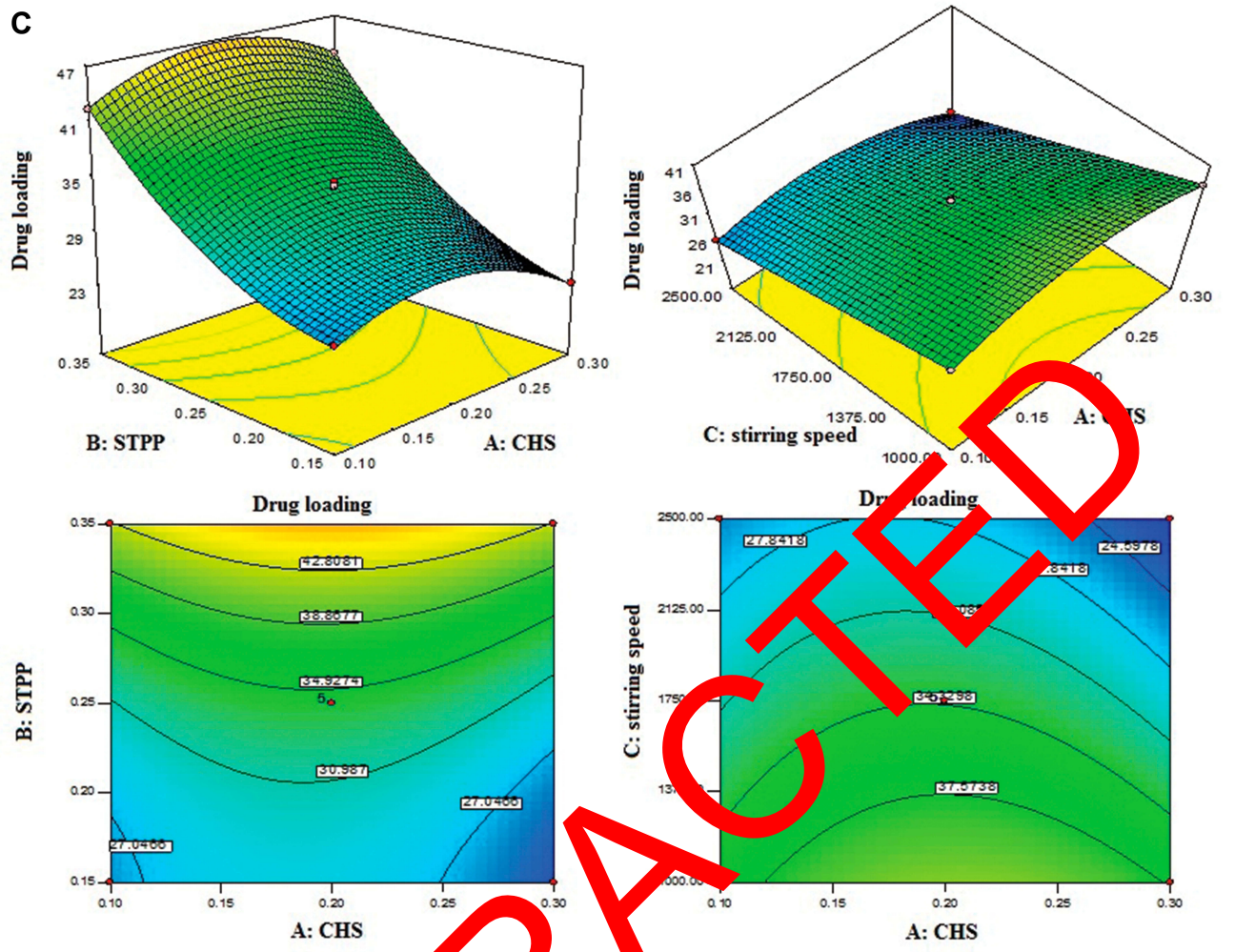

Figure 2 Effect of independent variables A, chitosan (CHS); B, sodium tripolyphosphate (STP); C, stirring speed on dependent variable ((**A**) size as Y_1), ((**B**) encapsulation efficiency as Y_2) and ((**C**) drug load as Y_3).

in the normal scale of ocular tolerance pH, ie, 5–7.5 as well as for gelling.[46] The drug content was found in the range of 97.24 ± 1.6% to 98.6 ± 2.06% confirming the homogeneity of the drug in developed formulation.

Gelling Strength

The gelling strength means speed (time) and stability of gelation on contact with physiological tear fluid pH. The viscosity of the solution should have optimum viscosity so it can be easily instilled into the eye and later converted to gel form. The gelling strength of prepared sol-gel formulations was evaluated and marked with a negative and positive sign (Table 5). The formulation NSG1 graded with negative sign means no gelation whereas, positive sign found for formulations NSG2-NSG6. It indicates different gelation strength. The NSG5 and NSG6 have shown the highest gelation strength (gelation time 10sec, remain for a long time). NSG6 has shown greater gelation strength than NSG5. The formulations NSG3 and NSG4 have shown gelation time of 25 sec but have shown lesser stability (++, dissolve within few hours). NSG2 forms gel in 26 sec but immediately disappeared (in few minutes) due to a low concentration of carbopol. The gelation takes place by efficient ionization of carbopol functional group due to increase in the pH. When the pH of formulation increases in contact with physiological fluid, the electrostatic repulsion between adjacent –COOH group increases. Simultaneously the extension of polymer network takes place. Moreover, tough gel formation may be due to the hydrophobic nature of carbopol, leads a formation of interlinked block aggregation network.[47] It was observed that on increasing the carbopol concentration the gelling strength was increased (Table 5). The GTM-CHNPopt sol-gel (NSG5) showed the good gelation strength at

Figure 3 Particle size (**A**) and Zeta potential (**B**) of optimized gentamycin chitosan nanoparticles (GTM-CHNPopt).

carbopol concentration of 4.5%. Based on physiochemical characteristic optimized hydrogel (NSG5) selected as optimized formulation and used for further study.

Viscosity

The viscosity of the optimized hydrogel (NSG5) was evaluated by brook filled viscometer. It is a very important

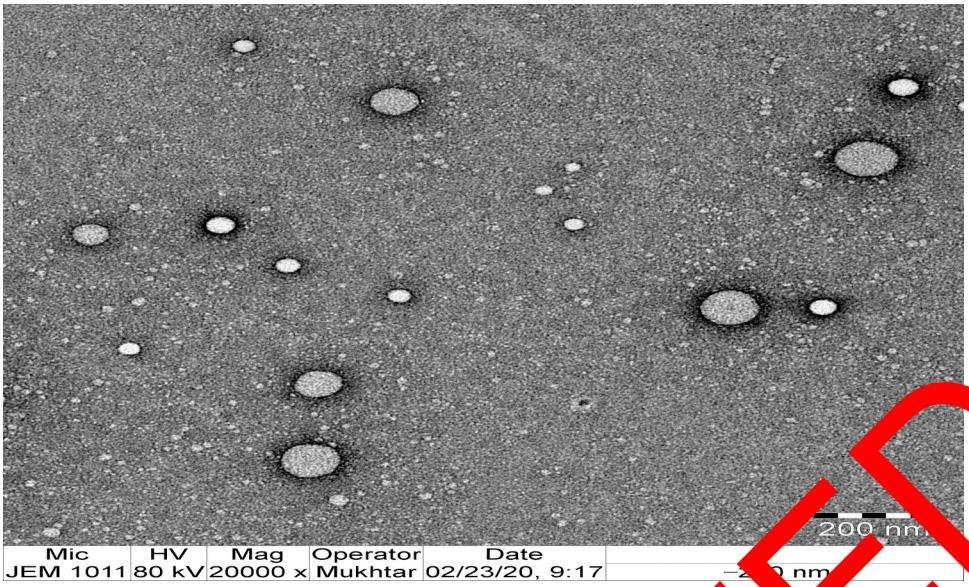

Figure 4 Transmission electron microscopic image of optimized gentamycin chitosan nanoparticles (GTM-CHNPopt).

parameter for increasing the corneal contact time (residence time). The formulation having the optimum viscosity (0.03 to 0.14S^{-1}) will not clear by eyelid blinking as well as with tear fluid turnover.[48] The viscosity also not disturbs the pseudoplastic behavior of tear film in the ey The rheological behavior of GTM-CHNPopt sol-gel (NS 5) depicted in Figure 7. It clearly shows that there is no more effect of shear stress on the rate of shear (sol system) means viscosity of the sol system not decreases significantly on increasing the force. On the other hand, the rate of share significantly changes (viscosity decreases) on increasing the shear stress, and the results indicate the pseudoplastic characteristic of in-situ gel systems. This behavior of the formulation would not hamper the blinking as well as patient compliance.[49]

Isotonicity Study

The isotonicity study of GTM-CHNPopt sol-gel (NSG5) was performed using the blood. Figure 8 shows that there

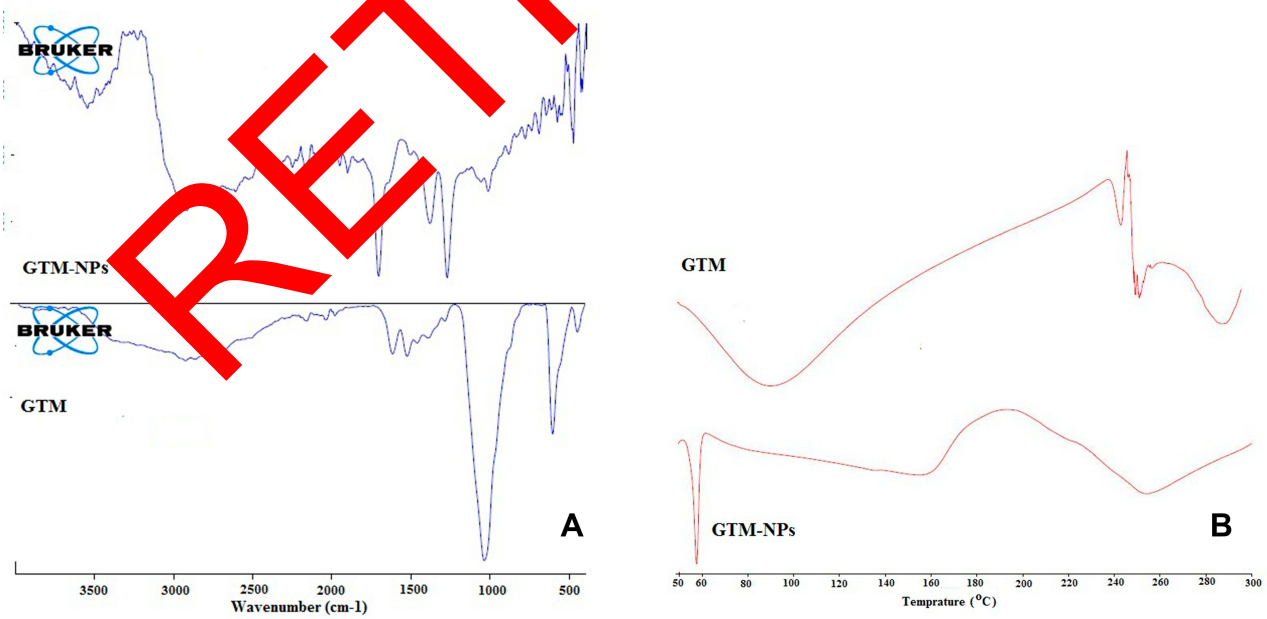

Figure 5 IR (**A**) and DSC (**B**) of GTM and optimized gentamycin chitosan nanoparticles (GTM-CHNPopt).

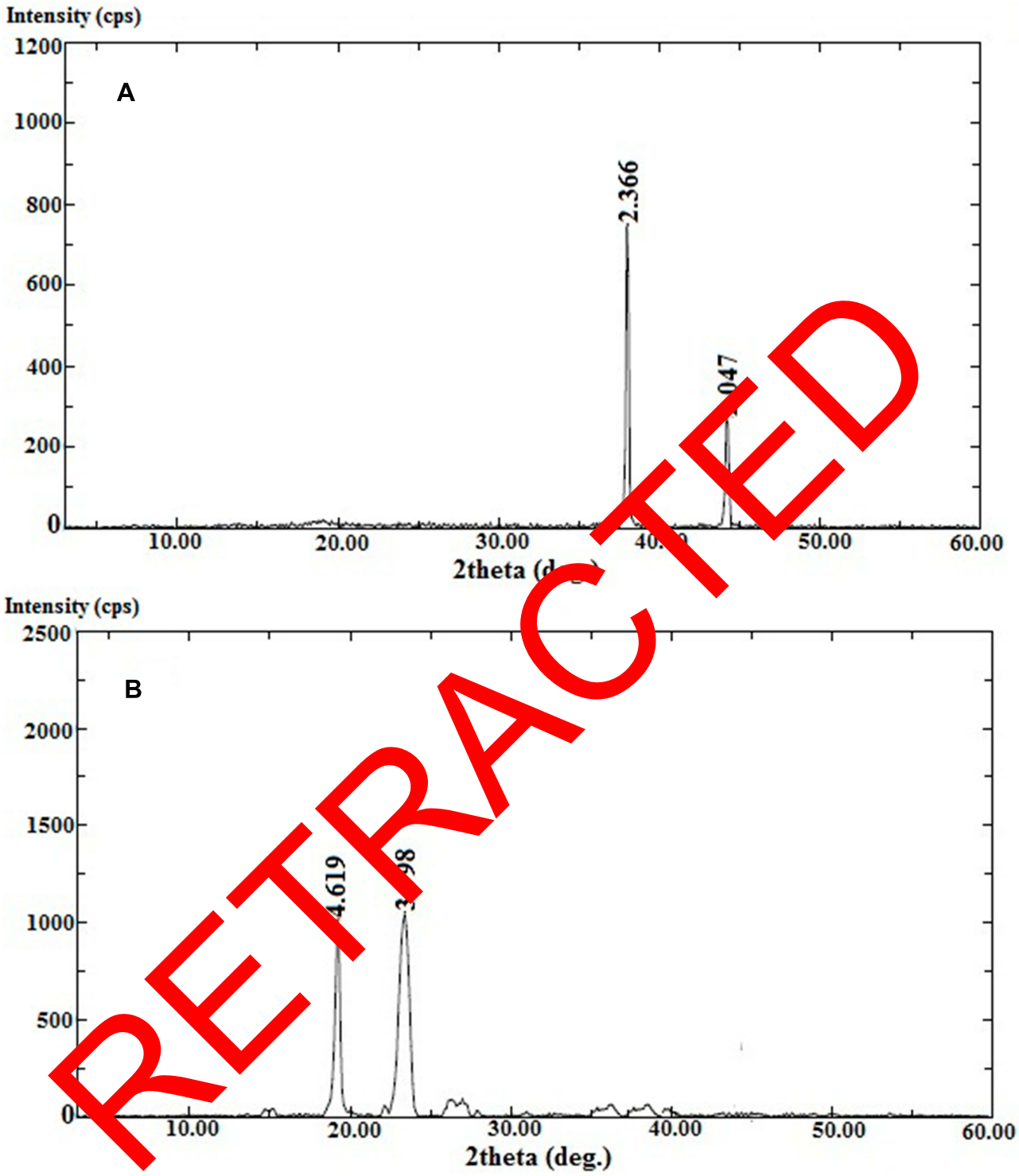

Figure 6 XRD of **(A).** GTM and **(B).** optimized gentamycin chitosan nanoparticles (GTM-CHNPopt).

is no any RBC ruptured after the addition of sol-gel (NSG5) and control (0.9% sodium chloride solution) in blood. It indicates that control (0.9% sodium chloride solution) was found to be isotonic and safe to blood.

Drug Release Study

The drug release study of GTM-CHNPopt, GTM-CHNPopt sol-gel (NSG5), and gentamycin eye drops (Gentacin) were performed and depicted in Figure 9. The

Table 5 Composition and Evaluation of GTM Nanoparticulate Sol-Gel

Formulation Code	Carbopol (%)	pH	Optical Transmittance (%)	Drug Content	Gelling Strength
NSG1	0.15	6.54±0.34	93.34±0.34	98.23±2.56	–
NSG2	0.2	6.43±0.54	94.56±0.54	97.45±1.76	+
NSG3	0.3	6.24±0.14	94.87±0.76	98.67±2.06	++
NSG4	0.4	6.16±0.25	96.34±0.32	96.87±2.65	++
NSG5	0.45	6.02±0.75	97.15±0.43	98.12±1.98	+++
NSG6	5	5.92±0.36	97.76±0.28	97.24±1.65	++++

Note: Each formulation contains GTM 0.3 %, CH 0.2%, TPP 0.25%, Stirring speed 1750rpm.

cumulative release profile showed that the marketed GTM eye drops releases approx 99% of GTM in 4h whereas GTM-CHNPopt showed 70.59±1.31% and GTM-CHNPopt sol-gel (NSG5) showed 58.99±1.28% release in 12h. There was a highly significant ($p<0.05$) difference in the release was observed in the [illegible] formulation with the marketed eye drop. Both the formulation showed slow drug release pattern due to the entrapment of GTM in chitosan polymer and gel matrix. The significant ($p<0.05$) difference in the release was also observed in GTM-CHNPopt and GTM-CHNPopt sol-gel (NSG5). The formulation GTM-CHNPopt sol-gel (NSG5) showed more slow and prolonged release behavior than the GTM-CHNPopt. The initial burst release was found in the first two hours then the slow release was observed. GTM-CHNPopt sol-gel (NSG5) showed a more sustained (prolonged) release of GTM because the first loosening of carbopol polymer takes place with the influence of pH and forms gel matrix. The drug first diffuses from the NSG5 in gel then diffused from the polymer gel matrix in dissolution medium.[50,51] The possible release pattern from the formulation, the release data were evaluated to check the goodness of fit for zero-order release kinetic, First-order release kinetic, Higuchi's matrix, and

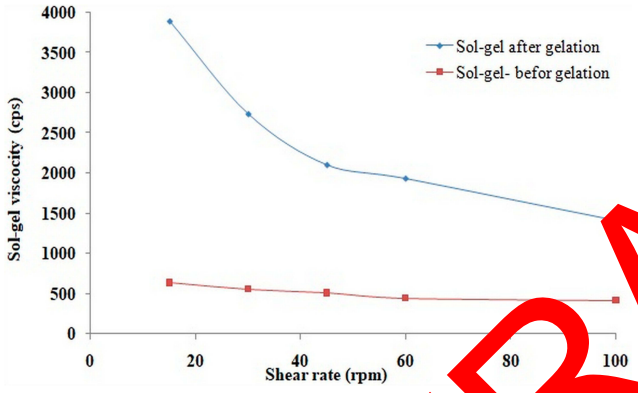

Figure 7 Viscocity of optimized gentamycin chitosan nanoparticle sol-gel (NSG5).

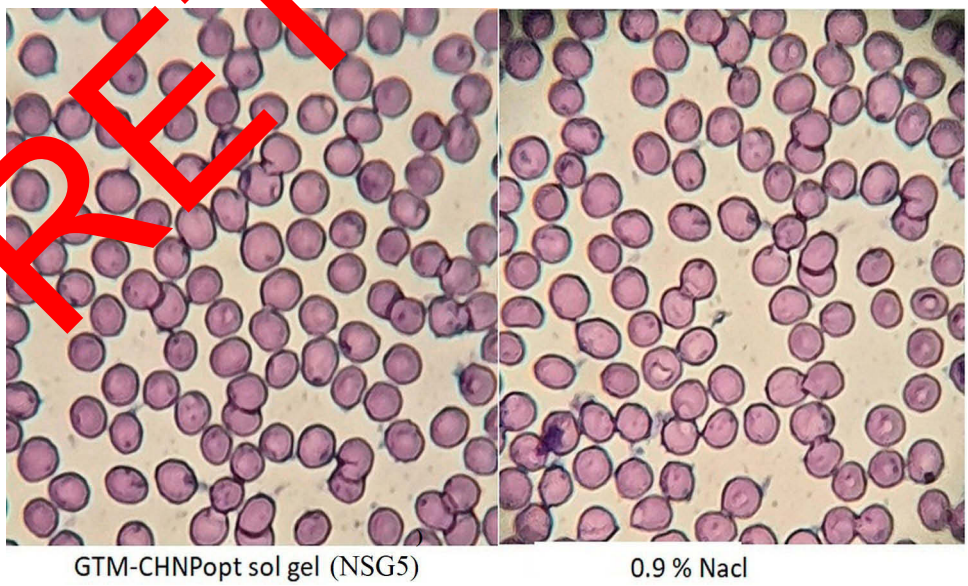

Figure 8 Isotonicity image of treated blood with optimized gentamycin chitosan nanoparticles sol-gel (GTM-CHNPopt sol-gel, NSG5) and control (0.9% NaCl).

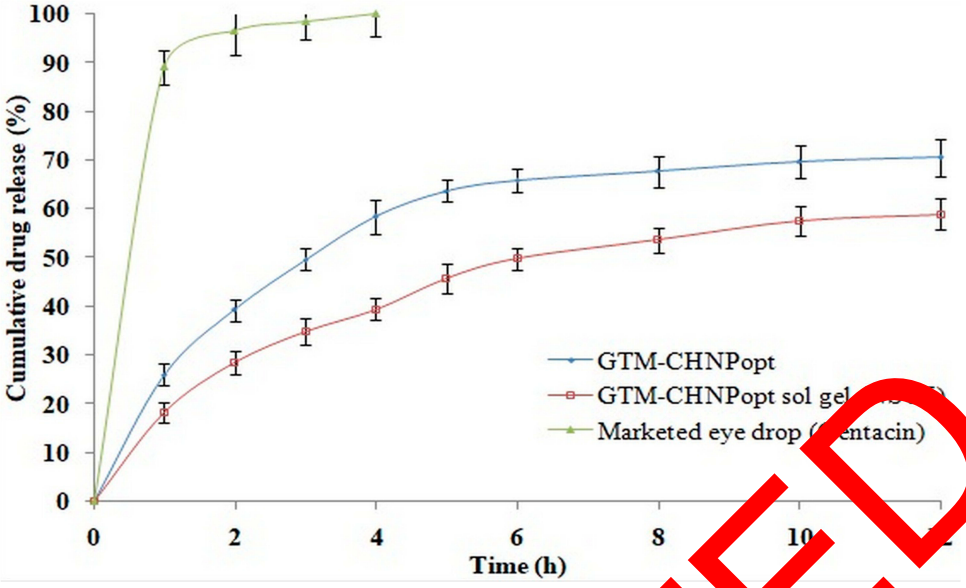

Figure 9 Comparative drug release profile of optimized gentamycin chitosan nanoparticles (GTM-CHN pt), optimized ntamyci hitosan nanoparticles sol-gel (GTM-CHNPopt sol-gel, NSG5) and marketed eye drop (Gentacin). .

Korsmeyer–Peppas model. The goodness of fit was evaluated by R^2 (correlation coefficient) values. The model showing the highest value was considered as the best model for release kinetic. The data exhibited the zero-order release model (0.994) is the best fit model.

Mucosdhesive Strength

The mucoadhesive strength of GTM HNPo sol-g (NSG5) was analyzed and found to be 065.2 ^{2}. This force is approx 7-fold more than the s r force of tear film exerted during the blinki 50 dyne/cm . The high mucoadhesive strength is due to t combined effect of chitosan (mucoadhesiv) as well as car ol 974P (gelling agent). The high m coadhe e strength indicates that the formulation will stay for a longer time on corneal tissue and not eliminated by tear fluid turn over as well as normal blinking.[25]

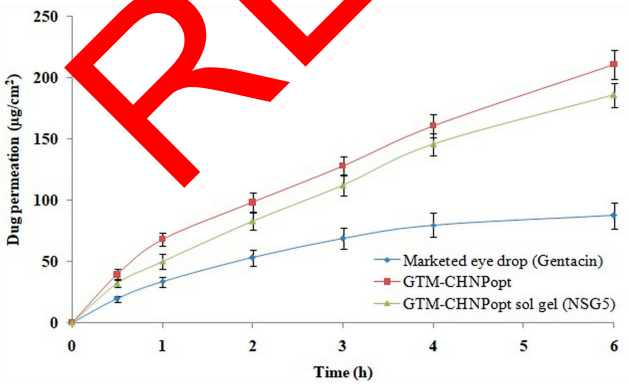

Figure 10 Comparative drug permeation profile of optimized gentamycin chitosan nanoparticles (GTM-CHNPopt), optimized gentamycin chitosan nanoparticles sol-gel (GTM-CHNPopt sol-gel, NSG5) and marketed eye drop (Gentacin).

Co l ermeation Study

The permeation study of GTM-CHNPopt, GTM-CHNPopt sol-gel (NSG5), and marketed eye drop (Gentacin) were performed on excised goat cornea and depicted in Figure 10. The marketed eye drop exhibited 87.29±2.34 µg/cm^2 (29.09 %) permeation in 6h. GTM-CHNPopt and GTM-CHNPopt sol-gel (NSG5) showed 210.62 µg/cm^2 (70.20 %) and 185.64 µg/cm^2 (61.88%) permeation, respectively. Both the formulation showed significant enhanced permeation ($P<0.0001$) as compared to marketed eye drop, whereas the difference between GTM-CHNPopt and GTM-CHNPopt sol-gel (NSG5) was significant ($P<0.05$). The permeation enhancement was found to be 2.41, and 2.12 fold higher than the marketed eye drop. The high corneal permeation is due to enhanced bioadhesion and penetration enhancing property of CH as well as the gelling property of corbopol. A similar type of finding was observed for ketoconazole nanoparticulate in situ gel[25] and dorzolamide HCl in-situ gel.[52] Moreover, GTM-CHNPopt exhibited high corneal permeation than GTM-CHNPopt sol-gel (NSG5). It indicates that the inclusion of GTM-CHNPopt into the carbopol polymer network slowed down the permeation. The flux of GTM-CHNPopt, GTM-CHNPopt sol-gel (NSG5), and marketed eye drop was calculated and found to be 27.10 µg/cm^2/h, 23.94 µg/cm^2/h and 5.89 µg/cm^2/h, respectively.

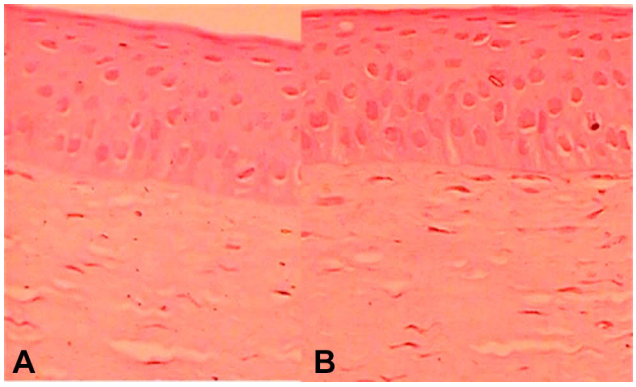

Figure 11 Comparative histopathology image of **(A).** optimized gentamycin chitosan nanoparticles sol-gel (GTM-CHNPopt sol -gel, NSG5) and **(B).** control (0.9% NaCl) - treated cornea.

Corneal Hydration Study

The corneal hydration test of GTM-CHNPopt sol-gel (NSG5) was performed by using goat cornea. The hydration was found to be 78.34±1.15% for GTM-CHNPopt sol-gel (NSG5). The value was found within the normal value of 75–80%.[39] It indicates that the formulation did not show any damage to the corneal tissue (epithelium or endothelium). This effect of formulation on goat cornea was further confirmed by histopathology.

Histopathological Study

Histopathological examination was performed f[illegible]ervation of internal damage or alteration in th[illegible] cornea after treatment with GTM-CHNPopt sol-gel (N[illegible]G5) v[illegible] control (0.9% NaCl). Figure 11A–B indicate[illegible] that there was no alteration in the anatomical as well as the morphological structure of GTM-CHNPopt sol-gel (NSG5)-treated cornea as compared to normal saline (0.9% NaCl, control). The results confirmed that GTM-CHNPopt sol-gel (NSG5) has not shown any toxicity and found safe for ocular administration. The result was agreed with previously published work ie, polymeric (CH and flaxseed gum) nanoparticulated delivery of timolol maleate[14] and gatifloxacin[19] for ocular administration.

Ocular Irritation

HET-CAM assessment is a well-est[illegible]lished [illegible]-vitro parameter for determination of ocul[illegible] tolerability [illegible]f the test sample,[53] and scores are depic[illegible]ed in [illegible]ble 6. It gi[illegible]es similar toxicity results like rabbit [illegible]njunctiva be[illegible]use [illegible]ick embryo has complete veins an[illegible] capillari[illegible]s.[37,54] Th[illegible] normal saline (0.9% NaCl, negati[illegible]e co[illegible]l and GT[illegible]-CHNPopt sol-gel (NSG5) showed [illegible]ero scores. [illegible]here [illegible] no sign of damage to blood vessel[illegible]ertili[illegible]d hen egg a[illegible]er incubation at appropriate condition (no heme[illegible]hage, nonirritant) (Figure 12). The positi[illegible]e control (0.1M NaO[illegible]H) showed the score 12.66 means hem[illegible]rrhage, vasc[illegible]ar lysis, and coagulation (served irritant). The [illegible]ore of HET[illegible]AM for GTM-CHNPopt sol-gel (NSG5) confirm[illegible]d that [illegible]is safe for ocular administration.

A[illegible]obial Assessment

The cup-plate method was employed for antimicrobial susceptibility of developed formulation against *S. aureus* and *E. coli*. The zone of inhibition (ZOI) for GTM-CHNPopt, GTM-CHNPopt sol-gel (NSG5), and marketed

Table 6 HET-CAM Test Score [illegible]r Ocular Irri[illegible]tion

Formulation	[illegible]gg	Effect	Scoring Time (min)				Overall Score
			0	0.5	2	5	
Normal saline (negative co[illegible]l)	Egg Egg 2 [illegible]	Vascular lysis Haemorrhage Coagulation	0 0 0	0 0 0	0 0 0	0 0 0	0
	Mean score		0	0	0	0	
0.1M NaOH (positive control)	Egg 1 Egg 2 Egg 3	Vascular lysis Haemorrhage Coagulation	0 0 0	5 7 2	3 5 7	1 3 5	12.66
	Mean score		0	4.66	5	3	
GNM-CHNPopt (NSG5)	Egg 1 Egg 2 Egg 3	Vascular lysis Haemorrhage Coagulation	0 0 0	0 0 0	0 0 0	0 0 0	0
	Mean score		0	0	0	0	

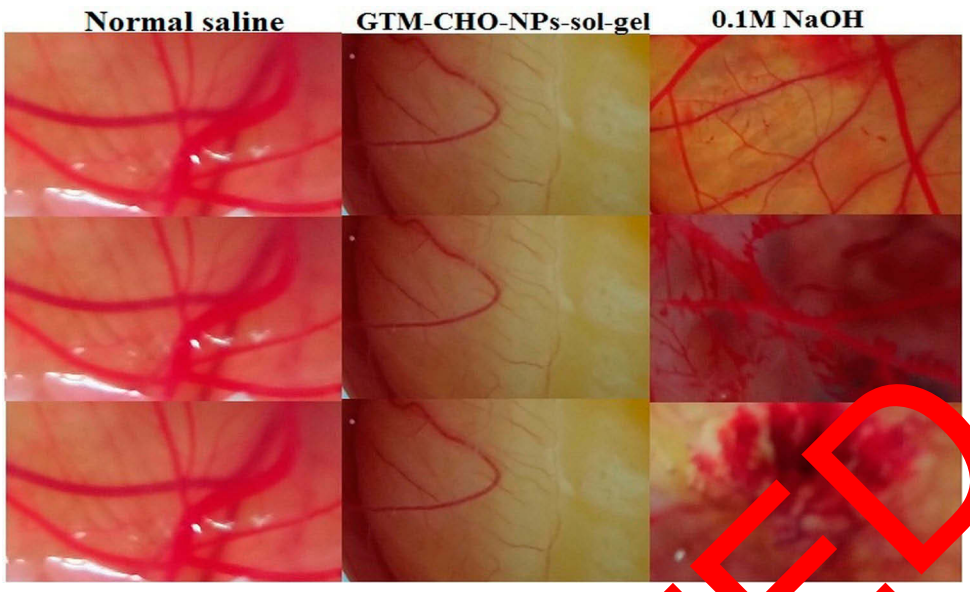

Figure 12 Comparative HET CAM image of optimized gentamycin chitosan nanoparticles sol-gel (GTM-CHNPopt sol-gel (NSG5), 1 M NaOH (positive control) and normal saline (control)-treated egg.

eye drop (Gentacin) was found to be 12.11 ± 1.12 mm, 15.78 ± 0.58 mm and 11.23 ± 1.36 mm against *S. aureus*. Further, the formulations were tested against the microorganism *E. coli*, and ZOI was found 11.54 ± 0.98 mm, 14.32 ± 0.32 mm, and 10.46 ± 0.29 mm. There was no zone of inhibition was observed for the normal saline solution (0.9%, normal control). GTM-CHNPopt sol-gel (NSG5) exhibited higher ZOI than GTM-CHNPopt and marketed eye drop. The significant-high ZOI value due to the sustained release of GTM and could assist the minimum inhibitory concentration (MIC) of GTM for the extended (prolonged) period.

Conclusion

Chitosan nanoparticles of gentamycin was successfully prepared and optimized by box-Behnken statistical design. The prepared GTM-CHNPs showed the particle size within <200 nm with positive zeta-potential. The optimized GTM-CHNPopt sol-gel (NSG5) exhibited high mucoadhesive strength (1065.2 dyne/cm^2) due to the presence of chitosan as well as carbopol as a mucoadhesive polymer. It exhibits significant ($p<0.05$) sustained release profile as well as corneal permeation (185.64 µg/cm^2, 61.88%) as compared to marketed eye drop (87.29 µg/cm^2, 29.09%). Finally, the significant enhanced ($p<0.05$) antimicrobial activity was found than the marketed eye drop. Our finding revealed the chitosan nanoparticles laden sol-to-gel can be successfully used for the treatment of bacterial conjunctivitis.

Disclosure

The authors report no conflicts of interest in this work.

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