THERMOSENSITIVE SOL-GEL SYSTEM CONTAINING VANCOMYCIN-LOADED THIOMERIC NANOPARTICLES FOR ENHANCED PERMEATION ACROSS NASAL MEMBRANE

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During oral delivery, vancomycin (VAN) is prone to enzymatic degradation and therefore, an intra-nasal highly mucoadhesive system, having enhanced nasal residence time, is considered in this study as an alternative way for vancomycin delivery. It was aimed to develop highly mucoadhesive *in-situ* gelling nanoparticles (NPs) by combining two mucoadhesive technologies: *i.e.* thiolation and thermosetters for nasal delivery of vancomycin. For this purpose, thiolated chitosan was synthesized, and the degree of thiolation was determined *via* Ellman's test. In the next step, vancomycin-loaded nanoparticles based on chitosan (CS-NP) and thiolated chitosan (TCS-NP-PL) were prepared and coated with 20% (w/w) solution of pluronic to form thiolated thermosensitive *in-situ* gelling systems. Nanoparticles were assessed for particle size (PS), zeta potential (ZP), morphology, gelling time, viscosity, swelling index, and gelling temperature. Likewise, CS-NP and TCS-NP-PL were evaluated for mucoadhesion, *ex-vivo* drug permeation across the nasal membrane, and *in-vitro* drug release. TCS-NP-PL showed gelation at 32 ± 0.5 °C in 45 ± 2 seconds. TCS-NP-PL showed a maximum mucoadhesion of up to 210 min. *In vitro* drug release studies revealed that CS-NP, TCS-NPs, and TCS-NPs-PL showed 55%, 54.6%, and 52.1% drug release, respectively, during 24 h. TCS-NP-PL exhibited 6.40-fold enhanced permeation across nasal mucosa, as compared to free drugs. TCS-NP-PL significantly increased nasal residence time and permeability of VAN, supporting the concept of combined use of thiolation and thermo *in-situ* gelling system for prolonged contact with nasal mucosa, providing increased transport across nasal membrane.

Keywords: thiomer, biomolecules, *in-situ* gelling, nano-carriers, chitosan, enhanced permeation

INTRODUCTION

Vancomycin (VAN) is a biomolecule-based antibiotic used to treat serious infections in patients either having serious hypersensitivity or resistance to β-lactam antibiotics, *e.g.*, cephalosporins and penicillins. On the one hand, intravenous administration of vancomycin is associated with serious side effects, including hyperallergic shocks and convulsions, and on the other, oral administration of VAN is limited because of acidic and enzymatic degradation in the gastrointestinal tract, resulting in low

epithelial permeability, and thus, making it impossible to use as the first line of defense against resistant strains in patients having non-compliance with the intravenous route.[1] Keeping this in view, the nasal route can be a possible route of administration for systemic delivery of VAN.[2]

The intranasal route shows many advantages, such as non-invasive, painless drug delivery, without the need for sterile preparation, and lower toxic adverse effects, compared to parenteral and oral routes.[3] The presence of numerous microvilli delivers extensive surface area for the absorption of drugs; hence, relatively lower doses of drugs are required to achieve a therapeutic response when delivered through the nose.[4] Furthermore, this mode of administration bypasses the hepatic first-pass metabolism of drugs.[5,6] Therefore, the intranasal delivery of VAN seems to be a suitable strategy to avoid the aforesaid drawback related to the oral and parenteral routes. Despite all of these advantages, the nasal canal is prone to mechanical stress because of the movement of the nasal villi that leads to sneezing and any applied formulation can flow out. To overcome this shortcoming, a highly mucoadhesive delivery system is needed that can withstand such abruptive mechanical stress.[6] Thiolated polymers are known for their highly mucoadhesive ability, while thermosensitive polymers can form a mucoadhesive gel at body temperature. To achieve a high degree of mucoadhesion, especially for the nasal route, a combination of two technologies: *i.e.* thiolation and thermosensitive gelling, will be a promising approach. Moreover, nanosystems have already proved their potential for the systemic delivery of peptide-based drugs *via* the nasal route due to the increased contact time like insulin and exenatide.[7] Therefore, it was aimed to prepare nanoparticles of thiolated chitosan and suspend them in the polymeric solution of pluronic to form a thiolated thermosensitive system for nasal delivery of VAN.[8]

For this purpose, thiolated chitosan was prepared and the degree of thiolation was determined by Ellman's reagent. The formed nanoparticulate systems were studied for their size, morphology, structure *via* FTIR, swelling index, and drug loading efficiency. The developed nanoparticles were suspended in a 20% (w/w) polymeric solution of pluronic. This system was further studied for its mucoadhession, drug release, and *in-vivo* pharmacokinetics.

EXPERIMENTAL

Reagents

Vancomycin hydrochloride was gifted from Remington Pharma. Pluronic F-127, also known as poloxamer 407, was acquired from Sigma Aldrich, Germany. LMW chitosan (CS) (DD 85% and viscosity of 50-100mPa s), hydroxylamine, thioglycolic acid 99%, EDAC (1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide), phosphate buffer, dialysis membrane (cut-off value 12-14 KD), acetic acid and hydrochloric acid were also purchased from Sigma Aldrich, Germany.

Synthesis of thiolated chitosan

The ionic gelation method was used to prepare thiolated chitosan from chitosan. For this purpose, 1 g of chitosan was dissolved in 1% (w/v) acetic acid solution under continuous stirring for 30 minutes at 600 rpm, 40 °C. To attach the thiol group, 1 g of thioglycolic acid (TGA) was added to the chitosan solution and dissolved completely. After dissolution, 50 mM of EDAC and 50 mM of hydroxylamine were added by adjusting the pH to 5 using 1M HCl solution. The resulting solution was further stirred for 3 hours to complete the reaction.[9] Afterward, it was dialyzed for 24 h against 5 mM HCl and 24 h with 1% NaCl and 5 mM HCl.[10] In the end, the purified thiolated chitosan (TCS) was lyophilized (Scanvac Coolsafe, 110-4) and stored at 4 °C for further processing.

Thiol content determination

Ellman's reagent was prepared by dissolving 2.5 mg of TCS in 250 µL of deionized water and used in spectrophotometric analysis of chitosan to determine the linked thiol groups. To this solution, 500 µL Ellman's reagent and 250 µL of phosphate buffer solution (pH 8.0) were added. The sample was kept at ambient temperature for 3 h, then, the supernatant was separated and poured onto a 96-well plate. A microtiter plate reader was used to measure absorbance at 430 nm. The thiol groups of the prepared polymer grafts were determined by using a standard TGA solution.[11]

Disulphide bonds determination

First, 2.5 mg of the polymer was added to 650 µL of phosphate buffer (pH 6.8) and 350 µL of deionized water. The resultant solution was left for 30 minutes, followed by the addition of sodium borohydride solution (4% w/v). After incubation at 37 °C for 1 h, the remaining sodium borohydride was decomposed by adding 200 µL of 5.0 M HCl. The resultant solution was neutralized with phosphate buffer (pH 8.5) and 100 µL of Ellman's reagent was added. After 1 h, a microtiter-plate reader was used to determine the free thiol group by shifting 300 µL of aliquot onto the microplate and analyzing the absorbance at 430 nm. Free thiol groups were determined by subtracting grafted thiol groups from total thiol groups that were present inactive on the polymer.[12]

Preparation of drug-loaded polymeric nanoparticles

The polymeric nanoparticles were prepared by the ion gelation technique, as presented in Table 1. Briefly, a 1% acetic acid solution was used to prepare 0.2% w/v solution of thiolated chitosan and chitosan separately to prepare two different nanoformulations based on thiolated chitosan and chitosan. The prepared mixture of 20 mg vancomycin (VAN) was added in each of these, followed by a dropwise addition of 0.2% w/v aqueous solution of tri-polyphosphate at the rate of 2 mL per minute, with continous stirring at 600 rpm. A formulation without VAN was prepared by the same method. All formulations were centrifuged at 4 °C for 60 minutes at 15000 rpm, followed by re-dispersion in deionized water, freezing and lyophilizing. The lyophilized NP was then stored at 4 °C until further use.[13]

Table 1
Composition of the developed nanoformulations

Formulation	0.2% w/v CS (mL)	0.2% w/v TCS (mL)	0.2% w/v TPP (mL)	VAN (mg)
Blank CS-NP	5	-	1.35	-
Blank TCS-NP	-	5	1.55	
CS-NP	5	-	1.35	20
TCS-NP	-	5	1.55	20

Blank CS-NP = chitosan based nanoparticles without vancomycin; Blank TCS-NP = thiolated chitosan based nanoparticles without vancomycin; CS-NP = chitosan nanoparticles with vancomycin; TCS-NP = thiolated chitosan nanoparticles with vancomycin

Physical characterization

Drug loading (DL) capacity and drug entrapment efficiency (EE)

EE (%) was calculated by the indirect ultracentrifugation method, where 10 mL of VAN-loaded NP were centrifuged at 4000 rpm for about 45 minutes. After centrifugation, the supernatant was collected, and VAN concentration was assessed by UV-Vis spectroscopy at 280 nm by using a validated method reported previously.[14] The entrapped drug was calculated by Equation (1), using the variations between the free drug in the supernatant and the initial drug content:[15]

$$\%EE = \frac{W_t - W_f}{W_t} \times 100 \quad (1)$$

where the free non-entrapped drug is denoted as W_f and the total added drug is denoted as W_t.

DL (%) was calculated as follows. Briefly, a certain weight of drug-loaded nanoparticles was dissolved in ethanol. Afterward, the quantity of VAN in solution was determined by UV-visible spectroscopy at 280 nm. Every sample was assessed three times by external standardization. The drug loading of nanoparticles was calculated according to Equation (2):[15]

$$\%DL = \frac{W_e}{W_n} \times 100 \quad (2)$$

where W_e is the amount of entrapped drug, W_n is the total weight of drug-loaded nanoparticles.

Zeta potential and particle size distribution

Zeta potential (ZP), mean particle size, and polydispersity index (PDI) of VAN-loaded chitosan and thiolated chitosan NPs were calculated by a Malvern Zetasizer (HAS 3000; Malvern Instruments Malvern, UK). The sample was prediluted with double distilled water (1:200) to minimize the iridescence and to adjust the conductivity. Measurements were done at 25 °C.[16]

Scanning electron microscopy

A scanning electron microscope (JSM-6490A, Tokyo Japan) was used to analyze structural morphology, mean diameter, and surface characteristics of the prepared nanoparticles at magnifications of ×250 and ×100. The samples were diluted 10-fold, air-dried, and placed onto a glass slide for analysis.[17]

Fourier transform infrared spectroscopy

The CS, TCS, VAN, and TCS-NP samples underwent FTIR analysis using a Bruker FTIR instrument (Tensor 27 series; Bruker Co., Germany). Before analysis, the instrument underwent vacuum drying to ensure the accuracy of the analysis. Absorption spectra were acquired within a frequency range of 500-4000 cm^{-1}, with a resolution of 4 cm^{-1}.[18]

Swelling studies

Swelling studies were carried out to assess the swelling index of CS-NPs and TCS-NP in phosphate buffer. The weighed amount of dried NPs was compressed in tablet form at a pressure of 1000 psi for 2 minutes. The compressed tablets were put in 30 mL of phosphate buffer at room temperature. The same compression parameters were used for all formulations studied. At different time intervals, the tablet was removed, excessive surface water was removed with the tip of filter paper, and the tablet was weighed again. The swelling index was determined by

calculating the weight change according to Equation (3):[19]

$$\text{Swelling index} = \frac{W_s - W_d}{W_d} \times 100 \quad (3)$$

Preparation of pluronic Sol-Gel

Sol-Gel was formulated using thermo-reversible Pluronic F-127 by a cold technique. 20% w/w of Pluronic F-127 solution was prepared by slowly adding 250 mg of polymer in 25 mL of cold deionized water. The solution was gently stirred with a magnetic stirrer. This gentle mixing and slow addition resulted in hydrated flakes remaining on the surface. Therefore, the rate of solution was improved. The container was placed in a refrigerator at 4 °C overnight to form a complete solution and kept in the refrigerator until further use.[20]

Evaluation of thermosensitive gelling behavior

The test tubes containing the prepared formulation were placed in a water bath at 4 °C, and gelation temperature was determined at the rate of 1 °C rise in temperature every 2 minutes. The temperature where there was no flow of formulation upon tilting the test tube at 90° was considered the endpoint.[21]

Rheological analysis

The prepared gel formulations were evaluated in terms of change in their viscosities with temperature using a Brookfield viscometer rheometer (Model RVDVE 330, Brookfield Eng. Lab, USA) at 30 °C (gelation temperature) and 35 °C.[20]

Preparation of drug-loaded pluronic gel of polymeric nanoparticles

The thermo-reversible Pluronic gel loaded with TCS-NP nanoparticles was developed by the cold technique, as reported by Schmolka *et al*.[22] 100 mg of TCS-NP were suspended in 1 mL of 20% w/w of pluronic solution and mixed under constant stirring. Afterward, the resulting mixture was sonicated for good distribution of TCS-NP and freeze-dried at -80 °C. Lyophilized TCS-NP-PL nanoparticles were stored at 4 °C for further use.

Mucoadhesion of prepared gel

The mucoadhesion study was carried out by the already reported method, with little modifications.[23] CS-NP, TCS-NP, and TCS-NP-PL were analyzed to determine mucoadhesion strength in an *ex-vivo* goat nasal mucosal template. A goat head was purchased from the local slaughterhouse and nasal mucosa was separated carefully from the nasal cavity and stored in normal saline. To avoid bacterial growth, gentamycin sulfate injection was dropped into a normal saline solution. Before use, cartilage associated with mucosal membrane and blood was removed carefully.[24] The compressed tablet of CS-NP, TCS-NP, and TCS-NP-PL was placed on the nasal mucosa, and these pieces of mucosa were then dipped on a basket tube rack of disintegration apparatus covered entirely with aluminum foil with double tape, with the basal side of the mucosa contacting simulated nasal fluid present in the chamber of the disintegration apparatus. Mucoadhesion strength was determined by studying the mean residence time (MRT) of samples (CS-NP, TCS-NP, and TCS-NP-PL).[25]

***In-vitro* drug release and kinetic modeling**

The release study was performed with 500 mL of phosphate buffer solution (pH 6.4) used as a dissolution medium. The weighed amount of CS-NP, TCS-NP, and TCS-NP-PL corresponding to 20 mg of vancomycin, was topped up in a dialyzing tube (molecular weight cutoff 12-14 kDa), with simulated nasal fluid as a dissolution medium. 3 mL of samples were removed from the medium and fresh simulated nasal fluid was replaced at predetermined time intervals. All the samples were subjected to a UV-visible spectrophotometer (Shimadzu Corporation) for analysis at 280 nm to determine the amount of vancomycin in the samples.[20] Zero-order, first-order, Korsmeyer-Peppas, and Higuchi models of drug release kinetics were studied on the drug release data to find out the best-fit model for pharmacokinetics of the encapsulated drug.[26]

***In-vitro* drug permeation study**

The optimized vancomycin *in-situ* gel was used for the transnasal permeation study using goat nasal membrane by the Franz cell diffusion technique.[24] The mucosal membrane of the goat was soaked in simulated pH 6.4 nasal fluid for 1 hour to keep sink conditions. Then, this mucous tissue was kept on the receptor cell of the diffusion assembly. CS-NP, TCS-NP, and TCS-NP-PL containing an equivalent amount of 2 mg of VAN were placed in the donor chamber, and 9 mL of diffusion medium was filled in the receptor compartment of the Franz cell. The drug permeation study was performed at 37±0.5 °C for 6 hours. The sampling of 0.5 mL was done at predetermined time intervals and fresh diffusion media replaced the medium. The determination of permeated drug concentrations was done by a UV-visible spectrophotometer (Shimadzu Corporation) at 280 nm. The percentage cumulative drug permeation and time graphs were plotted by calculating the diffused amount of drugs at different time intervals.[27] Apparent permeability Papp was calculated by the following formula:

$$\text{Papp} = (dQ/dt) / (C^0 \times A) \quad (4)$$

where the transport rate or flux (J) (µg/min) is denoted as dQ/dt across the biological membrane, C^0 (µg/mL) is the initial concentration of the drug in the donor chamber, and A is the surface area (cm^2).

Statistical analysis

The data are presented as the mean standard deviation of 3 independent experiments; $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Synthesis and characterization of TCS

TCS was prepared from chitosan with the EDAC coupling reaction and confirmed via FTIR analysis (details are discussed in the FTIR analysis section); 387 ± 31 µmol/g of polymer thiol groups was immobilized on the chitosan; 132 ± 20 µmol disulfide linkages were immobilized per gram of TCS. It appears as a white fibrous material after lyophilization.

Preparation, EE, DL, PS, and ZP of polymeric NPs

CS-NP and TCS-NP were successfully prepared by using TPP as an ionic crosslinking agent by the dropwise addition into the VAN-loaded polymeric solution until the translucent blue color appeared. The entrapment efficiency of CS-NP and TCS-NP was calculated to be 64.18 ± 3.1% and 89.3 ± 4.2% with drug loading of 58.35 ± 2.1% and 70.14 ± 4.4%. It was observed that TCS-based nanoparticles exhibited improved entrapment efficacy and VAN loading, as compared to CS-based nanoparticles CS-NP, which may be attributed to the existence of disulfide bonds in the TCS-NP resulting in a crosslinked matrix, and thus increased probability of VAN entrapment and immobilization within the polymeric matrix.

The prepared nanoparticles were then centrifuged and redispersed in deionized water. For the analysis of particle size and zeta potential, the prepared nanoparticles were diluted 10 times with deionized water and subjected to analysis by using Malvern Nano ZS Zeta Sizer, and the results of particle size, zeta potential, and PDI are presented in Table 2. The particle size of blank CS-NP and blank TCS-NP was found to be 295 ± 12 and 260 ± 9, respectively, with a zeta potential of 19.2 19.2 ± 2 and 20.3 ± 6, whereas the particle size of VAN-loaded CS and TCS-based nanoparticles, CS-NP and TCS-NP was 363.1 ± 18 and 290.2 ± 13, respectively, with zeta potential of 21.7 ± 4 and 21.24 ± 3. The increased particle size of CS-based nanoparticles, as compared to that of TCS-based nanoparticles, might be due to the enhanced viscosity of the polymeric solution. Both chitosan and thiolated chitosan solutions were prepared at the same concentration of 0.2% w/v. However, the inherent structural differences in thiolated chitosan can lead to variations in solution viscosity due to factors such as increased hydrophilicity and the presence of thiol groups, which can influence the polymer's interactions in solution. This can result in the observed differences in nanoparticle diameter.

Table 2

Particle size and zeta potential (ZP) of vancomycin-loaded chitosan NPs and thiolated chitosan NPs of the formulation

Formulation composition	PS (nm)	ZP (mV)	PDI	EE (%)	DL (%)
Blank CS-NPs	295± 12	19.2±2	0.62	-	-
Blank TCS-NPs	260±9	20.3±6	0.71	-	-
CS-NPs	363.1±18	21.7±4	0.55	64.18±3.1	58.35±2.1
TCS-NPs	290.2±13	21.24±3	0.512	89.3±4.2	70.14±4.4

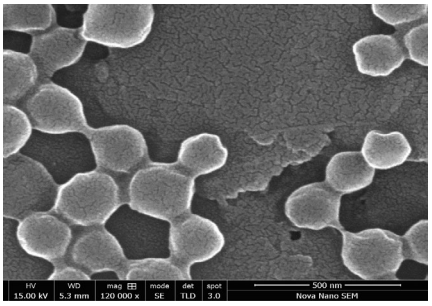

Figure 1: SEM image of TCS-NP at 20000X magnification, indicating the nano-size and round morphology

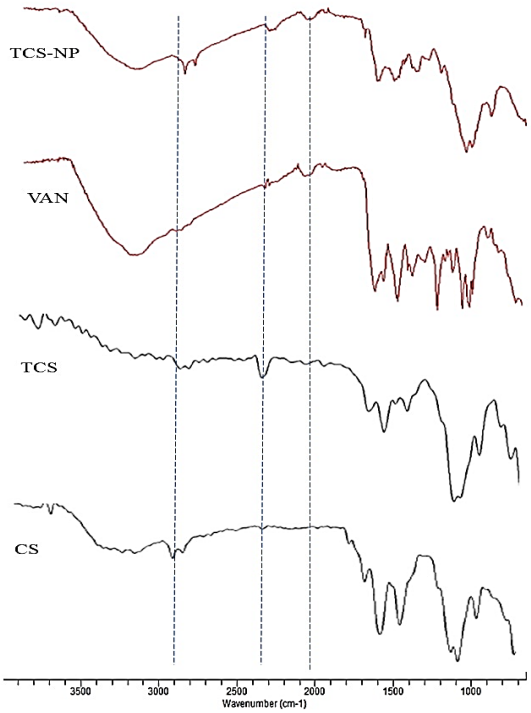

Figure 2: FTIR spectra of CS (chitosan), TCS (thiolated chitosan), VAN (vancomycin), TCS-NP (VAN loaded TCS based nanoparticles), representing the attachment of thiol groups via amide bond formation and lack of incompatibility between drug and polymeric excipients

Scanning electron microscopy

Scanning electron microscopy (SEM) confirmed the round surface topography of the developed TCS-NP, as shown in Figure 1. The particle size indicated by the SEM image is slightly lower compared to that observed by zeta analysis, and it might be because zeta represents the hydrodynamic size, whereas the SEM represents the size of the dried nanoparticles.

Fourier transform infrared spectroscopy

The FTIR study of chitosan (CS), thiolated chitosan (TCS), vancomycin (VAN), and vancomycin-loaded thiolated chitosan-based nanoparticles (TCS-NP) was carried out and the resultant spectra are presented in Figure 2. The spectrum of CS shows peaks at 560 cm^{-1} (out-of-plane bending of C–O), 700 cm^{-1} (out-of-plane bending of NH), 1180 cm^{-1} (C–O–C stretching), 2845 cm^{-1} (CH_2 stretching), and 3572 cm^{-1} (−OH stretching). In TCS, the characteristic peaks occurred at 1641 cm^{-1} (C=O stretching amides), and the signal was deformed at 3211 cm^{-1} (-NH amide in stretching), which confirmed the successful modification of the CS backbone via amide bond formation, leading to the immobilization of thiol groups, as indicated by the absorption peak at 2488 cm^{-1}.[28] The peaks at 801 cm^{-1} and 1230 cm^{-1} were due to the existence of the S-S disulfide bond and C-SH stretching, respectively, in thiolated chitosan, confirming the presence of mercaptans. The FTIR spectrum of vancomycin displayed peaks at 3387.38 cm^{-1}, 2935.51 cm^{-1}, 2842.19 cm^{-1}, 1632.81 cm^{-1} and 1093.52 cm^{-1}, confirming –COOH bond, $–CH_2$-CH_3 stretching, R-NH-R, $CO-NH_2$ and R-O-R, respectively. No interactions were observed between the active drug and the polymers, as no additional peak appeared in the TCS-NP.

Swelling studies

The swelling index gives an estimation of the ability of nanoformulations to absorb water, which is directly linked to the drug release behavior and mucoadhesion properties. Similarly, for the polymeric matrix to show mucoadhesion, its chains must be mobilized to interact with mucous glycoproteins, which is possible only if they are in hydrated form. A graphical representation of the swelling index of CS-NP and TCS-NP is presented in Figure 3.[29] Initially, no significant difference was observed up to the first 5 h, where the swelling index was found to be 60% ± 12 and 78% ± 16 in the case of CS-NP and TCS-NP, respectively. However, from 6 h to 24 h, TCS-NP exhibited a significantly increased

swelling index, compared to that of CS-NP. CS-NP might have lost their weight due to surface erosion of polymers, as compared to the TCS-NP, which might be due to the presence of a cross-linked structure of disulfide bond formation by the thiol groups, resulting in a crosslinked matrix having the ability to maintain integrity for a longer time.

Analysis of thermo-responsive TCS-NP-PL

Gelation time and gelation temperature

The two major parameters for the development of *in-situ* gels are gelation time and temperature. The temperature range for the nasal cavity is between 32–35 °C, which impacts sol to gel transformation. The gelation temperature of TCS-NP-PL was 32 ± 0.5 °C, which is almost close to nasal temperature. The gelation time of TCS-NP-PL was 45 seconds. Both temperature of gelation values seem to be promising for the formation of gel on the nasal mucosal surface.

Rheological analysis

The viscosity measurements for the prepared TCS-NP-PL are shown in Figure 4. The sol-gel phase transition was observed at 32.1 °C and the maximum viscosity observed was 29.2 mPa.s. After this temperature, the saturation phase started, in which there was no further increase in viscosity.

Mucoadhesion study

The mucoadhesion study was performed on disintegration apparatus and the results are shown in Figure 5. The time of adherence of CS-VAN-NPs, TCS-NP, and TCS-NP-PL on the mucous membrane was noted in the nasal medium. The time for mucoadhesion of CS-NP, TCS-NPs, and TCS-NPs-PL on the mucosal membrane was 75 minutes, 120 minutes, and 210 minutes, respectively. The increased mucoadhesion of TCS-NP might be due to the ability of thiol groups to develop covalent linkage with the mucus glycoproteins via the disulfide exchange mechanism. The cystine-rich sub-units of mucous glycoproteins contain thiol groups, which might be directly involved in the preparation of disulfide linkage with thiol groups of the TCS.

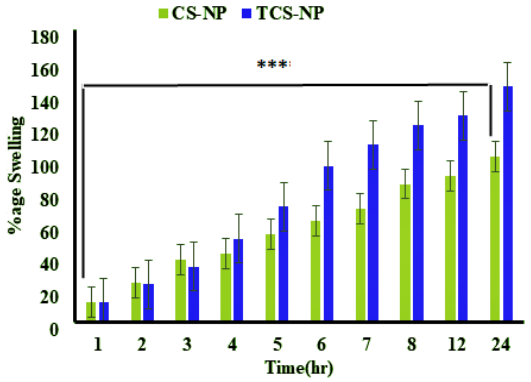

Figure 3: Kinetic swelling for CS-VAN-NPs and TCS-VAN-NPs, showing efficient swelling of thiomer-based formulation up to 24 h within nasal fluid

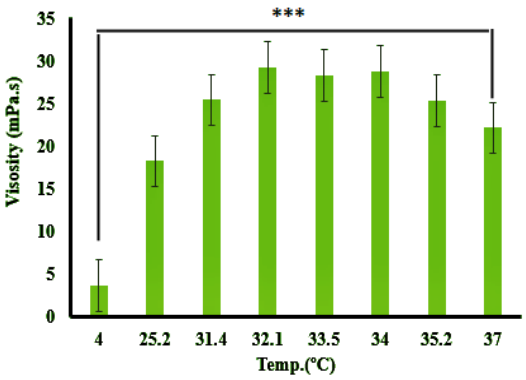

Figure 4: Viscosity of vancomycin-loaded TCS-NPs *in situ* nasal gel formulation showing increased viscosity and phase transition from solution to gel at 32.1 °C

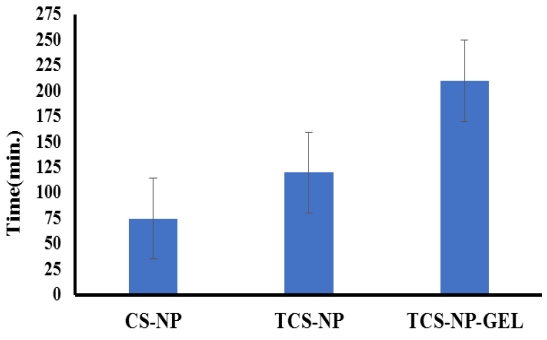

Figure 5: Mucoadhesion of vancomycin-loaded chitosan NPs, thiolated chitosan NPs, and thiomer-based *in-situ* nasal gel of vancomycin

Similarly, the TCS-NP-PL gel showed maximum mucoadhesion due to the combined effect of the thiol and pluronic gel, and the TCS-NP-PL present at the surface remains in contact with the mucous membrane for a 2.8 fold and 1.9 fold longer period of time, in comparison with CS-NP and TCS-NP, respectively.

***In-vitro* drug release and kinetic modeling**

The formulation of vancomycin-loaded NPs resulted in a sustained release pattern of vancomycin. The drug release profiles of CS-NPs, TCS-NPs, and TCS-NPs-PL are shown in Figure 6, indicating 55%, 54.6%, and 52.1% drug release, respectively. Kinetic models were applied to the data obtained from *in-vitro* drug release and the results are presented in Table 3. The formulations exhibited release patterns inclined towards first-order release as a concentration-dependent behavior. The Korsmeyer-Peppas model indicates the Fickian type of VAN release, as indicated by the N<0.45.

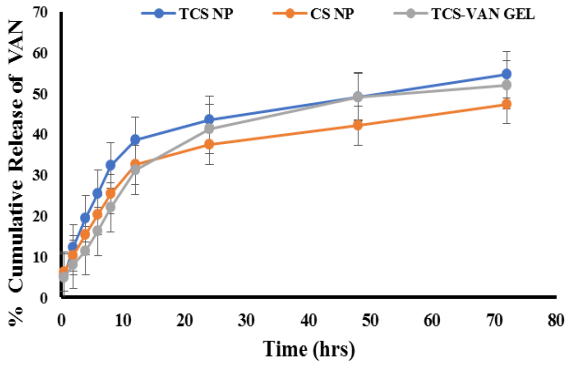

Figure 6: Percentage drug release of CS-NP, TCS-NP and TCS-NP gel, indicating a sustained release pattern

Table 3
Drug release kinetic modeling of *in vitro* drug release profile of vancomycin for determining drug release mechanism

Formulation	Zero-order			First-order			Korsmeyer Peppas			Higuchi		
	R^2	K^o	R	R^2	K^1	R	R^2	N	R	R^2	KH	R
CS-NPs	-0.08	0.86	0.871	0.27	0.01	0.913	0.95	0.35*	0.974	0.84	6.58	0.954
TCS-NPs	-0.19	1	0.843	0.27	0.02	0.902	0.93	0.34*	0.972	0.79	7.73	0.938
TCS-NPs-gel	0.435	0.94	0.898	0.7	0.02	0.945	0.95	0.44*	0.981	0.93	6.99	0.968

*N<0.45 = Fickian diffusion

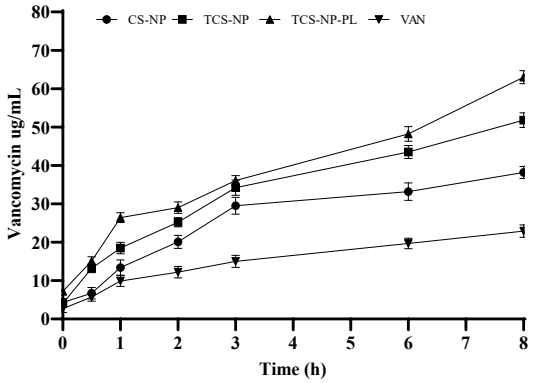

Figure 7: *In-vitro* drug permeation of vancomycin from CS-NP, TCS-NP, and TCS-gel evaluated by Franz Diffusion cell for 24 h

Table 4
In-vitro permeation enhancement in terms of concentration transported across the goat nasal membrane (model membrane) and apparent permeability along with enhanced permeability ratio of formulations CS-NPs and TCS-NPs and TCS-NPs-PL

Formulation	Apparent permeability coefficient (Papp×10^{-6} (cm/s)	Enhanced ratio	Efflux J (μgcm^{-2}min^{-1})
Vancomycin	2.28	-	0.2423
VAN loaded CS-NP	8.05	3.53	0.8502
VAN loaded TCS-NP	10.05	4.40	1.0682
VAN loaded TCS-NP-PL	14.60	6.40	1.5514

***In-vitro* drug permeation study**

Thiolated polymers are well known for their permeation-enhancing abilities via paracellular transport by opening tight junctions. The purpose of this study was to enhance the permeation of VAN by designing nanoparticles based on the thiolated polymer, supported by the increased contact tie due to their mucoadhesive nature. *In-vitro* permeation studies shown in Figure 7 indicated that thiomer-based nanoparticles in combination with pluronic (TCS-NP-PL) were highly effective in permeating the nasal mucosa, as compared to the pure drug, CS-NP and TCS-NP. Table 4 contains data on the apparent permeability and improvement in permeability ratio of all formulations, where TCS-NP-PL exhibited maximum apparent permeability (Papp) of 14.60 (cm/s) x 10^{-6} as compared to VAN and CS-NP, with Papp of 2.28 (cm/s) x 10^{-6} and 8.05 (cm/s) x 10^{-6}, respectively. Thus, TCS-NP-PL exhibited 6.40-fold enhanced permeability, as compared to that of VAN. TCS-NPs exhibited a permeability less than TCS-NP-PL, indicating a 4.40-fold improved permeation compared to VAN. The decreased permeability of VAN in the case of TCS-NP, as compared to TCS-NP-PL, might be due to the lower adherence time of TCS-NPs on the mucosa surface before becoming available for absorption at the nasal mucosa.

CONCLUSION

In the present study, thiolated thermosensitive chitosan nanoparticles were developed for nasal delivery of VAN. The synthesized thiolated chitosan showed a thiol content of 387 ± 31 μmol/g of polymer, indicating successful modification. Additionally, the presence of 132 ± 20 μmol/g of disulfide linkages per gram of TCS was confirmed. Thiolated chitosan was used to prepare NP having a size in the range of 290.2 ± 13nm loaded with VAN. TCS-NP exhibited 1.3-fold enhanced swelling in comparison with CS-NP. TCS-NP-PL Gel exhibited 6.40-fold enhanced permeation across nasal mucosa, as compared to the free drug, and 2.8-fold increased mucoadhesion in comparison with CS-NP and TCS-NP, respectively. The results suggested that combining thiolation with thermosetting gels serves as a promising approach in delivering vancomycin through nasal routes and might open the way for other peptide drugs as well.

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