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Research Article

FORMULATION AND IN-VITRO EVALUATION OF SUSTAINED RELEASE TROPICAMIDE LOADED CHITOSAN NANOPARTICLES FOR OCULAR DRUG DELIVERY

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ABSTRACT

The present research work deals with the formulation and evaluation of chitosan nanoparticles containing Tropicamide drug. The goal of present work is to enhance the residence time of drug in eye, enhancing bioavailability and reducing dosing frequency. The nanoparticles were formulated by ionic gelation method using chitosan as polymer and sodium TPP as cross-polymer. Nanoparticles were optimized using (2^2) factorial design and characterized by their particle size analysis, drug entrapment efficiency and percentage yield. In-vitro drug release studies were performed with Modified Franz Diffusion Cell. The nanoparticles formed were spherical in shape and size ranged between 402.4 nm to 604.5 nm. Drug entrapment efficiency, percentage drug release of optimized formulation was found to be 54.9% and 30.44% respectively. The drug release pattern of nanoparticles revealed its sustained release properties. The results of present work reveal that nanoparticles are promising drug delivery for enhancing the residence time by mucoadhesion which leads to enhancement of bioavailability of the tropicamide drug.

Keywords: Chitosan, Tropicamide, Nanoparticles, Sodium Tripolyphosphate (TPP), Ionic Gelation.

INTRODUCTION

Ophthalmic drug delivery systems using nanotechnology have the potential to enhance the corneal residence time of the drug to reduce the faster clearance of drug from eyes. Many factors like tear flow, blinking and epithelial barriers lead to low bioavailability of drugs in case of ophthalmics[1]. So many attempts were performed in the history to fix this problem in ophthalmic drug delivery. Nanoparticles are most promising method for ocular drug delivery. Many pharmaceutically active compounds faces problems like fast metabolism, slow absorption, higher toxicity profile etc. and these problems can be solved by using nanotechnology. Nanotechnology has properties like smaller size range, enhanced surface area and easily suspending in liquids, various optical and magnetic properties are offered by nanotechnology than conventional drug delivery systems[2].

The residence time of drugs in the precorneal area can be enhanced by use of mucoadhesive polymers. Among them, chitosan shows several important biological properties, like biodegradability, low toxicity, biocompatibility and mucoadhesiveness. Chitosan is a deacetylated form of chitin, after cellulose it is the second most abundant polymer in nature. Chitin is hydrophobic but in contrast, chitosan is soluble in acidic solutions which make it useful for pharmaceutical applications in development of drug delivery systems. The ionic interaction between positively charged amino group of chitosan and negative charged sialic acid residue in mucus lead to mucoadhesion mechanism. These unique properties makes chitosan a versatile biopolymer and useful for development of ophthalmic drug delivery systems[3].

Tropicamide (BCS Class-II drug) is an antimuscarinic agent used to produces mydriasis. It is frequently used during eye examinations for eye surgery, funduscopic examination, cycloplegic retinoscopy, cycloplegia. It blocks receptor in the muscles of the eye and control the size of pupil and lens shape. Tropicamide produces mydriasis due to blockage of receptors[4]. It has the fastest (25-45 min.) and briefest (4-6 hours) action. It is used as a short acting mydriatic in fundoscopy[5].

Calvo and Coworkers developed Ionic gelation method for the preparation of chitosan nanoparticles. In this method, polymer solutions and polyanion solutions were incorporated to prepare nanoparticles. The mechanism involved in nanoparticles formation was the interactions between positively charge amino groups of polymer and negatively charge anion. The positive or negative charge of the hydrophilic polymer was complexed with a multivalent cationic or anionic to form viscous gel particles having size range of nanometer[6]. The goal of present work is to enhance the residence time of drug in eye by mucoadhesion which can leads to enhance the bioavailability and reducing dosing frequency.

MATERIALS AND METHODS

Chemicals and Drug

Tropicamide was purchased from Optica Pharmaceutical Pvt. Ltd (Yamunanagar, India). Chitosan was obtained from Sigma-Aldrich Chemical Pvt Limited, Sodium tripolyphosphate from Central Drug House (P) Ltd. New Delhi, sodium hydroxide from Loba Chemie Pvt. Ltd., Mumbai and All other reagents and chemicals used were of analytical grade.

DRUG EXCIPIENTS COMPATIBILITY STUDIES

FT-IR Study

The drug excipients compatibility study was performed by using FT-IR Spectrophotometric method (FT-IR Bruker 1206 0280, Germany). The powdered Drug and Polymer were taken in the ratio of 1:1. FT-IR analysis was performed over a range of 4000-400 cm^{-1} by taking above mixture. The spectrum (Figure 1 & 2) obtained was compared for the presence of any peak in the mixture[7] (Figure 3).

DSC Study

DSC analysis was performed by using (DSC Q10 V9.9 Build 303, US instrument). 2 mg of each Tropicamide (Figure 4), and chitosan (Figure 5) were taken in closed aluminium pain respectively. Each pain containing drug and chitosan were heated separately from 20^o to 300^0C in an atmosphere of Nitrogen gas passing at a flow of 60 ml/min. An empty aluminium pan was taken as reference pan.

Preparation of Nanoparticles

Ionic gelation method was used for the preparation of chitosan nanoparticles**.** Tropicamide was taken at fixed amount (100 mg). 1% w/v solution of chitosan was prepared by dissolving chitosan in (1% v/v) acetic acid. The pH of the chitosan solution was adjusted to pH 5.5. Tropicamide 100 mg was dissolved in the chitosan solution. The drug and chitosan solution was sonicated for 30 minutes. Sodium tripolyphosphate (0.5% w/v) solution in distilled water (40 ml) was added dropwise in the chitosan drug solution (100 ml) under high speed stirring (3000 rpm) for 30 minutes. An opalescent suspension was obtained. The obtained suspension was centrifuged at 15000 rpm for 30 minutes to obtain the nanoparticles. The nanoparticles were freeze dried at -80^o C for 4 h followed by lyophilisation for 24 hours using mannitol (1%, w/v) as cryoprotectant[4, 7, 8].

Optimization Studies

The optimization process provides not only efficient use of resources, but also a method to obtain a mathematical model which can be used to characterize and optimize a formulation or process. Furthermore, by accurately defining the whole system, optimization techniques are a useful aid to process validation[12].
The factor is an assigned variable like concentration, temperature, drug treatment etc. the factorial level are the values assign to the factor. The notation used to denote factorial experiments conveys the following information:
When the design is 2^2 factorial, it denotes:
1. Number of factors (2)
2. Level of each factor (2)
3. Experimental conditions present in the design (2^2 =4)

The amount of polymers used in the study was taken in two levels; minimum and maximum as following:

Table 1: Amount of Chitosan and Sodium TPP in Different Batches

Formulation No.	Experiment	Drug (mg)	Chitosan (conc. w/v)	Sodium TPP (conc. w/v)
1	(1)	100	1	0.5
2	A	100	2	0.5
3	B	100	1	1
4	AB	100	2	1

Followings are the equations used to calculate the effects of factor A (Equation 1), effect of factor B (Equation 2) and magnitude of interaction of factors (Equation 3):

Effects of factor A = $\frac{1}{2}[(ab + a) - (b + 1)]$ …… Equation (1)

Effects of Factor B = $\frac{1}{2}[(ab + b) - (a + 1)]$ ……Equation (2)

Magnitude of Interaction = $\frac{1}{2}[(ab + 1) - (a + b)]$….Equation (3)

If the combined effect of two factors had to produce a greater effect than that produce by individual factor, the interaction is said to be synergistic[13, 14, 15].

The amounts of chitosan and Tripolyphosphate used in factorial design were determined from literature and maximum and minimum amount were fixed by preparing the nanoparticles as trail formulation. Four formulations were prepared (Table 1) according to the factorial design (2^2) by taking maximum and minimum concentrations of chitosan and sodium TPP. The amount of drug was fixed to 100 mg. Amount of chitosan was taken minimum (1mg/ml) to maximum (2mg/ml) while sodium TPP was taken minimum (0.5mg/ml) to maximum (1 mg/ml). Entrapment efficiency was calculated and on the basis of entrapment efficiency (Table 3), the effect of factors and their interactions (Table 4) were calculated with the help of above equations (1,2,3).
Further six formulations were prepared on the basis of effects of factors. The entrapment efficiency and percentage yield of all the formulations were calculated and on the basis of maximum entrapment efficiency, optimized formulation was selected for further in-vitro studies (Table 5).

Entrapment Efficiency and Percentage Yield

The entrapment efficiency was calculated by centrifugation (R-24, REMI high speed centrifuge, REMI Corporation, India) of the nanosuspension at 17000 rpm for 30 minutes. 10 ml of supernatant was filtered through 0.45µm whatman's filter paper and then diluted up to 100 ml with freshly prepared phosphate buffer pH 7.4. 10 ml of the sample was taken and analysed by UV spectrophotometer (UV- 1800, Shimadzu Corp., Japan) at λ_{max} 254 nm. The amount of free drug in supernatant was calculated[9,10].
Percentage yield was calculated to determine whether the method for loading the drug into polymer was efficient or not. The raw material, active ingridients, and other parameter process were a factor to produce product when the manufacturing process of nanoparticles. The produced results were determined by weigheing the nanoparticles and determined the percentage result of added materials weight that were the weight of drug and polymer which were added[22].
Entrapment efficiency and percentage yield of nanoparticles were calculated by using formulas given below: (Table 3):

$$\text{Entrapment Efficiency} = \frac{\text{Total amount of drug} - \text{Free drug}}{\text{Total amount of drug}} \times 100$$

$$\text{Percentage Yield} = \frac{\text{Total nanoparticles weight}}{\text{Total solid weigth}} \times 100$$

In-vitro Drug Release Study

In-vitro drug release was studied using modified Franz diffusion cell. Dialysis membrane was used between donor and receptor compartments of modified Franz diffusion cell. Before using, membrane was treated by soaking in distilled water for 15 minutes. Then membrane was boiled for 10 minutes at 80^o C in 1 Litre of Sodium bicarbonate solution. Transfer the membrane

in 1 mM Na_2EDTA solution for 10 minutes. Membrane was washed with distilled water and stored in 50 ml of Ethanol. Membrane was rinsed before use. Pre- treated dialysis membrane was taken (Specifications: Av. Flat width- 31.13 mm, Av. Diameter- 21.5mm, Av. Capacity- 3.63 ml/cm.) and it was fixed between donor and receptor compartments of modified Franz diffusion cell. The freshly prepared nanosuspension (5 ml) was filled in donor compartment and phosphate buffer (pH 7.4) was filled in receptor compartment. The sample (3 ml) was withdrawn from receptor compartment every hour and an equal amount of medium was replaced to maintain the total amount of medium. The samples taken were analysed by UV Spectrophotometer (UV- 1800, Shimadzu Corp., Japan) at 254 nm to calculate the amount of drug release[4,16].

CHARACTERIZATION OF NANOPARTICLES

FT-IR Analysis

The FT-IR of the nanoparticles was performed on (FT-IR Bruker 1206 0280, Germany) instrument by KBr disc technique. The spectrum was recorded (Figure 7) over the range of 4000-400 nm. The obtained spectrum was than interpreted**.**

DSC Analysis

DSC analysis (Figure 8) was performed by using (DSC Q10 V9.9 Build 303, US) instrument. 2 mg of the nanoparticles taken in closed aluminium pain was heated from 30^o to 250^o C in an atmosphere of Nitrogen gas passing at a flow of 60 ml/min. An empty aluminium pan was taken as reference pan**.**

Morphological Examination

Tropicamide loaded chitosan nanoparticles were identified for the morphology through the facility of scanning electron microscope (Figure 9). The optimized batch was used for the SEM analysis (Zeiss EVO 50).

Mucoadhesive Assay

Nanoparticles were evaluated for mucoadhesiveness through swelling index parameter. Mucoadhesive formulations have the property of water absorption through pores and capillary space which leads to increased weight and volume of material. Thus the swelling index of nanoparticles was calculated through their weight variation by using following formula[12].

$$\text{Swelling Index} = \frac{Wt-Wo}{Wo} \times 100$$

Where Wt = weight of nanoparticles at time t
Wo = weight of nanoparticles before placing in liquid medium

Drug Release Kinetic Study

The in-vitro drug release study was fitted to various kinetic equations i.e Zero order (Figure 10), First order (Figure 11), Korsmeyer Peppas (Figure 12) and Higuchi Model (Figure 13) to understand the mechanism and kinetic of drug release. Correlation Coefficient (R^2) values of the optimized batch was noted down[18] (Table 9).

FORMULATION OF EYE DROP CONTAINING NANOPARTICLES

An eye drop was prepared to check the sterility and other parameters for ocular use of the formulation. The nanoparticles equivalent to one dose (1% w/v) were washed twice with distilled water for eliminating any impurity and centrifuged at 17000 rpm for 15 minutes to recollect them. Other excipients used were listed below in table 2:

Table 2: Amount of Excipients Used for Eye Drop Formulation[19,20]

Category	Name of Excipient	Amount
API	Tropicamide Nanoparticles	Equivalent to one dose (1% w/v)
Buffering agent	Disodium Hydrogen Phosphate	20 mg
Isotonic agent	NaCl (0.9 %)	37.5 ml
Chelating agent	EDTA	0.01 %
Antimicrobial agent	Benzalkonium Chloride	0.01 %
Dilution Media	Water for Injection	Upto 100 ml

All the solid materials were dissolved in 50 ml of water for injection. Nanoparticles equivalent to 1% of tropicamide were dissolved into the above solution. Tonicity modifying agent NaCl (0.9%) 37.5 ml was added and volume was made upto 100 ml with water for injection[19,20].

Quality Control of Eye Drop

Following tests were performed for quality control of eye drop formulation:

Foreign Particulate Matter Analysis (Visual Inspection)

The eye drop was transferred into a transparent container free of any foreign particle. Visual inspection of the solution was performed to check the presence of any foreign particulate matter.

Determination of pH

The pH meter was used to determine the pH of eye drop formulation. The fluctuation in the pH was adjusted by using Disodium Hydrogen Phosphate buffer pH 7.4.

Tonicity determination

The ophthalmic solution should be isotonic to the tears. A solution's tonicity often directly correlates with the osmolality of the solution. Osmolality describes the total solute concentration of the solution. Osmolality is the ratio of moles of total solute particles per kg water. The unit for this measurement of concentration is osmolal (Osm), where 1 Osm is equal to one mole of total solute per kg water[21].

$$\text{Osmolality (Osm)} = \frac{Moles\ Total\ Solute}{Solvent\ mass\ (kg)}$$

Sterility Testing

For Bacteria

The sterility testing of the eye drop was performed by using Fluid Thioglycollate medium. The following culture medium was found to be suitable for the test. Fluid thioglycollate medium is primarily intended for the culture of anaerobic bacteria; however, it will also detect aerobic bacteria[23].
Pancreatic digest of casein: 15 gm
Yeast extract: 5 gm
Dextrose: 5.500 gm
Sodium chloride: 2.500 gm
L- Cystin: 0.500 gm
Sodium thioglycollate: 0.500 gm
Agar: 0.750 gm

Final pH : 7.1± 0.2

The media was prepared by dissolving all the solids in distilled water and warm gently for some time and cool to room temperature and maintain the pH of 7.1 ± 0.2. The medium was poured into suitable container and sterilised in an autoclave at 121° for 20 minutes. The test must be carried out under aseptic conditions designed to avoid accidental contamination of the product during testing. For achieving these conditions, grade A laminar airflow cabinet or an isolator is recommended. The test environment has to be adapted to the way in which the tests are performed.

Transfer the quantity of the preparation under examination directly into the culture medium so that the volume of the preparation under examination is not more than 10 % of the volume of the medium. Incubate the media for 7 days. Observe the culture media periodically during the 7 days of incubation[10].

For Fungi

Sabouraud dextrose agar medium was used for presence of any fungal growth. Following is the composition of Sabouraud dextrose agar medium.

Peptones: 10.0 g

Dextrose monohydrate :40.0 g

Agar :15.0 g

Water :up to 1000 ml

Proceed as described in the test for bacteria. Water was heated in water bath at 48 to 55^0 C and all the solid materials were dissolved in it with occasional steering. The medium was poured into suitable container and sterilised in an autoclave at 121 ºC for 20 minutes. Adjust the pH so that after sterilisation it is 5.6 ± 0.2. Incubate the medium containing formulation at 25^o C for 7 days[10].

RESULTS AND DISCUSSION

Drug Excipients Compatibility Study

FT-IR Study

FTIR analysis of Tropicamide, chitosan polymer and their physical mixture was performed. The characteristic peaks of drug (Figure 1), chitosan (Figure 2) and their physical mixture (Figure 3) were evident in spectra i.e 1024-1035 for C-O group, 1416 for C-H group, 3316-3399 for O-H group, 2970-3405 for N-H (amine) , 1067 for =C-H (alkene), 3706 cm^{-1} for O-H broad stretch respectively. The characteristic peaks of Tropicamide and chitosan were also present in their physical mixture. The shifting of some peaks in the spectrum of 1:1 mixture of drug and polymer might be due to the presence of weak van-der wall interaction between drug and polymer. This confirmed that there was no chemical interaction between drug and polymers[4].

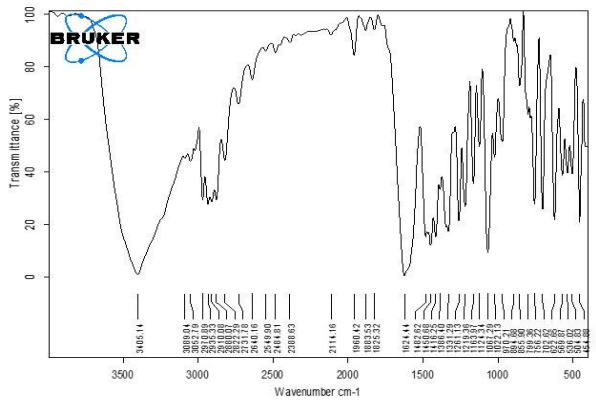


Figure 1: FT-IR Spectrum of Tropicamide

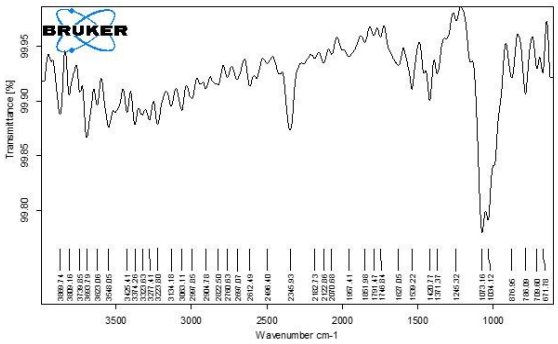


Figure 2: FT-IR Spectrum of Chitosan

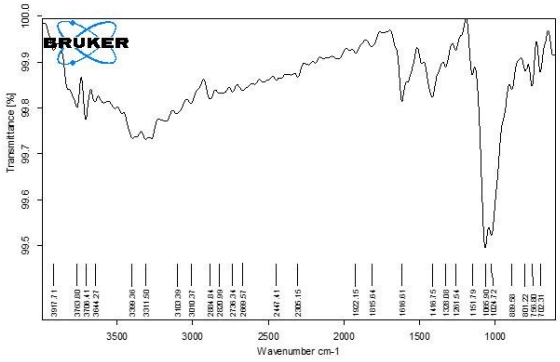


Figure 3: FT-IR Spectrum of Physical Mixture

DSC Study

The DSC thermogram of drug (Figure 4) showed a sharp endothermic peak at 103.16^0 C which indicated its melting point. It confirmed the purity of drug sample used for DSC study. A broader peak was obtained in the thermogram of chitosan (Figure 5), it reveal amorphous form of chitosan.

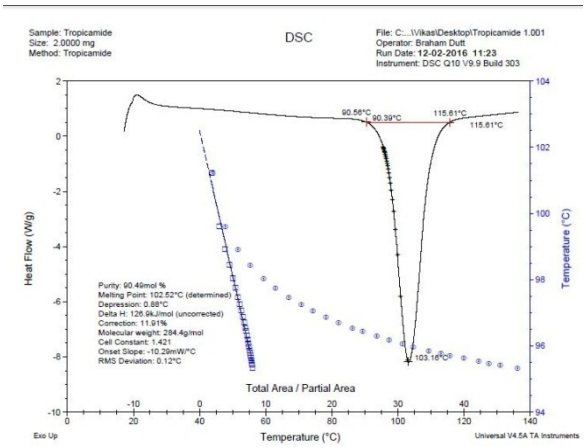


Figure 4: DSC Thermogram of Tropicamide

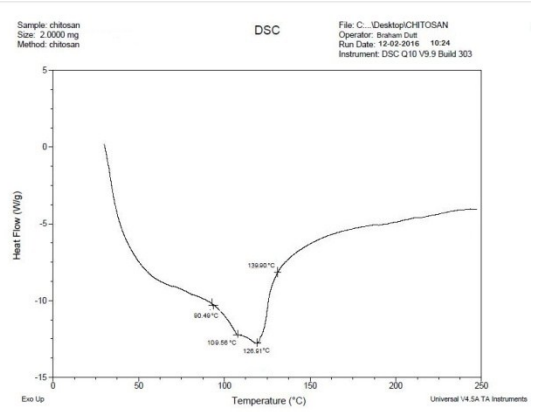


Figure 5: DSC Thermogram of Chitosan

Optimization Technique

Table 3: Entrapment Efficiency of Different Formulations

Formulation Code	Chitosan (conc. w/v)	Sodium TPP (conc. w/v)	EE* (%)
A1	1	0.5	20.53 ± 1.25
A2	2	0.5	36.97 ± 1.38
A3	1	1	22.95 ± 1.67
A4	2	1	54.99 ± 1.19

*values are mean of triplicate ± SD

The effect of factors and their magnitude of interactions were calculated by using equations (1, 2 and 3):

Table 4: Effects of Factor and their Interactions

Factors	Effects of factors
Effect of Factor A	24.24
Effect of Factor B	10.22
Magnitude of Interaction	7.8

On the basis of above results (Table 4) both factors were significant but factor A (chitosan) was more significant than factor B (sodium TPP). So the amount of sodium TPP was fixed to its minimum level and the amount of chitosan was varied between maximum and minimum level to obtain the optimized formulation (Table 5).

Table 5: Composition of Different Formulation and their Entrapment Efficiency

Formulation Code	Drug (mg)	Chitosan (conc. w/v)	Sodium TPP (conc. w/v)	EE* (%)	Percent Yield* (%)
F1	100	1.00	0.5	22.95 ± 1.33	27.68 ± 2.13
F2	100	1.25	0.5	33.47 ± 2.10	22.80 ± 1.51
F3	100	1.50	0.5	48.34 ± 1.59	24.64 ± 1.73
F4	100	1.75	0.5	51.24 ± 2.43	19.11 ± 0.67
F5	100	2.00	0.5	54.99 ± 0.86	21.20 ± 1.68
F6	100	2.25	0.5	44.11 ± 2.11	21.38 ± 1.25

*values are mean of triplicate ± SD

The formulation F5 was selected (Table 5) as optimized formulation because of high entrapment efficiency and good drug release profile, so optimized formulation was characterized for other parameters.

In- vitro Drug Release Study

The process variables used for the in-vitro drug release study are following (Table 6):

Table 6: Process Variables Used for In-vitro Drug Release Study

Parameter	Specifications
Formulation	F5 (Optimized)
Nanosuspension	5 ml
Dialysis Membrane	(Av. Flat width- 31.13 mm, Av. Diameter- 21.5mm, Av. Capacity- 3.63 ml/cm.)
Dissolution medium	Phosphate buffer pH 7.4
Diffusion Apparatus	Modified Franz Diffusion Cell
Sampling time	12 hours
Sample Amount	3 ml/ hour
Number of samples	12

The samples (3 ml) were collected per hour and an equal amount of buffer solution was added consequently to maintain the fixed amount of dissolution medium. The sample was analysed spectrophotometrically (UV- 1800, Shimadzu Corp., Japan) (17). The amount of drug release with time was reported in table (7) and shown in figure (6).

Table 7: Amount of Drug Release per Hour

Cumulative percent drug release after each time interval*						
Time (hr)	F1	F2	F3	F4	F5	F6
1	2.42 ± 1.31	2.34± 1.01	3.43± 2.13	5.89± 1.54	6.17± 2.07	4.61± 2.04
2	5.00 ± 1.65	5.02± 2.11	6.18± 1.61	8.75± 1.23	9.19± 1.56	7.60± 1.53
3	9.01 ± 2.33	8.40± 1.63	9.01± 1.82	11.74± 1.58	12.45± 1.44	10.69± 2.34
4	13.40 ± 1.69	10.36±2.11	12.01±0.36	14.89± 2.56	15.93± 3.21	13.95± 1.68
5	15.27 ± 2.21	12.21± 2.57	15.38± 1.02	15.19± 0.89	17.21± 0.96	16.32± 2.56
6	18.36 ± 3.22	14.36± 1.59	18.24± 1.25	18.27± 2.51	20.40± 1.57	19.41± 3.11
7	20.14 ± 2.41	16.76± 2.37	21.57±2.10	21.27± 0.67	24.31± 2.11	22.07± 1.58
8	23.92 ± 1.88	19.10± 1.29	24.71± 1.48	25.03± 3.00	26.61± 3.20	25.63± 2.39
9	25.71 ± 2.34	21.43± 2.14	25.62± 2.14	27.14± 1.29	28.11±1.35	26.58± 1.05
10	27.55 ± 2.56	23.56± 1.31	26.24± 3.12	28.18± 2.52	29.89± 2.31	27.20± 3.00
11	28.02 ± 1.91	24.16± 2.91	27.04± 1.68	28.82± 2.13	30.29± 1.83	28.16± 2.33
12	28.21 ± 3.01	24.41± 1.58	27.21± 3.11	29.08± 1.68	30.40± 2.45	28.44± 1.69

*values are mean of triplicate ± SD

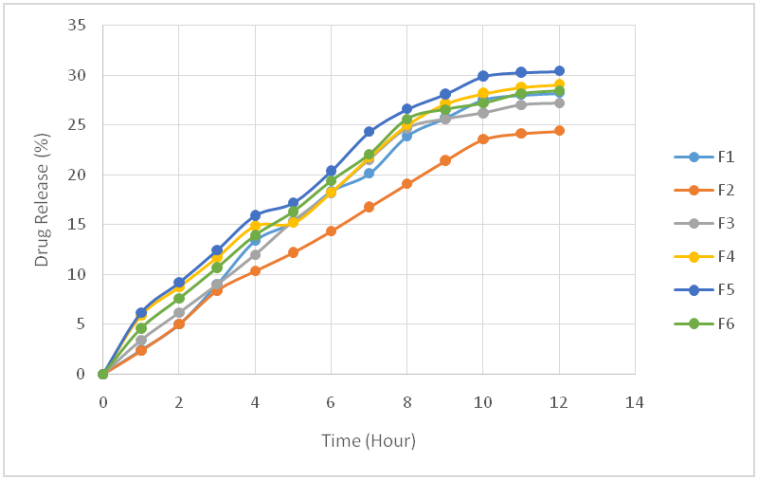


Figure 6: Drug Release profile of Different Formulations

CHARACTERIZATION OF NANOPARTICLES

FT-IR Analysis

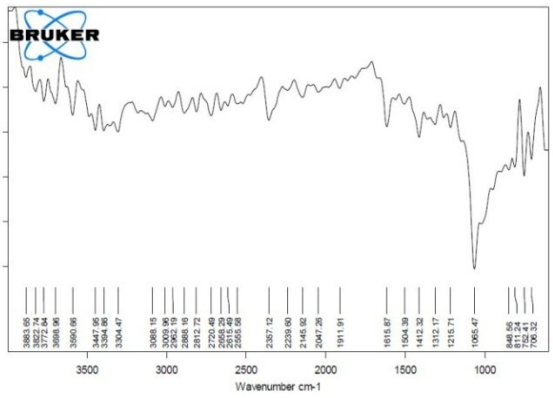


Figure 7: FT-IR Spectrum of Optimized Formulation

Spectrum of optimized formulation showed peaks at 752-848 due C-N stretch. The presence of N-H group of amines was confirmed by peak at 1616, small peak at 3088 reveals =C-H stretch. Peak at 1412 nm showed presence of C-H bending vibration of CH_3. The COOH group present in chitosan showed interaction with cations through shifting the peak of OH stretching of COOH in chitosan from 3231 cm^{-1} to 3170 cm^{-1} in ionically gelled nanoparticles. Presence of peaks in the range of 1900–1250 cm^{-1} can be attributed to overlapping contribution of C–N stretching of amides and C O group of tropicamide and C O group of ester and C–H bending vibration of chitosan[4].

DSC Analysis

The thermogram of Nanoparticles showed melting point at 115.20° C (Figure 8). In the thermogram of nanoparticles, the corresponding peaks of drug (Figure 4) and polymer (Figure 5) were absent, instead a broad peak at 115.20° C, this showed that drug was entrapped within polymer in the form of nanoparticles.

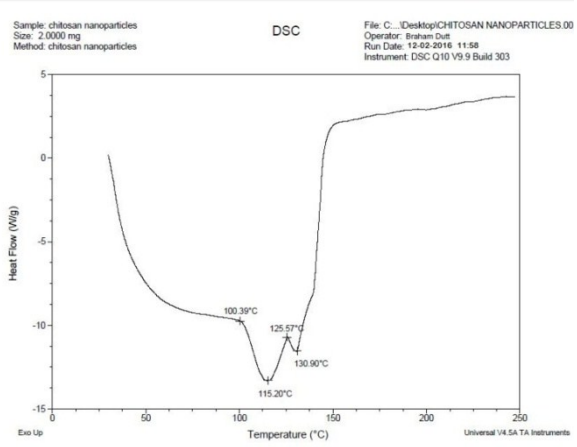


Figure 8: DSC Thermogram of Nanoparticles

Particles Size Analysis

Tropicamide loaded chitosan nanoparticles were identified for the morphology with the facility of scanning electron microscope (Zeiss EVO 50). The optimized batch was used for the SEM analysis. The morphology of nanoparticles were determined at 20.00 kV.

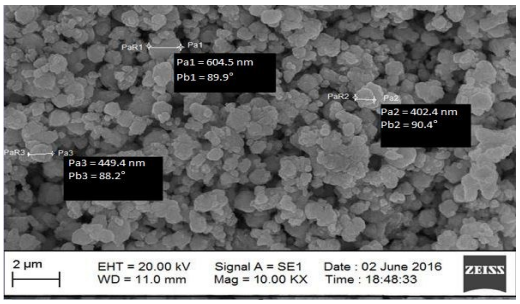


Figure 9: SEM Analysis of Nanoparticles

The minimum and maximum size was found to be 402.4 nm and 604.5 nm respectively (Figure 9).

Mucoadhesion Study

Nanoparticles (100 mg) were packed in dialysis membrane and placed in dissolution apparatus USP Type II containing phosphate buffer pH 7.4 rotating at 25 rpm speed. Nanoparticles were removed every hour upto 5 hrs. The swelling index was calculated by determining the change in weight of nanoparticles.

Table 8: Swelling Index of Nanoparticles

Time (hours)	Swelling Index*
1	0.0726 ± 0.56
2	0.1043 ± 1.20
3	0.1271 ± 1.09
4	0.1359 ± 1.95
5	0.1386 ± 1.84

*values are mean of triplicate ± SD

The change in weight and swelling index data revealed the mucoadhesive property of nanoparticles.

Drug Release Kinetic Study

The in-vitro drug release study was fitted to various kinetic equations i.e Zero order, First order, Korsmeyer Peppas and Higuchi Model to understand the mechanism and kinetic of drug release. Correlation Coefficient (R^2) values of the optimized batch was noted down (Table 9) (18). The Correlation Coefficient (R^2) value of Korsmeyer Peppas Release Kinetic Model was found to be maximum (Table 9).

Table 9: Correlation coefficient (R^2) Value of Various Kinetic Models

Release Kinetics Model	Zero Order	First Order	Korsmeyer Peppas Model	Higuchi Model
Correlation Coefficient Value (R^2)	0.9658	0.9748	0.9927	0.9865

This release kinetic model derived a relationship which described drug release from a polymeric system. In the Korsmeyer peppas model, data obtained from in vitro drug release studies were plotted as log cumulative percentage drug release versus log time. Korsmeyer peppas model indicated the mechanism of drug release i.e Fickian diffusion release (molecular diffusion of drug due to chemical potential gradient) or case II transport (diffusion associated with stresses or polymer disentanglement or erosion)[18].

FORMULATION OF EYE DROP CONTAINING NANOPARTICLES

Eye drop of nanoparticles was formulated by using the excipients given in the table 2. The prepared eye drop was seen transparent and clear and evaluated for quality control tests.

Quality Control of Eye Drop

Following tests were performed for quality control of eye drop formulation:

Foreign Particulate Matter Analysis (Visual Inspection):

Visual inspection of the solution was performed to check the presence of any foreign particulate matter. No visual foreign particle was seen in the formulation.

Determination of pH

The pH of eye drop was found to 7.4 which is the ideal pH for ophthalmic preparations. The fluctuation in the pH was adjusted by using Disodium Hydrogen Phosphate buffer pH 7.4.

Tonicity determination

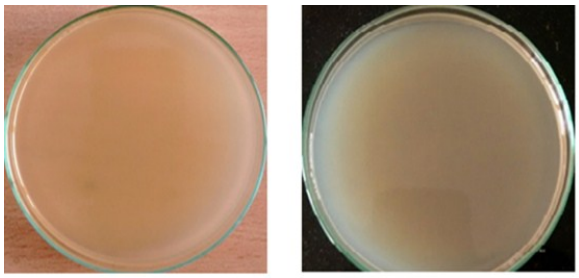
The ophthalmic solution should be isotonic to the tears. Osmolality of the eye drop was calculated using following formula:

The osmolality of the formulation was found to be 0.3 Osm.

Sterility Testing

For Bacteria

The sterility testing for presence of bacteria was performed by using Fluid Thioglycollate medium. The medium containing formulation was incubated for 7 days. The culture media was observed for 7 days of incubation[10]. No microbial growth was seen in the medium after seven days (Figure 10).



A B

Figure 10: Petri dish containing Thioglycollate medium and formulation at Day 1 (A) and Day 7 (B)

For Fungi

Sabouraud dextrose agar medium was used for presence of any fungal growth. Incubate the medium containing formulation at 25° C for 7 days[10]. No fungal growth was seen in the medium after seven days (Figure 11).

A B

Figure 11: Petri Dish Containing Sabouraud Dextrose Agar Medium and Formulation at Day 1 (A) and Day 7 (B)

SUMMARY

Chitosan polymer based nanoparticles have ability to interact and remain adhered to the ocular mucosa for longer time period, thus become a suitable drug carriers for enhanced and controlled drug release to the ocular surface. Recent researches are focussing on investigation in further detail, to know how the interaction of these particles occurs with mucosal membranes or components and their toxicity profile on repeated dose administration. The half-life of the tropicamide is very short, so to enhance the residence time of the drug in the eye, nanoparticles were prepared. The effect of various concentrations of polymer and cross polymer was studied through optimization technique (Table 5). It reveals that if we increased the amount of polymer the entrapment efficiency also increased but upto a limit, further increase in polymer concentration lead to decreased entrapment efficiency, may be due to failure of complexation with cross polymer or decreased complexation efficiency of sodium TPP because of its low concentration. The basic mechanism involved in the formation of nanoparticles is the electrostatic interactions between positively charged amino groups present in polymer and negatively charged anion. In other words it can be seen that in the ionic gelation method, due to interaction between the materials undergoes transition from liquid to gel phase. The obtained chitosan nanoparticles generally are of small size in the range of 200-500nm (Figure 9). The drug excipients compatibility study was performed spectrophotometric (Figure 3) and thermal analysis (Figure 4 & 5). The characteristic peaks of drug (Figure 1), chitosan (Figure 2) and their physical mixture (Figure 3) are evident in spectra (1024-1035, 1416, 3316-3399, 2970- 3405, 1067, 3706 cm^{-1}). This confirms that no physical interaction has taken place. The drug release patterns (Figure 6) of nanoparticles showing sustain release of drug. Particle size analysis (Figure 9) of optimized formulation (F5) revealed the minimum and maximum particle size 402.4nm and 604.5 nm respectively.

CONCLUSION

The present study concludes that Tropicamide can be entrapped in the form of nanoparticles using chitosan polymer to achieve sustained release action. The mucoadhesive property of chitosan and sustain release action of nanoparticles may contribute to enhance corneal residence time of Tropicamide by formulating its eye drop. Further In- vivo bioavailability studies are required to co-relate the results in body and confirm the findings.

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