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Research Article

# CHITOSAN LOADED MICROSPHERES OF TROPICAMIDE AS CONTROLLED RELEASE OF DRUG FOR OCULAR DRUG DELIVERY SYSTEM

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**ABSTRACT**

Ocular drug delivery via conventional eye drop is a convenient route of administration but it has serious limitations due to rapid clearance of the formulation from the eye. This study aimed to prepare and evaluate the sustained/controlled release of tropicamide microspheres with chitosan to increase ocular residence time of drug and also improve the bioavailability. The tropicamide loaded chitosan microspheres were prepared by ionic gelation technique using different ratios of polymer i.e. chitosan and cross-linking polymer i.e. sodium TPP. Microspheres were optimized by employing ($2^2$) factorial design and evaluated for particle size, entrapment efficiency, surface morphology and drug release profile. The entrapment efficiency was found to be higher with increase in the chitosan concentration. The increasing concentration of chitosan also showed the sustained release effect on the drug release. The optimized formulation showed the smooth morphology and average particle size was found to be about 15µm.The *in-vitro* release profile of tropicamide loaded microspheres showed a sustained release pattern as compared to pure drug. The results revealed that microspheres would be the promising candidates for enhancing the residence time by mucoadhesion which leads to enhancement of bioavailability of the tropicamide drug.

**Keywords:** Microspheres, Tropicamide, Chitosan, Sodium TPP, Sustained release, Ionic gelation technique.

## INTRODUCTION

Ocular administration of drug is primarily associated with the need to treat ophthalmic diseases. The majority of ocular disorders are treated by topical drug preparations in the form of solutions, suspensions, *in-situ* gels and ointments. These systems like *in-situ* gels and ointments may improve the pre-corneal residence time and penetration through the cornea but can cause blurred vision[1,2]. Unfortunately, these conventional forms have a disadvantage of poor ocular bioavailability, because of various factors like tear flow, blinking, various anatomical and physiological barriers present in the eye[3]. So many attempts were performed in the history to fix this problem in ophthalmic drug delivery. The literature survey reveals that the intraocular bioavailability of topically applied drugs is ranging from 5-10% of total administered, which is extremely poor[4,5]. Therefore, there is a need for controlled or sustained ocular drug delivery system. Controlled release systems (microspheres, nanoparticles and liposomes etc.) are prepared to obtain prolonged or controlled drug delivery, to improve bioavailability and to target drug at specific sites.

Microspheres are the most promising method for ocular drug delivery. Microsphere, as carrier for drug, is one of the various approaches of drug delivery which maximizes the drug concentration at the target site[6].Many pharmaceutically active compounds faces problems like fast metabolism, slow absorption, higher toxicity profile etc. and these problems can be solved by using microspheres. These submicron particles are better than conventional ophthalmic dosage forms to enhance bioavailability without blurring the vision. In addition, drug loaded microspheres have the ability of targeting at site of action, leading to a decrease in the dose required and side effects[7]. The report by Duarte et al., (2007) showed that ophthalmic drug delivery systems of Acetazolamide using Eudragit RS 100 and RL 100 exhibited slower release than single drug[8].

The residence time of drugs in the precorneal area can be enhanced by use of mucoadhesive polymers. Chitosan, a cationic polysaccharide polymer, has some important properties such as mucoadhesion, biocompatibility, nontoxicity and good ocular tolerance[9]. The mucoadhesive character of the polymer increased the drug residence time due to the capacity to adhere at mucin coat covering the conjunctiva and the corneal surfaces of the eye by non-covalent bonds forming the basis of ocular mucoadhesion. Chitosan is a fiber product that is obtained from deacetylation of chitin. Chitin and chitosan are most abundant naturally occurring polymers, ranked second after cellulose. 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 make chitosan a versatile biopolymer and useful for development of ophthalmic drug delivery systems[10].

Tropicamide, a BCS class-II drug (Low solubility, high permeability) is an antimuscarinic agent used to produce short acting mydriasis and cycloplegia. It is typically used during eye examinations and also be used before or after eye surgery. 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[11]. It has the fastest (25-45 min.) and briefest (4-6 hours) action[12]. Low dose (0.5%) is useful in producing mydriasis with only slight cycloplegia and stronger concentration (1%) paralyzes accommodation[13].

The aim of present study is to prepare tropicamide loaded chitosan microspheres by ionic gelation method. In this method, a hydrophilic polymer is complexed with a multivalent cationic or polyanionic to form highly viscous gel particles. The resulting microspheres were formed due to electrostatic interactions between positively charged group and negatively charged anion[14].The goal of present work is to enhance the residence time of drug in eye by mucoadhesion which can lead to enhance the bioavailability and reduce dosing frequency.

## MATERIALS AND METHODS

### Materials

The drug Tropicamide was supplied by Optica Pharmaceuticals (Yamunanagar, India). Chitosan was obtained from Sigma Aldrich Chemical Pvt. Limited. Sodium TPP was purchased from Loba Chemie Pvt. Ltd., Mumbai (India). All other chemicals and solvents used were of analytical grade.

### Preparation of Tropicamide Loaded Chitosan Microspheres

Microspheres were prepared by ionic gelation method. In this method, chitosan in different concentrations (as shown in Table 1) was dissolved in 1% v/v glacial acetic acid and pH of the solution was adjusted to pH 5.5. Different concentrations of sodium TPP solution was prepared in distilled water. The drug tropicamide added to solution of chitosan and sonicated for 30 minutes. After that, the solution of Sodium TPP was added into a solution of chitosan at 1 drop/sec with continuous stirring. An opalescent suspension was obtained. The obtained suspension was centrifuged at 15000 rpm for 30 minutes to obtain the microspheres. The microspheres were freeze dried at −80 ˚C for 4 hours followed by lyophilization for 24 hours using mannitol (1%, w/v) as cryoprotectant[15,16]. The prepared microspheres was passed through the sieve and stored in well closed container

### Optimization Studies

It is a process of finding the best way of using the existing resources while taking in to the account of all the factors that influences decisions in any experiment. It is 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[17].

A $2^2$ factorial design was used to optimize the amount of chitosan and sodium tripolyphosphate against the fixed amount of drug tropicamide. A 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:

i. Number of factors = 2
ii. Level of each factor = 2
iii. Experimental conditions present in the design ($2^2$ =4)

The following equations are used to calculate the effects of factors A i.e. chitosan (Equation 1), effect of factor B i.e. sodium tripolyphosphate (Equation 2) and magnitude of interaction of factors (Equation 3):

Effects of factor A = ½ [(ab+a)-(b+1)]…… Equation (1)
Effects of Factor B = ½ [(ab+b)-(a+1)]…….Equation (2)
Magnitude of Interaction = ½ [(1+ab) – (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[18,19,20].

The amount of polymers i.e. chitosan and sodium tripolyphosphate was taken each at two levels i.e. maximum (3% w/v for chitosan and 2% w/v for sodium tripolyphosphate) and minimum (1.5% w/v for chitosan and 1% w/v for sodium tripolyphosphate) for fixed amount of tropicamide drug to obtain four formulations as shown in Table-1. The amounts of chitosan and sodium tripolyphosphate used in factorial design were determined from literature and maximum and minimum amount were fixed by preparing the trial formulations. Trial formulation showed that when the concentration of chitosan was below minimum value (1.5% w/v), the yield of the microspheres was very low and a polymer film was formed when concentration of chitosan above from maximum level (3.0% w/v). Four formulations were prepared according to the factorial design ($2^2$) by taking maximum and minimum concentrations of chitosan and sodium TPP. The drug tropicamide was taken at fixed concentration 500 mg. The effect of factors (concentrations of chitosan and sodium TPP) was calculated on the basis of entrapment efficiency and determined the most important factor responsible for the response.

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

| Formulation Sr. No. | Experiment | Chitosan (w/v) | Sodium TPP (w/v) |
|---|---|---|---|
| 1. | (1) | 1.5 % | 1.0 % |
| 2. | A | 3.0 % | 1.0 % |
| 3. | B | 1.5 % | 2.0 % |
| 4. | AB | 3.0 % | 2.0 % |

### Characterization of Microspheres

### FTIR Analysis

The FTIR of drug, polymer, physical mixture and microspheres was performed by FTIR Spectrophotometric method (FT-IR Bruker 1206 0280, Germany). The drug polymer compatibility study was performed by taking both in the ratio of 1:1. Now the samples were finely ground with infra-red grade KBr then pressed into pellet and IR spectra were taken in transmission over the range of 4000-400 $cm^{-1}$ at ambient temperature. The obtained spectrum was then interpreted[15].

### DSC Analysis

Thermal behaviour and compatibility studies of drug, polymer and microspheres was analyzed by using (DSC Q10 V9.9 Build 303, US) instrument. 2 mg of the sample (drug, polymer) taken in closed aluminum pan was heated from 40 ˚C to 250 ˚C in an atmosphere of nitrogen gas passing at a flow of 60 ml/min. An empty Aluminium pan was taken as reference pan.

**Determination of Entrapment Efficiency**

Entrapment efficiency is the percentage of actual drug content present in chitosan microspheres, relative to the theoretical drug content and was calculated according to following equation:

$$\text{Entrapment efficiency} = \frac{\text{Actual drug content in microspheres}}{\text{Theoretical drug content}} \times 100$$

The amount of tropicamide entrapped in chitosan microspheres was estimated by dissolving 200mg of prepared microspheres in HPLC grade Ethanol by stirring for 2h, and the solution was ultra-sonicated for 30 min. This colloidal dispersion was filtered through 0.45µm whatman's filter paper and then diluted with freshly prepared phosphate buffer pH 7.4 and measured the absorbance using UV spectrophotometer at 254 nm. The actual amount of drug present in prepared microspheres was calculated[11,21].

**Surface Morphology of Microspheres**

The surface morphology and size of microspheres were determined by scanning electron microscopy. The particle size of optimized batch was determined by SEM analyzer (Zeiss EVO 50).

***In-vitro* drug dissolution and release studies**

*In-vitro* dissolution of Tropicamide-loaded microspheres was carried out using a USP II dissolution apparatus (Dissolution Test Apparatus, LABINDIA DS 8000) on samples of microspheres equivalent to one dose of drug. The tests were performed using phosphate buffer pH 7.4 as dissolution medium in following conditions 900 ml, 37±0.5 ˚C and 100 rpm. Aliquots (5.0 ml) of the medium were withdrawn at fixed interval of time by using the sampler and replaced with fresh medium and absorbance was measured at 254 nm by UV spectrophotometer and calculated for the drug release [22].

**Drug release kinetics study**

The drug release study or release kinetics of optimized formulation was determined by various drug release kinetics models such as 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[23].

**Swelling Index**

Microspheres (100 mg) were packed in dialysis bag and placed in dissolution apparatus USP Type II containing phosphate buffer pH 7.4 rotating at 25 rpm speed. Microspheres were removed every hour up to 7 hrs. Thus, the swelling index of microspheres was calculated through their weight variation by using following formula[17]:

**$S.I = (W_t - W_o)/ W_o$**

Where S.I = Swelling Index

$W_t$ = Weight of the microspheres at time t,

$W_o$ = Weight of the microspheres before placing in dissolution apparatus

## RESULTS AND DISCUSSIONS

**Preparation of Microspheres**

Microspheres were prepared by ionic gelation method with different concentrations of chitosan and sodium TPP according to $2^2$ factorial design. Their composition was reported in Table-1. Tropicamide was taken at fixed concentration (500mg) for each formulation. The prepared microspheres were spherical in shape with accurate size and having free flowing properties. The prepared microspheres were stored in well closed air tight container and evaluated for the further parameters.

**Entrapment Efficiency**

The entrapment efficiency of microspheres was calculated for the prepared microspheres according to the Table-1 and results shown in the Table-2. Microspheres with minimum polymer concentration showed the lowest entrapment efficiency whereas higher concentration showed the highest entrapment efficiency.

***In-vitro* drug release studies**

*In-vitro* drug release profile of the prepared microspheres was calculated and the results are shown in the Table-3. The drug release study revealed that formulation A1 i.e. low polymer concentration showed the maximum drug release whereas formulation A4 i.e. high polymer concentration showed the minimum drug release.

**Table 2: Entrapment Efficiency of Different Formulations**

| Formulation Sr. No. | Experiment | Drug (mg) | Chitosan (w/v) | Sodium TPP(w/v) | Entrapment Efficiency*(%) |
|---|---|---|---|---|---|
| A1 | (1) | 500 | 1.5 | 1.0 | 28.72 ± 1.40 |
| A2 | A | 500 | 3.0 | 1.0 | 44.08 ± 0.75 |
| A3 | B | 500 | 1.5 | 2.0 | 37.43 ± 2.14 |
| A4 | AB | 500 | 3.0 | 2.0 | 58.76 ± 3.82 |

*Results were expressed in average of triplicate ± SD

**Table 3: *In-vitro* Drug Release Study**

| Percentage cumulative drug release profile of different Formulations | | | | |
|---|---|---|---|---|
| **Time (min.)** | **A1 (%)** | **A2 (%)** | **A3 (%)** | **A4 (%)** |
| 15 | 10.91± 1.42 | 9.59± 3.73 | 13.31± 2.56 | 7.28± 2.18 |
| 30 | 14.44± 2.17 | 15.17± 1.64 | 17.67± 1.72 | 11.61± 1.93 |
| 60 | 19.83 ± 3.72 | 17.60± 1.87 | 25.05± 1.28 | 15.19± 1.42 |
| 120 | 23.98± 2.91 | 24.14± 1.05 | 28.97± 1.91 | 19.01± 0.38 |
| 180 | 29.13± 0.23 | 25.66± 0.56 | 32.62± 0.77 | 20.84± 1.37 |
| 240 | 34.19± 1.58 | 28.39± 1.81 | 35.78± 1.83 | 23.91± 0.92 |
| 300 | 36.28± 0.68 | 31.30± 3.40 | 37.94± 0.78 | 26.07± 1.23 |
| 360 | 37.86± 2.38 | 33.62± 0.61 | 40.60± 1.24 | 27.98± 2.14 |
| 420 | 40.60± 0.92 | 35.21± 1.92 | 41.93± 1.88 | 30.88± 0.33 |
| 480 | 45.48± 1.17 | 39.92± 0.62 | 43.51± 0.85 | 32.88± 0.86 |

*Results were expressed in average of triplicate ± SD

**Table 4: Effect of Factors and their Interactions**

| Factors | Effect of Factors |
|---|---|
| Effect of Factor A | 18.35 |
| Effect of Factor B | 11.69 |
| Magnitude of Interaction | 2.98 |

**Table 5: Different Formulations and their Entrapment Efficiency**

| Formulation Sr. No. | Drug (mg) | Chitosan (% w/v) | Sodium TPP (% w/v) | Entrapment Efficiency* (%) |
|---|---|---|---|---|
| F1 | 500 | 1.5 | 2.0 | 37.43 ± 1.26 |
| F2 | 500 | 2.0 | 2.0 | 40.64 ± 0.73 |
| F3 | 500 | 2.5 | 2.0 | 44.77 ± 1.61 |
| F4 | 500 | 3.0 | 2.0 | 58.76 ± 2.45 |

*Results were expressed in average of triplicate ± SD

**Table 6: Drug Release Profile of Different Formulations and Pure Drug**

| Cumulative percent drug release of different Formulations* | | | | | |
|---|---|---|---|---|---|
| **Time (min.)** | **F1** | **F2** | **F3** | **F4** | **Pure Drug** |
| 15 | 13.31 ± 1.28 | 8.85± 1.81 | 12.15± 2.17 | 7.28± 2.51 | 67.24± 3.54 |
| 30 | 17.67± 2.39 | 13.44±1.09 | 15.35± 1.38 | 11.61± 1.80 | 85.12± 2.83 |
| 60 | 25.05± 1.82 | 16.93±2.13 | 20.66± 1.73 | 15.19± 1.36 | 90.83± 1.47 |
| 120 | 28.97± 0.17 | 21.33± 1.42 | 22.91± 1.46 | 19.00± 1.05 | 94.00± 0.31 |
| 180 | 32.62± 0.85 | 25.23± 0.87 | 26.40± 0.74 | 20.84± 1.26 | 94.35± 0.75 |
| 240 | 35.78± 0.29 | 26.74± 0.55 | 28.48± 0.81 | 23.91± 0.48 | 95.34± 0.89 |
| 300 | 37.94± 0.97 | 32.44± 0.69 | 31.71± 1.32 | 26.07± 0.67 | 96.17± 0.66 |
| 360 | 40.60± 1.21 | 34.70± 0.81 | 33.46± 0.91 | 27.98± 0.29 | 96.83± 0.52 |
| 420 | 41.93± 0.14 | 37.85± 0.95 | 34.96± 0.27 | 30.88± 0.73 | 97.17± 0.48 |
| 480 | 43.51± 0.42 | 40.84± 0.57 | 36.87± 0.18 | 32.88± 0.45 | 98.33± 0.70 |

*Results were expressed in average of triplicate ± SD

**Table 7: Correlation coefficient ($R^2$) Value of Various Kinetic Models**

| Release kinetic model | Zero order | First order | Korsmeyer-Peppas model | Higuchi model | Hixson Crowell model |
|---|---|---|---|---|---|
| **$R^2$ Value** | 0.894 | 0.925 | 0.987 | 0.975 | 0.916 |

**Table 8: Swelling Index of Microspheres**

| Time (hours) | Swelling Index* |
|---|---|
| **1** | 4.51 ± 1.73 |
| **2** | 13.94 ± 2.61 |
| **3** | 17.86 ± 0.71 |
| **4** | 19.69 ± 0.82 |
| **5** | 21.71 ± 0.28 |
| **6** | 22.86 ± 0.66 |
| **7** | 22.98 ± 0.12 |

*Results were expressed in average of triplicate ± SD

**Optimization Technique**

On the basis of entrapment efficiency of the prepared formulations, the effect of factors and their magnitude of interactions were calculated by using above equations (1, 2 and 3) and their effects were shown in Table-4.

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 maximum level and the amount of chitosan was varied between maximum and minimum level to obtain the optimized formulation.

On the basis of effects of factors, further four formulations were prepared (Table 5) and entrapment efficiency and drug release was calculated for all the prepared formulations. The optimized formulation was selected on the basis of higher entrapment efficiency and sustained release of the drug.

The concentration of chitosan showed enormous effect on entrapment efficiency. The increasing concentration of chitosan (1.5% to 3.0%) at constant sodium TPP showed an increase in the entrapment efficiency from 37.43 to 58.76 respectively as shown in Table-5. Entrapment efficiency was found to be dependent on the concentration of the chitosan so more entrapment efficiency was obtained with higher polymer concentration. The similar results were reported by Nagarajan et al., (2015) that entrapment efficiency of Lansoprazole loaded chitosan nanoparticles increased from 39.3±2.6% and 85.6±1.2% as the amount of chitosan increased[24]. Patil et al., (2014) formulated doxorubicin loaded spray dried chitosan nanocarriers and concluded that particles formed with maximum chitosan concentration resulted in increased entrapment efficiency[25].

***In-vitro* drug release studies**

The *in-vitro* drug release profile from different drug-loaded chitosan microspheres was presented in table, in comparison with the drug release profile of pure drug. The rate of release of pure drug was quite fast: more than 65% drug is dissolved in about 15 min, whereas, the microspheres released about 32.88 to 43.51 % drug release in 8 hrs. So, the microspheres were considered to be satisfactory candidates for controlled delivery of drug.

During drug release study it was found that, the release of drug from microspheres depends upon the chitosan concentration. High concentration of chitosan showed the slow release of the drug from microspheres because the higher cross linking due to high polymer prevented the release of drug from microspheres.

The same behaviour was observed by Nagarajan et al., (2015) that the drug content for the Lansoprazole loaded chitosan nanoparticles varied from 69.5±7.2% to 87.9±1.2%. The release drug content was decreased with increase in chitosan concentration[24].Sriramet al., (2013) studied that the results of the *in-vitro* dissolution studies of formulations F1 to F5 showed 94.92 % cumulative drug release at the end of 12 h while F5 formulation showed 78.67 % cumulative drug release at the end of 12 h. This is because with increase in polymer concentration the free drug concentration on the surface of microspheres will be decreased and more drug get entrapped in the microspheres and drug gets released slowly[26].

**Characterization of Optimized formulation**
**FT-IR Study**

Tropicamide, chitosan, their physical mixture and microspheres was analyzed using FT-IR spectrophotometer for characteristic absorption bands. The IR spectra of tropicamide (Figure 2) showed several characteristic peaks at 1487 $cm^{-1}$for C=C, Aromatic C=O vibrations at 1660 $cm^{-1}$, 2905 $cm^{-1}$ correspond to C-H Aliphatic vibrations, 1256 $cm^{-1}$ belongs to C-N (Nitrile), 1660 $cm^{-1}$for C=N (Imine), 3650 $cm^{-1}$for O-H Aromatic.

The IR spectra of chitosan (figure 3) was characterized by the intense peak at 3625 $cm^{-1}$ correspond to the combined peak of O-H stretching and intermolecular hydrogen bonding. Specific peaks of chitosan were observed at3079 for N-H (2º Amide), 3350 for $NH_2$ (2º Amine), 1656 for O=C-$NH_2$ (Carbonyl amide), 2881 for C-H Aliphatic, 3625 for O-H Aromatic. The symmetric stretch of C-O-C (Ether) was found at 1073 $cm^{-1}$.

The peaks present in polymer mixture (figure 4) were 1134 for C-N (Nitrile), 2369 for N-H (Amine), 3396 for $NH_2$ Stretching 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[11].

The FT-IR spectrum of microspheres (figure 5) was different from that of physical mixture and each pure component. This spectra changed may be attributed to the electrostatic interaction between chitosan and TPP. Spectrum of formulation (Figure 7) showed peaks at 1463 $cm^{-1}$ for C=C, 2953 $cm^{-1}$ for C-H, 3068 $cm^{-1}$ belongs to C-H, 3678 $cm^{-1}$ for O-H, 1261 $cm^{-1}$ for C-N, 1694 $cm^{-1}$ for C=N, 1647 $cm^{-1}$ for C=O. 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 microspheres.

**Differential Scanning Calorimetry**

The DSC thermogram of drug (Figure 6) showed a sharp endothermic peak at 101.89℃ which indicated its melting point. A broader peak was obtained in the thermogram of chitosan (Figure 7) it revealed amorphous form of chitosan. The thermogram of microspheres (Figure 8) showed melting point at 115.48℃. In the thermogram of microspheres, the corresponding peaks of drug and polymer were absent, instead a broad peak at 115.48℃, this showed that drug was entrapped within polymer in the form of microspheres.

**Surface Morphology of Microspheres**

The morphology of tropicamide loaded microspheres (Figure 9) was identified by Scanning Electron Microscopy at 10.0 kV acceleration voltages. The optimized batch was used for the SEM analysis. The particle size was found to be 15µm and spherical in shape. Similar results were obtained by researchers. Gaviniet al., (2004) prepared PLGA microspheres for the ocular delivery of vancomycin having particle size 11µm[22].Selvarajet al., (2011) prepared chitosan microspheres for ocular delivery of acyclovir and resulted in micron size particles of 2-12 µm[27].

**Drug Release Kinetic Study**

To determine the exact mechanism of present drug delivery system from the microspheres, the release data of prepared tropicamide loaded chitosan microspheres in phosphate buffer (pH 7.4) were fitted to various drug release kinetics models such as Zero order, First order Korsmeyer-Peppas, Higuchi model and Hixson Crowell model to understand the mechanism and kinetic of drug release. The Correlation Coefficient ($R^2$) value of Korsmeyer-Peppas model was found to be high.

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) [23].

**Swelling Index**

Microspheres 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. The swelling index was calculated by determining the change in weight of microspheres and shown in Table-8.The change in weight and swelling index data revealed the mucoadhesive property of microspheres.

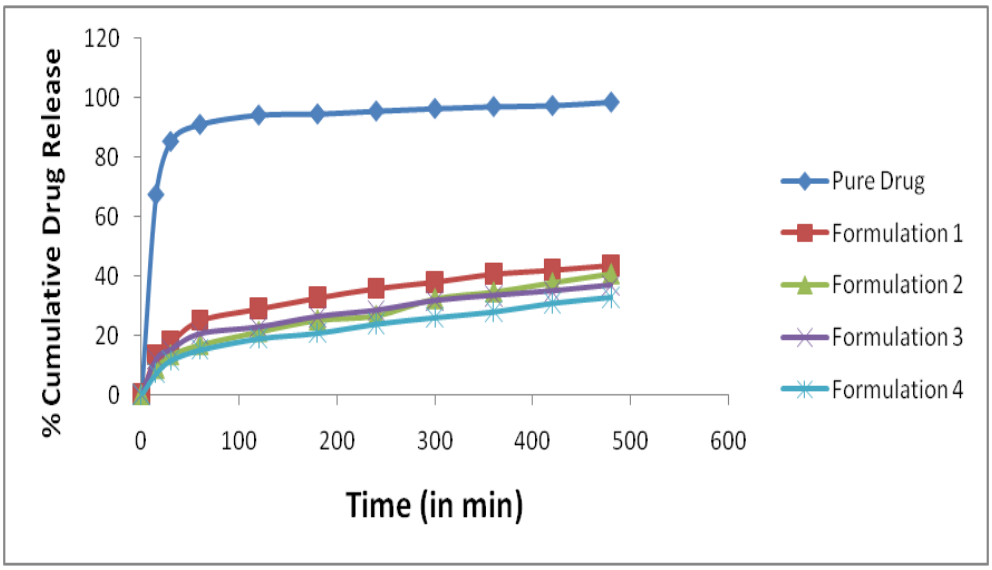


**Figure 1: Drug Release Profile of Pure Drug and Different Formulation**

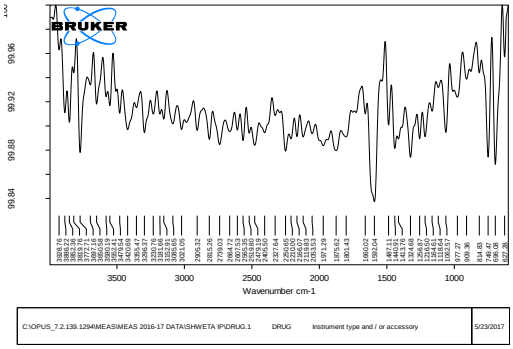


**Figure 2: FT-IR Spectra of pure drug (Tropicamide)**

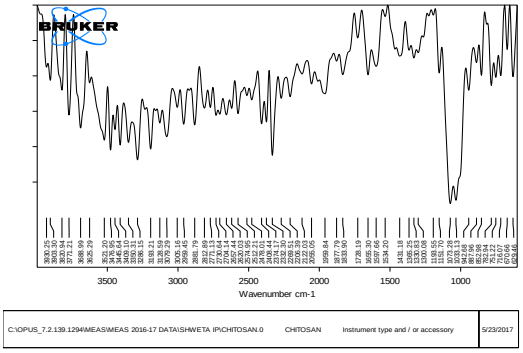


**Figure 3: FT-IR Spectra of Polymer (Chitosan)**

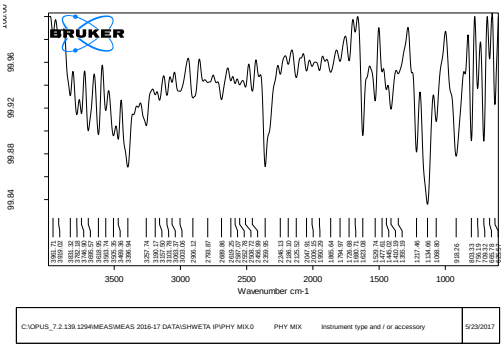


**Figure 4: FT-IR Spectra of Physical Mixture (Drug and Polymer)**

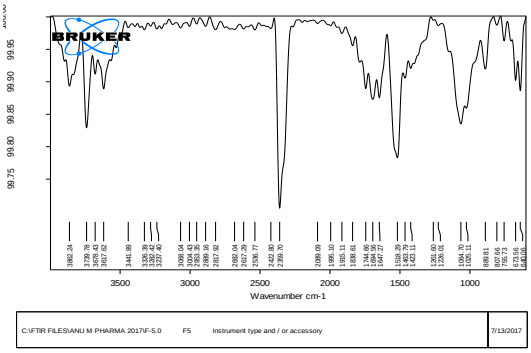


**Figure 5: FT-IR Spectra of Formulation**

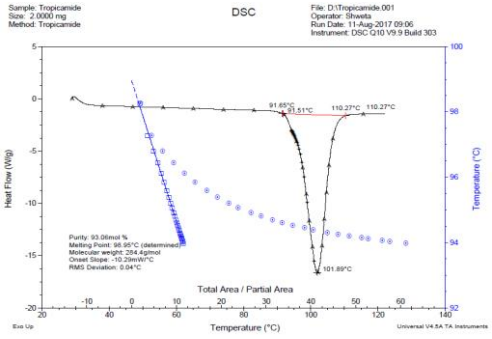


**Figure 6: DSC analysis of pure drug (Tropicamide)**

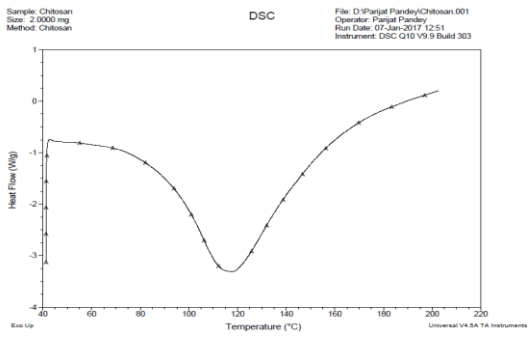


**Figure 7: DSC Analysis of Polymer (Chitosan)**

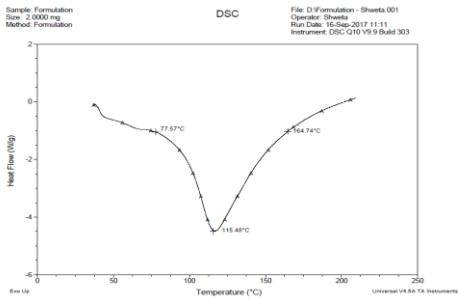


**Figure 8: DSC of Tropicamide loaded Chitosan Microspheres**

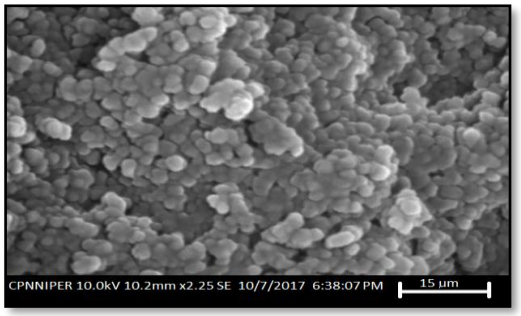


**Figure 9: SEM of Tropicamide loaded Chitosan Microspheres**

## CONCLUSION

Microspheres of tropicamide drug were prepared with an objective to increase the ocular residence time and bioavailability, to prolong the drug release and to improve the permeation across the corneal membrane. Chitosan was used as a primary mucoadhesive polymer and Sodium tripolyphosphate as cross linking polymer, in different ratios according to $2^2$ factorial design selecting two factors (Chitosan and NaTPP) and two level (min. and Max.). The microspheres were subjected for evaluation of various parameters such as entrapment efficiency, *in-vitro* drug release, particle size and swelling index. It was noticed that on increasing the fraction of chitosan, the entrapment efficiency of microspheres was also increased. *In-vitro* drug release studies were carried out using USP type-II apparatus. The release profile of tropicamide loaded chitosan microspheres showed a sustained release as compared to pure drug and drug release from the microspheres decreased with increase in chitosan concentration. The entrapment efficiency and drug release profile of microspheres appeared to depend on the concentration of chitosan, which is the most significant factor for the response. The mucoadhesive property of chitosan and sustain release action of microspheres may contribute to enhance corneal residence time of tropicamide. Further, in- vivo bioavailability and stability studies are required to co-relate the results in the body and confirm the findings.

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