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# Formulation and evaluation of Trospium chloride Microspheres for sustained drug delivery

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## ABSTRACT

The objective of the present study was to prepare and evaluate microspheres for the sustained release of Trospium Chloride by using various ratio of 1.5%w/v chitosan polymer, 2.5% w/v chitosan polymer, 3.5%w/v chitosan polymer. It is used to treat the Urge urinary incontinence and overactive bladders are common conditions often associated with profound impairment of the health and quality of life of the patient. It mainly acts as the M3 receptor antagonist that is present on sphincter muscle which is present at the urinary bladder opening. Trospium Chloride loaded Chitosan microspheres were prepared by a solvent evaporation method. The resultant microspheres were evaluated for average particle size, drug entrapment, *In vitro* drug release. FTIR, DSC and SEM were used to investigate the physical state of the drug in the microspheres. The mean particle size of the microspheres was influenced by varying drug Polymer ratio while drug loading was dependent on drug : polymer ratio. The entrapment efficiency was 98.23%. The release of drug from the microspheres extended upto 8 to 12hrs. The results of FTIR indicated the stable character of Trospium Chloride loaded Chitosan microspheres and also absence of drug polymer interaction. The Trospium Chloride loaded Chitosan microspheres prepared under optimized conditions showed good sustained release characteristics & were stable under the conditions studied.

**Keywords:** Microspheres; Trospium Chloride; Chitosan; Solvent evaporation method.

## INTRODUCTION

Trospium chloride is a quaternary ammonium compound with the chemical name of spiro [8-azoniabicyclo [3, 2, 1] octane-8,1'-pyrrolidinium]-3-[ (hydroxy diphenyl-acetyl)-oxy]chloride (1α, 3β, 5α)-(9Cl). Overactive bladder (OAB), a common problem of the urinary tract, can be described as urinary urgency, frequency, orurge urinary incontinence (UI). OAB can present with a constellation of symptoms or as a symptom syndrome, which may include urgency and frequency (voiding more than eight times in a 24-hour period) along with nocturia. Patients may describe a sudden, compelling desire to urinate that may be difficult to defer until a later time (Radihejazi and mansooramiji et al., 2013)

There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microspheres as carriers for drugs. Microspheres are characteristically free flowing powders consisting of protiens or synthetic polymers which are biodegradable in nature and ideally having a particle size less than 200 μm.

Microspheres can be defined as solid, approximately spherical particles ranging in size from 1 to 1000 μm. They are made of polymeric, waxy or other protective materials, that is, biodegradable synthetic polymer and modified natural products. Such as starches, gums, proteins, fats and waxes (Agnihotrisa et al., 2014).

The development of new delivery systems for the controlled release of drugs is one of the most interesting fields of research in pharmaceutical sciences. Microparticles can be used for the controlled release of drugs, vaccines, antibiotics, and hormones. For example, by taking advantage of the characteristics of microspheres, beyond the basic benefits, the microspheres could provide a larger surface area and possess an easier estimation of diffusion and mass transfer behavior also the encapsulated small molecules could diffuse out of the barrier with precise kinetics modeling and control-release of drugs to the body fluid. Among the polymer systems employed, the Ethyl cellulose, a weak cationic polysaccharide, has many advantages for

developing micro-particles in drug release applications (Kashyap N et al., 2007).

The use of controlled release systems has certain advantages compared with conventional dosage forms, as they can minimize side effects, and prolong the efficacy of the drug. These release forms regulate the drug release rate and can reduce the frequency of administration of the drug, thus assuring better patient compliance. Pulsatile delivery systems based on chitosan have also been described, which are interesting with regard to adjusting drug release to physiological needs of the body, as in the case of hormone release (Sinhav.R et al., 2014).

### ADVANTAGES

1. Reliable means to deliver the drug to the target site with specificity, if modified, and to maintain the desired concentration at the site of interest without untoward effects.

2. Solid biodegradable microspheres have the potential throughout the particle matrix for the controlled release of drug.

3. Microspheres received much attention not only for prolonged release, but also for targeting of anticancer drugs to the tumour.

4. The size, surface charge and surface hydrophilicity of microspheres have been found to be important in determining the fate of particles *in vivo*.

5. Studies on the macrophage uptake of microspheres have demonstrated their potential in targeting drugs to pathogens residing intra cellularly.

6. **Blood flow determination**: Relatively large microspheres (10-15 µm in diameter) are useful for regional blood flow studies in tissues and organs. In most cases the microspheres are injected at desired locations in the circulatory system and eventually lodge in the capillaries. The microspheres and fluorescent dyes they contain are first extracted from the tissue sample, and then fluorescence is quantitated on a pectrofluorometer or fluorescence microplate reader. Traditionally, this type of study has been carried out using radiolabelled microspheres; however fluorescent microspheres have been shown to be superior in chronic blood flow measurements (Park et al., 2002).

7. A highly vascularised sub-epithelial layer allowing for rapid and direct absorption into the systemic circulation, avoiding first-pass hepatic metabolisum.

8. A less hostile environment than the gastro-intestinal tract, resulting in reduces drug denaturing.

9. Improved patient compliance and comfort compared with intravenous administration.

10. Rapid absorption, higher bioavailability.

11. Voidance of liver first pass metabolism. Avoidance of irritation of the gastrointestinal membrane.

### LIMITATIONS

➤ The nasal route of delivery is not applicable to all drugs. In spite of high permeability of the nasal mucosa, many drugs may not be sufficiently absorbed (Radiheizi et al., 2003).

➤ Lack of adequate aqueous solubility is often a problem. Note that the entire dose is to be given in a volume of 25–200 ml which can cause nasal irritation.

➤ Others may undergo metabolic degradation in the nasal cavity. The nasal route is less suitable for chronically administered drugs.

➤ For example, insulin for the treatment of type I diabetes may not be an appropriate drug candidate for nasal delivery because patients will need to administer this drug daily for the remainder of their lives.

➤ If a chronic drug is to be administered much less frequently, its nasal delivery may still be a viable option.

### MATERIALS AND METHODS

Trospium chloride (Hetero Hyderabad), Heavy liquid paraffin, (M.R.Silicone industries, mumbai), Light liquid paraffin, chitosan, (India sea foods, cochin, India), Glutaraldehyde (Lindyunganhond iaimp.exp. co. ltd, China) Span80 (Zibo – zhocuntianhe chemicals co. ltd, china).

### Method

For optimizing the polymer concentration nine formulae were prepared by taking drug polymer ratio of TCM 1 (0.1:1.5),TCM 2 (0.2:1.5), TCM 3 (0.3:1.5),TCM 4 (0.1:2.5), TCM 5 (0.2:2.5),TCM 6(0.3:2.5),TCM 7(0.1:3.5),TCM 8(0.2:3.5),TCM 9 (0.3:3.5). The drug and chitosan microspheres were prepared by making 2.5%w/v chitosan solution in a aqueous acetic acid(1%v/v) and the drug was added and then this dispersed phase was added with stirring to continuous phase(125ml) consisting of liquid paraffin and heavy liquid paraffin in the ratio of 1:1 containing 1.0%w/v span80 to form a water in oil (w/o) emulsion. Stirring was continued at 3000rpm using a triple blade propeller stirrer. Solution of measured quantity of (2.5ml each) of toluene saturated glutaraldehyde (2.5%v/v) was added in drop wise at 15, 30, 45and 60 minutes. Stirring was continued for 2.5h to obtain microspheres which were separated by filtration under vaccum and washed first with petroleum ether and then with distilled water to remove the adhered liquid paraffin and glutaraldehyde respectively. The microspheres were then finally dried in a desicator. The final preparation was free flowing powder consisting of spherical micron sized particles (Jayakrishan et al., 1992)

## Compatibility studies

### IR studies

In the preparation of drug and polymer may interact as they are in close contact with each other, which could lead to the instability of drug pre-formulation studies regarding the drug – polymer interaction are therefore very critical in appropriate polymer. FT – IR Spectroscopy was employed to ascertain the compatibility between trospium chloride and the chitosan polymer. Perkin Elmer Jasco FTIR- 401, Japan, (Prakash Goudanayar et al., 2008).

### Differential scanning calorimetry

The output of a DSC is a plot of heat flux (rate) versus temperature at a specified temperature rate. DSC provides information about the physical properties of the sample as crystalline or amorphous nature and demonstrates a possible interaction between drug and polymers in formulations. According to the thermo grams.

### Surface morphology

The morphology, surface appearance of Chitosanmicrospheres was found by Scanning Electron Microscopy (SEM). The particles were freeze dried, coated with gold palladium to achieve a film of 20nm thickness and observed microscopically (P. Ile et al., 1999).

### In vitro evaluation of microspheres

### Percentage production yield (PY).

The percentage production yield was calculated as follows:

$$PY\% = \frac{Practical\ mass\ of\ microspheres}{Theoretical\ mass} \times 100$$

Each formulation was carried out in triplicate and the PY (%) was calculated.

### Drug loading capacity and encapsulation efficiency

Loading capacity is the maximum amount of drug that can be incorporated in the microspheres. The encapsulation efficiency is the amount of added drug (in percent) that is encapsulated in the formulation of microspheres (Inoue K et al., 1996).

$$Percentage\ drug\ loading = \frac{Mact}{Mms} \times 100$$

$$Percentage\ encapsulation\ efficiency = \frac{Mact}{Mthy} \times 100$$

Where Mact is the actual drug content in a weighed quantity of microspheres, Mms is the weighed quantity of microspheres and Mthy is the theoretical amount of drug in microspheres calculated from the quantity added in the process.

## Particle size determination

Particle size of Microspheres was determined using an optical microscopy method. Approximately 100 microspheres were counted for particle size. The distribution of particle size was measured by suspending in water. (Inoue K et al., 1996)

### Equilibrium swelling studies of microspheres

A preweighed amount (100 mg) of microspheres was placed in Phosphate buffer (pH7.4) and allowed to swell to a constant weight. The microspheres were removed and blotted with filter paper, and their changes in weight were measured. The degree of swelling (a) was then calculated from the following formula. (Inoue K et al., 1996)

$$\alpha = \frac{Wg - Wo}{WO}$$

Where

Wo is the initial weight of the microspheres and

Wg is the weight of the microspheres at equilibrium swelling in the medium.

### Drug content determination

50mg of Trospium chloride microspheres was crushed and suspended in water to extract the drug from the microspheres. After 24 h, the filtrate was assayed spectrophotometrically at 215nm for drug content against water as blank.

### Mathematical modeling of release kinetics

The in vitro drug release data were fitted to various release kinetic models, namely first order ( Ln Qo- k1 t), zero order Q=(Qo_ko t), Higuchi equations (Q=kh 2)1/2, Korsemeyer-Peppas (log Q tvs log t). (Iims. T et al., 2000)

Where

Qt, is the cumulative amount of drug released at time t and

$Q_0$ is the initial amount of drug present in microspheres;

k0 is the zero order release rate constant,

k1 is the first order release rate constant, and

kh is the diffusion rate constant.

## RESULTS AND DISCUSSION

### Compatibility studies

### IR studies

The IR spectrum of the pure Trospium chloride sample recorded by FTIR spectrometer. This was compared with standard functional group frequencies of Trospium chloride as shown in Table 2

From FTIR study, the characteristic peaks of drug such as of OH (3130.57), CH Stretching Aromatic

Table 1: Formulations of trospium chloride microspheres

| S.No | Ingredients | TCM1 | TCM2 | TCM3 | TCM4 | TCM5 | TCM 6 | TCM 7 | TCM 8 | TCM 9 |
|------|-------------|------|------|------|------|------|-------|-------|-------|-------|
| 1 | Trospium chloride | 0.1 | 0.2 | 0.3 | 0.1 | 0.2 | 0.3 | 0.1 | 0.2 | 0.3 |
| 2 | Chitosan | 1.5 | 1.5 | 1.5 | 2.5 | 2.5 | 2.5 | 3.5 | 3.5 | 3.5 |
| 3 | Glutaraldehyde | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| 4 | Liquid paraffin (light) | 65 | 65 | 65 | 65 | 65 | 65 | 65 | 65 | 65 |
| 5 | Liquid parraffin | 65 | 65 | 65 | 65 | 65 | 65 | 65 | 65 | 65 |
| 6 | Span80 | qs | qs | qs | qs | qs | qs | qs | qs | qs |

Table 2: IR Interpretations for Pure drug and Polymer

| Funtional Groups | Trospium Chloride | Trospium Chloride + Chitosan |
|------------------|-------------------|------------------------------|
| OH | 3130.57 | 3140.12 |
| CH Stretching(Aromatic) | 3003.27 | 3020.34 |
| CHStretching(Aliphatic) | 2963.12 | 2862.10 |
| C=O | 1749 | 1630 |
| C=C | 1600 | 1560 |
| Al-CH-bend | 1454.3 | 1334.2 |
| Ar-CH (In plane Bending) | 1091.75 | 1082 |
| Ar-CH ( Out plane Bending) | 920.08 | 900.21 |
| c-o-c (Ether inkage) | 1193.98 | 1082.23 |
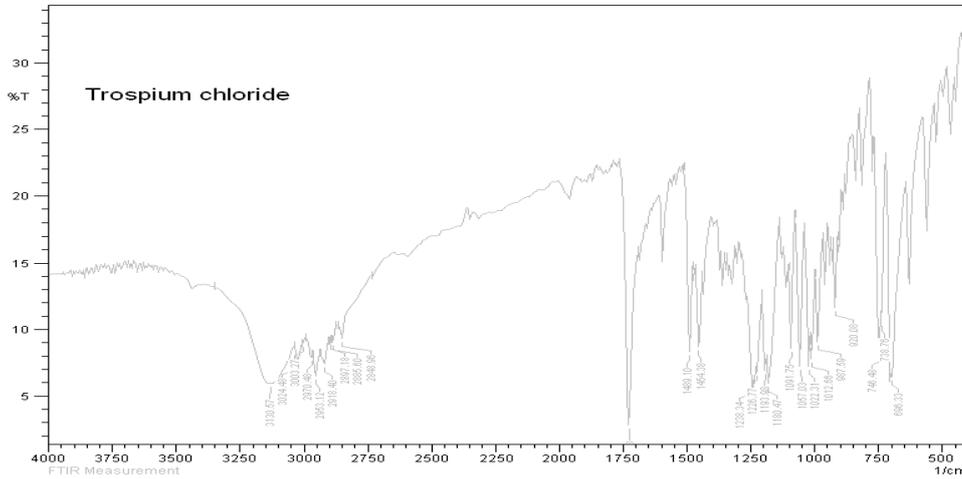


Figure 1: FT – IR of Trospium chloride
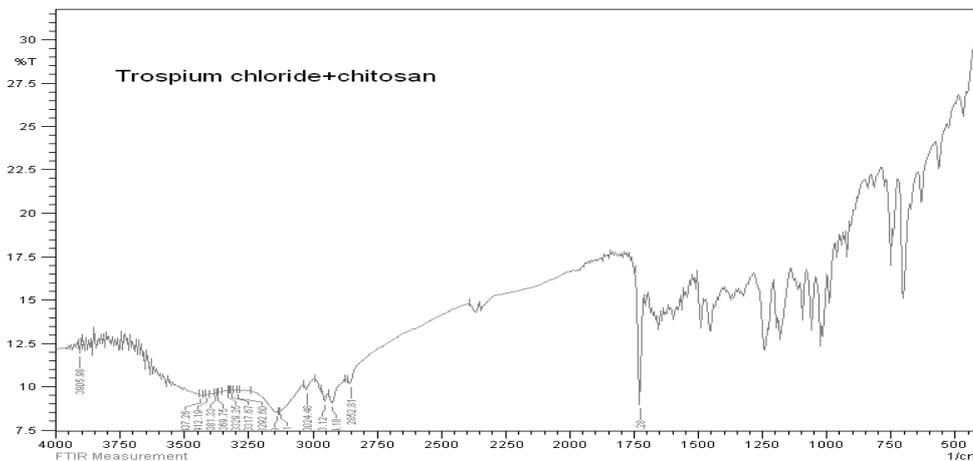


Figure 2: FT –IR of Trospium chloride + Chitosan

(3003.27cm$^{-1}$), CH Stretching Aliphatic (2963.12cm$^{-1}$), C=O(1749cm$^{-1}$), Al-CH-bend(1454.3cm$^{-1}$), Ar-CH In plane Bending(1091.75cm$^{-1}$), Ar-CH Out plane Bending (920.08cm$^{-1}$), c-o-c Ether inkage (1193.98cm$^{-1}$) appeared for the pure drug trospium chloride. For SLN all peaks which have been obtained for the pure drug were available at same wave length for OH (3130.57), CH Stretching Aromatic (3003.27cm$^{-1}$), CH Stretching Aliphatic (2963.12cm$^{-1}$), C=O(1749cm$^{-1}$), Al-CH-bend (1454.3cm$^{-1}$), Ar-CH In plane Bending(1091.75cm$^{-1}$), Ar-
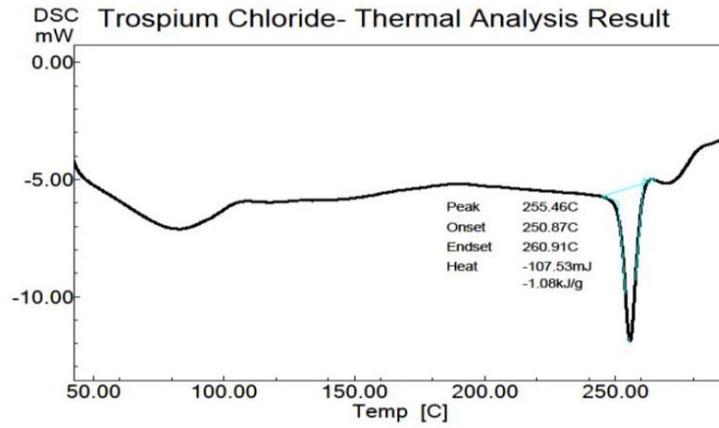
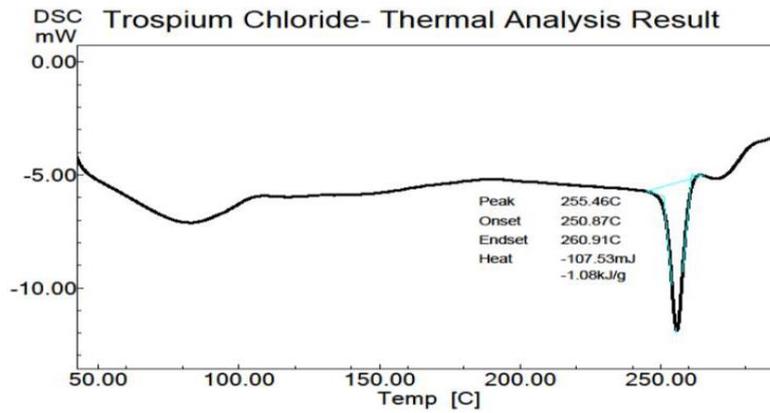**Figure 3: DSC of Trospium chloride**



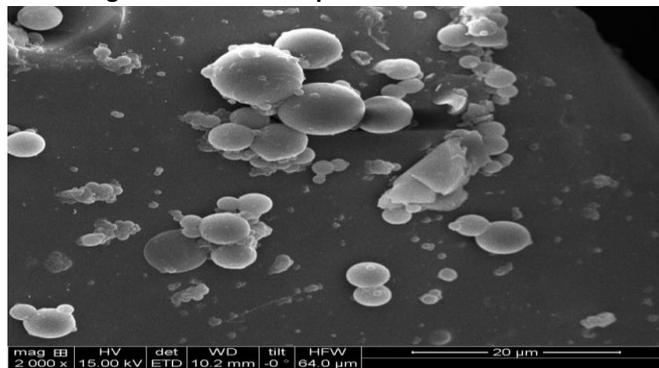**Figure 4: DSC of Trospium chloride + Chitosan**


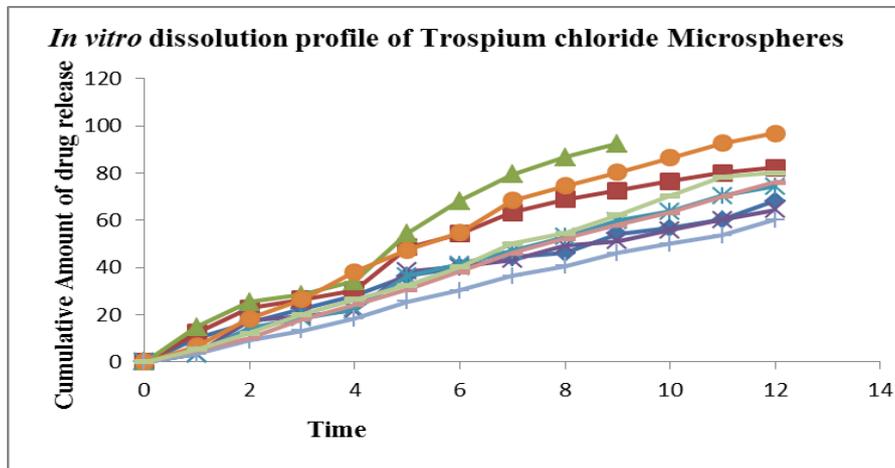
**Figure 5: SEM PICTURES OF FORMULATION TCM6**



**Figure 6: In Vitro Dissolution Release of Trospium chloride Microspheres**

**Table 3: Characterization of Trospium chloride microspheres**

| F. code | Percentage yield (%) | Percentage drug content | Drug entrap-ment efficiency (%) | Particle size(μm) | Cumulative Percentage Release (in 12hrs) | Swellability Studies |
|---------|---------|---------|---------|---------|---------|---------|
| TCM1 | 42.3. | 38.11 | 60.56 | 30.12 | 68.24 | 0.7345 |
| TCM2 | 46.9 | 42.31 | 64.5 | 33.25 | 82.3 | 0.7823 |
| TCM3 | 50.1 | 51.64 | 69.32 | 38.7 | 92.4 | 0.4534 |
| TCM4 | 56.6 | 81.40 | 92.67 | 40.6 | 64.5 | 0.5678 |
| TCM5 | 74.2 | 85.34 | 95.54 | 54 | 74.3 | 0.3456 |
| **TCM6** | **81.25** | **87.2** | **98.23** | **37.4** | **96.8** | **0.9234** |
| TCM7 | 62.3 | 63.54 | 72.27 | 103.6 | 60.2 | 0.6987 |
| TCM8 | 69.8 | 75.14 | 74.3 | 120.6 | 76.2 | 0.4455 |
| TCM9 | 72.1 | 78.58 | 76.1 | 142.3 | 80.1 | 0.2342 |

**Table 4: Cumulative % drug releases of Trospium chloride microspheres**

| F.Code | Cumulative % Drug Release in 1hr time intervals | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | TCM1 | TCM2 | TCM3 | TCM4 | TCM5 | TCM6 | TCM7 | TCM8 | TCM9 |
| 1 | 10.2 | 12.4 | 14.8 | 3.2 | 3.2 | 6.5 | 3.2 | 4.8 | 5.2 |
| 2 | 16.8 | 22.6 | 25.3 | 17.6 | 14.6 | 18.2 | 9.12 | 10.16 | 12.12 |
| 3 | 22.4 | 26.5 | 28.4 | 19.4 | 18.5 | 26.4 | 13.2 | 18.12 | 20.12 |
| 4 | 28.2 | 30.5 | 34.2 | 21.83 | 22.4 | 38.2 | 18.4 | 24.2 | 26.4 |
| 5 | 36.5 | 48.5 | 54.4 | 38.4 | 36.3 | 47.5 | 25.5 | 30.6 | 32.4 |
| 6 | 40.2 | 54.3 | 68.3 | 40.3 | 41.2 | 54.8 | 30.2 | 46.2 | 40.2 |
| 7 | 44.4 | 63.4 | 79.4 | 43.2 | 47.1 | 68.4 | 36.5 | 52.4 | 50.2 |
| 8 | 46.2 | 68.9 | 86.7 | 49.2 | 52.9 | 74.6 | 40.6 | 58.12 | 54.6 |
| 9 | 54.2 | 72.6 | 92.4 | 51.3 | 59.7 | 80.3 | 46.2 | 63.3 | 62.2 |
| 10 | 56.8 | 76.5 | -- | 55.8 | 63.8 | 86.6 | 50.1 | 70.1 | 70.45 |
| 11 | 60.2 | 80.2 | -- | 60.3 | 70.4 | 92.7 | 53.7 | 76.2 | 78.3 |
| 12 | 68.24 | 82.3 | -- | 64.5 | 74.3 | 96.8 | 60.2 | -- | 80.1 |

**Table 5: Release kinetics of Trospium chloride Microspheres**

| Formulation code | Zero order | | First order | | Higuchi | | Krosmayer | |
|---|---|---|---|---|---|---|---|---|
| | $R^2$ | M | $R^2$ | M | $R^2$ | M | $R^2$ | M |
| TCM1 | 0.9883 | 5.011 | 0.9818 | 2.0027 | 0.9905 | 5.4827 | 0.0917 | 0.0653 |
| TCM2 | 0.963 | 6.706 | 0.9887 | 0.0672 | 0.9603 | 6.706 | 0.869 | 0.0686 |
| TCM3 | 0.9791 | 10.912 | 0.7641 | 0.1759 | 0.9791 | 10.912 | 0.9563 | 0.1015 |
| TCM4 | 0.9899 | 6.4462 | 0.779 | 0.1097 | 0.9889 | 6.4462 | 0.9832 | 0.0417 |
| TCM5 | 0.9668 | 7.155 | 0.9911 | 0.0673 | 0.9668 | 7.155 | 0.7957 | 0.088 |
| TCM6 | 0.984 | 8.3857 | 0.913 | 0.12 | 0.8149 | 0.088 | 0.8149 | 0.088 |
| TCM7 | 0.9971 | 5.1648 | 0.9906 | 0.0343 | 0.9971 | 5.1648 | 0.8485 | 0.0968 |
| TCM8 | 0.9983 | 6.5878 | 0.9736 | 0.052 | 0.9983 | 6.5878 | 0.8691 | 0.0953 |
| TCM9 | 0.9966 | 7.0889 | 0.9966 | 7.0889 | 0.966 | 7.0889 | 0.8701 | 0.0937 |

CH Out plane bending (920.08cm$^{-1}$), c-o-c Ether inkage (1193.98cm$^{-1}$) remaining peaks also either shifted or replaced in the IR spectrum of formulation shown in Fig. 1 to 2.

### DSC studies

The pure drug Trospium chloride shown as an endothermic peak at 260.91ºC. The peak neither is nor shifted in the case of DSC of the Trospium chloride microspheres formulation containing Trospium chloride. The DSC of physical mixture of the Trospium chloride and chitosan as showed an endothermic peak at 260.91ºC. The DSC spectra as shown in fig. 1 to 2

### MORPHOLOGY OF THE PARTICLES

The following methods are used to determine particle size, size distribution, and morphology of Chitosan microspheres.

### SEM

Morphology and structure of stealth Microspheres were determined using scanning electron microscopy

(SEM) (Hitachis- 2600N Japan), and photomicrographs were taken at suitable magnifications. The photographs of the optimized TCM6 formulation taken by Scanning electron microscopy are shown in the fig. 3

## EVALUTION OF TROSPIUM CHLORIDE MICROSPHERES

## PERCENTAGE YIELD

The production yield of microspheres of trospium chloride using chitosan. The TCM 1 (42.3%), TCM 2 (46.9%), TCM3 (50.1%), TCM4 (56.6%), TCM 5 (74.2), TCM6 (81.25%) TCM 7(62.3%), TCM 8 (69.8%), TCM 9 (72.1%) results as shown in Table 3.

## Drug loading capacity and encapsulation efficiency

## Drug entrapment efficiency (%EE)

Percentage entrapment efficiency of TCM 1 (60.56%), TCM 2 (64.5%), TCM3 (79.32%), TCM4 (92.67%), TCM 5 (95.54%), TCM6 (98.23%), TCM 7(97.27%), TCM 8 (74.3%), TCM 9(76.1 %) respectively. The TCM 6 shows the good formulation & high efficiency. Results as shown in Table 3.

## Particle size

Particle size distribution of Microspheres represented it indicated that particles are in nanometric range suitable for over active bladder. TCM 1 (30.12μm), TCM 2 (33.25μm), TCM3 (38.7μm), TCM4 (40.6μm), TCM 5 (54μm), TCM6 (37.4μm), TCM 7(103.6μm), TCM 8 (120.6μm), TCM 9(142.3μm) Formula as shown in given Table 3.

## Percentage drug content

Drug content distribution of Microspheres represented it indicated that drug content is in nanometric range suitable for over active bladder. TCM 1 (38.11%), TCM 2 (42.31%), TCM3 (51.64%), TCM4 (81.40%), TCM 5 (85.34%), TCM6 (87.2%), TCM 7 (63.54%), TCM 8 (75.14 %), TCM 9(78.58%) Formula as shown in given Table 3.

## Equilibrium swelling studies of microspheres

A pre-weighed amount (100 mg) of microspheres was placed in Phosphate buffer (pH7.4) and allowed to swell to a constant weight. The microspheres were removed and blotted with filter paper, and their changes in weight were measured results as shown in Table 3.

## In vitro drug release kinetics

For understanding the mechanism of drug release rate kinetics of the drug from dosage forms, the invitro drug diffusion data obtained was fitted to various mathematical models such as zero order, First order, Higuchi matrix, Krosmeyer Peppas model .The values are complied in. The % drug release with data to various kinetic models for different microspheres formulations is presented in Fig. And Table

## CONCLUSION

Sustained release microspheres of Trospium chloride an anti-muscarnic drug was developed to investigate the influence of variables such as the amount of drug and the amount of polymer on drug release. Trospium chloride microspheres (TCM) were prepared by w/o emulsion solvent evaporation method using chitosan as a release retarding polymer. Nine formulae were prepared using varying concentration of chitosan (1.5% w/v, 2.5% w/v and 3.5% w/v and increasing concentrations of drug (1:1, 1:2 and 1:3). The morphology, particle size distribution, drug content uniformity and entrapment efficiency and in vitro drug release studies were conducted. The mean particle size of optimized microspheres (TCM 4 to TCM 6) was found in a range of 37.4 μm to 54.0 μm. The drug content was found to be in the range of 38.11 to 87.2 %. The drug release patterns were studied by using 0.1N HCL for 2 hrs and in 7.4 phosphate for 12 hours using USP XIII apparatus 2 at $37^0$C at 100rpm. Microspheres were characterised by Fourier transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC) to confirm the absence of chemical interactions between drug and polymer. The optimised batch TCM 6 released 96.8 % of drug at 12 h Phosphate buffer pH7.4. With regard to release kinetics, the data of the optimized formula were best fitted with the Higuchi model ($r^2$=0. 8149) and showed zero order release ($r^2$=0.984) with Fickian diffusion mechanism The findings of the present study conclusively state that chitosan (1:3 of drug to polymer ratio) microspheres of trospium chloride is potential for the sustained drug delivery of trospium chloride in urinary tract infections.

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