DEVELOPMENT AND EVALUATION OF MUCOADHESIVE MICROSPHERES OF TETRACYCLINE BY USING A SPRAY DRYING TECHNIQUE

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ABSTRACT
Tetracycline an antibiotic was selected as drug for the experiment. Chitosan microspheres of Tetracycline were prepared by spray drying technique using chitosan as a rate controlling polymer and the microspheres were evaluated. Chitosan microspheres were spherical, discrete, free flowing and multinucleate monolithic type. The mean size range was found for formulation in the range of 17.24 to 19.02 μ. Entrapment efficiency was in the range of 82.862 to 89.758 %. The FTIR and DSC study confirmed that no chemical interaction took place during entrapment process. The X-ray diffraction study indicates the amorphous dispersion of the drug after entrapment in to microspheres. Zeta potential study shows positive result. SEM study has revealed that the spheres were almost spherical in shape with rough outer surface. The effect of polymer on release profile of drug was calculated. Tetracycline release from the chitosan microspheres was slow over 10 hr. and dependant on core: coat ratio and size of microspheres. Drug release was by higuchi release kinetic mechanism. Good linear relationship was observed between core: coat ratio of the microspheres and release rate. Chitosan microspheres of Tetracycline exhibited good controlled release characteristics and were found suitable for once a day oral controlled release products.

Keywords: microspheres, Control release, spray drying.

1. INTRODUCTION
Oral controlled release systems continue to be most popular one among all the drug delivery systems due to their several advantages over the conventional systems like:
1. Improved patient compliance and convenience due to less frequent dosing of drug required.
2. Reduction in fluctuation of steady state plasma level and therefore helps in better control of disease condition.
3. Better control of plasma levels of highly potency drugs.
5. Reduction in health care cost through improved therapy, shorter treatment period and less frequency of dosing.1,2
6. Patentability, and opportunity for extending product life-cycle.3

However, the problem frequently encountered with controlled release dosage forms is the inability to increase the residence time of the dosage form in the stomach and proximal portion of the small intestine, due to the rapid gastrointestinal transit phenomenon of the stomach which may consequently diminish the extent of absorption of many drugs since almost of the drug entities are mostly absorbed from the upper part of the intestine. Therefore it would be beneficial to develop sustained release formulation which remain at the absorption site.
for an extended period of time. Several approaches have been immersed to prolong the residence time of the dosage form at the absorption site and one of these is the development of oral controlled release bioadhesive system. In the early 1980’s, Professor Joseph, R. Obinson at the University of Wisconsin pioneered the concept of bioadhesion as a new strategy to prolong the residence time of various drugs on the ocular surface. Various gastrointestinal mucoadhesive disease forms, such as discs, microspheres, and tablets, have been prepared and reported by several research groups.

1.1 Bioadhesion: 5-7
American society of testing and materials has defined ‘Adhesion’ as the state in which two surfaces are held together by interfacial forces, which may consist of valence forces, interlocking action, or both. Good define ‘Bioadhesion’ as the state wherein two materials out of which at least one of biological origin, are held together for an extended period of time by interfacial forces. Alternatively it can also be defined as the ability of a material to adhere to biological tissue for an extended period of time. In biological systems, four types of bioadhesion can be distinguished.

1. Adhesion of a normal cell to another normal cell.
2. Adhesion of a cell with a foreign substances.
3. Adhesion of a normal cell to a pathological cell.
4. Adhesion of an adhesive to a biological substrate.

Bioadhesion are classified into three types based on phenomenological observation, rather than on the mechanism of bioadhesion:

Type I: Bioadhesion is characterized by adhesion occurring between biological objects without involvement of artificial material e.g. cell fusion and cell aggregation.

Type II: Bioadhesion can be represented by cell adhesion onto culture dishes or adhesion to a variety of substances including metals, woods and other synthetic materials.

Type III: Bioadhesion can be described as adhesion of artificial substances to biological substrates such as adhesion of polymer to skin or other soft tissues.

A term ‘Bioadhesive’ is define as a substances that is capable of interacting with biological materials and being retained on them or holding them together for extended period of time.

For drug delivery purpose, the term bioadhesives implies attachment of a drug carrier system to a specified biological location. The biological surface can either be an epithelial tissue or it can be the mucous coat on the surface of a tissue. If adhesive attachment is to a mucous coat, the phenomenon is referred to as ‘Mucoadhesion’. Leung and Robinsone describe mucoadhesion as the interaction between a mucin and a synthetic or natural polymer.

1.2 Theories of bioadhesion: 5-7
For adhesion to occur, molecules must bond across the interface of mucus. These bonds can arise in the following ways:

Ionic bonds: two oppositely charged ions attach each other via electrostatic interactions to form a strong bond (e.g. in a salt crystal).

Covalent bonds: electrons are shared, in pairs, between the bonded atoms in order to fill the orbital in both. These are also strong bonds.

Hydrogen bonds: a hydrogen atom, when covalently bonded to electron negative atoms such as oxygen, fluorine or nitrogen, carries a partial positive charge and is therefore is attracted to other electronegative atoms. The bond formed is generally weaker than ionic or covalent bonds.

Van der Waals bonds: are some of the weakest of interaction that arise from dipole-dipole and dipole-induced dipole attractions in polar molecules, and dispersion forces with non-polar substances.

Hydrophobic bonds: more accurately described as the hydrophobic effect, these are indirect bonds that occur when non-polar groups are present in an aqueous solution. Water molecules adjacent to non-polar groups from hydrogen bonded structures, which lower the system entropy. There is therefore an increase in the tendency of non-polar groups to associates with each other to minimize this effect.

For bioadhesion to occur, a succession of following phenomenon is required. The first stage involves an intimate contact between a bioadhesive and a membrane, either from a good wetting of the bioadhesive surface or from the swelling of the bioadhesive. In second stage, once contact is established, penetration of the bioadhesive into the crevice of tissue surface or interpenetration of the chain of the bioadhesive with that of the mucus take place.

On a molecular level, mucoadhesion can be explained on the basis of molecular interactions.
The interaction between two molecules is composed of attraction and repulsion. Attractive interaction arise from van der Waals forces, electrostatic attraction, hydrogen bonding, and hydrophobic interaction. Repulsive interaction occur because of electrostatics and steric repulsion. For mucoadhesion to occur, the attractive interaction should be larger than nonspecific repulsion.

Spray drying is extensively employed in the pharmaceutical industry to produce raw drugs or excipients or in the microentrapment process. Spray drying technique is based on the drying of the mist of the polymer and drug in air. One of major advantage of spray drying technique is feasibility of operation under aseptic condition, which is rapid, requiring single step operation and suitable for both batch and bulk manufacturing.

**MATERIALS AND METHODS**

Tetracycline Hydrochloride was received as a gift sample from Glenmark pharmaceutical Ltd., Nashik. Chitosan was received as a gift sample from Central Institute of Fisheries Technology, Cochin. All the reagents and solvents used were of analytical grade satisfying Pharmacopoeial standards.

**Preparation of tetracycline encapsulated chitosan microspheres by spray drying technique**

Encapsulation of Tetracycline in chitosan microspheres was carried out by spray drying method. Required volume (200 ml) of chitosan solution was prepared using glacial acetic acid solution (1% W/V) by overnight swelling. The required volume of tetracycline was dissolved in 20 ml of methanol. The tetracycline solution was then added to aqueous chitosan solution and homogenized at 400 rpm for 30 min using universal motors Mumbai stirrer. The different drug polymer ratios used. Spray drying was then performed using a Lu-222 spray drier (Labultima-Mumbai) with a standard nozzle with a peristaltic pump, atomization occurred by the force of the compressed air, disrupting the liquid into small droplets. The droplets together with hot air, were blown in to a drying chamber. The products was then collected in the collector. In standard conditions, the atomization pressure 2 kg cm⁻² and vaccume 120 mm respectively (Table 1).

**Evaluation of microspheres**

The prepared microspheres were evaluated for percentage yield, encapsulation efficiency, particle size and in vitro drug release.

1. **Percentage yield**

The percentage yield of microspheres was determined from the ratio of the weight of solidified microspheres obtained to the weight of solid materials used in the inner phase.

Practical yield

\[ \text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100 \]

2. **Encapsulation efficiency**

20 mg of microspheres were accurately weighed and crushed by using mortar and pestle. Crushed microspheres were suspended in 10 ml methanol and stirred for half an hour. Then the suspension was filtered through whatman filter paper No. 44. Then 1 ml of this solution was diluted to 100 ml with distilled water and absorbance was measured at 280 nm against distilled water as a blank. The drug content was determined from the standard curve. Encapsulation efficiency was calculated from following relationship.

\[ \text{Encapsulation efficiency} = \frac{\text{Estimated drug content}}{\text{Theoretical drug content}} \times 100 \]

3. **Particle size analysis of microspheres**

Average particle diameter and size distribution of microspheres were determined by laser diffractometer using a Mastersizer Micro Version 2.19 (Malvern Instruments, Malvern, UK). Approximately 10 mg of microspheres were stirred in 10 ml distilled water. Then aliquot of the microspheres suspension was added into recirculation unit, which was subsequently circulated 3500 times per minute. Particle size was expressed as equivalent volume diameter. The particle size distribution was also expressed in terms of SPAM factor determined as:

\[ \text{SPAM} = \frac{d_{90} - d_{50}}{d_{10}} \]

Where \(d_{10}\), \(d_{50}\) and \(d_{90}\) are the diameter sizes and the given percentage value is the percentage of particles smaller that that size. A high SPAM value indicate a wide size distribution.

**Bulk density and tapped density**

Both loose bulk density (LBD) and tapped bulk density (TBD) was determined. A quantity of 2 g of microspheres from each batch was introduced in a
10 ml measuring cylinder. After the initial volume was observed, the cylinder was allowed to fall on to a hard surface from a height of 2.5 cm at 2 sec intervals (Table 2). The tapping was continued till no volume change was noted. LBD and TBD were determined by following formula.

\[
LBD = \frac{\text{weight of powder in gms}}{\text{volume of packing in ml}} \\
TBD = \frac{\text{weight of powder in gms}}{\text{tapped volume of packing in ml}}
\]

**Compressibility index**
Carr developed this method of measuring powder flow from bulk densities. The percentage compressibility of a powder is a direct measure of the potential powder arch or bridge strength and is calculated according to following equation.

\[
\% \text{Compressibility} = \frac{LBD - TBD}{LBD} \times 100
\]

**In vitro drug release study of Tetracycline microspheres**
Microspheres equivalent to 500 mg of tetracycline were filled in a capsule and in vitro drug release was studied using USP Apparatus II with 900 ml of dissolution medium at 37.5 ± 0.1 °C for 10 hrs at 100 rpm. 0.1 N HCl (pH 1.2) was used as dissolution medium. 10 ml of sample was withdrawn after every hour, and was replaced with an equal volume of fresh dissolution medium. Collector sample were analysed at 280 nm by spectrophotometrically. The study was performed in triplicate (Tabla 3). Dissolution study was also conducted for marketed capsule Dissolution data for different formulations of microspheres are reported in Table 4

**Micromeritics study**
Particle size of drug affects flow characteristics, tableting as well as release from delivery system. Micromeritics study of the drug powder was done by Malvern mastersizer which works on the principle of laser diffraction. The study was conducted using chloroform. The drug was found to be sparingly soluble in chloroform hence the study could be conducted in chloroform. Obscuration of laser beam by the particle was found to be 12.8% which was sufficient obscuration for the study.

**FTIR analysis**
The Infra-red spectroscopy analysis was performed by Fourier Transformation Infrared Spectrophotometer 8400 (Shimadzu), with a resolution of 8 cm⁻¹ , in the range of 4000-5000 cm⁻¹, KBr pellet.

**Differential scanning calorimetry (DSC)**
The DSC analysis of pure drug, drug-loaded microspheres, and polymer were carried out using Shimadzu DSC 60 to evaluate any possible drug-polymer interaction. The analysis was performed at a rate 10.0⁰ C min⁻¹ to 300 °C temperature range under nitrogen flow of 25 ml min⁻¹

**X-Ray diffraction studies (XRD)**
Drug, polymer formulated microspheres were analysed by XRD in order to check effect of compression on crystallinity of ingredients as well as to check any interaction between the excipients. Powder X-ray diffraction patterns were obtained by a diffractometer (PW 3710, Philips)

**Scanning electron microscopy (SEM)**
The shape and surface morphologies of the drug-loaded microspheres were investigated using scanning electron microscopy (XL 30 ESEM Philips)

**Zeta Potential**
The microparticles were dispersed in deionized water at pH 6.0 and the surface charge (zeta potential) was measured by laser doppler anemometry using a Zetamaster (Malvern, UK).

**RESULTS AND DISCUSSION**

**a) Effect on drug encapsulation efficiency**
The method showed good encapsulation efficiency. Percent drug encapsulated was found to be in a range of 76-93% for chitosan microspheres. It was observed that with increase in polymer concentration drug encapsulation efficiency was increased. Drug encapsulation efficiency was slightly increased as the concentration Tween 80 was increased because dispersing agent decrease the interfacial tension between the lipophilic and hydrophilic phases of the emulsion and simplify the formation of microspheres also this dispersing agent provides a thin protective layer around the droplets and reduces the extent of their collision and coalescence.
b) Effect on particle size
   Particle size for chitosan microsphere was found in the range of 18-19 μm with SPAN factors ranging between 8-9.

c) Kinetic treatment of dissolution data
   All the formulations except TC3 followed Higuchi's kinetics with R² ranging from 0.9633-0.9809 while TC3 fitted to Hixon equation with R² 0.9851. The value of kinetic constant showed a decreased trend from 4.87 to 3.83 with increased polymer concentration.

d) Effect on drug release
   In vitro dissolution results showed that the microspheres prepared with a different core-coat ratio gave better-sustained action. Chitosan gave sustained action over 10 hrs. Table 1 clearly illustrates that the rate of drug release from the microspheres depended on the polymer concentration of the prepared devices. An inverse relationship was observed between polymer content and drug release rate from the prepared microspheres. In all cases of polymers, it was seen that microspheres containing 25% polymer released the drug more rapidly, while those with 100% polymers exhibited a relatively slower drug release profile.

Physical properties of optimized microspheres
   It was found that all batches of microspheres were discrete, free flowing and spherical. Bulk density was found to be 0.555, 0.652 gm/cm³ for TC4 and TP4 respectively which showed good flow characteristics of microspheres. Tapped density was found to be 0.652, 0.789 gm/cm³ for TC4 and TP4 which showed good flow characteristics of microspheres. A value of angle of repose was 26.96°, 25.32° for TC4 and TP4. Indicated good flow properties of microspheres. Compressibility index was found to be 17.47%, 21.01% for TC4 and TP4, resulted in good to excellent flow properties of microspheres. It is due to the fact that the microspheres membrane a more open structure.

e) Surface topography of the optimized microspheres
   Optimized microspheres TC4, TP4 were analyzed for surface characterization using Scanning Electron Microscopy. TC4 microspheres were found to be spherical, discrete, non-porous structure with rugged polymeric surface. TP4 microspheres were found to be spherical, discrete, with distinct pores on the surface.

Compatibility study of optimized microspheres
   The compatibility study of Tetracycline hydrochloride with excipients was done by UV spectroscopy, X ray diffractometry, FTIR, and DSC.

   I. By UV Spectroscopy
   The UV spectrum of pure drug solution of Tetracycline was obtained at 280 nm, TC4, TP4 microspheres were found at 280 nm, which showed that there was no interaction between drug and excipients.

   II. By X-ray diffractometry
   Characteristic crystalline peaks of Tetracycline hydrochloride were observed at 2θ of 11.082, 13.649, 15.288, 15.823, 19.796, 20.729, 21.801, 24.245, 28.521, 30.125, 32.538 (Figure 15) indicating the presence of crystalline Tetracycline hydrochloride. Peaks of Tetracycline are also present in chitosan, microspheres even if reduced in intensity. Typical diffraction patterns of Tetracycline hydrochloride loaded chitosan, microspheres are shown in Figure 1-3. The decreased intensity of peaks is due to decrease in drug crystallinity. This indicates that Tetracycline hydrochloride is present in the chitosan, microspheres with reduced crystallinity.

   III. By FTIR
   The characteristic peaks of aromatic NH₂, aliphatic NH, aliphatic OH and aromatic c=c of pure drug were almost identical with those of chitosan, microspheres which indicated that absence of any polymer drug interaction. Typical FTIR patterns of Tetracycline hydrochloride loaded chitosan, microspheres are shown in Figure 4-6.

   IV. By DSC
   The characteristic endothermic peak for Tetracycline hydrochloride was obtained at 241.81 °C, which was also obtained in chitosan, microspheres with slight change which showed that drug is dispersed in microspheres. Typical DSC
patterns of Tetracycline hydrochloride loaded chitosan microspheres are shown in Figure 7-9.

**SEM**

Prepared microspheres were spherical and completely covered with polymer coat. The surface of the drug–loaded microspheres had small pores on their surfaces, which will be responsible for control drug release (figure 10).

**Zeta Potential**

The microspheres were suspended in Phosphate buffer (pH 1.2) for 30 minutes. The suspension (2% w/v) was employed for the determination of zeta potential (figure 11).

**CONCLUSION**

Chitosan polymer was found suitable as microencapsulating agent and the chitosan coated microspheres exhibited good controlled release characteristics and were found suitable for oral controlled release products. The spray drying technique found to be an excellent approach in the design of controlled release microspheres of Tetracycline.

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**Fig. 1, 2 & 3**: XRD spectrum of Tetracycline, Chitosan, Drug loaded chitosan microspheres
Fig. 4, 5 & 6: FTIR Spectrum of Tetracycline, Chitosan and drug loaded chitosan microspheres

Fig. 7, 8 & 9: DSC curves of Tetracycline, Chitosan and drug loaded chitosan microspheres
Fig. 10: Scanning electron microscopy of Tetracycline, chitosan and drug loaded chitosan microspheres at 2000 magnification

Table 1: Batch specifications of the Tetracycline encapsulated chitosan microspheres

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Drug : polymer</th>
<th>Inlet Temperature</th>
<th>Outlet Temperature</th>
<th>Pump Speed</th>
<th>Nozzle Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>1:0.25</td>
<td>110°C</td>
<td>80°C</td>
<td>2 rpm</td>
<td>1.5 mm</td>
</tr>
<tr>
<td>A2</td>
<td>1:0.5</td>
<td>110°C</td>
<td>80°C</td>
<td>2 rpm</td>
<td>1.5 mm</td>
</tr>
<tr>
<td>A3</td>
<td>1:0.75</td>
<td>110°C</td>
<td>80°C</td>
<td>2 rpm</td>
<td>1.5 mm</td>
</tr>
<tr>
<td>A4</td>
<td>1:1</td>
<td>110°C</td>
<td>80°C</td>
<td>2 rpm</td>
<td>1.5 mm</td>
</tr>
</tbody>
</table>
Table 2: Physical properties of Tetracycline Hydrochloride microspheres

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Bulk density g/cm³</th>
<th>Tapped Density g/cm³</th>
<th>Compressibility Index (%)</th>
<th>Angle of Repose</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC1</td>
<td>0.576</td>
<td>0.681</td>
<td>18.22</td>
<td>29.02</td>
</tr>
<tr>
<td>TC2</td>
<td>0.555</td>
<td>0.652</td>
<td>17.47</td>
<td>27.46</td>
</tr>
<tr>
<td>TC3</td>
<td>0.535</td>
<td>0.625</td>
<td>16.82</td>
<td>28.08</td>
</tr>
<tr>
<td>TC4</td>
<td>0.555</td>
<td>0.652</td>
<td>17.47</td>
<td>26.96</td>
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</table>

Table 3: In vitro drug release study of Tetracycline microspheres

<table>
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<tr>
<th>Time (Hrs)</th>
<th>Cumulative % drug release (mean ± SD, n=3)</th>
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<tbody>
<tr>
<td>TC1</td>
<td>TC2</td>
</tr>
<tr>
<td>1</td>
<td>30.89</td>
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<tr>
<td>2</td>
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<tr>
<td>3</td>
<td>45.85</td>
</tr>
<tr>
<td>4</td>
<td>52.57</td>
</tr>
<tr>
<td>5</td>
<td>60.74</td>
</tr>
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<td>6</td>
<td>71.31</td>
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<tr>
<td>7</td>
<td>78.79</td>
</tr>
<tr>
<td>8</td>
<td>87.77</td>
</tr>
<tr>
<td>9</td>
<td>...</td>
</tr>
</tbody>
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Table 4: Evaluation of different formulations of Tetracycline microspheres

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Evaluation Parameters</th>
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<tbody>
<tr>
<td>TC1</td>
<td>% Yield 60.17 ± 0.548%</td>
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<tr>
<td>TC2</td>
<td>65.52</td>
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<tr>
<td>TC3</td>
<td>70.12</td>
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<tr>
<td>TC4</td>
<td>73.29</td>
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REFERENCES