Development and Evaluation of Mucoadhesive Microspheres of Tetracycline by using a Spray Drying Technique

Hire NN*, Derle DV, Deore AB, and Nathe KR

Department of Pharmaceutics, M.V.P. Samaj’ College of Pharmacy, Shivaji Nagar, Gangapur Road Nashik, M.S. India.

ABSTRACT

Tetracycline an antibiotic drug was selected as drug for the experiment. Polyethylene oxide microspheres of Tetracycline were prepared by spray drying technique using Polyethylene oxide as a rate controlling polymer and the microspheres were evaluated. Polyethylene oxide microspheres were spherical, discrete, free flowing and multinucleate monolithic type. The mean size range was found for formulation in the range of 13.71 to 15.48 μ. Entrapment efficiency was in the range of 93.827 to 91.407 %. 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. The effect of polymer on release profile of drug was calculated. Tetracycline release from the Polyethylene oxide microspheres was slow over 12 hr and dependant on core: coat ratio and size of microspheres. Drug release was by higuchi mechanism. Good linear relationship were observed between core: coat ratio of the microspheres and release rate. Polyethylene oxide microspheres of Tetracycline exhibited good controlled release characteristics and were found suitable for once a day oral controlled release products.

Keywords: Microspheres, spray drying, controlled release. PEO
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:

- Improved patient compliance and convenience due to less frequent dosing of drug required.
- Reduction in fluctuation of steady state plasma level and therefore helps in better control of disease condition.
- Better control of plasma levels of highly potency drugs.
- Maximum utilization of drug enabling reduction in total amount of dose administered.
- Reduction in health care cost through improved therapy, shorter treatment period and less frequency of dosing \([1, 2]\).
- 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 remains at the absorption site for an extended period of time. Several approaches have been immerged 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 bioadhesive as a new strategy to prolong the residence time of various drugs on the ocular surface.\([4]\)

Various gastrointestinal mucoadhesive dosage forms, such as discs, microspheres, and tablets, have been prepared and reported by several research groups.\([5]\)

Bioadhesion:

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 valency 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.

- Adhesion of a normal cell to another normal cell.
- Adhesion of a cell with a foreign substance.
- Adhesion of a normal cell to a pathological cell.
- 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 bioadhesion 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.

Spray drying is extensively employed in the pharmaceutical industry to produce raw drugs or excipients or in the microentrainment process. Spray drying technique is based on the drying of the mist of the polymer abd 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.[8]

**MATERIALS AND METHODS**

Tetracycline Hydrochloride was received as a gift sample from Glenmark pharmaceutical Ltd., Nashik. Polyethylene oxide was received as a gift sample from Lubrizol Advanced Materials India PVT. Ltd., Mumbai. All the reagents and solvents used were of analytical grade satisfying Pharmacopoeial standards.
Preparation of Tetracycline Encapsulated Polyethylene Oxide Microspheres by Spray Drying Technique:

Encapsulation of tetracycline in polyethylene oxide microspheres was carried out by spray drying method. Required volume (200 ml) of polyoxyethylene solution was prepared. Then tetracycline was dissolved in 20 ml of methanol. The tetracycline solution was then added to aqueous polyoxyethylene 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 in to small droplets.The droplets together with hot air, were blown in to a drying chamber. The products were then collected in the collector. In standard conditions, the atomization pressure 2 kg cm\(^{-2}\) and vaccum 120 mm respectively.

Evaluation of microspheres:

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

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.

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

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.

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

Particle size analysis of microspheres:

Average particle diameter and size distribution of microspheres were determined by laser diffractometer using a Master sizer 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:

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

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.

**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).
Antimicrobial Susceptibility Test:

The microspheres were able to entrap the drug at high levels and sustain its release over a prolonged time. Microspheres prepared with tetracycline loaded antibiotic antimicrobial susceptibility test was carried out and compared with the control disc of the tetracycline. The diameter of the zones of complete inhibition are measured, including the diameter of the disc. Zones are measured to the nearest whole millimeter, using sliding caliper.

RESULTS AND DISCUSSION

Effect on drug encapsulation efficiency:

The method showed good encapsulation efficiency. Percent drug encapsulated was found to be in a range of 82-95% for Polyethylene oxide. 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.

Effect on particle size:

Particle size for Polyethylene oxide microsphere was found in the range of 13-15 μm with SPAN factors ranging between 5 and 6.

In all above batches of Tetracycline hydrochloride microspheres it was observed that the size of microspheres increased as the concentration of inner phase polymer was increased.

Kinetic treatment of dissolution data:

All the formulations except followed zero order kinetics with R² ranging from 0.9826-0.9941 where as TP fitted to Koresmeyer equation with R² 0.9977.

Effect on drug release:

In vitro dissolution results showed that the microspheres prepared with a different core-coat ratio gave better-sustained action. Polyethylene oxide gave sustained action over 12 hrs Table: 02 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 0.652 gm/cm$^3$, TP4 which showed good flow characteristics of microspheres.

Tapped density was found to be 0.789 gm/cm$^3$ TP4 which showed good flow characteristics of microspheres.

A value of angle of repose was 25.32° TP4 indicated good flow properties of microspheres. Compressibility index was found to be 21.01% and TP4 resulted in good to excellent flow properties of microspheres. Is due to the fact that the microspheres membrane a more open structure.

Surface topography of the optimized microspheres:

Optimized microspheres TP4 were analyzed for surface characterization using Scanning Electron Microscopy.

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, DSC and zeta potential.

By UV Spectroscopy:

The UV spectrum of pure drug solution of Tetracycline was obtained at 280 nm whereas UV spectra of pure drug solution of which showed that there was no interaction between drug and excipients.

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 4) indicating the presence of crystalline Tetracycline hydrochloride. Peaks of Tetracycline are also present in Polyethylene oxide microspheres even if reduced in intensity. Typical diffraction
patterns of Tetracycline hydrochloride loaded Polyethylene oxide microspheres are shown in Figure 03.

Fig 4: Scanning electron microscopy of Tetracycline, Polyethylene Oxide and drug loaded Polyethylene Oxide microspheres at 5000 magnification

Fig 3: XRD spectrum of Tetracycline, Polyethylene Oxide , Drug loaded Polyethylene Oxide microspheres.

Table 3: 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>TP1</td>
<td>0.6</td>
<td>0.714</td>
<td>19</td>
<td>25.72</td>
</tr>
<tr>
<td>TP2</td>
<td>0.625</td>
<td>0.75</td>
<td>20</td>
<td>26.51</td>
</tr>
<tr>
<td>TP3</td>
<td>0.681</td>
<td>0.714</td>
<td>4.84</td>
<td>27.12</td>
</tr>
<tr>
<td>TP4</td>
<td>0.652</td>
<td>0.789</td>
<td>21.01</td>
<td>25.32</td>
</tr>
</tbody>
</table>

The decreased intensity of peaks is due to decrease in drug crystallinity. This indicates that Tetracycline hydrochloride is present in the Polyethylene oxide microspheres with reduced crystallinity.

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 Polyethylene oxide microspheres which indicated
that absence of any polymer drug interaction. Typical FTIR patterns of Tetracycline hydrochloride loaded Polyethylene oxide microspheres are shown in fig: 02.

![FTIR spectrum](image)

**Fig 2:** Stokes disc diffusion technique showing inhibition zones of control and test organism against shigella flexneri organism.

![Stokes disc diffusion technique](image)

**Fig 2:** FTIR Spectrum of Tetracycline, Polyethylene Oxide and drug loaded Polyethylene Oxide microspheres.

**Table 2:** In vitro drug release study of Tetracycline Polyethylene oxide microspheres

<table>
<thead>
<tr>
<th>Time (Hrs)</th>
<th>Cumulative % drug release (mean ± SD., n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TP1</td>
</tr>
<tr>
<td>1</td>
<td>11.04</td>
</tr>
<tr>
<td>2</td>
<td>16.15</td>
</tr>
<tr>
<td>4</td>
<td>32.69</td>
</tr>
<tr>
<td>6</td>
<td>46.53</td>
</tr>
<tr>
<td>8</td>
<td>55.47</td>
</tr>
<tr>
<td>10</td>
<td>78.11</td>
</tr>
<tr>
<td>12</td>
<td>96.40</td>
</tr>
</tbody>
</table>

**By DSC:**

The characteristic endothermic peak for Tetracycline hydrochloride was obtained at 241.81 °C, which was also obtained in Polyethylene oxide microspheres with slight change. which showed, that drug is dispersed in microspheres. Typical DSC patterns of Tetracycline hydrochloride loaded Polyethylene oxide microspheres are shown in Figure 01.
Fig 1: Stokes disc diffusion technique showing inhibition zones of control and test organism against salmonella typhi organism.

Fig 1: DSC curves of Tetracycline, Polyethylene Oxide and drug loaded Polyethylene Oxide microspheres

Table1: Batch specifications of the Tetracycline encapsulated polyethylene oxide 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>TP1</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>TP2</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>TP3</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>TP4</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>

V 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. The results are presented in fig05.
Fig 5: Zeta potential result of drug loaded Polyethylene Oxide microspheres

Zeta Potential of Selected Microsphere Formulations

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline Microspheres</td>
<td>20.24</td>
</tr>
</tbody>
</table>

VI. Antimicrobial Susceptibility Test:

The sizes of the zones of inhibition are interpreted by referring to (Zone Diameter Interpretative Standards and equivalent Minimum Inhibitory Concentration Breakpoints) the NCCLS M100-S12: Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Informational Supplement, and the organisms are reported as either susceptible, intermediate, or resistant to the agents that have been tested. Some agents may only be reported as susceptible, since only susceptible breakpoints are given.

Zone Size Interpretative Chart

(Based on Results obtained using Mueller Hinton Agar)

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Antimicrobial Agent</th>
<th>Symbol</th>
<th>Disc Content</th>
<th>Interpretative Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tetracycline</td>
<td>TE</td>
<td>30 mcg</td>
<td></td>
</tr>
<tr>
<td>SD037</td>
<td>Enterobacteriacease.</td>
<td></td>
<td></td>
<td>Sensitive mm or more</td>
</tr>
<tr>
<td></td>
<td>Aci.robacter, staphylococcus, Enterococcus, N.meningitidis, H. influenzae, N.gonorhoease, Streptococcus spp</td>
<td></td>
<td></td>
<td>Intermediate mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Resistant mm or less</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15</td>
<td>12-14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>19</td>
<td>15-18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>29</td>
<td>26-28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>23</td>
<td>19-22</td>
</tr>
</tbody>
</table>
Reading Plates and Interpreting Results

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Antimicrobial Agent</th>
<th>Disc content</th>
<th>Control disc mm</th>
<th>Test disc mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC 25922</td>
<td>Shigella flexneri</td>
<td>30 mcg</td>
<td>25</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Salmonella typhi</td>
<td>30 mcg</td>
<td>30</td>
<td>28</td>
</tr>
</tbody>
</table>

CONCLUSION

Chitosan polymer was found suitable as microencapsulating agent and the polyethylene oxide 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.

REFERENCES