PREPARATION AND IN-VITRO EVALUATION OF BIOADHESIVE BIODEGRADABLE ANTIBIOTIC IMPLANT

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ABSTRACT
The present study aimed at the preparation and iv-vitro evaluation of bioadhesive biodegradable antibiotic implant for local administration of drug. The Surgical incision need systemic administration of antibiotics is an inherently inefficient method for achieving high local tissue concentration, because the vascular system distributes antibiotics uniformly throughout the entire body, only a fraction of the given dose reaches at the site of infection and provide unnecessary drug loading to the various organs of the body. Therefore, a local method to deliver the antibiotic only to the localized area where the risk of infection is greatest, is necessary. The conventional implant has drawback that it requires surgical intervention to adhere to the skin at the site of infection. To overcome these limitations cefuroxime axetil was formulated as bioadhesive polymeric implant so that it get adhere to the skin and release the drug over the period of 4-5 days. Implants were prepared by compression technique using PEG 6000, Tween 80 as erosion enhancers and chitosan and glyceryl monostearate as degradable polymer and studied the effect of different bioadhesive polymers on bioadhesion duration of implants. The implants were characterized for bioadhesive strength, bioadhesive duration, drug content uniformity, weight variation and drug release study. The effect of chitosan, GMS, erosion enhancers on release profile of cefuroxime axetil was also assessed. Optimized formulation gave 96 h 50 min of bioadhesion duration and 89.17% drug release for 96 h. thus implant was found to be promising carrier system for cefuroxime axetil because of easy of preparation and maximum bioadhesion duration and stability for prolonged period.

Key words: Cefuroxime axetil, Glyceryl monostearate, chitosan.

INTRODUCTION
Subcutaneous implants have been increasingly recognized as a useful drug delivery system that provides greater assurance of patient compliance and a better therapeutic outcome than conventional drug therapies, particularly for chronic medication (Smart JD,1991).

Subcutaneous tissue is essentially a sheet of areolar tissue lying directly underneath skin. It is rich in fat, but poor in nerve network and hemoperfusion. Therefore, the subcutaneous tissue is an ideal location for implantation and prolonged drug administration because of its ready access to implantation, slow drug absorption, and low drug reactivity to the insertion of foreign material (Sinha VR et al.,1998).

The concept of bioadhesive was introduce in controlled drug delivery area, in early 1980s The term bioadhesion refers to any bond formed between either two biological surfaces or a bond between a biological and a synthetic surface. In case of bioadhesive drug delivery systems, the term bioadhesion is typically used to describe the adhesion between polymers, either synthetic or natural and soft tissues, in which bonds form between mucus or a mucus membrane and polymer, the term mucoadhesion is used synonymously with bioadhesion(Chickering DE et al.,2007; Hamah B, 2004).
Bioadhesive polymers are synthetic or natural polymers which interact with the mucus layer covering the mucosal epithelial surface and mucin molecule constituting the major part of mucus. Many different types of materials have been used for Implantable drug delivery systems, ranging from biodegradable collagen to non-biodegradable titanium metal. It is important that all materials used for implants be physically and chemically stable, but vitally important that the materials are biocompatible (Chien YW et al., 1987).

MATERIALS AND METHODS

Materials

Cefuroxime axetil was gift sample from Zim laboratory, Nagpur, HPC, HPMC-100, HPMC E-15, HPMC K, M Gift samples from Colorcon Asia Ltd., Goa. GMS was gift sample from Nitika Pharmaceuticals, Nagpur. All other ingredients used throughout the study were of analytical grade and were used as received.

METHOD OF PREPARATION OF IMPLANT

Glyceryl monostearate, chitosan in the specified quantity and the optimized concentration of PEG 6000 (47.5mg) and tween 80 (2.5mg) heated to 70°C on water bath under stirring with glass rod. The weighed quantity of drug was dispersed uniformly just before the solidification of the mass. The solidified blend was stored in a freezer for 1 h. The hard mass thus obtained was ground to fine powder and passed through #10. These granules were compressed by compression machine punch size 8 mm (flat) to form tablet shaped pellets and then prepared pellets were layered with weighted quantity of different bioadhesive polymers (Roy AA et al., 2012).

EVALUATION OF PREPARED IMPLANT

Formulation of preliminary trial batches and their optimization

Primary batches of implants were prepared for optimizing the formulation components like Tween 80, PEG 6000, chitosan and Glyceryl monostearate.

Weight variation test

Twenty pellets were selected randomly from each batch and weighed individually. The average weight of each batch of tablet was calculated. Individual weights of the implant were compared with the average weight. Since the tablets weighed over 250 mg, I.P. specifies that the tablets pass the test if not more than two of the individual weights deviate from the average weight by more than 5 % (Liberman HA et al., 1989).

Percentage deviation allowed under weight variation test as per I.P.

<table>
<thead>
<tr>
<th>Average weight of tablet (X mg)</th>
<th>Percentage deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>X ≤ 80 mg</td>
<td>10</td>
</tr>
<tr>
<td>80 &lt; X &lt; 250 mg</td>
<td>7.5</td>
</tr>
<tr>
<td>X ≥ 250 mg</td>
<td>5</td>
</tr>
</tbody>
</table>

Hardness test

The crushing strength (kg/cm²) of prepared implant was determined for implant of each batch by using Monsanto tablet hardness tester. Hardness indicates the ability of a tablet to withstand mechanical shocks while handling (Liberman HA et al., 1989).

Drug content in implants

Drug content uniformity in implants was determined, implant was placed in volumetric flask containing 10 ml of ethanol; the flask was vigorously shaken to extract the drug from the implant. 1 ml of resulting solution was taken and diluted to 100 ml with phosphate buffer pH 6.8. The absorbance of solution was measured spectrometrically at 281 nm. The polymeric solution without drug serves as a blank. The drug content was studied in triplicate and the mean was reported. (Shanti S et al., 2011).

Bioadhesive strength of implants

The pellets were evaluated by using apparatus for bioadhesive strength. For evaluation of bioadhesive strength a fresh membrane was securely placed, mucosal side upwards, on a solid block made of rubber within a water bath containing pH 6.8 isotonic phosphate buffer at (37+1) °c. The model mucosal surface used was goat membrane. The middle section of the membrane was selected by discarding 40-50mm at either end of the fresh membrane. It was then washed with isotonic phosphate buffer solution and then preserved in the same or used immediately. The mucosal surface was stabilized and the test disc was placed in contact with the mucosal surface to give sufficient contact area for adhesion. The weights were placed on the right hand side of the pan and the force required to detach the membrane was calculated.

This gives the bioadhesive strength in grams.

For each experiment a fresh mucosa was used. The mass in grams required to detach the formulation is calculated to determine the force of adhesion. (Gupta A et al., 1992).

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\text{Force of adhesion (N)} = \frac{\text{bioadhesive strength}}{1000} \times 9.81
\]

Bioadhesive duration of implants

The pellets were evaluated by using apparatus for bioadhesive duration. The membrane was securely placed, mucosal side downwards to a modified apparatus in a water bath. The isotonic phosphate buffer was supplied to the membrane at a constant rate from another
Further experimentation the membrane was brought to particular length in dimension. The mucosal surface to give sufficient contact area for adhesion. As soon as the adhesive joint between the test disc and the mucus membrane was detached it was recorded manually. For each experiment a fresh mucosal membrane was used. (Smart JD et al., 1994).

### In-vitro release study

**Gel simulating in vitro implantation (gel method)**

In vitro release was followed by placing the pellet/implant in agar gel simulating subcutaneous tissues condition with respect to viscosity and water content. The agar crystals were dissolved in boiling pH 6.8, 0.1 mol/Ltr phosphate buffer to prepare 1.5% agar solution, which was poured into petri dish and left to congeal. The plate was covered and placed in oven (37°C). Several agar plates implanted with cefuroxime axetil devices were prepared at the same time and the samples were collected at 6, 12, 24, 48, 72, 96hr. At each sampling time, one plate was removed from the oven.

The plate was divided into four sampling zones and three samples were removed from each zone using a cork borer size 4 (8 mm in diameter). The samples were accurately weighed and dissolved in boiling 25% NaCl buffer. The solution was cooled in an ice bath to precipitate the agar. The resultant suspension was weighed, sonicated, and then centrifuged to obtain a clear supernatant containing cefuroxime axetil. The supernatant was analyzed by UV assay to determine the concentration of cefuroxime axetil. (Mathur VB et al., 2010).

### Stability studies

Formulation was wrapped in aluminum foil, sealed and kept for stability studies as per ICH guidelines, under conditions 40 ± 2°C/75 ± 5% RH. Every month, sample from each formulation was withdrawn and estimated for drug concentration. Weighted formulation was crushed and dissolved in 0.1 mmol L⁻¹ HCl under stirring for 2 h and physical properties and drug concentration was calculated.

### Table 1. Formula for implants containing different bioadhesive polymers for layering the implants

<table>
<thead>
<tr>
<th>Ingredients used (mg)</th>
<th>Formulation code</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I₁</td>
</tr>
<tr>
<td>Cefuroxime axetil</td>
<td>100</td>
</tr>
<tr>
<td>GMS</td>
<td>100</td>
</tr>
<tr>
<td>PEG-6000</td>
<td>47.5</td>
</tr>
<tr>
<td>Tween 80</td>
<td>2.5</td>
</tr>
<tr>
<td>Chitosan</td>
<td>200</td>
</tr>
<tr>
<td>Sodium alginate</td>
<td>100</td>
</tr>
<tr>
<td>Pectine</td>
<td>-</td>
</tr>
<tr>
<td>Carbopol 981</td>
<td>-</td>
</tr>
<tr>
<td>HPMC K4M</td>
<td>-</td>
</tr>
<tr>
<td>HPMC E-15</td>
<td>-</td>
</tr>
<tr>
<td>HPMC K-100M</td>
<td>-</td>
</tr>
<tr>
<td>HPC</td>
<td>-</td>
</tr>
</tbody>
</table>

### Table 2. Physical properties of implants I₁-I₇

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Thickness (mm)</th>
<th>Weight uniformity</th>
<th>Hardness (kg/cm²)</th>
<th>Content uniformity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I₁</td>
<td>3.52 ± 0.17</td>
<td>551 ± 0.027</td>
<td>10.4 ± 0.21</td>
<td>97.48 ± 1.67</td>
</tr>
<tr>
<td>I₂</td>
<td>3.48 ± 0.24</td>
<td>551 ± 0.023</td>
<td>10.4 ± 0.23</td>
<td>98.23 ± 1.19</td>
</tr>
<tr>
<td>I₃</td>
<td>3.49 ± 0.07</td>
<td>550 ± 0.020</td>
<td>10.6 ± 0.17</td>
<td>98.87 ± 1.32</td>
</tr>
<tr>
<td>I₄</td>
<td>3.53 ± 0.19</td>
<td>553 ± 0.024</td>
<td>10.4± 0.12</td>
<td>98.45 ± 1.48</td>
</tr>
<tr>
<td>I₅</td>
<td>3.52 ± 0.25</td>
<td>551 ± 0.019</td>
<td>10.6 ± 0.09</td>
<td>98.13 ± 1.58</td>
</tr>
<tr>
<td>I₆</td>
<td>3.49 ± 0.18</td>
<td>553 ± 0.023</td>
<td>10.4 ± 0.22</td>
<td>96.25 ± 1.65</td>
</tr>
<tr>
<td>I₇</td>
<td>3.48±0.24</td>
<td>552 ±0.025</td>
<td>10.4 ±0.17</td>
<td>96.34±1.38</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SD(n=3)
RESULT AND DISCUSSION

The I₃ showed higher drug release (89.712 ± 0.08%) in 96 h as compared to other batches (Table 4, figure 1). More sustained drug release was obtained due to increased hardness (10.6 kg/cm²), as the compactness in the dosage form leading to the reduction in the porosity, the lower erosion and penetration of release medium in the dosage form. The I₃-I₇ showed comparatively low drug release (figure 1) due to decreased in hardness. The drug release initially is due to the solubility of erosion enhancers in the releasing medium and GMS which form hydrophobic matrix prolong the drug release.

Different bioadhesive polymers possess different bioadhesive properties. The bioadhesive...
strength of sodium alginate (I₁) posses higher bioadhesive strength (28.94g) as compared to pectin (I₂, 19.62g.) Table 3, although both polymers has COOH and OH groups but pectin being more cross linked provide lower hydrogen bonding between the polymer and mucous membrane. The presence of COONa groups in sodium alginate in addition to the above groups helps to provide higher bonding and hence the bioadhesive strength.

The I₆ (HPMC K-100M), I₄ (HPMC K4M), I₃ (HPMC E15) and I₁ (HPC) contains similar groups as CH₂OR and OR but I₇ (HPC) showed higher bonding strength (15.20g) in comparison to the I₆ (6.10g), I₄ (9.33g), I₃ (12.64g) Table 3, due to their difference in molecular weights. I₁₉ (HPC) being lower molecular weight posses higher solubility or hydrophilicity and hydrogen bonding formed during hydration of polymer. HPMC being higher molecular weight polymer showed lower solubility and longer duration for hydration leading to the lower bonding strength. Carbopol 981 (I₉) showed adhesive strength of (21.75g) due to the presence of hydrophilic groups COOH.

The duration of attachment of the dosage form to the mucus membrane is dependent on time of hydration of dosage form. On excessive hydration, polymers forms the mucilage which provides the slippery surface, consequently the dosage form gets detached from mucus surface. Among the cellulose derivatives both HPMC and HPC contains similar groups as CH₂OR and OR but the I₆ (HPMC K-100), I₄ (HPMC K4M), I₃ (HPMC E15) showed higher bioadhesive duration 17h 15min,1h 30min,8h 20min respectively in comparison I₁ (5h 24min), due to the variation in molecular weight (Table 3). HPMC K-100M being higher molecular weight showed lower solubility or hydrophilicity leads to delay in hydrogel formation consequently higher bioadhesive duration. The carbopol 981 (I₉) showed higher duration of bioadhesion 96h 50min due to their cross linking and higher compactness of the pellets, chain elongation takes longer duration for hydration or solubility leading to the higher bioadhesion duration. Among the natural polymers, the pectin (I₁) showed higher bioadhesive duration (15.33min) as compared to sodium alginate (I₁) which showed bioadhesion duration of 12.20min. The difference in bioadhesion is may be due to the crosslinking.

The in vitro release profiles were applied on various kinetic models in order to find out the mechanism of drug release. The n values were calculated, which describes the drug release mechanism. The value of n in agar gel method was 0.454 for I₁, indicating fickian transport (diffusion mechanism). The R² values for the Higuchi plot were 0.9690. This system was best presented by the Higuchi model.

In the present study, the optimized formulation I₃ (Table 5) was selected for stability studies. Stability studies of the drug formulations are performed as per ICH guidelines to ascertain whether the drug undergoes any degradation during its shelf life. The I₁₅ showed drug content as 98.87% and after 3 month the drug content was found to be 98.44% (Table 2). From the stability study data, it can be concluded that the optimized formulation is stable.

CONCLUSION
According to the result it can be concluded that GMS forms the hydrophobic matrix for the drug delivery system and release profile not only depend upon solubility of drug but also on concentration of erosion enhancers.

Different bioadhesive polymer possess different bioadhesive properties and concluded that more hydrophillic polymers due to theirs faster hydration, showed higher bioadhesive strength and lower bioadhesive duration, while lower hydrophillic polymer showed lower bioadhesive strength but higher bioadhesive duration. As the compactness of the implant increase the bioadhesion duration increases, while the bioadhesive strength approximately remain the same.

Furthermore, the in vitro release profile rate suggests that drug release kinetics of the batches of bioadhesive implant follows higuchi model and mechanism of drug release from all batches indicated fickian diffusion transport. From the evaluation, among the various formulations I₃ was found to be optimum, showed the sustained drug release profile up to 96 h and maximum bioadhesion duration.

Finally it may be concluded that, this drug delivery system offers a valuable dosage form, provides a better option and reliability to prevent post operative infection at the site of surgery which allows a better control of fluctuations.

REFERENCES