FORMULATION AND EVALUATION OF MUCOADHESIVE MICROSPHERE OF ESOMEPRAZOLE

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Abstract: The gastro retentive drug delivery systems can be retained in the gastrointestinal track for longer period of time and improve the oral bioavailability of drug. In the present research work, mucoadhesive microspheres of esomeprazole were prepared and evaluated. The mucoadhesive microspheres were prepared by solvent evaporation method. Coating of microsphere is done by method as described by Gopferich Achim et al. The surface morphological characteristics of mucoadhesive microspheres were investigated using scanning electron microscopy. The scanning electron microscopy of formulation code F14 reveals that the microspheres were spherical, discrete with rough texture. The highest percentage mucoadhesion was found 14% in formulation F14. By, above results it was concluded that formulation code F14 showed reproducible results, with good Mucoadhesive properties and good surface morphology.

Keywords: Mucoadhesive, Microsphere, Esomeprazole, Gastro Retentive Drug Delivery.
INTRODUCTION

Several approaches have been studied to increase the residence time of the dosage form including floating drug delivery system (DDS), swelling and expending drug delivery system, mucoadhesive DDS and high density DDS. Various gastrointestinal mucoadhesive dosage forms, such as bilayered tablets, microspheres, have been thoroughly prepared and reported by several research groups. Among the number of approaches, mucoadhesive drug delivery system is one of the promising delivery system in which drug delivery system adheres to the mucous layer of stomach and thus remains in stomach for longer period of time.

Mucoadhesion is defined as a state in which two components, of which one is of biological origin, are held together for extended periods of time by the help of interfacial forces. Generally speaking, bioadhesion is a term, which broadly includes adhesive interactions with any biological or biologically derived substance, and mucoadhesion is used when the bond is formed with a mucosal surface, while the term cytoadhesive implies adhesion to cells. Thus Mucoadhesive drug delivery systems are type of gastro retentive drug delivery systems.

In the development of oral controlled-release dosage forms, considerable benefits may ensue from the use of bioadhesive or mucoadhesive polymers providing relatively short-term adhesion between the drug delivery system and the mucus or epithelial cell surface of the gastrointestinal tract. So Binding will therefore involve secondary forces, like hydrogen bonds or Vander Waals forces. Mucoadhesives may, therefore, be regarded as a specific class of bioadhesives. When mucoadhesive polymers are utilized, the residence time of dosage forms on the mucosa can be significantly prolonged, allowing a sustained drug release at a given target site.

Microspheres may be defined as solid, spherical particles that range in size 1-1000 μm and which are made up of polymeric, waxy or other protective materials such as biodegradable synthetic polymers and modified natural products such as polysaccharides, gums, proteins, fats and waxes. For example, natural polymers include albumin and gelatin. Similarly the synthetic polymers include polylactic acid and polyglycolic acid. Microspheres are small in size and possess large surface to volume ratio. The lower sized microspheres have colloidal properties. The interfacial properties of microspheres are extremely important, often dictating their activity.

Esomeprazole is Anti-Ulcer Agent, Proton-pump Inhibitors and Antihistamines. It is absorbed completely (90%) after oral administration and the protein binding of esomeprazole is 97%. Esomeprazole is extensively metabolized in the liver by the cytochrome P450 (CYP) enzyme system and approximately 80% of an oral dose of esomeprazole is excreted as inactive metabolites in the urine and the remainder is found as inactive metabolites in the feces.
MATERIALS & METHODS:

Materials:

The following ingredients are used in present investigation:

Table 1: List of ingredients used

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Manufacture name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esomeprazole magnesium trihydrate</td>
<td>Heliox pharma</td>
</tr>
<tr>
<td>Ethyl cellulose</td>
<td>Cental drug house (P) Ltd.</td>
</tr>
<tr>
<td>Hpmc k100m</td>
<td>Cental drug house (P) Ltd.</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Changsu yangyuan chemical, china</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>Finar chemical Ltd., Ahemdabad</td>
</tr>
<tr>
<td>Light paraffin liquid</td>
<td>Finar chemical Ltd., Ahemdabad</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>Thomas beaker</td>
</tr>
<tr>
<td>Cellulose acetate phthalate</td>
<td>Cental drug house (P) Ltd.</td>
</tr>
</tbody>
</table>

Methods:

Preparation of mucoadhesive microsphere\textsuperscript{10-13}:

The mucoadhesive microspheres were prepared by solvent evaporation method. Ethanol and dichloromethane was taken in 1:1 ratio in 10 ml beaker. Weighed amount of HPMC K100M in the above solution and dissolved completely. Then weighed amount of ethyl cellulose added into the beaker and dissolved completely, and then weighed amount of esomeprazole was added and stirred with glass rod and dissolved. 25 ml of light liquid paraffin was taken in 100ml beaker and stirred by stirrer at 1000 rpm. Then the drug-polymer solution was added drop wise drop in LPL with the help of syringe (21 no needle) and starring continued for 1 hr. Then after 1 hr the prepared microspheres were separated with the help of Whatman grade filter, washed several time with petroleum ether. Then the microspheres were dried at room temperature for 12 hrs. The microspheres are prepared at different concentration of ethyl cellulose, HPMC K100M, ethanol, dichloromethane and different temperature range\textsuperscript{39}.
Coating of mucoadhesive microspheres

Coating of microsphere is done by method as described by Gopferich Achim et al. 100 mg of dried core microspheres were directly dispersed by vortexing for 5 sec into 1 ml of ethanol and acetone (1:1 ratio) solution containing 20 mg coating polymer (cellulose acetate phthalate). The coated microspheres were filtered by whatman grade filter paper and dried.

**Table 2: Formulae for first batch of mucoadhesive microsphere of esomeprazole**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug (mg)</th>
<th>Ethyl cellulose (mg)</th>
<th>HPMC K100M (mg)</th>
<th>Ethanol (ml)</th>
<th>Dichloromethane (ml)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>30</td>
<td>15</td>
<td>15</td>
<td>1.2</td>
<td>1.2</td>
<td>37</td>
</tr>
<tr>
<td>F2</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>1.2</td>
<td>1.2</td>
<td>37</td>
</tr>
<tr>
<td>F3</td>
<td>30</td>
<td>60</td>
<td>30</td>
<td>1.2</td>
<td>1.2</td>
<td>37</td>
</tr>
<tr>
<td>F4</td>
<td>30</td>
<td>30</td>
<td>60</td>
<td>1.2</td>
<td>1.2</td>
<td>37</td>
</tr>
<tr>
<td>F5</td>
<td>30</td>
<td>30</td>
<td>90</td>
<td>1.2</td>
<td>1.2</td>
<td>37</td>
</tr>
<tr>
<td>F6</td>
<td>30</td>
<td>90</td>
<td>30</td>
<td>1.2</td>
<td>1.2</td>
<td>37</td>
</tr>
</tbody>
</table>

**Table 3: Formulae for second batch of mucoadhesive microsphere of esomeprazole**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug (mg)</th>
<th>Ethyl cellulose (mg)</th>
<th>HPMC K100M (mg)</th>
<th>Ethanol (ml)</th>
<th>Dichloromethane (ml)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F7</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>0.5</td>
<td>0.5</td>
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<td>F8</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>1.1</td>
<td>1.1</td>
<td>37</td>
</tr>
<tr>
<td>F9</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>1.2</td>
<td>1.2</td>
<td>37</td>
</tr>
<tr>
<td>F10</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>1.4</td>
<td>1.4</td>
<td>37</td>
</tr>
<tr>
<td>F11</td>
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<td>30</td>
<td>30</td>
<td>1.5</td>
<td>1.5</td>
<td>37</td>
</tr>
<tr>
<td>F12</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>2</td>
<td>2</td>
<td>37</td>
</tr>
</tbody>
</table>
Table 4: Formulae for third batch of mucoadhesive microsphere of esomeprazole

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug (mg)</th>
<th>Ethyl cellulose (mg)</th>
<th>HPMC K100M (mg)</th>
<th>Ethanol (ml)</th>
<th>Dichloromethane (ml)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F13</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>1.2</td>
<td>1.2</td>
<td>35</td>
</tr>
<tr>
<td>F14</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>1.2</td>
<td>1.2</td>
<td>37</td>
</tr>
<tr>
<td>F15</td>
<td>30</td>
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<td>40</td>
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<tr>
<td>F16</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>1.2</td>
<td>1.2</td>
<td>43</td>
</tr>
</tbody>
</table>

EVALUATION OF MUCOADHESIVE MICROSPHERES

1. Identification study of drug\textsuperscript{18-21}

A. Physical description:

The procured sample of drug was characterized for its physical characteristics like appearance and odour.

B. Melting point:

The normal melting point of a solid can be defined as the temperature at which the solid and liquid are in equilibrium at a total pressure of 1 atmosphere. Experimentally, melting point is actually recorded as the ‘range of temperatures’ in which the first crystal starts to melt until the temperature at which the last crystal just disappears. Melting point was determined with the help of melting point apparatus made by prefit India.

For the determination of melting point a capillary tube was taken. One end of capillary tube was sealed by heating the one end with the help of candle. The drug was inserted from another end of capillary tube. The capillary tube and a thermometer inserted in meting point apparatus. The melting point range was determined.

2. Solubility analysis\textsuperscript{22, 23}

The solubility of esomeprazole was determined in various solvents. The study was performed using shake flask method. Saturated solution of esomeprazole was prepared in respective solvent media and stirred for 24 hours. The solution was then centrifuged for 15 min over 10,000 rpm and filtered through whatmann filter paper (#44). The concentration of
esomeprazole was determined using UV-visible spectrophotometer (UV-1800, Shimadzu corporation) against methanol as blank.

3. **Partition Coefficient**

Partition coefficient is a measurement of drug’s lipophilicity and its ability to cross cell membrane. Partition coefficient of esomeprazole was determined at 37 ± 0.5 °C by taking 10 ml of 1-octanol and 10ml distill water in separating funnel.10 mg of drug was taken in separating funnel. Separating funnel was shaken for 10 min. After shaking, the system remained undisturbed for half an hour and stand for 24 hrs. After 24 hrs two layers were separated through separating funnel and the amount of esomeprazole solubilized in octanol was determined by measuring the absorbance at 301 nm against methanol through double beam UV/Vis spectrophotometer.

Subtracting this amount from the total amount of drug gave the amount of drug present in the aqueous phase. Partition co-efficient was determined as ratios of amount of drug in octanol to the amount of drug in distill water and its logarithm value was taken for log P.

\[
K_{O/W} = \frac{\text{Concentration of drug in non-aqueous phase}}{\text{Concentration of drug in aqueous phase}}
\]

4. **Determination of absorption maxima of Esomeprazole**

A. **Determination of absorption maxima of in methanol**

10 mg accurately weighed drug was transferred into a 100 ml volumetric flask and dissolved in sufficient quantity of methanol. The volume was made up to 100 ml with the solvent. 1 ml of the above solution was diluted up to 10 ml with same methanol (10 µg/ml) and was scanned under UV-Visible Spectroscope (UV 1800, Shimadzu corporation) for determination of \(\lambda_{\text{max}}\) (absorption maxima). The same solution was reread three times. The above procedure was repeated for one more solution of same concentration. All the readings gave same value of \(\lambda_{\text{max}}\).

B. **Determination of absorption maxima of esomeprazole in pH 6.8 buffer**

10 mg accurately weighed drug was transferred into a 100 ml volumetric flask and dissolved in sufficient quantity of PH 6.8 buffer. The volume was made up to 100 ml with the solvent. 1 ml of the above solution was diluted up to 10 ml with same PH 6.8 buffer (10 µg/ml) and was scanned under UV-Visible Spectroscope (UV 1800, Shimadzu corporation) for determination of \(\lambda_{\text{max}}\) (absorption maxima). The same solution was reread three times. The above procedure was
repeated for one more solution of same concentration. All the readings gave same value of $\lambda_{\text{max}}$.

C. **Determination of absorption maxima of in 1N NaOH**

10 mg accurately weighed drug was transferred into a 100 ml volumetric flask and dissolved in sufficient quantity of 1N NaOH. The volume was made up to 100 ml with the solvent. 1 ml of the above solution was diluted up to 10 ml with same 1N NaOH (10 µg/ml) and was scanned under UV-Visible Spectroscope (UV 1800, Shimadzu corporation) for determination of $\lambda_{\text{max}}$ (absorption maxima). The same solution was reread three times. The above procedure was repeated for one more solution of same concentration. All the readings gave same value of $\lambda_{\text{max}}$.

D. **Determination of absorption maxima of esomeprazole in 0.1 N HCl**

10 mg accurately weighed drug was transferred into a 100 ml volumetric flask and dissolved in sufficient quantity of 0.1 N HCl. The volume was made up to 100 ml with the solvent. 1 ml of the above solution was diluted up to 10 ml with same 0.1 N HCl (10 µg/ml) and was scanned under UV-Visible Spectroscope (UV 1800, Shimadzu corporation) for determination of $\lambda_{\text{max}}$ (absorption maxima). The same solution was reread three times. The above procedure was repeated for one more solution of same concentration. All the readings gave same value of $\lambda_{\text{max}}$.

5. **preparation of calibration curves of esomeprazole**

For the determination of the content of esomeprazole, standard plots of the drug were prepared in methanol, 1NNaOH, pH 6.8 buffer, 0.1 N HCl in a suitable concentration range. Stock solutions of the esomeprazole in the respective solvents having concentration of 100µg/ml were prepared and diluted serially to attain a concentration range between 2-28µg/ml. These were analyzed spectrophotometrically at 301, 305, 300, 278 nm respectively using UV spectrophotometer.

6. **Determination of percent yield of microspheres**

Thoroughly dried microspheres were collected and weighed accurately. The percentage yield was then calculated using the formula given below:

% yield = Mass of microspheres obtained / Total weight of drug and polymer used × 100
7. Determination of Entrapment Efficiency\(^{32,33}\)

The entrapment efficiency of the microspheres or the percent entrapment can be determined by allowing washed microspheres to lyse. The lysate is then subjected to the determination of active constituents as per monograph requirement. The percent encapsulation efficiency is calculated using following equation:

\[
% \text{Entrapment} = \frac{\text{Actual content}}{\text{Theoretical content}} \times 100
\]

8. Particle size analysis of microsphere\(^{9,34,35}\)

Particle size analysis of drug loaded microspheres was determined by optical microscopic method using a compound microscope. At least 100 microspheres were analyzed for each preparation and the mean particle size was determined by using Edmondson’s equation

\[
D_{\text{mean}} = \frac{\Sigma nd}{\Sigma n}
\]

Where \(n\) = number of microspheres observed and \(d\) = mean size range

9. Scanning electron microscopy\(^{35,36,37}\)

A scanning electron microscope was used to characterize the surface topography of the microspheres after gold coating. A scanning electron photomicrograph of drug-loaded microspheres was taken. A small amount of microspheres was spread on glass stub. Afterwards, the stub containing the sample was placed in the scanning electron microscope (JEOL, JSM-6360) chamber. The scanning electron photomicrograph was taken at the acceleration voltage of 10 kV, chamber pressure of 0.6 mm Hg, original magnification 500.

10. Flow properties\(^{12,38}\)

It is defined as the maximum angle possible between the surface of pile of the powder and the horizontal plane. The angle of repose was determined by the funnel method. A funnel was secured with its tip at a given height (h), above a flat horizontal surface on which a graph paper was placed. Microspheres were carefully poured through a funnel until the apex of the conical pile just touches the tip of funnel. The angle of repose was then calculated using the formula.

\[
\theta = \tan^{-1}(h/r)
\]

Where, \(\theta\) = angle of repose, \(h\) = height of pile, \(r\) = radius of the base of the pile.
11. *In vitro* mucoadhesion test\(^{31,34}\)

In the present study, the eggshell membrane was used to substitute the animal stomach mucosa in the mucoadhesion evaluation of microspheres, based on the similarity between the eggshell membrane and the stomach mucus with respect to its composition and thickness. The good correlation between *in vitro* data from the eggshell membrane and *in vivo* mucoadhesion studies demonstrated the potential of the eggshell membrane as substitute for the gastric mucosa. The eggshell membranes were obtained from fresh chicken eggs. After emptying the egg of its content, the external shell was removed, and the underlying membrane was isolated. A piece of egg membrane was tied on to a glass slide. Approximately 50 microspheres were spread onto the wet membrane and the prepared slide was hung on one the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that membrane specimen was given regular up and down movements in a beaker containing the simulated gastric fluid USP (pH 6.8). At the end of 1, 5 and 10h, the microspheres still adhering onto the membrane was counted.

12. *In vitro* release studies\(^{38,39}\)

Coated microsphere equivalent to 40 mg of esomeprazole magnesium (formulation F14) were taken and the release rate of drug from coated microsphere was determined using USP type 1 apparatus. The microspheres were taken in basket. Then the basket was immersed in pH 1.2 HCl buffer for 2hr followed by phosphate buffer of pH 6.8 for 10 hr maintained at 37°C± 0.5°C and was rotated at the speed of 100 rpm. Sample aliquots of 5ml were withdrawn at every hour up to 12hrs and estimated for its drug content using UV spectroscopy at 300 nm.

**RESULT AND DISCUSSION:**

**Identification of drug:**

**Physical description:** The drug was found yellowish in colour and odourless.

**Melting point:** Melting point was found 178-183°C.
FT-IR studies

FT-IR of drug

Figure 1: FT-IR Spectrum of Esomeprazole

FT-IR of HPMC K 100 M

Figure 2: FT-IR Spectrum of HPMC K 100 M

FT-IR of ethyl cellulose

Figure 3: FT-IR of ethyl cellulose
FT-IR data of drug and polymer

![FT-IR data](image)

**Figure 4: FT-IR data of drug and polymer**

**FT-IR of Drug and polymer**

**Table 5: Interpretation of FT-IR spectra**

<table>
<thead>
<tr>
<th>Functional group</th>
<th>Reference peak</th>
<th>Observed peak of drug</th>
<th>Observed peak of ethyl cellulose</th>
<th>Observed peak of HPMC K100M</th>
<th>Observed peak of drug and polymer mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-H (hetero-aromatic ring)</td>
<td>3080-3000</td>
<td>3070.46</td>
<td>-</td>
<td>-</td>
<td>3068.53</td>
</tr>
<tr>
<td>N-H</td>
<td>3500-3220</td>
<td>3413.77</td>
<td>-</td>
<td>--</td>
<td>3421.48</td>
</tr>
<tr>
<td>C=S</td>
<td>700-600</td>
<td>636.47, 696.25</td>
<td>661.54</td>
<td>-</td>
<td>636.47, 696.25</td>
</tr>
<tr>
<td>S=O</td>
<td>1080-1030</td>
<td>1076.21</td>
<td>-</td>
<td>-</td>
<td>1076.21</td>
</tr>
<tr>
<td>CH$_3$-O-Ar</td>
<td>1275-1200</td>
<td>1226.64, 1272.93</td>
<td>-</td>
<td>-</td>
<td>1226.64, 1251.72, 1272.93</td>
</tr>
<tr>
<td>C=C(hetero-aromatic ring)</td>
<td>1600-1430</td>
<td>1434.94, 1477.37, 1569.95, 1591.16</td>
<td>-</td>
<td>-</td>
<td>1434.94, 1477.37, 1569.95, 1591.16</td>
</tr>
<tr>
<td>C=N (hetero-aromatic ring)</td>
<td>1600-1430</td>
<td>1434.94, 1477.37, 1569.95, 1591.16</td>
<td>-</td>
<td>-</td>
<td>1434.94, 1477.37, 1569.95, 1591.16</td>
</tr>
<tr>
<td>Aryl akyl ether</td>
<td>1275-1200</td>
<td>-</td>
<td>1234.36</td>
<td>1265.22</td>
<td>1226.64, 1251.72, 1272.93</td>
</tr>
<tr>
<td>C-O-C</td>
<td>1150-1085</td>
<td>-</td>
<td>1110.92</td>
<td>1116.71</td>
<td>1114.78</td>
</tr>
</tbody>
</table>
Identification of drug and polymer:

The drug and polymer are identified by FT-IR as the observed peak of drug and polymer was matched with reference peak of drug and polymer respectively.

Drug and polymer compatibility study:

The compatibility between drug and polymer was determined by FT-IR. The position of peak in FT-IR spectra of pure drug and polymers were compared with those in FT-IR spectra of drug-polymer mixture. No disappearance or significant shift in the peak position of drug and polymer in the spectra was observed, which proves that the drug and polymers used for the study are compatible.

Solubility study

The solubility study of esomeprazole in different solvent suggests that the drug is maximum soluble in methanol and minimum soluble in light liquid paraffin than other solvent.

Descending order of solubility in different solvents-

Methanol > Ethanol > pH 7.4 buffer > Choloform > Acetone > Tween 80 > Span 80 > n-haxane > light liquid paraffin.

Table 6: Solubility of esomeprazole

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Solvent</th>
<th>Solubility (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methanol</td>
<td>247.027027</td>
</tr>
<tr>
<td>2</td>
<td>Ethanol</td>
<td>230.2702703</td>
</tr>
<tr>
<td>3</td>
<td>Acetone</td>
<td>53.24324324</td>
</tr>
<tr>
<td>4</td>
<td>Light liquid paraffin</td>
<td>0.190540541</td>
</tr>
<tr>
<td>5</td>
<td>n-haxane</td>
<td>0.202702703</td>
</tr>
<tr>
<td>6</td>
<td>Choloform</td>
<td>99.45945946</td>
</tr>
<tr>
<td>7</td>
<td>Span 80</td>
<td>4.243243243</td>
</tr>
<tr>
<td>8</td>
<td>Tween 80</td>
<td>8.324324324</td>
</tr>
<tr>
<td>9</td>
<td>pH 7.4 buffer</td>
<td>103.030303</td>
</tr>
</tbody>
</table>
Partition Coefficient:

The partition coefficient of esomeprazole between octanol and water was found to be 4.52 and log P value was 0.655368, which showed that drug is lipophillic in nature.

**Determination of absorption maxima of esomeprazole:**

The absorption maxima of esomeprazole was found 301nm in methanol, 300 nm pH 6.8 buffer, 305nm in 1N NaOH.

**Calibration curve of esomeprazole in different solvents:**

Calibration curve of esomeprazole in methanol was prepared and R² value was found 0.9885.

Calibration curve of esomeprazole in pH 6.8 buffer was prepared and R² value was found 0.9968.

Calibration curve of esomeprazole in 1N NaOH was prepared and R² value was found 0.9844.

Calibration curve of esomeprazole in 0.1 N HCl was prepared and R² value was found 0.993.

**Determination of absorption maxima of in methanol:**

*Table 7: Determination of absorption maxima of esomeprazole in methanol*

<table>
<thead>
<tr>
<th>Solution ID</th>
<th>Concentration</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>λ&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Abs</td>
<td>λ&lt;sub&gt;max&lt;/sub&gt;</td>
</tr>
<tr>
<td>Solution 1</td>
<td>10 µg/ml</td>
<td>301</td>
<td>0.392</td>
<td>301</td>
</tr>
<tr>
<td>Solution 2</td>
<td>10 µg/ml</td>
<td>301</td>
<td>0.392</td>
<td>301</td>
</tr>
</tbody>
</table>

**Figure 5: Determination of absorption maxima of esomeprazole in methanol**
Table 8: Determination of absorption maxima of esomeprazole in pH 6.8 buffer

<table>
<thead>
<tr>
<th>Solution ID</th>
<th>Concentration</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\lambda_{\text{max}}$</td>
<td>Abs</td>
<td>$\lambda_{\text{max}}$</td>
</tr>
<tr>
<td>Solution 1</td>
<td>10 $\mu$g/ml</td>
<td>300</td>
<td>0.361</td>
<td>300</td>
</tr>
<tr>
<td>Solution 2</td>
<td>10 $\mu$g/ml</td>
<td>300</td>
<td>0.361</td>
<td>300</td>
</tr>
</tbody>
</table>

Figure 6: Determination of absorption maxima of esomeprazole in pH 6.8 buffer

Table 9: Determination of absorption maxima of esomeprazole in 1N NaOH

<table>
<thead>
<tr>
<th>Solution ID</th>
<th>Concentration</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\lambda_{\text{max}}$</td>
<td>Abs</td>
<td>$\lambda_{\text{max}}$</td>
</tr>
<tr>
<td>Solution 1</td>
<td>10 $\mu$g/ml</td>
<td>305</td>
<td>0.473</td>
<td>305</td>
</tr>
<tr>
<td>Solution 2</td>
<td>10 $\mu$g/ml</td>
<td>305</td>
<td>0.473</td>
<td>305</td>
</tr>
</tbody>
</table>

Table 10: Determination of absorption maxima of esomeprazole in 0.1N HCl

<table>
<thead>
<tr>
<th>Solution ID</th>
<th>Concentration</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\lambda_{\text{max}}$</td>
<td>Abs</td>
<td>$\lambda_{\text{max}}$</td>
</tr>
<tr>
<td>Solution 1</td>
<td>10 $\mu$g/ml</td>
<td>278</td>
<td>0.478</td>
<td>278</td>
</tr>
<tr>
<td>Solution 2</td>
<td>10 $\mu$g/ml</td>
<td>278</td>
<td>0.478</td>
<td>278</td>
</tr>
</tbody>
</table>
Figure 7: Determination of absorption maxima of esomeprazole in 0.1 N HCl

Calibration curve of esomeprazole in Methanol

Table 11: Absorbance data for calibration curve of esomeprazole in methanol

<table>
<thead>
<tr>
<th>S.no</th>
<th>Concentration (µg/ml)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0.056</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0.1</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0.135</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>0.232</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>0.309</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>0.392</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>0.475</td>
</tr>
<tr>
<td>8</td>
<td>14</td>
<td>0.558</td>
</tr>
<tr>
<td>9</td>
<td>16</td>
<td>0.641</td>
</tr>
<tr>
<td>10</td>
<td>18</td>
<td>0.724</td>
</tr>
<tr>
<td>11</td>
<td>20</td>
<td>0.807</td>
</tr>
<tr>
<td>12</td>
<td>22</td>
<td>0.890</td>
</tr>
<tr>
<td>13</td>
<td>24</td>
<td>0.973</td>
</tr>
</tbody>
</table>
Figure 8: Calibration curve of esomeprazole in methanol

Calibration curve of esomeprazole in pH 6.8 buffer

Table 12: Absorbance data for calibration curve of esomeprazole in pH 6.8 buffer

<table>
<thead>
<tr>
<th>S.no.</th>
<th>Concentration µg/ml</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0.062</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0.091</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0.146</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>0.202</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>0.309</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>0.361</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>0.432</td>
</tr>
<tr>
<td>8</td>
<td>14</td>
<td>0.492</td>
</tr>
<tr>
<td>9</td>
<td>16</td>
<td>0.560</td>
</tr>
<tr>
<td>10</td>
<td>18</td>
<td>0.603</td>
</tr>
<tr>
<td>11</td>
<td>20</td>
<td>0.680</td>
</tr>
<tr>
<td>12</td>
<td>22</td>
<td>0.757</td>
</tr>
<tr>
<td>13</td>
<td>24</td>
<td>0.834</td>
</tr>
<tr>
<td>14</td>
<td>26</td>
<td>0.911</td>
</tr>
<tr>
<td>15</td>
<td>28</td>
<td>0.988</td>
</tr>
</tbody>
</table>
Figure 9: Calibration curve of esomeprazole in pH 6.8 buffer

Calibration curve of esomeprazole in 1N NaOH

Figure 10: Calibration curve of esomeprazole in 1N NaOH
Table 13: Absorbance data for calibration curve of esomeprazole in 1N NaOH

<table>
<thead>
<tr>
<th>S.no.</th>
<th>Concentration (µg/ml)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>0.115</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0.197</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>0.316</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>0.408</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>0.473</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>0.553</td>
</tr>
<tr>
<td>7</td>
<td>14</td>
<td>0.676</td>
</tr>
<tr>
<td>8</td>
<td>16</td>
<td>0.674</td>
</tr>
<tr>
<td>9</td>
<td>18</td>
<td>0.892</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>0.975</td>
</tr>
</tbody>
</table>

Calibration curve of esomeprazole in 0.1 N HCl

![Graph showing the calibration curve with the equation y = 0.0392x + 0.0311, R² = 0.9935.]

Figure 11: Calibration curve of esomeprazole in 0.1 N HCl

Table 14: Absorbance data for calibration curve of esomeprazole in 0.1 N HCl

<table>
<thead>
<tr>
<th>S.no.</th>
<th>Concentration (µg/ml)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>0.092</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0.163</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>0.263</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>0.343</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>0.487</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>0.520</td>
</tr>
</tbody>
</table>
Determination of percent yield of microspheres

Table 15: Percent yield of microspheres

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Formulation code</th>
<th>% production yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>68.33</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>65</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>69.16</td>
</tr>
<tr>
<td>4</td>
<td>F4</td>
<td>59.58</td>
</tr>
<tr>
<td>5</td>
<td>F5</td>
<td>74</td>
</tr>
<tr>
<td>6</td>
<td>F6</td>
<td>81.86</td>
</tr>
<tr>
<td>7</td>
<td>F7</td>
<td>49.55</td>
</tr>
<tr>
<td>8</td>
<td>F8</td>
<td>85</td>
</tr>
<tr>
<td>9</td>
<td>F9</td>
<td>65</td>
</tr>
<tr>
<td>10</td>
<td>F10</td>
<td>89.55</td>
</tr>
<tr>
<td>11</td>
<td>F11</td>
<td>71.77</td>
</tr>
<tr>
<td>12</td>
<td>F12</td>
<td>96.11</td>
</tr>
<tr>
<td>13</td>
<td>F13</td>
<td>65</td>
</tr>
<tr>
<td>14</td>
<td>F14</td>
<td>96.88</td>
</tr>
<tr>
<td>15</td>
<td>F15</td>
<td>86.22</td>
</tr>
<tr>
<td>16</td>
<td>F16</td>
<td>76.88</td>
</tr>
</tbody>
</table>

In first batch highest percentage yield was found in formulation code F6. In second batch the highest percentage yield was found in formulation code F12. In third batch the highest percentage yield was found in formulation code F14.

Determination of Entrapment Efficiency

Table 16: Entrapment Efficiency

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Theoretical drug content (mg)</th>
<th>Practical drug content (mg)</th>
<th>% Drug content</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>7.31</td>
<td>5.23</td>
<td>71.54</td>
</tr>
<tr>
<td>F2</td>
<td>5.12</td>
<td>5.11</td>
<td>99.80</td>
</tr>
<tr>
<td>F3</td>
<td>3.61</td>
<td>1.6652</td>
<td>46.12</td>
</tr>
<tr>
<td>F4</td>
<td>4.19</td>
<td>1.52</td>
<td>36.42</td>
</tr>
<tr>
<td>F5</td>
<td>2.70</td>
<td>1.24</td>
<td>46.03</td>
</tr>
<tr>
<td>F6</td>
<td>2.44</td>
<td>1.52</td>
<td>62.48</td>
</tr>
</tbody>
</table>
In first batch the highest entrapment efficiency was found in formulation code F2. In second batch the highest entrapment efficiency was found in formulation code F9. In third batch the highest entrapment efficiency was found in formulation code F13.

**Particle size analysis of microsphere**

**Table 17: Particle size analysis of microsphere**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>F2</th>
<th>F9</th>
<th>F14</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32.5</td>
<td>24</td>
<td>32.4</td>
</tr>
</tbody>
</table>

The mean particle size of microsphere was found 32.5 µm, 24 µm, 32.5 µm of formulation F2, F9, F14 respectively.

**Scanning electron microscopy**

The scanning electron microscopy of formulation code F14 reveals that the microspheres were spherical, discrete with rough texture.

![Figure 12: Scanning electron microscopy of formulation F14](image)
In vitro mucoadhesion test

Table 18: In vitro mucoadhesion test

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Time (Hr)</th>
<th>No. of microsphere adhere</th>
<th>% Mucodhesion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F2</td>
<td>F9</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>35</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

The table shows that some of microspheres were adhere to the membrane even after 10hrs. The highest percentage mucoadhesion was found 14% in formulation F14.

Flow properties

Table 19: Angle of repose of formulation F2

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Height of pile (cm)</th>
<th>Radius of the base of the pile (cm)</th>
<th>Angle of repose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>h₁=0.5, Average</td>
<td>r₁=0.9, Average</td>
<td>=29°</td>
</tr>
<tr>
<td>2</td>
<td>h₂=0.5, height</td>
<td>r₂=0.9, radius</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>H₃=0.5, =0.5cm</td>
<td>r₃=0.9, =0.9cm</td>
<td></td>
</tr>
</tbody>
</table>

Table 20: Angle of repose of formulation F9

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Height of pile (cm)</th>
<th>Radius of the base of the pile (cm)</th>
<th>Angle of repose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>h₁=0.7, Average</td>
<td>r₁=1.1, Average</td>
<td>=34.15°</td>
</tr>
<tr>
<td>2</td>
<td>h₂=0.4, height</td>
<td>r₂=0.8, radius</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>H₃=0.8, =0.633cm</td>
<td>r₃=0.9, =0.933cm</td>
<td></td>
</tr>
</tbody>
</table>

Table 21: Angle of repose of formulation F14

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Height of pile (cm)</th>
<th>Radius of the base of the pile (cm)</th>
<th>Angle of repose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>h₁=0.7, Average</td>
<td>r₁=0.9, Average</td>
<td>=36.25°</td>
</tr>
<tr>
<td>2</td>
<td>h₂=0.6, height</td>
<td>r₂=0.9, radius</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>H₃=0.7, =0.66cm</td>
<td>r₃=0.9, =0.9cm</td>
<td></td>
</tr>
</tbody>
</table>
Angle of repose less than or equal to 40° indicates free flowing properties of the microspheres. However angle of repose greater than 40° indicates poor flow of material. It can be observed from table that the angle of repose for various batches of the microspheres is found to be less than 40°, it indicates good flow properties of the microspheres.

**In vitro release studies**

**Table 23: Percentage cumulative drug release**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Time (hrs)</th>
<th>Percentage cumulative drug release</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>18.46154</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>27.21795</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>39.79866</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>46.15472</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>55.27214</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>64.43881</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>72.9729</td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>80.87063</td>
</tr>
<tr>
<td>10</td>
<td>9</td>
<td>84.0373</td>
</tr>
<tr>
<td>11</td>
<td>10</td>
<td>87.90093</td>
</tr>
<tr>
<td>12</td>
<td>11</td>
<td>88.9536</td>
</tr>
<tr>
<td>13</td>
<td>12</td>
<td>89.96212</td>
</tr>
</tbody>
</table>

**Figure 13: percentage cumulative drug release**
Drug release kinetics

1. Zero order

![Zero order graph](image)

\[ y = 7.444x + 13.493 \]
\[ R^2 = 0.9431 \]

![Fig 14: Zero order](image)

2. First order

![First order graph](image)

\[ y = -0.0895x + 2.0395 \]
\[ R^2 = 0.9867 \]

![Fig 15- first order](image)
3. Higuchi model

\[ y = 29.293x - 7.7507 \]
\[ R^2 = 0.9783 \]

![Higuchi model graph](image)

**Fig 16- Higuchi model**

4. Koresmayer Peppas model

\[ y = 1.1855x + 0.8284 \]
\[ R^2 = 0.6902 \]

![Koresmayer Peppas model graph](image)

**Fig 17- Koresmayer Peppas model**
Table 24: Drug release kinetics

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Zero order</th>
<th>higuchi</th>
<th>First order</th>
<th>Peppas</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.943</td>
<td>0.978</td>
<td>0.986</td>
<td>0.690</td>
</tr>
</tbody>
</table>

The value of $r^2$ (0.986) was found to be highest for the First order model, which also indicates that the test product follows First order release kinetics. The $n$ value was found 0.828. Values of $n$ between 0.45 and 0.89 can be regarded as an indicator of both phenomena (drug diffusion in the hydrated matrix and the polymer relaxation) commonly called anomalous transport.

CONCLUSION:

Ethycellulose was used as matrix polymer in which drug was dispersed because of its hydrophobic characteristic. Hydroxy propyl methyl cellulose (K 100M) was used as Mucoadhesive polymer. By observing above all evaluation parameters, it was concluded that formulation code F14 showed reproducible results, with good Mucoadhesive properties and good surface morphology. The formulation follows first order kintecs and anomalous transport.

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