Preparation and Characterization of Mucoadhesive microspheres containing Clopidogrel

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Clopidogrel bisulphate has been the most widely used as antithrombotic. Clopidogrel has greater solubility in acidic pH and it is absorbed through gastrointestinal region. The aim of the present research work was to develop the mucoadhesive micro-spheres containing clopidogrel. The micro-spheres were prepared by ionotrophic gelation method. The microcapsules were prepared with HPMC K4M, Xanthan gum and pectin along with sodium alginate. The prepared micro-spheres were characterized for various physicochemical properties such as, particle size distribution, entrapment efficiency, in vitro dissolution, in vivo radiographic study, DSC, FTIR and SEM study. In vitro drug release study showed that the drug release was extended up to 14 hours and the drug release was mainly depend on the polymer ratio and polymer concentration. In vivo radiographic study revealed that the prepared micro-spheres retained in the stomach for more than 6 hours. DSC and FTIR study showed no drug polymer interaction. SEM study showed the spherical and porous nature of the prepared micro-spheres.

Keyword: Mucoadhesive, Micorspheres, Clopidorel, HPMCK4M, Xanthan gum, Pectin

1. Introduction
Multiple unit dosage forms such as microspheres or beads have gained popularity as oral drug delivery systems because of more uniform distribution of the drug in the gastrointestinal tract, more uniform drug absorption, reduced local irritation, and elimination of unwanted intestinal retention of polymeric material, when compared to nondisintegrating single-unit dosage form1,2.

Microencapsulation by various polymers and their applications are well known. 3,4 Microencapsulation and resulting microcapsules have gained good acceptance as a process to achieve controlled-release drug targeting. Mucoadhesion is a topic of current interest in the design of drug delivery systems to prolong the residence time of the dosage form at the site of application or absorption and to facilitate intimate contact of the dosage form with the underlying absorption surface to improve and enhance the bioavailability of the drug5,6,7.

Clopidogrel bisulfate is a thienopyridine class inhibitor of P2Y12 ADP platelet receptors. Chemically it is methyl (+)-(S)-α-(2-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetate sulfate (1:1). The empirical formula of clopidogrel bisulfate is C16H16ClNO2S•H2SO4 and its molecular weight is 419.98. 
It is an oral, thienopyridine class antiplatelet agent used to inhibit blood clots in coronary artery disease, peripheral vascular disease, and cerebrovascular disease. The main objective is to prepare the mucoadhesive microspheres of clopidogrel using different polymers such as HPMC, Xanthan gum and pectin.

2. Materials and methods

2.1 Materials

Clopidogrel was obtained as a gift sample from Alkem laboratories Ltd (Mumbai, India). Sodium alginate was obtained from Signet Chemical Corporation, Xanthan gum was obtained from Kachabo gums, Navi Mumbai, Maharashtra, HPMC K4M was obtained from Colorcon Asia Pvt Ltd, Pectin was obtained from CDH Chemical Pvt Ltd, New Delhi. All other chemicals and reagents used in the study were of analytical grade.

2.2 Methods

2.2.1 Preparation of Microspheres

Mucoadhesive microspheres of clopidogrel were prepared using ionotropic gelation method. The cross linking polymer sodium alginate and mucoadhesive polymer were soaked in the water for 24 hours. The pure drug such as clo pidogrel was dissolved in 10 ml of water and mixed with the above polymer mixture. The above solution was added drop wise using 24 gauge syringe to the Al$_2$(SO$_4$)$_3$ containing the pectin 5%. The formed microspheres were allowed for 30 minutes in the above solution under stirring condition for the completion of reaction and for the formation of spherical microspheres. The prepared microspheres were filtered, washed with distilled water and finally dried at 45 °C. The dried microspheres were stored in air tight container.

2.2.2 Evaluation of Microspheres

Encapsulation Efficiency (EE) [9]

Drug loaded micro capsules (100 mg) were powdered and suspended in 0.1 N HCl. Then the contents suspended in the water were kept for sonication (Power sonic 505, HWASHIN technology co) for about 20 minutes and shaking using mechanical shaker (ORBITECH, Scigenics biotech) for about 20 mts for the complete extraction of drug from the microcapsules. The resultant solution was filtered through 0.45 µm membrane filter (MILLIPORE). Drug content was determined by UV- visible spectrophotometer (Schimadzu, UV-1700 E 23) at 254 nm. The percent entrapment was calculated by using the following formula.

\[
\text{Encapsulation Efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100
\]

2.3 Particle size distribution [10]

Particle size analysis of the microcapsules was done by sieving method using Indian Standard Sieves # 16, #20, #30, #40, #60 and #80. The results of particle size distribution were given in the Table.


The FT-IR spectra acquired were taken from dried samples. A FT-IR (Thermo Nicolet 670 spectrometer) was used for the analysis in the frequency range between 4000 and 400 cm$^{-1}$, and 4 cm$^{-1}$ resolution. The results were the means of 16 determinations. A quantity equivalent to 2 mg of pure drug and drug loaded micro capsules were selected separately.

Differential scanning calorimetry (DSC) study of drug loaded microcapsules was performed using a Diamond DSC (Mettler Star SW 8.10) to determine the drug excipient compatibility study. The analysis was performed at a rate 5 °C min$^{-1}$ from 500 °C to 2000 °C temperature range under nitrogen flow of 25 ml min$^{-1}$.

2.5 Scanning Electron Microscopy (SEM)

Morphological characterization of the microcapsules was done by using Scanning electron microscope (JEOL JSM -5200). The samples were coated to 200Å° thickness with
gold-palladium using prior to microscopy. Microcapsules before dissolution study were only subjected to SEM study.

2.6 In Vitro Drug Release Studies
The in vitro dissolution studies were performed using USP type I dissolution apparatus (LABINDIA, DISSO-2000, Mumbai, India) at 75 rpm. The micro capsules were weighed and filled in the empty capsule shells and placed in the basket. The dissolution medium consisted of 0.1N hydrochloric acid (900 mL), maintained at 37°C ± 5°C. An aliquot (5 mL) was withdrawn at specific time intervals and drug content was determined by UV-visible spectrophotometer (Shimadzu, UV-1700 E 23) at 254 nm. The release studies were conducted in triplicate.

2.7 Determination of Stability of the Microcapsules
The microspheres prepared in the present study were filled in the hard gelatin capsules and then filled in HDPE containers and stored at the following conditions like 40°C/75 RH for 3 about months as per ICH guidelines. The samples were characterized for % drug content. The results were summarized in the table.

2.8 In vitro wash-off test: [12]
The mucoadhesive properties of the microspheres was evaluated by in vitro wash-off test which is a simple and quick method reported by Lehr et al [3] as follows: pieces of tissue (pig stomach, about 2 x 5 cm), and small intestine, (about 2 x 15 cm), obtained from slaughter house and stored in Tyrodes solution) were tied onto a plastic slide (about 2 x 15 cm) using rubber bands. Microspheres were spread (25 No) onto each wet, rinsed tissue specimen, and counted. Immediately thereafter, the prepared two slides were connected with suitable support onto one of the groves of a USP tablet disintegrating test apparatus, permitting a slow, regular up and down movement (~30min⁻¹) in a test fluid (0.1N HCl, pH 1.2) kept at 37°C. At given intervals, the motor was stopped and number of microspheres still adhering onto the tissue was counted. The results obtained can be used as a measure of bioadhesion.

2.9 X-Ray studies [13]
To determine transit behavior of chitosan dispersed alginate multiple unit systems through gastrointestinal tract, radio opaque barium sulfate was encapsulated in multiple unit systems. After administration to human volunteer, X-ray photographs were recorded at predetermined time intervals.

<table>
<thead>
<tr>
<th>S No</th>
<th>Ingredients</th>
<th>F-1</th>
<th>F-2</th>
<th>F-3</th>
<th>F-4</th>
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<tr>
<td></td>
<td>In mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Clopidogrel</td>
<td>500</td>
<td>500</td>
<td>500</td>
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<tr>
<td>2</td>
<td>Na Alginate</td>
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<tr>
<td>3</td>
<td>HPMCK4M</td>
<td>150</td>
<td>400</td>
<td>900</td>
<td>1400</td>
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</table>
3. Results and Discussion

The mucoadhesive microspheres of Clopidogrel were prepared with an aim to improve its bioavailability (Table1). The average size of the prepared microspheres with HPMC K 4 M was 650 -820 μm. The average size of the prepared microspheres with Xanthangum was 750 -800 μm. The average size of the prepared microspheres with Pectin was 600 -700 μm. The particle size of microspheres was increased with increase in the polymer concentration. The entrapment efficiency of the prepared microspheres with HPMC K 4 M was found 68.11- 84.55 %. The entrapment efficiency of the prepared microspheres with Xanthangum was found 69.89- 89.18 %. The entrapment efficiency of the prepared microspheres with Pectin was found 59.67- 75.45 %. Highest entrapment was observed for the microspheres prepared with Xanthangum. The entrapment efficiency was least in the Pectin when compared with the other formulations. In vitro dissolution form the microspheres prepared with low concentration HPMC K 4 M was released completed drug in 10 hours. The release was extended up to 13 hours for the formulation with increased polymer concentration (Figure-1).

Table 2 : Entrapment efficiency and release kinetics of clopidogrel microspheres

<table>
<thead>
<tr>
<th>Code</th>
<th>EE (%)</th>
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<th>First order</th>
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<tr>
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<td>F-2</td>
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<tr>
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<tr>
<td>F-4</td>
<td>85.45</td>
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<tr>
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<tr>
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<td>0.7919</td>
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<tr>
<td>F-7</td>
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<td>0.9161</td>
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<td>F-12</td>
<td>75.45</td>
<td>0.9898</td>
<td>0.9121</td>
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</table>

Figure 1: Dissolution plot of the Microspheres Prepared with HPMC K 4 M
The in vitro dissolution of the microspheres prepared with the xanthan gum was extended the drug release up to 14 hours and released the complete drug (Figure 2). The in vitro dissolution of the microspheres prepared with the Pectin was extended the drug release up to 13 hours (Figure 3). The release was retarded more with the xanthan gum than pectin than HPMC. In vitro release kinetic study revealed that the prepared microspheres followed zero order release from all the formulations with diffusion mechanism.

Figure 2: Dissolution plot of the Microspheres Prepared with Xanthangum

Figure 3: Dissolution Plot of the Microspheres Prepared with Pectin
In vitro wash off study was carried out as specified in the methods section. The results of the wash of study showed that highest mucoadhesive strength was observed for the microspheres prepared with Xanthan gum. The mucoadhesive strength was increased with increase in the polymer proportion.

**Figure 4:** DSC thermograms of (A) Pure Clopidogrel, (B) Microspheres prepared with HPMC K 4 M (C) Microspheres prepared with Xanthangum, (D) Microspheres prepared with Pectin.

DSC thermograms of pure clopidogrel showed sharp endothermic peak at 182 °C. Similar sharp endothermic peaks were observed in the formulations prepared with the HPMC K 4, Xanthangum and Pectin. This clearly indicated no drug polymer interaction. (Figure-4). FTIR spectrum peaks points of pure drug were similar with the spectrum peak points of the prepared mucoadhesive microspheres further confirms no drug polymer interaction. (Figure-5).

**Figure 5:** FTIR Spectra of (A) Pure Clopidogrel, (B) Microspheres prepared with HPMC K 4 M (C) Microspheres prepared with Xanthangum, (D) Microspheres prepared with Pectin.
Figure 6: SEM images of (A) Microspheres prepared with HPMC K 4 M (B) Microspheres prepared with Xanthangum, (C) Microspheres prepared with Pectin.

SEM images of the prepared microspheres showed spherical with porous surface. (Figure-6)

In vivo radiographic study showed that the prepared microspheres remained stick to the gastric mucosa for about 6 hours. This indicates the good gastric residence time of the prepared microspheres. (Figure-7)

Figure 7: X-Ray images of (A) After 30 minutes (B) After 6 Hours

4. Conclusions
The microsphere prepared with HPMC, Xanthangum and Pectin by ionotrophic gelation method exhibits spherical, free flowing, good entrapment efficiency and exhibit good mucoadhesive property hence these microsphere was slow and extended release over prolonged periods of time and depended on composition of the polymer used. Drug release was diffusion controlled and followed zero order kinetics.

These mucoadhesive microspheres are thus suitable for oral controlled release of Clopidogrel.

5. References
2. Bodmeier R, Chen H, Tyle P, Jarosz P., Pseudoephedrine HCl microspheres formulated into


