Comparative Evaluation of Bromhexine HCl Mucoadhesive Microspheres Prepared by Anionic, Cationic and Nonionic Polymers

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Abstract
The purpose of this study was to formulate and assess the mucoadhesive microspheres of bromhexine hydrochloride, a mucolytic agent, using three different types of polymers to achieve gastric retention for improved solubility and bioavailability of the drug. The mucoadhesive formulation was prepared because it dissolved in the pH range of 1 to 4. The characteristics of the prepared microspheres were evaluated by determining the particle size, percent drug loading, surface morphology, swelling behavior, mucoadhesive bond strength and drug entrapment efficiency. The in vitro dissolution was studied using the USP dissolution apparatus I in 0.1N HCl (pH 1.2) media for 8 hours. The release kinetics were analyzed by using zero order, first order, Higuchi, Korsmeyer-Peppas and Hixon-crowell equations to explain the release mechanism from the microspheres. The microspheres exhibited good swelling index and the drug entrapment efficiency was above 79 % for all the formulations. All the formulations showed drug release above 25%, 35%, 50% and 75% after 2 hrs, 4 hrs, 6 hrs and 8 hrs of dissolution respectively. The mucoadhesive bond was observed up to 8 hrs in acidic media. The surface morphology of the prepared microspheres was studied by Scanning Electron Microscope (SEM) and no interaction was found between drug and polymer from the FTIR study.

Keywords: Bromhexine Hydrochloride, mucoadhesive microspheres, gastric retention, solvent evaporation, bioavailability, release kinetic, surface morphology.

Introduction
The maintenance of drug content at the site of action is the primary concern with any dosage formulation design. Some conventional dosage forms provide poor management of plasma drug concentrations. Drug-level fluctuations due to frequent administration and variations in their absorption or metabolism can result in toxic effects or render the drugs less effective. These problems can be solved by designing new drug-delivery systems that can provide steady-state plasma concentrations of the drug(s) administered. Recently, extensive efforts have been dedicated to developing controlled-release drug-delivery systems. These dosage forms are designed to release the drugs constantly over an extended period (Gaura et al., 2014).

The most feasible method for achieving a prolonged and predictable drug delivery in the GI tract is to control the gastric residence time by gastro retentive and control release dosage forms that have some benefits in safety and efficacy over conventional release systems. This method of application is especially helpful in the delivery of poorly soluble and insoluble drugs. It is acknowledged that the time available for drug dissolution becomes less adequate, the solubility of a drug decreases, and so the transit time becomes an important factor affecting drug absorption in drugs.
with lower solubility (Ganesan and Kanth, 2013). The concept of bioadhesion or more specifically mucoadhesion is one of the concepts to increase gastric retention of drugs. The side effects of conventional drug delivery systems have been attenuated by designing the drug in the form of mucoadhesive microspheres which provides various advantages like, maximized absorption rate due to improved drug protection by polymer encapsulation, intimate contact with the absorbing membrane and longer gut transit time resulting in extended periods for absorption (Yadav et al., 2016).

Bromhexine hydrochloride is considered as BCS Class II drug, i.e. low soluble with high permeability as per Biopharmaceutical Classification System (BCS). Bromhexine hydrochloride (BRX-HCl), possesses mucolytic and mucokinetic activities. The oral bioavailability of bromhexine hydrochloride is 20%, and it undergoes extensive first-pass metabolism in the liver. It requires administration of the drug four times a day which causes compliance problems to patients. The drug causes gastric irritation upon oral administration (Sahin and Arslan, 2008). Being BCS class II drug, it dissolves in the pH range of 1 to 4, i.e. in the stomach and shows low solubility in the lower region of the gastrointestinal tract (Harikumar and Sharma, 2012).

The poor bioavailability of bromhexine hydrochloride was the drug selection criteria, which could be increased by prolonging the gastric retention time. The present study was focused on the development of a gastro-retentive mucoadhesive microspheres using various mucoadhesive polymers like Gelatin, Carbopol 971P and HPMC K4M and to study the effect of these polymers on physical properties and drug release profile of bromhexine hydrochloride. The microspheres were prepared by solvent evaporation method. By preparing mucoadhesive microspheres of bromhexine hydrochloride, it is possible to retain the drug in the stomach or acidic pH for a prolonged period of time and thus to improve the solubility and bioavailability of the drug.

**Materials and Methods**

**Materials**: Bromhexine Hydrochloride was received as a gift sample from Incepta Pharmaceuticals Ltd., Bangladesh. Methocel K4M was collected from Colorcon Asia Pvt. Ltd, USA. Carbopol 971P, Gelatin, Ethanol, Light Liquid Paraffin, Tween 80 and n-hexane were supplied by Merck, Germany. All the chemicals used were of analytical grade. Distilled water was collected from the research laboratory.

**Preparation of microspheres**: Bromhexine hydrochloride mucoadhesive microspheres were prepared by using the non-aqueous solvent evaporation method by taking drug and different polymers in different proportions. The internal phase was prepared by dissolving drug and polymer in ethanol. The ratio of drugs to polymers used to prepare the different formulations was 1:1, 1:2 and 1:3. The external phase was prepared by dispersing 2% tween 80 in light liquid paraffin. The internal phase was then slowly poured to the external phase. The mixture was stirred with a propeller at 350 rpm for 3 hours at room temperature. The light liquid paraffin was then decanted, and the microspheres were separated by filtration and washed three or four times with n-hexane (50ml). These microspheres were air-dried for 24 h and stored in a desiccator for further use. The compositions of the different formulations are shown in table 1.

| Table 1. Composition of different microspheres formulations. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Formulation code | F1 (mg) | F2 (mg) | F3 (mg) | F4 (mg) | F5 (mg) | F6 (mg) | F7 (mg) | F8 (mg) | F9 (mg) |
| Drug (mg)        | 100      | 100      | 100      | 100      | 100      | 100      | 100      | 100      | 100      |
| Gelatin (mg)     | 100      | 200      | 300      | -        | -        | -        | -        | -        | -        |
| Carbopol 971P (mg)| -        | -        | -        | 100      | 200      | 300      | -        | -        | -        |
| HPMC K4M(mg)     | -        | -        | -        | -        | -        | -        | 100      | 200      | 300      |
| Total weight (mg)| 200      | 300      | 400      | 200      | 300      | 400      | 200      | 300      | 400      |
**Particle size analysis:** The particle size and size distribution of the microspheres were determined using the sieving method. Weighed microspheres of each formulation were placed separately on a set of standard sieves (ISO Nominal Aperture 3310-1) and shaken for 5 minutes using mechanical sieve shaker (Behera et al., 2008). Microspheres that were retained on each sieve were collected and weighed, and the average particle size was calculated based on the following formula:

\[
\text{Average particle size} = \frac{\sum (\% \text{ Retained} \times di)}{\sum \% \text{ Retained}}
\]

Where \( di \) is the arithmetic means of the upper and lower openings of the sieve on which the portion of microspheres was retained (Abdallah et al., 2012).

**Percentage yield:** Thoroughly dried microspheres were collected and weighed accurately. The % yield was calculated using the formula given below (Gupta et al., 2014).

\[
\% \text{ yield} = \frac{\text{Weight of microspheres obtained}}{\text{Weight of drug + polymer}} \times 100\%
\]

**Estimation of drug entrapment efficiency:** A weighed quantity of 15 mg of the microspheres was taken. The amount of drug entrapped was estimated by dissolving the microsphere in methanol and then extracting the drug in 0.1N HCl of pH 1.2. The volume was made up to 100 mL using 0.1N HCl. The solution was filtered, and from the filtrate, 1 ml of the sample was taken and further diluted to 10 ml and the absorbance was measured at 245 nm (Harikumar and Sharma, 2012).

\[
\text{Entrapment Efficiency} = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug load expected}} \times 100\%
\]

**Swelling measurement:** Around 15 mg of microspheres from each batch were placed separately in a vessel which contains 100 ml of 0.1N HCl of pH 1.2 at a temperature 37 ± 0.5°C. The microspheres were periodically removed at an interval of 1 hour. The swollen microspheres were removed from the media and weighed at 1st hour, 2nd hour, 3rd hour, 4th hour, 5th hour, 6th hour, 7th hour and 8th hour after removing excess water with the help of filter paper. Fluid sorption was calculated from the difference between the initial weight of the microspheres and the weight at the time of determination. (Erum et al., 2016).

\[
\% \text{ swelling} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100
\]

**Ex-vivo mucoadhesion test:** The mucoadhesive strength of the prepared microspheres were determined according to the method described by (Bhowmick et al., 2019). A fresh goat intestinal mucosa was collected within 1 hour of slaughter and was cleaned by washing with isotonic saline solution. The mucosal membrane was separated from the underlying fat and loose tissues and then washed with distilled water. The membrane was cut into pieces. Two pieces of goat stomach mucosa were attached on the bottoms of two glass vials separately. One of the glass vials was fixed on the surface of the floor, and the other piece was attached with the balance on the left hand side. A glass beaker was kept on the right side of the balance. The right and left sides were balanced by adding extra weight on both the sides of the balance. Approximately 50 mg of microsphere from each batch was placed between the two glass vials, and 2-3 drops of acidic solution (0.1N HCl of pH 1.2) were added on it for wetting. The vials were gently pressed to remove the presence of air, and the balance was kept in this position for 5 minutes. The microspheres formed a single layer along the surface of the disk. Water was added slowly at 1ml/min to the beaker placed at the right side of the balance until the microspheres were separated from the goat stomach mucosal membrane. The water in ml (1 ml equivalent to 1 gram) required to separate the microsphere from the mucosal surface gave the measure of mucoadhesive strength. The strength of the mucoadhesive microspheres was calculated using the following equations:

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

\[
\text{Bond strength (N/m2)} = \frac{\text{Force of adhesion}}{\text{Surface area of vial}}
\]
Drug-excipient compatibility studies by FTIR:
FTIR study was performed by using Fourier transformed infrared spectrophotometer (Prestige-21, Shimadzu, Japan). FTIR spectra of pure drug, pure polymers and formulations containing both drug and polymers were performed to study the drug-polymer interaction (Chaturvedi et al., 2012).

Scanning electron microscopy for surface morphology study: The prepared microspheres were coated with a thin layer of gold by sputtering and then the microstructures were observed in a Scanning Electron Microscope (SEM) that operated at an acceleration voltage of 20 kV. The microspheres were dried completely before SEM examination was done at different magnifications of 20 kV X 50, 20 kV X 200 (Masaelia et al., 2016).

In-vitro dissolution study: The dissolution studies of the microspheres were carried out in a type I USP dissolution test apparatus (basket type) with 100 rpm in 0.1N HCl as dissolution medium (900 ml) maintained at 37 ± 0.5°C. Microspheres equivalent to 15 mg of the pure drug were used. At specific time intervals, up to 8 h, aliquots were withdrawn and analyzed at 245 nm spectrophotometrically against 0.1N HCl as blank. The withdrawn volume was replaced with an equal volume of 0.1N HCl to maintain sink conditions. All experiments were performed in triplicate. The percentage of drug release was plotted against time. The average of the percentage of release was calculated for each batch to find the percentage of release (Harikumar and Sharma, 2012).

Kinetic data analysis: The drug release kinetic studies carried out for microspheres of bromhexine HCl was evaluated using the linear regression method:
(1) Zero-order release kinetic model – cumulative % of drug released versus time (T);
(2) First-order release kinetic model – log cumulative percent drug remaining versus time (T);
(3) Higuchi release kinetic model – cumulative percent drug release versus square root of time (T);
(4) Korsmeyer-Peppas release kinetic model – log cumulative percent drug released versus log time (T) and
(5) Hixson-Crowell model – (Cube root of initial amount - Cube root of drug remaining) versus time (T) (Sikdar et al., 2019).

Results and Discussion
Particle size analysis and percentage yield:
Percent yield is the percent ratio of actual yield to the theoretical yield. Usually, percent yield is lower than 100% because the actual yield is often less than the theoretical value. Reasons for this include incomplete or competing reactions and loss of sample during recovery. The effects of polymeric concentration on microsphere particle size and the percentage yield of all the formulations are represented in table 2.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Mean particle size (μm)</th>
<th>Percentage yield (%)</th>
<th>Drug entrapment efficiency (%)</th>
<th>Swelling at 8 hours (%)</th>
<th>Mucoadhesion bond strength (N/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>380.67</td>
<td>84.45</td>
<td>79.59</td>
<td>180.14</td>
<td>850.00</td>
</tr>
<tr>
<td>F2</td>
<td>394.48</td>
<td>88.50</td>
<td>82.89</td>
<td>175.42</td>
<td>875.98</td>
</tr>
<tr>
<td>F3</td>
<td>410.36</td>
<td>87.67</td>
<td>81.79</td>
<td>163.21</td>
<td>935.14</td>
</tr>
<tr>
<td>F4</td>
<td>322.54</td>
<td>89.65</td>
<td>82.05</td>
<td>179.69</td>
<td>847.43</td>
</tr>
<tr>
<td>F5</td>
<td>341.94</td>
<td>93.20</td>
<td>84.36</td>
<td>198.53</td>
<td>853.38</td>
</tr>
<tr>
<td>F6</td>
<td>381.41</td>
<td>91.47</td>
<td>85.30</td>
<td>202.06</td>
<td>900.00</td>
</tr>
<tr>
<td>F7</td>
<td>319.16</td>
<td>86.95</td>
<td>83.47</td>
<td>185.27</td>
<td>1011.42</td>
</tr>
<tr>
<td>F8</td>
<td>339.19</td>
<td>89.83</td>
<td>84.30</td>
<td>175.59</td>
<td>1039.71</td>
</tr>
<tr>
<td>F9</td>
<td>368.17</td>
<td>90.52</td>
<td>88.70</td>
<td>205.41</td>
<td>1045.31</td>
</tr>
</tbody>
</table>

Table 2. Mean particle size, percentage yield, drug entrapment efficiency, swelling index and mucoadhesion bond strength of bromhexine hydrochloride microspheres.
According to the definition of the microsphere, the size of microspheres should be between 1-1000 μm. In all formulations, the mean particle size of the prepared microspheres ranged from (319.16-410.36) μm. So, it can be said that the prepared formulations were microsphere in terms of their particle size. The particle size of microsphere increases as the concentration of polymeric ratio increases (Jagtap et al., 2012). F3 is larger in particle size while F7 is smaller in particle size. This could be due to the accumulation of more polymers in the formulation leading to larger size particles. Besides, rpm may play a vital role in increasing or decreasing the particle size of the microspheres. More rpm causes the size of the particles to be small.

From table 2, the yield was found to be satisfactory, with the result ranging from 84.45 to 93.2%. F1 shows the lowest percentage yield, whereas F5 has the highest percentage yield of microspheres. Overall three formulations showed a yield above 90%. All formulations were above 75% yield. This outcome reveals that during the preparation of microspheres, fewer amounts of polymers were lost as the washing procedure was done carefully.

**Drug entrapment efficiency (DEE):** The entrapment efficiency of all the formulations is presented in table 2. The entrapment efficiency of the prepared microspheres formulation ranges from 79.59- 88.70%. Formulation F9 exhibited the best result in terms of %DEE of 88.70% and F1 exhibited minimum %DEE of 79.59%. Considering the amount of polymers used in F6 and F9, current investigation suggests that drug entrapment efficiency increases with increasing polymeric concentration.

**Swelling measurement:** The percent of swelling is represented in table 2. Percent swelling is more in case of F9 as compared to other formulations and lowest in F3. The % swelling was found in the range of 163.21% to 205.41%.

**Mucoadhesive bond strength:** From the table 2, it is clear that F4 has the weakest bond and F9 has the strongest bond with mucous layer of goat stomach. So F9 has excellent mucoadhesive properties compared to others.

**Surface morphology study by scanning electron microscope (SEM):** Bromhexine hydrochloride microspheres prepared by using solvent evaporation technique were observed by Scanning Electron Microscope (SEM) to see the morphological changes. The shape and surface morphology is an important consideration for microsphere characterization. The shape of microspheres reflects the uniformity of ingredients per unit of the particle as well as the property and dissolution configuration of the microsphere. Presence of holes was considered as the area of quick-release since these types of area are supposed to facilitate the entrance of dissolution medium to the microsphere. The presence of such holes might cause leaching of the matrix system of the particles.

Nature of the surface is another parameter to analyze. If the surface is rough, then there is more chance of wetting and contact of water with it than the smoother one. The uneven texture might cause holding of moisture at crests, cracks or ridges as variably seen from formulation to formulation. The persistence of moisture for more time can cause weakening of the matrix system.

**In-vitro Dissolution Analysis:** Calibration curve was plotted for bromhexine hydrochloride based on the data obtained in UV-Spectrophotometer. In vitro dissolution analysis was performed in type I USP dissolution test apparatus (rotating basket type) with
900 ml of dissolution medium (0.1N HCl, pH 1.2) at 37 ± 0.5°C stirred with specific rpm. The release profile of bromhexine hydrochloride from the prepared microspheres were analyzed spectrophotometrically.

Figure 1. Surface morphology of prepared Bromhexine Hydrochloride mucoadhesive microspheres. (A) Magnification at × 50, (B) Magnification at × 200, (C) Magnification at × 500.

Figure 2 (A). FTIR spectrum of Bromhexine HCl. (B). FTIR spectrum of Bromhexine HCl + Gelatin. (C). FTIR spectrum of Bromhexine HCl + Carbopol 971P. (D). FTIR spectrum of Bromhexine HCl + Methocel K4M.

The highest percentage of drug release was obtained from the formulation F7 (90.31%) which contains the nonionic polymer HPMC K4M, and the lowest percentage of drug release was obtained from the formulation F3 (79.41%) containing the cationic polymer gelatin as an increase amount of polymer causes slow dissolution of the drug from the formulation. The presence of lower amount of
nonionic polymer in F7 than in F9 resulted in smaller particle size of microspheres and ensured higher release from the formulation. In case of F3, the polymer ratio is more than F1, so the drug release gradually decreases from F1 to F3 as gelatin concentration increase. A similar case was observed in F4, F5 and F6. That is, the drug release of bromhexine hydrochloride gradually decreases with the increase of carbopol 971P in formulation F4 to F6.

![Graph](image1.png)

Figure 3 (A). Zero order plot of release kinetics of Bromhexine HCl microspheres. (B). Zero order plot of release kinetics of Bromhexine HCl microspheres.

![Graph](image2.png)

Figure 4 (A). Korsmeyer-Peppas plot release kinetics of Bromhexine HCl microspheres. (B). Korsmeyer-Peppas plot release kinetics of Bromhexine HCl microspheres.

Table 3. Release profiles of Bromhexine hydrochloride from microspheres in various models.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Zero Order</th>
<th>First Order</th>
<th>Higuchi</th>
<th>Hixson- Crowell</th>
<th>Korsmeyer-Peppas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R^2</td>
<td>K_0</td>
<td>R^2</td>
<td>K_f</td>
<td>R^2</td>
</tr>
<tr>
<td>F1</td>
<td>0.986</td>
<td>9.67</td>
<td>0.957</td>
<td>-0.19</td>
<td>0.945</td>
</tr>
<tr>
<td>F2</td>
<td>0.990</td>
<td>9.58</td>
<td>0.939</td>
<td>-0.19</td>
<td>0.937</td>
</tr>
<tr>
<td>F3</td>
<td>0.966</td>
<td>8.70</td>
<td>0.879</td>
<td>-0.16</td>
<td>0.901</td>
</tr>
<tr>
<td>F4</td>
<td>0.977</td>
<td>9.85</td>
<td>0.909</td>
<td>-0.21</td>
<td>0.928</td>
</tr>
<tr>
<td>F5</td>
<td>0.981</td>
<td>9.75</td>
<td>0.920</td>
<td>-0.21</td>
<td>0.937</td>
</tr>
<tr>
<td>F6</td>
<td>0.982</td>
<td>9.71</td>
<td>0.939</td>
<td>-0.20</td>
<td>0.938</td>
</tr>
<tr>
<td>F7</td>
<td>0.983</td>
<td>10.51</td>
<td>0.955</td>
<td>-0.26</td>
<td>0.976</td>
</tr>
<tr>
<td>F8</td>
<td>0.977</td>
<td>10.55</td>
<td>0.956</td>
<td>-0.25</td>
<td>0.966</td>
</tr>
<tr>
<td>F9</td>
<td>0.975</td>
<td>10.02</td>
<td>0.932</td>
<td>-0.23</td>
<td>0.960</td>
</tr>
</tbody>
</table>
Kinetics data analysis: The coefficient ($R^2$) was calculated for all batches using MS-excel and graphs were plotted. The value of rate constant and coefficient are shown in Table 3. The zero order and Korsemeyer-Peppas plot were represented in figures 3 (A-B) and 4 (A-B) respectively. Based upon the regression coefficient, zero order drug release of bromhexine hydrochloride from prepared microspheres was observed. Korsemeyer-Peppas equation was used to analyze the release pattern of the drug from the polymeric system. The drug release exponent was found to be 0.62 to 0.72 in korsemeyer peppas plot which was less than 0.89 but more than 0.45. This indicates that the drug transport system followed Anomalous (non-Fickian) transport.

Conclusions
Bromhexine hydrochloride loaded mucoadhesive microspheres had been successfully prepared by non-aqueous solvent evaporation technique for prolonged as well as controlled action of bromhexine hydrochloride. Three different types of polymers; Gelatin, Carbopol 971P and HPMC K4M were used to produce the microspheres which showed excellent mucoadhesive property. Each polymeric microsphere had different effects on the physico-chemical properties of the prepared microspheres. It was observed that with the increase in polymer concentration the release rate decreased. From the in vitro drug release studies, it is concluded that by changing the polymers and the ratio of polymers in the formulation the release of bromhexine hydrochloride mucoadhesive microspheres can be controlled.

References