Comparative study of in vitro release and mucoadhesivity of gastric-compacts composed of multiple unit system/bilayered discs using direct compression of metformin hydrochloride

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Abstract

Introduction: Metformin is an oral anti-diabetic drug in the biguanide class. The goal of this study was to develop gastric-retentive MH discs in order to prolong the retention of drug in gastric mucosa.

Methods: Two groups of metformin hydrochloride (MH) mucoadhesive gastroretentive discs were prepared: (a) bilayered discs prepared by direct compression of powders containing polymers as Carbopol 934P (CP, mucoadhesive polymer) and ethylcellulose (EC, rotundant polymer), (b) multiple unit system (microparticle) discs prepared by the emulsification, solvent evaporation, and compression technique from microparticles using polymers CP and EC. Gastric-mucoadhesive compacts were evaluated by investigating their release pattern, swelling capacity, mucoadhesion property, surface pH, and in vitro gastro-retentive time. Discs formulation was subjected to disintegration and dissolution tests by placing in 0.1 M hydrochloric acid for 8 h. Results: The production yield showed F1 microparticles of 98.80%, mean particle size of 933.25 μm and loading efficiency of 98.44%. The results showed that prepared microparticle discs had slower release than bilayered discs (p<0.05). The bilayered discs exhibited very good percentage of mucoadhesion. The results also showed a significant higher retention of mucoadhesive bilayered discs in upper gastrointestinal tract (F1, 1:2 ratio of CP:EC). Histopathological studies revealed no gastric mucosal damage.

Conclusion: Mucoadhesive multiple unit system/bilayered discs interact with mucus of gastrointestinal tract and are considered to be localized or trapped at the adhesive site by retaining a dosage form at the site of action as well as improving in the intimacy of contact with underlying absorptive membrane to achieve a better therapeutic performance of anti-diabetic drug.

Introduction

In order to deliver drugs in a predictable time frame, oral controlled release delivery systems are designed. These systems enhance the efficacy, minimize the adverse effects and increase the bioavailability of drugs. In the present research, an attempt was made to develop oral mucoadhesive controlled release Metformin Hydrochloride (MH) microparticles using ethylcellulose (EC) and carbomer 934P (CP). Mucoadhesive drug delivery is a topic of 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 thereby to facilitate the intimate contact of dosage form, thus to improve and enhance the bioavailability. The mechanism of adhesion of certain macromolecules to the epithelium of a mucous tissue is understood. They are characterized with an epithelial level whose surface is protected by mucus. The mucus contains glycoproteins, lipids, inorganic salts and 95% water by mass, making it a highly hydrated system. Mucin is the significant glycoprotein of mucus and is responsible for its structure. The principle functions of mucus are covering and lubricating the epithelium and some other functions based on the epithelium protection. Mucus width can change from 50-450 μm in the stomach to less than 1 μm in the oral cavity. 1 The mucous area, majorly used for the drug administration and absorption, is gastrointestinal mucus.2 The mucoadhesion ought to extend over the substrate to initiate the close connection, enhance the surface contact, and in turn increase the diffusion of its chains inside the mucus. Attraction and
repulsion forces increase, and for a mucoadhesion to be successful, the attraction forces ought to dominate. Each step can be facilitated by the type of dosage form and the manner it is administered. For example, a partly hydrated polymer can be adsorbed with the substrate through the attraction by the surface water. The mechanism of mucoadhesion is usually separated in two stages, the contact stage and the consolidation stage. The first stage is characterized by the contact between the mucoadhesion and the mucous membrane, with covering and swelling of the formulation and initiating its deep contact with the mucous level. In addition, it is not feasible to directly attach the formulation over the mucous membrane in the gastrointestinal tract. Consequently, the particle must control this repulsive barrier. In the consolidation stage, the mucoadhesive substances are activated by the presence of moisture. Humidity plasticizes the system, permitting the mucoadhesive molecules to break freely and to join up by poor van der Waals and hydrogen bonds. Essentially, there are two theories explaining the consolidation stage: the diffusion theory and the dehydration theory. Depending on the diffusion theory, the mucoadhesive molecules and the mucous glycoproteins mutually interact by means of interpenetration of their chains and the structure of secondary bonds. According to the dehydration theory, materials that are able to easily jellify in an aqueous environment, when placed in relationship with the mucus, may reason its dehydration appropriate to the difference of osmotic pressure. Bioadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio, more intimate contact with the mucous layer, and certain targeting of drugs to the absorption site. Mucoadhesive microspheres that are retained in the stomach would increase the drug absorption and decrease the dosing frequency which provides better patient compliance as compared to conventional dosage forms. In the type 2 diabetic patients, MH reduces plasma glucose levels by lowering the insulin resistance. MH is the most commonly prescribed oral anti-diabetic drug in the world, which primarily helps by lowering blood glucose levels and preventing insulin resistance by virtue of its hepatoselective insulin-sensitizer action. MH has an oral bioavailability of 50–60% below fasting conditions, and is absorbed gradually. MH is not metabolized. It is removed from the body by kidneys and eliminated unchanged with the urine. The mean elimination half-life in plasma is 6.2 h. MH drug is distributed to (and appears to accumulate in) red blood cells, with a so longer excretion half-life of 17.6 h. Carbomers (derived from poly acrylic acid polymers) have not only negatively charged but are also mucoadhesive. In this condition, mucoadhesion is obtained from physicochemical processes, as hydrophobic interactions, hydrogen and van der Waals bonds, which are controlled by pH and ionic composition. CP chains are elastic and show non-irritant properties. In the partially hydrated state, the tissue damage caused by friction or tissue contact, is decreased as a result of hydration. Nonionic polymers, including hydroxypropyl methylcellulose, ethylcellulose and methylcellulose, present a weaker mucoadhesive force compared to anionic polymers. This polymer is often used as a rate-controlling membrane to modulate the drug release from dosage forms with organic or aqueous coating techniques. This paper describes the preparation of bilayered device comprising a drug containing mucoadhesive layer (CP) and a drug free backing layer (alone EC). The mucoadhesive layer was composed of a mixture of drug and CP with backing layer made of EC by direct compression in an attempt to develop a novel oral drug delivery system for the treatment of diabetes. The best formulation was selected based on the ex vivo mucoadhesive performance, drug release, and swelling index. Physical properties of the selected samples were determined.

Materials and methods

Materials

Metformin hydrochloride was purchased from Mahban chemical company (Excir, Iran). Other chemicals were carbopol 934P (B.F.G, USA), ethyl cellulose 48 CP (Sigma-Aldrich, USA), n-hexane, ethanol, span 80, and hydrochloric acid (Merck, Germany). All the chemicals used were of either laboratory or analytical grade. Glucophage Tablet® was supplied from Hexal pharmaceutical company (Germany).

Methods

Preparation of mucoadhesive buccal compacts by direct compression

Microparticles were formed after a series of steps such as emulsion solvent evaporation (Table 1): a) The MH microspheres prepared were filled into the die cavity and compressed to single-layer compacts. b) Bilayered compacts were prepared by a direct compression procedure involving two consecutive steps. In the first step, the backing membrane was created by blending the MH and CP by homogeneous mixing in mortar and pestle, and then the poor mucoadhesive polymer (EC) was poured on the medicated layer. Eight millimeters (in diameter) of EC polymer was then filled in the die cavity on previously obtained backing layer and was compressed (3 tonne) using flat faced punch (Erweka, Germany). The discs formulation was developed and manufactured through the direct compression process, the simplest, easiest and most economical method of manufacturing.

Physicochemical characterization of the discs

Weight variation was determined on 10 discs as per the requirement of discs with average weight <300 mg (limit ± 5% of average weight). Hardness of the discs was measured on six discs using Erweka hardness tester (Germany). Content uniformity of discs (containing microspheres or bilayers) was done by weighing the 3 discs and crushing with mortar and pestle. Then, 50 mg of mixture were
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After a particular time interval, discs were removed and wiped with tissue paper and weighed at the time intervals of 30, 60, 120, 240, 360 and 480 min (W2). The swelling index could be computed by using the formula:

\[
\text{Swelling Index} = \frac{W2 - W1}{W1} \times 100
\]

where \(W1\) is the initial weight of the disc and \(W2\) is the weight of the disc at the specified time.

Differential Scanning Calorimetry (DSC)
The physical state of drug in the microspheres was analyzed by Differential Scanning Calorimeter (Shimadzu, Japan). The thermo grams of the samples were obtained at a scanning rate of 10 °C/min conducted over a temperature range of 25-300 °C.

Swelling index
Swelling index was determined by placing the preweighed discs (W1) from each formulation in a beaker (containing 50 mL of HCl, pH 1.2) and the solution was maintained at 37 °C. After a particular time interval, discs were removed and wiped with tissue paper and weighed at the time intervals of 30, 60, 120, 240, 360 and 480 min (W2). The swelling index could be computed by using the formula:

\[
\text{Swelling Index} = \frac{W2 - W1}{W1} \times 100
\]

Surface pH
The surface pH of the discs was determined to investigate the possibility of any irritation side effects in vivo, because a more acidic or alkaline pH may cause irritation to the gastric mucosa. Therefore, the idea behind the test is to keep the surface pH as close to acidic pH as possible. For the determination of surface pH, three discs from each formulation (microspheres and bilayered) were kept in contact with 50 mL of 0.1 M HCl (pH 1.2) and pH was measured at time intervals of 0, 1, 2, 4, 6 and 8 h by using a glass electrode in contact with the discs on pH meter (Corning pH meter 120, USA). Excessive HCl was drained by bringing the electrode near the surface of the disc and allowing it to equilibrate for 1 min. The results were analyzed for mean and standard deviation.14

In vitro gastro-retention time
The mucoadhesive property of discs was evaluated by an ex vivo adhesion testing method. Freshly excised piece of stomach mucosa from rat (3 cm long) was mounted on the microscope slide with cyanoacrylate glue. Microscope slides were vertically attached to the arm of a USP tablet disintegration test machine. When the disintegration apparatus was operated, the tissue specimen was given a slow, regular up and down moment in the test fluid (900 mL of 0.1 M HCl) at 37±0.5 °C. At the end of one hour, and at the hourly intervals up to 8 h, the machine was stopped and test was carried out in triplicate.

In vitro mucoadhesion force
For this study, rat stomach mucosal membrane was used. A simple apparatus was worked out and designed to measure the minimum detachment force. A piece of mucosal membrane (2.0 cm × 1.5 cm), removed from newly sacrificed rat, was adhered to a glass vial which was fixed on a height-adjustable pan. The pieces of stomach were stored frozen in phosphate buffer, pH 7.4 and thawed to room temperature before use. After hydrating the mucosa with 150 ml of 0.1 M HCl, the disc was brought into contact with the mucosa by applying 300 mg for 2 min. The vial was then moved upwards at constant speed and was connected to the balance. Weights were added at a continual rate to the pan on the other side of the modified balance of the used device until the two vials were separated. The bioadhesive force, expressed as the detachment stress in g/cm², was determined by the minimal weights that detached the tissues from the surface of each formulation using the following equation.16

\[
\text{Detachment Stress (g/cm}^2\text{)} = \frac{m}{A}
\]

Where \(m\) is the weight added to the balance in grams and \(A\) is the area of tissue exposed. The vial containing 0.1 M HCl was weighed and the minimum detachment force was calculated accordingly. The test was performed at room temperature, and the mean of three measurements was

Table 1. Metformin Hydrochloride microparticle and bilayered discs formulation prepared by direct compression

| Formulations | Polymers (CP: EC) ratio | Internal organic phase (O₁/O₂) | External organic phase (O₂) |
|--------------|-------------------------|-------------------------------|-----------------------------|
|              |                         | MH (mg) Ethanol (ml) CP (mg) EC (mg) Liquid paraffin (ml) Span 80 (%w/w) |
| F₁           | 1:2                     | 500  20  225  450  125  3 |
| F₂           | 1:3                     | 500  20  225  675  125  3 |
| F₃           | 1:4                     | 500  20  225  900  125  3 |
| F₁ˊ          | 1:2                     | 500  -  225  450  -  -  - |
| F₂ˊ          | 1:3                     | 500  -  225  675  -  -  - |
| F₃ˊ          | 1:4                     | 500  -  225  900  -  -  - |

* EC (Ethylcellulose), CP (Carbomer 934p) and (MH) Metformin Hydrochloride. F₁, F₂ and F₃ microspheres formulation were compressed by single punch to 300 mg discs. F₁ˊ, F₂ˊ and F₃ˊ formulation were prepared by direct compressed to bilayered disc (300 mg).
DE is defined as an area under the curve of drug release from the discs. The kinetic models used were:

\[ Q = k_f t \]  
\[ \ln Q = \ln \left( \frac{Q_0}{R_i} \right) - k_i t \]  
\[ Q = S \cdot t^{0.5} = k_H \cdot t^{0.5} \]  

Where \( Q \) is the amount of drug release in the time \( t \), \( Q_0 \) is the initial amount of drug in the discs, \( S \) is the surface area of the discs and \( k_f, k_i, k_H \) are constant rates of drug release profiles of various disc formulations, \( R_i \) is the percentage dissolution of one formulation at a given time point and \( T_i \) is the percentage dissolution of the formulation to be compared at the same time point. The difference factor fits the result between 0 and 15 as the test and reference profiles are identical, and approaches above 15 when the dissimilarity increases.

Data obtained from in vitro release studies were fitted to various kinetic equations to find out the mechanism of drug release from the discs. The kinetic models used were:

\[ \int_1^n \frac{y dt}{100t} \]

Where \( y \) is the drug percent dissolved at the time \( t \). All dissolution efficiencies were obtained with \( t \) equal to 480 min. The in vitro release profiles of various disc formulations were compared with disc formulations using the difference factor (\( f_t \)), as defined by:

\[ f_t = \left( \frac{\sum_{n=1}^{\infty} |R_i - T_i|}{\sum_{n=1}^{\infty} R_i} \right) \times 100 \]

Where \( n \) is the number of time points at which percentage (%), dissolution was determined, \( R_i \) is the percentage dissolution of one formulation at a given time point and \( T_i \) is the percentage dissolution of the formulation to be compared at the same time point. The difference factor fits the result between 0 and 15 as the test and reference profiles are identical, and approaches above 15 when the dissimilarity increases.

Histopathological evaluation of gastric mucosa

Histopathological assessment of tissue incubated in 0.1 M HCl, pH 1.2, was compared with that treated with two groups of gastric mucoadhesive discs for 8 h. The tissue was fixed with 10% formalin, routinely processed, and embedded in paraffin. Paraffin sections were cut on microscope slides and stained with hematoxylin and eosin. A pathologist, blinded to the study, worked on detecting any damage to the tissue and examining the sections on light microscope.

In vitro dissolution analysis

The release rate of MH from the developed discs (multiple unit system/bilayered) was determined by using USP dissolution testing apparatus II (Paddle type). The discs were kept in inert, non reactive sinker. The dissolution test was performed using 900 ml 0.1 M HCl (pH 1.2), at 37 ± 0.5 °C and 100 rpm. A sample (5 ml) of the solution was withdrawn from the dissolution apparatus hourly for 8 h, and the samples were replaced with fresh dissolution medium. The samples were passed through filter after dilution, and the absorption of these solutions was measured at 205 nm by spectrophotometry (UV-160, Shimadzu, Japan). The cumulative percentage of drug release was calculated using software. Kinetic parameters were also obtained by the mathematical processing of drug release data. Evaluation of the influence of formulation variables on the release rate of constant \( k \) values was obtained for different groups of microsphere preparation.

In order to have a better comparison between different formulations of dissolution efficiency (DE), \( t_{\infty}, \% \) (dissolution time for 50% fraction of drug) and difference factor (\( f_t \), used to compare multipoint dissolution profiles) were calculated. DE is defined as an area under the dissolution curve up to a certain time, and \( t \) is expressed as a percentage of the area of the rectangle arising from 100% dissolution in the same time. The areas under the curve (AUC) were calculated for each dissolution profile by the trapezoidal rule. DE can be calculated by the following formula:

\[ \text{DE} = \int_1^n \frac{y dt}{100t} \]

Results

The mucoadhesive discs were prepared with MH microspheres, MH, and two polymers (EC and CP) by using direct compression. Results showed that an increase in the amount of EC increased the particle size of microspheres, unlike the percentage of mucoadhesion. However, the loading efficiency was decreased (p<0.05). At a 900 mg EC amount (\( F_t \)), the production yield, particle size and loading efficiency of microspheres were 85.74%, 1071.52 μm and 81.87%, respectively (Table 2). These discs were evaluated for the content uniformity, hardness and friability, pH, mucoadhesion force, swelling % and retentive time in the gastric mucosa. The results are shown in Table 3. Discs made of bilayered were physically stable for more than 50-97.50 min in 0.1 M HCl at 37 °C, and exhibited higher mucoadhesion on the gastric mucosa (2.70-3.99%) compared to all other discs (0.75-2.74%). Although more than 30% of the initial dimension of all
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Table 2. Effect of polymers (CP:EC) ratio on the loading efficiency, production yield and particle size of Metformin Hydrochloride microspheres

| Formulations | Carborner :EC ratio | Production yield (%±SD) | Theoretical drug content (%) | Mean drug Loading (%±SD) | Drug loading efficiency (%±SD) | Mean particle Size (µm±SD) |
|--------------|---------------------|-------------------------|-----------------------------|--------------------------|------------------------------|---------------------------|
| F₁           | 1:2                 | 89.64 ± 3.54            | 42.55                       | 33.47 ± 1.78             | 78.66 ± 4.19                | 794.33 ± 25.11            |
| F₂           | 1:3                 | 98.80 ± 6.07            | 33.33                       | 32.81 ± 2.49             | 98.44 ± 6.98                | 933.25 ± 10.47            |
| F₃           | 1:4                 | 85.74 ± 2.48            | 30.77                       | 25.19 ± 2.37             | 81.87 ± 7.73                | 1071.52 ± 10.30           |

Table 3. Physicochemical characteristics of gastric-mucoadhesive microparticles and bilayered discs

| Formulation code | F₁ | F₂ | F₃ | F₁ | F₂ | F₃ |
|------------------|----|----|----|----|----|----|
| Polymer (CP:EC) ratio | 1:2 | 1:3 | 1:4 | 1:2 | 1:3 | 1:4 |
| Weight variation (mg ± SD) | 298 ± 0.002 | 299 ± 0.005 | 298 ± 0.001 | 299 ± 0.001 | 298 ± 0.003 | 299 ± 0.004 |
| Hardness (N ± SD) | 24.28 ± 1.63 | 23.58 ± 2.01 | 22.29 ± 1.28 | 67.13 ± 1.03 | 61.52 ± 1.79 | 59.97 ± 1.51 |
| Friability (±SD) | 0.30±0.03 | 5±0.63 | 15±0.85 | 0.567±0.06 | 0.708±0.08 | 0.841±0.11 |
| Content uniformity (±SD) | 96.32 ± 0.62 | 95.95 ± 0.20 | 95.95 ± 0.20 | 96.32 ± 0.62 | 95.95 ± 0.20 | 95.45 ± 0.45 |
| pH surface (±SD) | 1.147 ± 0.01 | 1.162 ± 0.01 | 1.166 ± 0.01 | 1.288 ± 0.04 | 1.235 ± 0.04 | 1.252 ± 0.05 |
| *Swelling (±SD) | 90.16 ± 3.55 | 83.09 ± 2.24 | 83.09 ± 2.24 | 344.12 ± 3.55 | 276.04 ± 2.24 | 221.24 ± 2.24 |
| Mucoadhesive strength (g/m²±SD) | 2.74 ± 0.24 | 1.76 ± 0.27 | 0.75 ± 0.05 | 3.99 ± 0.27 | 2.99 ± 0.49 | 2.70 ± 0.29 |
| Residence time (min±SD) | 42.26 ± 0.36 | 51.51 ± 0.19 | 20.36 ± 0.35 | 97.50 ± 10.60 | 70.00 ± 14.14 | 50.00 ± 7.07 |

* All of results are related to 8th h.
on the mucosal surface up to 8 h as mentioned in Table 3. It was observed that the microparticle discs swelled slowly and produced lower mucoadhesive strength (as F₁ to F₃).

The microscopic observations indicated that the microparticles had no significant effect on the microscopic structure of mucosa. As shown in Fig. 3, no cell necrosis was observed.

**Effect of amount of EC used**

The effect of amount of EC was studied by using F₁ to F₃ (microparticle discs) and F’₁ to F’₃ (bilayered discs). Formulas F’₁, F’₂, and F’₃ were used to study the effect of polymer-polymer ratios. It was shown that the release was truly gradual in the first two hours in HCl solution (pH 1.2) while for F₁, F₂, and F₃ formulations (microparticle discs), the release was quicker. It was found that there was a significant (p<0.05) increase in the release of MH at microparticle discs as shown in Table 4. The rank order for the different formulations (microparticle discs) was as follows: F₃>F₂>F₁.

The release of MH from F₁ and F’₁ was significantly (p<0.05) higher than that from other formulations (Fig. 4). Accordingly, the release of MH from F’₃ was significantly (p<0.05) higher than that from F’₁, as seen in Fig. 3. This different rapid release was occurred in comparison with F’₃.

**Determination of release kinetics**

The release kinetics of MH from all the prepared discs was determined by finding the best fitting of the dissolution data to the mathematical models (1, 2 and 3). Besides, analysis of the trial data depending on the model 4, as well as explaining the corresponding release exponent values shows better understanding of the release mechanism from discs (Table 5).

**Discussion**

By the procedure of mucoadhesion, mucoadhesive polymers know wetting, swelling, and interdiffusing or understanding the mucus or surface layer. In this process, different polymers are believed to make strong entanglements and reside in the application site for a prolonged period of time. Coutinho et al.²⁰ showed that an increase in polymer concentration will cause an increase in the number of cross-linked chains. This in turn, will increase the gel mechanical strength and also its water loading efficiency.

This finding can be related to an alteration in particle size, which may accordingly affect mucoadhesion. As
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Figure 4. Cumulative percent release of MH from discs (prepared microparticles discs/ bilayered discs) with different polymers ratios.

Table 4. Comparison of various release characteristics of MH from different microsphere formulations, discs and commercial® tablet

| Formulation       | a Rel₂ (%) | b Rel₈ (%) | c DE  | d T₅₀% (h) | e f₁  |
|-------------------|------------|------------|-------|------------|-------|
| F₁                | 45.02±0.74 | 75.62±0.65 | 51.78 | 4          | 50.22 |
| F₂                | 37.25±0.90 | 73.98±1.07 | 48.21 | 4.4        | 54.34 |
| F₃                | 29.5±0.78  | 68.16±1.05 | 42.32 | 5          | 59.15 |
| F’₁               | 16.45±1.95 | 74.63±1.05 | 35.55 | 5.8        | 67.78 |
| F’₂               | 17.28±0.29 | 88.57±1.07 | 47.72 | 4.2        | 58.28 |
| F’₃               | 19.97±4.08 | 93.60±3.73 | 52.43 | 3.8        | 54.21 |
| Glucofage®Tab     | 105.81±3.78| 104.33±4.84| 98.97 | >0.5       | 0     |

* a Rel₂ = amount of drug release after 2 h; b Rel₈ = amount of drug release after 8 h; c DE = dissolution efficiency; d T₅₀% = dissolution time for 50% fractions; e f₁ = Differential factor.

Table 5. Fitting parameters of the in vitro release data to various release kinetics models

| Formulation | ORDER           | MPE%  | RSQ  | Slope | Intercept | K     |
|-------------|-----------------|-------|------|-------|-----------|-------|
| F₁          | Peppas          | 4.69  | 0.973| 0.318 | -2.389    | 0.0918|
| F₂          | Higuchi         | 6.3   | 0.968| 0.03  | 0.042     | 0.0304|
| F₃          | Linear- probability | 5.58  | 0.981| 0.003 | -0.849    | 0.0027|
| F’₁         | Linear- probability | 3.49  | 0.997| 0.004 | -1.452    | 0.0043|
| F’₂         | Linear- probability | 8.31  | 0.988| 0.006 | 1.623     | 0.0063|
| F’₃         | Linear- probability | 6.93  | 0.987| 0.007 | 1.585     | 0.0069|

the polymer ratio (CP:EC) decreases (F₁ and F’₁), the percentage of mucoadhesion conversely increases; since the greater amount of polymer results in a higher amount of free –OH (hydroxyl) groups,” which are responsible for binding to the sialic acid groups within the mucous network.

The DSC analysis of microspheres revealed negligible change in the melting point of MH indicating no modification or interaction between the drug and polymer (Fig. 2). Therefore, it resulted in an increase in the mucoadhesive characteristics of microspheres and bilayered discs. In vivo mucoadhesive tests showed that MH mucoadhesive bilayered discs adhered more strongly to the gastric mucosa and could be retained in the gastrointestinal tract for long period of time (Table 3).

In an acidic medium, the hydrogel exists in a collapsed state due to the hydrogen bond. Like most hydrogels, the viscosity of the hydrogel can be controlled by its polymer concentration. Higher polymer concentration leads to a more viscous gel with higher elasticity.¹⁶ This ability is due to its hydrophilic nature, highly cross-linked structure, and quick swelling due to high water uptake.²² Table 3 shows the effect of CP/EC ratios on the swelling property of MH. In the acidic medium, the swelling index increased significantly (p<0.05) due to the hydrophilic character of CP so that the percentage of water uptake enhanced on increasing its concentration.²³,²⁴ The ability of CP to uptake water is adequate to the presence of hydrophilic groups (-COOH).²⁵ Discs made with microspheres showed gradual swelling in 0.1 N HCl, whereas bilayered discs showed
more swelling due to their dissolution characteristics. The extent of swelling shown by microspheres discs (F1 to F4) after 8 h was 75.26, 83.09 and 90.16%, respectively. During the research on control and maintenance of integrity of the discs prepared with various ratios of CP and EC, it was obtained that the incorporation of about 4:1 ratio EC to CP into microsphere matrix did not improve the swelling exactly or prolong the dissolution of discs. On the other hand, the discs were disintegrated within the first one hour with a mucoadhesion force of 2.74 g/cm².

Swelling of discs involves the absorption of liquid resulting in an increase in the weight and volume. The liquid uptake by the particles could be due to the saturation of capillary distance inside the particles or hydration of microparticles/bilayered discs. The liquid takes the particles inside pores and joins to big particles through breaking the hydrogen bonds and thus resulting in the swelling of microparticles/bilayered discs. Water uptake by cross-linked hydrogels (carbomer 934P) may occur initially through metastable pores, and as swelling proceeds, mechanism is replaced by diffusion.26 Swelling is related with the polymer concentration, the Ionic power, and the presence of water. In the case of microparticles, it suggests that the incorporation of water-insoluble polymers such as EC leads to a rigid structure.27 Mucoadhesive bilayered discs are anticipated to take water from the underlying mucosal tissue by adsorbing, swelling, and capillary effects, and accordingly leading to a considerable stronger adhesion.21 This perhaps occurs as slow swelling avoids the formation of over hydrated structure which loses its mucoadhesive property before reaching the target. On the other hand, the highest swelling observed in bilayered discs (F2) could be due to the presence of high amount of carbomer 934P (1:2 ratio) at pH 1.2, which is capable of absorbing a high amount of water.21

According to in vitro mucoadhesion test performed by Nakanishi et al.,28 mucoadhesive force depends on the hydrogen bond between the carbosyl group in the polymer and mucus. Sandri et al.29 have highlighted the use of polyacrylic acid in the bilayered formulation for the MH formulation which is used in diabetes. It forms an ionic complex with hyaluronic acid which provides higher binding power. The formulation also includes gelatin that improves the mucoadhesion of polyacrylic acid by negating the effect of medium ionic strength. It also improves the ability of polyacrylic acid in controlling the drug release rate as well as in resisting the discharge by gastric fluid.

The degree of swelling is related to both drug release kinetics and mucoadhesion. Rapidly swollen discs are mucoadhesive. Excessive swelling again leads to the reduced mucoadhesion, because water molecules bind the polymer carbosyl groups required for adhesion.30 F1’, F2’ and F3’ formulations containing the same levels of CP but different levels of EC demonstrated a respective decrease in the amount of residence time. Thus, EC had a negative effect on in vitro residence time. A similar effect has been demonstrated in the buccal patch of sumatriptan succinate by Shidhaye et al.31 It was observed that the effect of concentration of EC on the in vitro residence time was significant, with discs containing high proportion of EC eroding rapidly and giving short residence time (F3’, 1:4 ratio).

CP of the polymers showed a significant level of mucoadhesive interaction with the gastric mucosa which was much predictable. Binding and sticking properties of CP also contribute to the mucoadhesion. Furthermore, the high plastic deformation property of CP makes it suitable as a binder-filler for direct compression. Bilayered discs showed the highest mucoadhesion in this study and did not dissolve in 0.1 N HCl for about 100 min.

The potential use of mucoadhesive systems as drug carriers lies in their prolongation of the residence time at the absorption site, allowing an intensified contact with the epithelial barrier.4 Therefore, a bioadhesive system controlling the drug release could improve the treatment of diseases and help in maintaining an effective concentration of the drug at the action site.6 Mucous membranes of human organisms are relatively permeable and allow fast drug absorption.32 It has also been reported that polyanionic polymers (CP polymer) are more effective as bioadhesives than polycationic polymers or nonionic polymers. Some reports showed a direct relationship between swelling and mucoadhesion while others did not.33,34 The strength was dependent on the property of bioadhesive polymers, which on hydration, adheres to mucosal surface, and on the concentration of the polymer used, as well. The bioadhesive property of carbopol is reported to be due to the carboxyl groups’ presence on its acrylic acid backbone, which possesses an ability to interact with sialic acid molecules present in the mucous layer.35 This high bioadhesive strength of carbopol may be due to the formation of secondary bioadhesive bonds with mucin and interpenetration of the polymer chains in the interfacial region, in comparison with other polymers that only undergo superficial bioadhesion. Bilayered discs containing a high CP polymer (F1’) had a faster hydration rate and achieved a maximum swelling at a shorter period which could promote the penetration of polymer chains with the tissue.

CP polymer containing a greater portion of hydroxyl groups could provide the ability to form hydrogen bonds and could bind more strongly with the oligosaccharide chains of mucin.16 Therefore, the higher bioadhesive performance of negatively charged polymers may be related to the good balance between the available hydrogen bonding sites and an open expanded conformation.36 For non-ionic polymers (cellulose derivatives), the absence of proton donating carboxyl groups reduces its ability to form hydrogen bonds.37 The suggested mucoadhesion of cellulose derivatives resulted mainly from the pressure developed by their swollen gels against mucin gels.38 Cellular membrane was intact and no damage was observed in the used rat stomach mucosa. Consequently, formulation containing microparticles seemed to be safe
Comparative study of in-vitro release and mucoadhesivity

Variables affecting the dissolution profile of MH discs

Effect of polymers’ ratio

It was seen that the in vitro release of MH depended on the swelling behavior of the discs. The release was occurred very fast in first 2 h in HCl solution (pH 1.2) because the charge density of CP was sufficiently high and the ionic interactions were increased, leading to the formation of much stronger network. While at the next 6 h, the release was slower because the ionic interaction of MH and negatively charged polymers of CP was greatly reduced, forming a loose network with increased porous surface which allows greater part of dissolution media along with counterions. It was found that there was an important (p<0.05) acceleration in the release of MH as the amount of CP enhanced in the complex as observed in Fig. 4. In fact, carbomer hydrogel is formed in release conditions and in formulations with larger amounts of CP, close networks of CP are formed in comparison with formulations containing low amounts and thus diffusion of drugs is decreased. The same result was obtained on studying different CP:EC hydrogels for modified release of amoxicilline, when the release of amoxicilline decreased with increasing the ratio of CP:EC. When the amount of CP increased in the complex, the release rate and swelling index increased due to the hydrophilic nature of CP so that the percentage of water uptake increased on increasing its concentration. In similar studies conducted on the nifedipine or clarithromycin matrix tablets consisting of CP 974P, HPMC K4M and NaCMC with the less quantum of EC, the effect of types of excipients is observed. Also, EC has a low permeability to drug which results from its high intermolecular attraction. The pores present in EC polymer acts as a channeling agent for the entrance of the liquid medium through the microparticles’ wall, causing it to swell. Hydrogen bonds between the hydroxyl groups of the carboxylic moiety and the carbonyl oxygen of ester group increase the degree of solidity of the polymer and decrease its porosity and permeability. Thus, by varying the ratio of polymers (CP: EC) in the MH microparticles, the rate of release of MH can be controlled.

Kinetics of drug release

The dissolution profile of the optimized batch was fitted to various models, as mentioned above, to a certain kinetic modeling of drug release. The least value of sum of square of residuals (RSQ) and mean percent error (MPE) were used to select the most appropriate kinetic model. A high correlation was observed between the linear-probability order models (Table 5). Linear-probability model in F, showed the highest RSQ (0.997) and the least MPE (3.49). The mechanism of MH release from the formulated discs from microspheres (F1 to F3) was by Fickian diffusion (n= 0.318, 0.41 and 0.296, respectively) and for bilayered discs, F1 to F3, was by anomalous non-Fickian diffusion, that is, diffusion coupled with erosion (kinetic exponent, n=0.570, 0.809 and 0.755, respectively).

Conclusion

Prepared gastro-retentive discs of MH by direct compression of CP and EC showed superior bioadhesive properties compared to microparticle discs. The adhesive force was significantly affected by the mixing ratio of CP: EC in the discs. The studies show that the bilayered discs will undergo sol-gel transition at a lower concentration of EC polymer (higher concentration of CP polymer) compared to microparticle discs, which might suggest that there is much mucoadhesion. Our research also showed that bilayered discs and microparticle discs have the unlike gel strength and drug release profile. The rheological data of this study further showed that the bilayered discs have a higher viscosity compared to microparticle discs which help in minimizing the leakage during administration of the formulation.

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Ethical issues

The present study was performed in accordance with the ethical guidelines of the 1975 Declaration of Helsinki of Tabriz University of Medical Sciences, Tabriz-Iran.

Competing interests

Authors certify that no actual or potential conflict of interests exists in relation to this article.

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