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Research Article

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, rotardant 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 F_2 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 (F'_1, 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

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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.[3] 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.[4] 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.[1] 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.[1] 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.[1] 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.[5,6] 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-line 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.[7] 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.[8] Nonionic polymers, including hydroxypropyl methylcellulose, ethylcellulose and methylcellulose, present a weaker mucoadhesive force compared to anionic polymers.[9] This polymer is often used as a rate-controlling membrane to modulate the drug release from dosage forms with organic or aqueous coating techniques.[10-12]

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):[13] 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 weighting the 3 discs and crushing with mortar and pestel. Then, 50 mg of mixture were

dissolved in 100 ml of 0.1 M HCl. Content uniformity of discs was done by extracting the drug in 0.1 M HCl. Two milliliters of this solution was filtered and the filtrate was diluted up to 100 ml with 0.1 M HCl. Next, 2 ml from this solution was picked up; this filtrate was diluted up to appropriate dilution (10 ml), and the drug concentration was measured with spectrophotometer (UV-160, Shimadzu, Japan) at 205 nm against 0.1 N HCI as a blank.

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.[14] 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).[15] The swelling index could be computed by using the formula:

Swelling Index = W2-W1/ W1×100

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 from the tubes with a tissue paper and the pH was noted 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.[16] 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/cm^2, was determined by the minimal weights that detached the tissues from the surface of each formulation using the following equation:[16]

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

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	Emulsion (O_1/O_2)					
		Internal organic phase (O_1)				External organic phase (O_2)	
		MH (mg)	Ethanol (ml)	CP (mg)	EC (mg)	Liquid paraffin (ml)	Span 80 (%w/w)
F_1	1 :2	500	20	225	450	125	3
F_2	1:3	500	20	225	675	125	3
F_3	1:4	500	20	225	900	125	3
F'_1	1 :2	500	-	225	450	-	-
F'_2	1 :3	500	-	225	675	-	-
F'_3	1 :4	500	-	225	900	-	-

* EC (Ethylcellulose), CP (Carbomer 934p) and (MH) Metformin Hydrochloride. F_1, F_2 and F_3 microspheres formulation were compressed by single punch to 300 mg discs. F'_1, F'_2 and F'_3 formulation were prepared by direct compressed to bilayered disc (300 mg).

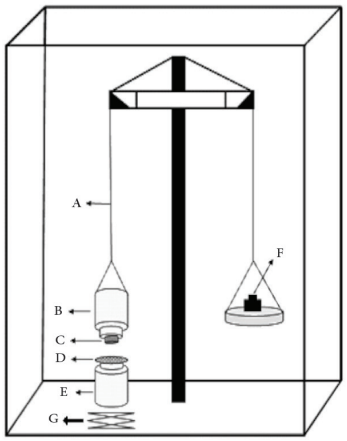

Fig. 1. Bioadhesive force measuring device: (A) modified balance; (B, E) glass vial; (C) MH discs; (D) rat tissue; (F) Weights; (G) height-adjustable pan.

used as the mucoadhesive strength of the discs (Fig. 1).

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.[16]

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.[17] 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_{50}% (dissolution time for 50% fraction of drug) and difference factor (f_1, used to compare multipoint dissolution profiles) were calculated.[18] 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:

$$DE = \int y \frac{dt}{100t}$$

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_1), as defined by:[18]

$f_1 = \{[\Sigma_{t=1}^{n} |R_t - T_t|] / [\Sigma_{t=1}^{n} R_t]\} \times 100$

Where *n* is the number of time points at which percentage (%) dissolution was determined, R_t is the percentage dissolution of one formulation at a given time point and T_t 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:

$Q_t = k_0 t$ (zero-order equation)

$\ln Q_t = \ln Q_0 - k_1 . t$ (first-order equation)

$Q_t = K . S . t^{0.5} = k_H . t^{0.5}$ (Higuchi equation based on Fickian diffusion)

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_0, k_1 and k_H are constant rates of zero order, first order and Higuchi equation, respectively. Besides these basic release models, the release data was fitted to the Peppas and Korsemeyer equation (power law):

$M_t/M_\infty = k.t^n$

Where M_t is the amount of drug release at the time t and M_∞ is the release amount at the time $t = \infty$; thus M_t/M_∞ is the fraction of drug released at the time t, k is the kinetic constant, and *n* is the diffusion exponent which can be used to characterize the mechanism of drug release.[19]

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_3), 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

discs were retained for about 8 h in 0.1 N HCl, bilayered discs showed the highest adhesive strength (3.99 g/cm^2) amongst all the others formulations. Better retention and mucoadhesion of discs F'_1 containing 225 mg CP and 450 mg EC (1:2 ratio of polymers) could be attributed to more amount of CP than F'_2 and F'_3 discs. CP was chosen for the preparation of mucoadhesive microspheres, owing to its good mucoadhesive characteristics and EC was used as a carrier polymer. Different amounts of EC, from 500 mg to 900 mg, were found to have a significant influence on the percentage of retention observed (i.e., percentage of microspheres/bilayered discs adhered and remained on the gastric mucous layer), particle size and drug entrapment efficiency.

One of the important factors related to the microspheres (F_3), as reported by Lee *et al.*,[3] is the viscosity of the polymer solution. A 1:2 mixture of CP and EC was found to be suitable as the polymers' ratio. Polymer concentration (EC) had a negative effect on the percentage of mucoadhesion. It was found that on increasing the concentration of polymer (EC), the particle size increases (1:4 ratio). The mean particle size of microspheres increased from 794.33 µm to 1071.52 µm with an increase in the concentration of polymer from 38.3% to 55.38%. The particle size of microspheres enhanced with the increase in the concentration of polymer; hence at higher concentrations the polymer solution dispersed into larger globules. At concentrations lower than the appreciate level, the solution got less viscous and dispersed into different fine globules that easily coalesced, resulting in bigger microspheres. An increase in the EC showed a decrease in the percentage of mucoadhesion and particle size, but an increase in the drug entrapment efficiency of microspheres.

Pure MH shows a sharp melting endotherm around 231.27 ˚C (Fig. 2). It is evident from thermograms that the DSC curves of physical mixtures of drug with polymers as well as the microparticle formulations are almost the same. This endotherm of the drug is present in most of the thermograms F_1, F_2 and F_3 at 223.86 ˚C, 220.27 ˚C and 221.41 ˚C, respectively (Fig. 2). However, the intensity of the drug fusion peak for the microparticle formulations was lower than that of the pure drug and physical mixtures.

Swelling properties, surface pH and in vitro mucoadhesive strength determination studies

The swelling study results as indicated in Table 3 shows that the bilayered formulation (F'_1) has sufficient swelling feature which is essential for good mucoadhesive properties, as the more the swelling, the greater the exposure of formulation to the biological surface and the more the mucoadhesion. It was seen that as the amount of EC increased in CP: EC ratio, the swelling index also significantly decreased (p>0.05).

In the acidic medium, it was seen that as the amount of CP increased (F_1 and F'_1), the swelling index significantly increased, too (p<0.05). The surface pH of the optimized formulations was found to be in the range of gastric pH which indicated that there will be no irritation due to the formulation on the stomach surface. Also, force-based and time-based mucoadhesive strength determination studies as shown in Table 3 indicated that bilayered formulation showed good mucoadhesive strength.

The *in vitro* mucoadhesive test results (see Table 3) showed that the bilayered discs represented the maximum mucoadhesion (p<0.05) in comparison with microparticle discs (multiple unit system). F'_1 discs indicated the highest mucoadhesive strength. Carbomer 934P polymer showed sufficient mucoadhesive power required to retain the drug

Table 2. Effect of polymers (CP:EC) ratio on the loading efficiency, production yield and particle siz e of Metformin Hydrochloride microparticles

Formulations	Carbomer :EC ratio	Production yield (%±SD)	Theoretical drug Content (%)	Mean drug Loading (%±SD)	Drug loading efficiency (%±SD)	Mean particle Size (µm±SD)
F_1	1:2	89.64 ± 3.54	42.55	33.47 ± 1.78	78.66 ± 4.19	794.33 ± 25.11
F_2	1:3	98.80 ± 6.07	33.33	32.81 ± 2.49	98.44 ± 6.98	933.25 ± 10.47
F_3	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	F_1	F_2	F_3	F'_1	F'_2	F'_3
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/m^2±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.

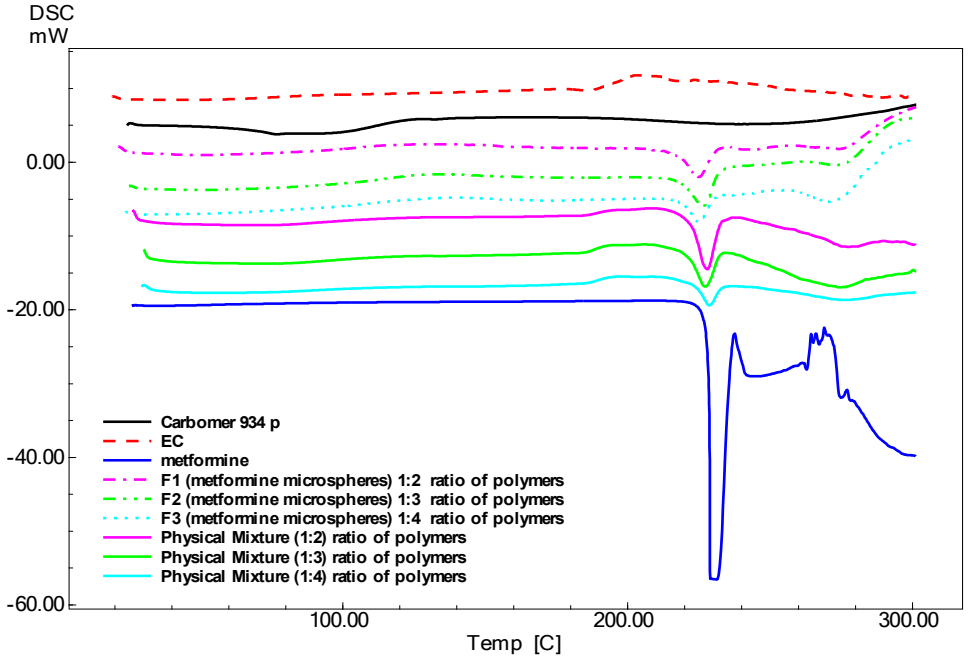

Fig. 2. DSC thermogram of Carbomer 934 p; (EC) Ethylcellulose; metformin hydrochloride (MH); microspheres of F_1, F_2, F_3 and physical mixture of F_1, F_2 and F_3, respectively.

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 F_1 to F_3). 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 F_1 to F_3 (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 F_1, F_2 and F_3 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: F_1>F_2> F_3.

The release of MH from F_1 and F'_1 was significantly (p<0.05) higher than that from other formulations (Fig. 4). Accordingly, the release of MH from F'_1 was significantly (p<0.05) higher than that from F'_3 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.[8] 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

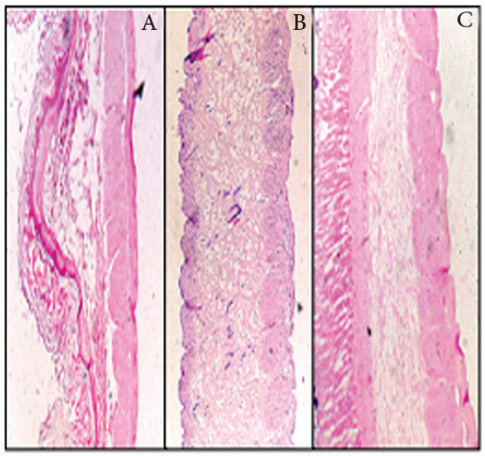

Fig. 3. Histopathological evaluation of sections of rat gastric mucosa (A) un-treated (B) treated with microparticles discs (C) bilayered discs containing MH (magnitude X).

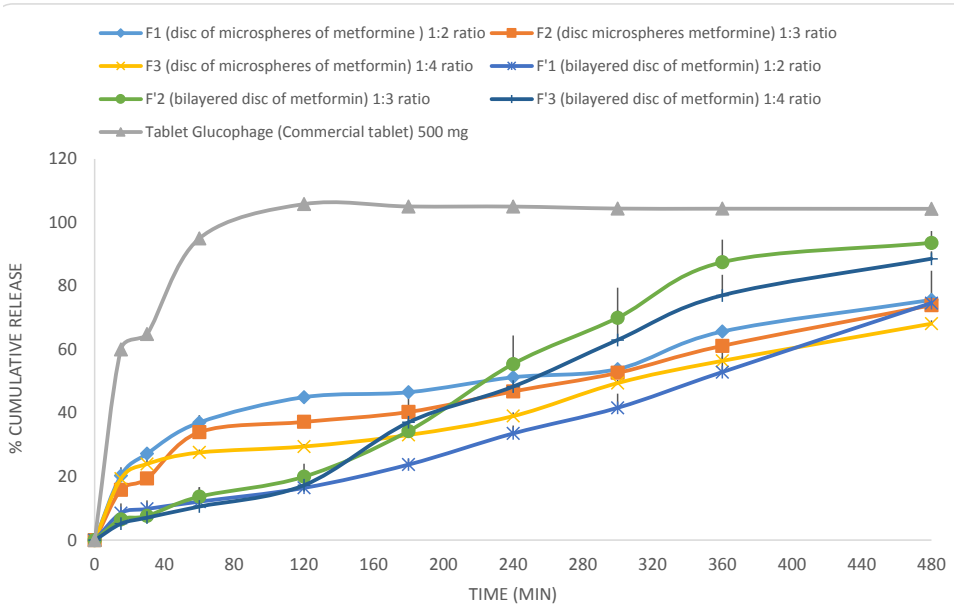

Figure 4. Cumulative percent release of MH from discs (prepared microparticles discs/ bilayered discs) with different polymers ratios.

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

Formulation	[a]Rel_2 (%)	[b]Rel_8 (%)	[c]DE	[d]$T_{50\%}$ (h)	[e]f_1
F_1	45.02±0.74	75.62±0.65	51.78	4	50.22
F_2	37.25±0.90	73.98±1.07	48.21	4.4	54.34
F_3	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] Rel_2 = amount of drug release after 2 h; [b] Rel_8 = amount of drug release after 8 h; [c]DE = dissolution efficiency; [d]t 50% = dissolution time for 50% fractions; [e] f_1 = Differential factor.

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

Formulation	ORDER	MPE%	RSQ	Slope	Intercept	K
F_1	Peppas	4.69	0.973	0.318	-2.389	0.0918
F_2	Higuchi	6.3	0.968	0.03	0.042	0.0304
F_3	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 (F_1 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,[21] 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 vitro* 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 (F_1 to F_3) 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/cm^2.

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 up water from the underlying mucosal tissue by absorbing, 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 (F'_1) 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 carboxyl 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 carboxyl groups required for adhesion.[30]

F'_1, F'_2 and F'_3 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 (F'_3, 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.[8] 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 (F'_1) had a faster hydration rate and achieved a maximum swelling at a shorter period which could promote the interpenetration 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 entire and no damage was observed in the used rat stomach mucosa. Consequently, formulation containing microparticles seemed to be safe

with the consideration of oral regimen (Fig. 3).

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 camparison 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.[39] 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 excepients is observed.[40] 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'_1 showed the highest RSQ (0.997) and the least MPE (3.49). The mechanism of MH release from the formulated discs from microspheres (F_1 to F_3) was by Fickian diffusion (n= 0.318, 0.41 and 0.296, respectively) and for bilayered discs, F'_1 to F'_3 was by anamolous 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.

References

1. Smart JD. The basics and underlying mechanisms of mucoadhesion. *Advanced drug delivery reviews* **2005**;57:1556-68.
2. Junginger HE, Thanou M Verhoef JC,. Drug Delivery: Mucoadhesive Hydrogels. In: Swarbrick S, editor. *Encyclopedia of Pharmaceutical Technology*. New York: CRC Press;**2002**. p. 1848-63.
3. Lee JW, Park JH, Robinson JR. Bioadhesive-based dosage forms: The next generation. *J Pharm Sci* **2000**;89:850-66.
4. Hägerstrom H, Edsman K. Limitations of the rheological mucoadhesion method: the effect of the choice of conditions and the rheological synergism parameter. *Eur J Pharm Sci* **2003**;18:349-57.
5. Sharma HK, Pradhan SP, Sarangi B. Preparation and in-vitro evaluation of enteric controlled release pantoprazole loaded microbeads using natural mucoadhesive substance from Dillenia Indica L. *Int J Pharm Tech Res* **2010**;2:542-51.
6. Parmar H, Bakliwal S, Gujarathi N, Rane B, Pawar S. Different methods of formulation and evaluation of mucoadhesive microsphere. *Int J Appl Bio PharmTech* **2010**;1:1157-67.
7. Woodley J. Bioadhesion. *Clin pharmaco* **2001**;40:77-84.
8. Huang Y, Leobandung W, Foss A, Peppas NA. Molecular aspects of muco-and bioadhesion: Tethered structures and site-specific surfaces. *J control Rel* **2000**;65:63-71.

9. Mortazavi SA, Moghimi HR. Effect of surfactant type and concentration on the duration of mucoadhesion of carbopol 934 and HPMC solid compacts. *Iran J Pharm Res* **2010**;2:191-9.
10. Lin SY, Lin KH, Li MJ. Formulation design of double-layer in the outer shell of dry-coated tablet to modulate lag time and time-controlled dissolution function: studies on micronized ethylcellulose for dosage form design (VII). *The AAPS Journal* **2004**;6:1-6.
11. Siepmann F, Hoffmann A, Leclercq B, Carlin B, Siepmann J. How to adjust desired drug release patterns from ethylcellulose-coated dosage forms. *J control Rel* **2007**;119:182-9.
12. Neau SH, Howard MA, Claudius JS, Howard DR. The effect of the aqueous solubility of xanthine derivatives on the release mechanism from ethylcellulose matrix tablets. *Int J pharm* **1999**;179:97-105.
13. Jelvehgari P, F Khansari M. Mucoahhesive microspheres for gastroretentive delivery of metformine hydrochloride: In vitro evaluation. *Res Pharm Sci* **2012**;7:S356.
14. Shidhaye SS, Thakkar PV, Dand NM, Kadam VJ. Buccal drug delivery of pravastatin sodium. *AAPS Pharm Sci Tech* **2010**;11:416-24.
15. Satyabrata B, Ellaiah P, Choudhury R, Murthy KVR, Bibhutibhusan P, Kumar MS. Design and evaluation of methotrexate buccal mucoadhesive patches. *Int J Pharm Biomed Sci* **2010**;1:31-6.
16. Ch'Ng HS, Park H, Kelly P, Robinson JR. Bioadhesive polymers as platforms for oral controlled drug delivery II: Synthesis and evaluation of some swelling, water-insoluble bioadhesive polymers. *J Pharm Sci* **1985**;74:399-405.
17. Chiao CSL, Price JC. Formulation, preparation and dissolution characteristics of propranolol hydrochloride microspheres. *J Microencapsul* **1994**;11:153-9.
18. Peh KK, Wong CF. Polymeric films as vehicle for buccal delivery: swelling, mechanical, and bioadhesive properties. *J Pharm Pharm Sci* **1999**;2:53-61.
19. Youan BBC, Benoit MA, Baras B, Gillard J. Protein-loaded poly (epsilon-caprolactone) microparticles. I. Optimization of the preparation by (water-in-oil)-in water emulsion solvent evaporation. *J Microencapsul* **1999**;16:587-99.
20. Coutinho DF, Sant SV, Shin H, Oliveira JT, Gomes ME, Neves NM. *et al.* Modified Gellan Gum hydrogels with tunable physical and mechanical properties. *Biomaterials* **2010**;31:7494-502.
21. Dhaliwal S, Jain S, Singh HP, Tiwary AK. Mucoadhesive microspheres for gastroretentive delivery of acyclovir: in vitro and in vivo evaluation. *The AAPS Journal* **2008**;10:322-30.
22. Yadav VK, Gupta AB, Kumar R, Yadav JS, Kumar B. Mucoadhesive polymers: means of improving the mucoadhesive properties of drug delivery system. *J chem pharm Res* **2010**;2:418-32.
23. Zate SU, Kothawade PI, Mahale GH, Kapse KP, Anantwar SP. Gastro retentive bioadhesive drug delivery system: A review. *system* **2010**;5:6.
24. Khan GM. Controlled release oral dosage forms: Some recent advances in matrix type drug delivery systems. *The sciences* **2001**;1:350-4.
25. Peppas NA, Khare AR. Preparation, structure and diffusional behavior of hydrogels in controlled release. *Advanced drug delivery reviews* **1993**;11:1-35.
26. Stoy VA, Kliment CK. *Hydrogels: Specialty Plastics for Biomedical, Pharmaceutical and Industrial Applications*. Basel: Technomic; **1990**.
27. Rajput G, Majmudar F, Patel J, Thakor R, Rajgor NB. Stomach-specific mucoadhesive microsphere as a controlled drug delivery system. *Systematic Reviews in Pharmacy* **2010**;1:70.
28. Nakanishi T, Kaiho F, Hayashi M. Use of sodium salt of Carbopol 934P in oral peptide delivery. *Int J Pharm* **1998**;171:177-83.
29. Sandri G, Poggi P, Bonferoni MC, Rossi S, Ferrari F, Caramella C. Histological evaluation of buccal penetration enhancement properties of chitosan and trimethyl chitosan. *J Pharm Pharmaco* **2006**;58:1327-36.
30. Deasy PB, Collins AEM, Maccarthy DJ, Russell RJ. Use of strips containing tetracycline hydrochloride or metronidazole for the treatment of advanced periodontal disease. *J Pharm Pharmacol* **1989**;41:694-9.
31. Shidhaye SS, Saindane NS, Sutar S, Kadam V. Mucoadhesive bilayered patches for administration of sumatriptan succinate. *AAPS Pharm Sci Tech* **2008**;9:909-16.
32. Jasti B, Li X, Cleary G. Recent advances in mucoadhesive drug delivery systems. *Pharma Tech* **2003**;194:6.
33. Bottenberg P, Cleymaet R, Muynck C, Remon JP, Coomans D, Michotte Y. *et al.* Development and testing of bioadhesive, fluoride containing slow release tablets for oral use. *J Pharm Pharmacol* **1991**;43:457-64.
34. Llabot JM, Manzo RH. Double-layered mucoadhesive tablets containing nystatin. *AAPS Pharm Sci Tech* **2002**;3:47-52.
35. Mortazavi SA, Smart JD. An investigation of some factors influencing the in vitro assessment of mucoadhesion. *Int J pharm* **1995**;116:223-30.
36. Bertram U, Bodmeier R. In situ gelling, bioadhesive nasal inserts for extended drug delivery: in vitro characterization of a new nasal dosage form. *Eur J Pharm Sci* **2006**;27:62-71.
37. Alireza Mortazavi S, Smart JD. An in-vitro method for assessing the duration of mucoadhesion. *J control Rel* **1994**;31:207-12.
38. Nafee NA, Ismail FA, Boraie NA, Mortada LM. Mucoadhesive delivery systems. I. Evaluation of mucoadhesive polymers for buccal tablet formulation. *Drug Develop Ind Pharm* **2004**;30:985-93.
39. Liu Z, Lu W, Qian L, Zhang X, Zeng P, Pan J. In vitro and in vivo studies on mucoadhesive microspheres of amoxicillin. *J Control Rel* **2005**;102:135-44.
40. Varshosaz J, Dehghan Z. Development and characterization of buccoadhesive nifedipine tablets. *Eur J Pharm Biopharm* **2002**;54:135-41.