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BILAYERED MUCO-ADHESIVE DELIVERY SYSTEM FOR EXTENDED RELEASE OF BISPHOSPHONATES

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ABSTRACT

Bisphosphonates are used against osteoporosis and Paget's disease. Despite their benefits; bisphosphonates suffers from poor oral bioavailability (1-2%). Bilayered multipolymeric films comprising of risedronate sodium with chitosan (85% deacetylated) and hydroxypropylmehtyl cellulose (HPMC 4KM) interpolymer complex of different ratios were prepared by solvent casting method. An impermeable backing membrane of ethyl cellulose was incorporated in to the films. The films were studied for swelling degree, moisture uptake, viscosity, folding endurance, water vapor transmission rate and mucoadhesive time. The prepared films demonstrated uniform and reproducible drug content (99.67±0.023-100.31±0.056%), thickness (0.286±0.032-0.443±0.051mm), mucoadhesivity (7.91±0.295-11.806±0.379 g) and sustained drug release profile up to 8 hours. The delayed release of the drug is probably as a result of combined effect of ethyl cellulose hydrophobicity, gel-forming property of HPMC and interpolymer complexation of chitosan and HPMC 4KM. The study confirms the potential of bilayered, interpolymer complex films as a promising candidate for buccal delivery of bisphosphonates.

KEY WORDS

Bilayered, bisphosphonate, chitosan, hydroxypropyl methylcellulose, swelling degree, mucoadhesive

INTRODUCTION

Buccal route of drug delivery provides direct access to the systemic circulation through the internal jugular vein bypassing the first pass metabolism leading to high bioavailability. Other advantages such as excellent accessibility, low enzymatic activity, suitability for drugs or excipients that mildly and reversibly damage or irritate the mucosa, painless administration, easy drug withdrawal, facility to include permeation enhancer/enzyme inhibitor or pH modifier in the formulation, versatility in designing as multidirectional or unidirectional release systems for local or systemic actions make buccal adhesive drug delivery system as promising option for continued research[1].

The selection of optimal polymers in a drug delivery system remains pivotal in the formulation of controlled release of buccal drug delivery system for enhancing mucoadhesivity and obtaining controlled drug release profiles. With homopolymeric systems one may find that a polymer such as chitosan, which has been shown to display excellent mucoadhesivity, is nevertheless unable to prolong drug release, while a polymer such as hydroxypropyl methyl cellulose which is not a good mucoadhesive is however ideal for prolonging drug release. Also, single polymers may not be able to provide desired drug release profiles or mucoadhesivity. Films with polymeric blends as a drug delivery system could be ideal for delivery of drugs in the oral cavity due to its flexibility and comfort[2].

Ethyl cellulose is one of the most widely used water-insoluble polymer in pharmaceutical film coating due to its convenient film formability, good physicochemical property and minimal toxicity[3].

Osteoporosis and Paget's disease of bone are major problems in women and geriatric patients where antiresorptive agents are normally recommended. Bisphosphonates have an established role in the treatment of osteoporosis. They are analogous to inorganic pyrophosphate, an endogenous regulator of bone turnover that inhibits bone resorption and mineralization *in vitro*. Bisphosphonates are also used in Paget's disease of bone, malignant hypocalcemia during myeloma, osteolytic bone metastasis and fibrous dysplasia of bone. Despite their benefits, bisphosphonates suffer from very poor oral bioavailability (1-2%). Higher localized concentration of bisphosphonates has resulted in severe gastro-intestinal side effects such as dysphagia, esophagitis and gastric ulceration.

For development of mucoadhesive bilayered buccal films, chitosan and HPMC-K4Minter polymer complex was used. Because of the properties such as hydrophobicity, low water permeability, drug impermeability and moderate flexibility ethyl cellulose was used as a backing membrane[4].

MATERIALS AND METHODS

Risedronate sodium was a gift sample from Fleming Laboratories, Hyderabad, India. Chitosan was procured from Indian Institute of Fisheries, Cochin, India. HPMC- K4Mand Ethyl cellulose was obtained commercially from SD Fine Chemicals. All other reagents and chemicals used were of analytical reagent grade.

(1) Preparation of Mucoadhesive Bilayered Buccal Films[5, 6]:

Backing Layer: A glass petri plate; of 9 cm diameter, was used for preparing the formulations as a casting surface. Initially,

backing membrane of ethyl cellulose was fabricated by slowly pouring a solution containing 1 g of ethyl cellulose with diethyl phthalate 2% w/w of the polymer as plasticizer in 20 ml of acetone to the glass petri plate and air dried for 2h.

Mucoadhesive layer containing Drug: Initially weighed quantity of chitosan was dissolved in specified volume of 1% v/v acetic acid under constant stirring till clear solution was obtained. HPMC- 4 KM was separately dissolved in specified quantity of purified water, to which the drug was added and dissolved. The drug-polymer solution was mixed to chitosan solution and stirred for uniform mixing. The resultant clear solution was then casted on the preformed backing layer of ethyl cellulose and allowed to dry uniformly undisturbed at room temperature by placing an inverted funnel onto the petri plate. The formed bilayered films were stored in a desiccator until further used.

Table 1
Formulations of buccal films containing Risedronate sodium

Ingredients	Batch code						
	FD1	FD2	FD3	FD4	FD5	FD6	FD7
Risedronate sodium (g)	0.280	0.280	0.280	0.280	0.280	0.280	0.280
Chitosan (g)	0.5	0.67	0.33	0.574	0.426	-	1.0
HPMC- K4M(g)	0.5	0.33	0.67	0.426	0.574	1.0	-
Ethyl cellulose (g)	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Propylene Glycol (ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Acetic acid 1% v/v (ml)	25	30	20	30	20	-	50
Purified water (ml)	25	20	30	20	30	50	-

(2) Evaluation studies

i) Mass uniformity and film thickness[5, 6]:

Assessment of mass uniformity was done with five different randomly selected films from each batch and thickness of each film was measured at five different locations using a screw. The mean and standard deviations were calculated.

ii) Viscosity determination[7]:

The solutions containing both polymer and plasticizer were prepared in the same concentration as that of the films. A model DV II + Brookfield viscometer attached to a helipath spindle number 4 was used. The viscosity was measured at 20 rpm at room temperature. The record values were the mean of three determinations.

iii) Swelling study[8, 9]:

Buccal films were weighed individually and placed separately on a 2% w/v agar gel plates, incubated at 37± 1^0 C and examined for any physical change. At regular time intervals, the sample films were removed from the gel plates and excess surface water was removed carefully using the filter paper. The swollen films were then reweighed and degree of swelling was determined according to the following equation.

Degree of swelling = Wet weight - Original dry weight / Original dry weight

iv) Folding endurance test[10, 11]:

Folding endurance of the films were determined by repeatedly folding one film at the same place till it broke or folded up to 200

times at the same place without breaking gave the value of folding endurance of the film.

v) Drug polymer compatibility study:

The drug: polymer interaction study was carried out by analyzing the pure drug, polymer and the drug: polymer physical mixture (1:1) using a KBr pellet and scanned from 400 to 4000cm^{-1} using FTIR (Shimadzu S-1601, Japan).

vi) Surface pH determination[12]:

A combined glass electrode was used for this purpose. The films were allowed to swell by keeping them in contact with 1 ml of pH 6.8 phosphate buffer for 2h at room temperature and pH was noted down by bringing the electrode in contact with the surface of the film, allowing it to equilibrate for one minute.

vii) Water vapor transmission rate[13]:

For the rate of water vapour transmission studies vials of equal diameter were used as transmission cells. These transmission cells were washed thoroughly and dried in an oven. About 1 g of anhydrous calcium chloride was taken in the cells and the sample films were securely fixed over the brim. The cells were accurately weighed, kept in a closed desiccator containing saturated solution of potassium chloride to maintain a humidity of 84% RH. The cells were taken out and weighed after 24 h. interval up to 5 days of storage. The amount of water vapour transmitted was calculated using the formula.

Water vapour transmission= WL/S

Where, W is the vapour transmission rate usually expressed as the grams of moisture, L is the thickness of the film in cm, S is the exposed surface area in cm^2. From the data obtained water vapour transmission was calculated.

viii) Uniformity of drug content:

The drug content was determined by sonicating and dissolving the drug from the film in 50 ml of phosphate buffer (pH 6.8) and filtering with whatmann filter paper (0.45 µm). The resultant filtrate was diluted to 250 ml and absorbance were recorded at 262 nm using UV- Spectrophotometer (Shimadzu UV 1700, Japan)

ix) Ex-vivo muco adhesive strength test[14, 15]:

Fresh porcine buccal mucosa was obtained from a local slaughter house and used within 2 hours of slaughter. The mucosal membrane was separated by removing the underlying fat and loose tissues. The membrane was washed with distilled water and then with phosphate buffer pH 6.8 at 37^0 C. Mucoadhesive strength of the film was measured using a fabricated mucoadhesive strength test apparatus (Figure 4). A buccal mucosa of 3 cm^2 was fixed to the immovable platform with cyanoacrylate adhesive. The sample film was glued to the movable platform. The exposed film surface was moistened with 15 µl of phosphate buffer and left for 30 s for initial hydration and swelling. The movable platform was then horizontally moved towards the fixed platform and brought in contact with the mucosal surface. A preload of 20 g was placed over the movable shaft for 3 min as initial pressure. On the pan attached with the movable platform, weights were added proportionately at a definite interval times. The total weight required for complete detachment of the film was recorded and the mucoadhesion force calculated per unit area of the film as follows:

Force of adhesion= Bioadhesive strength X 9.81/1000

Bond strength= Force of adhesion/ Disk surface area

The mass in grams required to detach the film from the mucosal surface gave the measure of mucoadhesive strength. Bond strength represents the area under the work or energy required for detachment of the two systems (mucin/polymeric film). The results of bioadhesive parameters are given in Table 3.

Figure 4
Mucoadhesive strength test apparatus

x) Ex-vivo mucoadhesion time[14]:

The *ex vivo* mucoadhesion time was evaluated after application of the films onto freshly cut porcine buccal mucosa. The fresh buccal mucosa was fixed in the inner side of the beaker, above 2.5 cm from the top bottom with cyanoacrylate glue. The drug layer side of each film was wetted with one drop of isotonic phosphate buffer pH 6.8 and pasted to the porcine buccal mucosa by applying a light force with a fingertip for 30 seconds. The beaker was filled with 200 ml of phosphate buffer pH 6.8 and was kept at 37± 1^0 C. After 2 minutes, a 50 rpm stirring rate was applied to simulate the buccal cavity environment, and film adhesion was monitored up to 12 h. The time required for the film to detach from the buccal mucosa was recorded as the mucoadhesion time.

xi) *Ex-vivo* permeation studies[14, 15]:

The permeation study was carried out through porcine buccal mucosa, using a Keshary Chien glass diffusion cell. The mucosa was mounted between the donor and receptor compartment. The formulation with drug layer was placed on the mucosa and the compartments clamped together. The donor compartment was filled with 1 ml of phosphate buffer pH 6.8. The receptor compartment (15 ml capacity) was filled with phosphate buffer pH 7.4 maintained at 37± 0.2^0 C and the hydrodynamics in the receptor compartment was maintained by stirring with a magnetic bead at 50 rpm. One ml sample was withdrawn at predetermined time interval and analyzed for drug content at 262 nm.

RESULTS AND DISCUSSION

In the present study, buccal films for controlled delivery of risedronate sodium were developed using chitosan and HPMC-K4Minterpolymer complex with ethyl cellulose as backing membrane. The films were prepared using different ratios of chitosan to HPMC-K4M(1:1, 2:1, 1:2, 4:3 and 3:4 batch codes FD1 to FD5) FD6 and FD7 are films with pure HPMC-K4M and chitosan with the backing membrane of ethyl cellulose, propylene glycol as plasticizer and as a permeation enhancing agent.

The prepared bilayered films were smooth in appearance, uniform in thickness, mass and drug content and showed no visible cracks. The films exhibited good folding endurance (more than 250 foldings). Film thickness ranged from 0.286±0.032 to 0.443±0.051 mm and mass ranged from 0.026±0.002 to 0.054±0.010 g. The drug content ranged between 99.67±0.023 to 100.31±0.056 %, indicating the favorable drug loading and uniformity with-in the film. The values of evaluation studies were shown in Table 2 and 3.

Table 2
Evaluation parameters of the mucoadhesive films

Batch code	Thickness (mm) ± S.D	Water vapor transmission (g/cm^2 /hr)	%Drug content ± S.D	Folding Endurance	Mass uniformity(g) ±S.D	Viscosity(cps) ± S.D	Surface pH± S.D
FD1	0.286±0.032	0.00264	99.72±0.026	>250	0.039±0.003	70.66±6.658	6.23±0.12
FD2	0.376±0.023	0.00368	99.79±0.033	>250	0.036±0.006	73.2±9.781	5.70±0.13
FD3	0.323±0.066	0.00495	99.80±0.029	>250	0.034±0.004	160.2±42.116	5.90±0.18
FD4	0.436±0.050	0.00520	100.23±0.062	>250	0.034±0.0005	73.7±9.021	6.02±0.14
FD5	0.33±.026	0.00392	99.97±0.034	>250	0.036±0.005	99.5±12.101	5.94±0.02
FD6	0.443±0.051	0.00224	100.31±0.056	>250	0.026±0.002	184.33±21.079	6.05±0.13
FD7	0.336±0.003	0.00636	99.67±0.023	>250	0.054±0.010	32.166±3.790	5.81±0.05

Table 3
Bioadhesive parameters of the mucoadhesive films

Batch code	*Ex-vivo* mucoadhesive strength (g) ± S.D	Force of adhesion (N)	Bond Strength ($N\ m^{-2}$)	Mucoadhesion time (hours) ± S.D
FD1	21±1.00	0.206	9.155	8.5±0.500
FD2	26.33±1.528	0.258	11.466	7.983±0.689
FD3	41±1.00	0.402	17.866	8.923±0.371
FD4	29±1.00	0.284	12.622	7.91±0.295
FD5	40.333±2.517	0.395	17.555	9.76±0.250
FD6	49.666±1.528	0.487	21.644	10.613±0.375
FD7	67.333±2.082	0.660	29.333	11.806±0.379

The prepared films were evaluated for the water vapour transmission rate, the amount of water vapours getting transmitted from the film to adsorbent were calculated by applying a suitable formula and it revealed that FD7 films with chitosan had the highest rate of vapour transmission. This may be due to the less viscosity of the chitosan solution in its uncombined form and also due to the erosion property of the chitosan films when comes in contact with the moisture. All the other formulations have considerable less rate of water vapour transmission, which may be the effect of interpolymer complexation between chitosan and HPMC4KM and also due to the hydrophobic effect of ethyl cellulose backing membrane. The water vapour transmission values are shown in Table 2.

Appropriate swelling behavior of a buccal adhesive system is the essential property for uniform and prolonged release of the drug and effective mucoadhesion. The swelling study indicated that the degree of swelling was higher in films with higher concentration of HPMC-4KM. The weak aqueous solubility of cationic polymer chitosan and the hydrophobicity of ethyl cellulose limited the swelling of the films. The swelling results may also be attributed to polymeric blending of HPMC-K4M and chitosan with ethyl cellulose. These films were reported to have swelled greatly at the initial period and then decreased in volume with increased time, similar to the profile obtained in this study. The swelling degree of these films may be considered not sufficient to cause discomfort. And also, the erosion data confirmed that the film could maintain its integrity for a prolonged period of time. Swelling behavior of films as a function of time is shown in Figure 3.

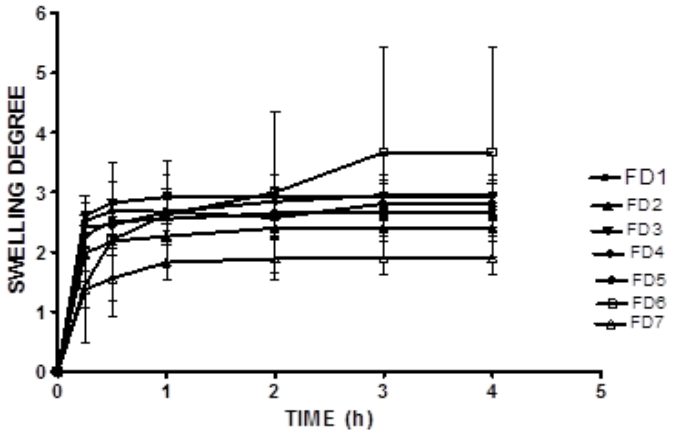

Figure 3
Swelling degree of the bilayered mucoadhesive films

The formulations were subsequently tested for mucoadhesive properties, as a prerequisite for buccal controlled drug delivery systems is adhesion on the oral mucosa. A measurement of the mucoadhesivity of the bilayered films formulated in this study was therefore of importance as it is intended to remain in contact with the buccal mucosa for a prolonged period to facilitate the controlled release of risedronate. Formulation FD7 with pure chitosan showed highest mucoadhesive strength and mucoadhesion time followed by FD3 and FD5 which contain a higher ratio of HPMC-4KM. The HPMC particles were finer and higher in quantity and so provided greater surface area for contact with the mucus membrane. The film had higher buccoadhesive strength because the moisture absorbed may just be the maximum required to produce maximum buccoadhesion of swollen films. All the formulations showed a good mucoadhesion time, minimum being 7.91±0.295 hours for batch FD4. The good mucoadhesion of the films may also be attributed to combined effect of chitosan and HPMC-4KM. The mucoadhesive properties of the films are shown in Table 3.

The result of the drug release studies carried out on the films is presented in Figure 2. This reveals a delayed and controlled release of risedronate from all the formulations. FD5 shows the slowest cumulative percentage release (43.922±5.713%) and FD2 shows highest cumulative percentage release (77.543± 1.837%) after 8 hours. The delayed release of the drug from the films may probably be as a result of combined effect of the ethyl cellulose hydrophobicity, the interpolymer complexation of chitosan and HPMC-K4Mand gel- forming property of HPMC-4KM. Polymers retard the drug release because increase in tortuosity as a result of swelling in contact with aqueous fluid increases the path length available for the drug to diffuse out from the swollen matrix. The formulation FD2 films with 2:1 ratio of chitosan and HPMC-K4M had the highest release which may be attributed to the nature of the network within the film which may be loose with consequent ease of penetration of the diffusion medium and diffusion of the risedronate from the film matrix. The release of risedronate was as a result of swelling of the matrices with batches FD3 and FD4. The slow release of drug from batch FD 5 (43.922±5.713% release after 8 hours) containing 3:4 chitosan and HPMC-K4M may be due to the effect interpolymer complexation of the polymers, high concentration of HPMC-K4M and also due to the effect of hydrophobic coating layer of ethyl cellulose as a backing membrane which does not allow the easy penetration of aqueous fluid

into the polymer matrix. The effect of high viscosity also plays a major role in slowing the release of the drug from batch FD 5. As the viscosity is related to the strength and durability of the gel layer, the diffusion of the drug will be easier in cases of formulations with less viscosity as seen in formulations FD2 and FD4 where the initial release of the drug was high.

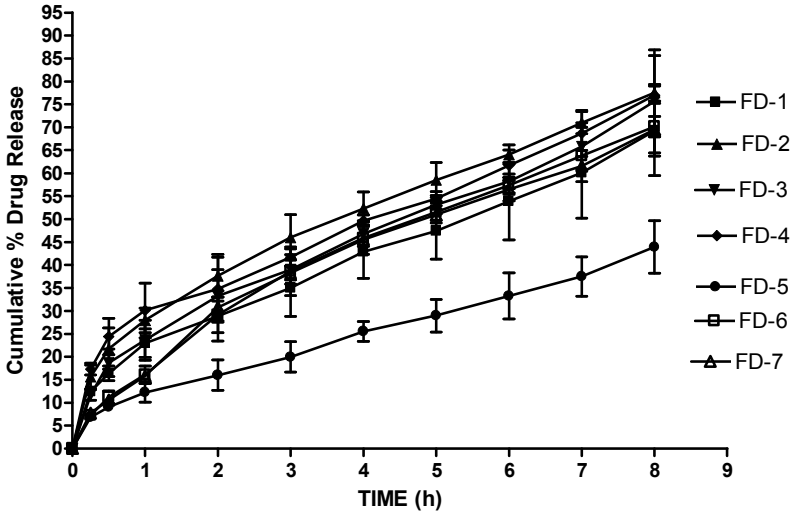

Figure 2
Comparative Ex-Vivo drug release of the bilayered mucoadhesive films

The comparison of FTIR spectra of pure drug, pure polymer with the drug polymer complex (Figure 1) showed that there is no interaction between the drug and polymer and they are compatible with each other.

Surface pH evaluation of oral mucosa dosage forms is an important characterization study, as an acidic or alkaline pH may cause irritation to the oral mucosa. It was therefore necessary to determine if any extreme surface pH changes occurred with the films during the drug release period under investigation. The surface pH of the films remained fairly constant at a pH of approximately 5.70±0.13 to 6.23±0.12 over the 8 hour test period, confirming that the surface pH of the films was within the neutral conditions of the saliva, (pH 5.8-7.1) and that no extremes in pH occurred throughout the test period. These results suggested that the polymeric blend identified was suitable for oral application owing to the acceptable pH measurement.

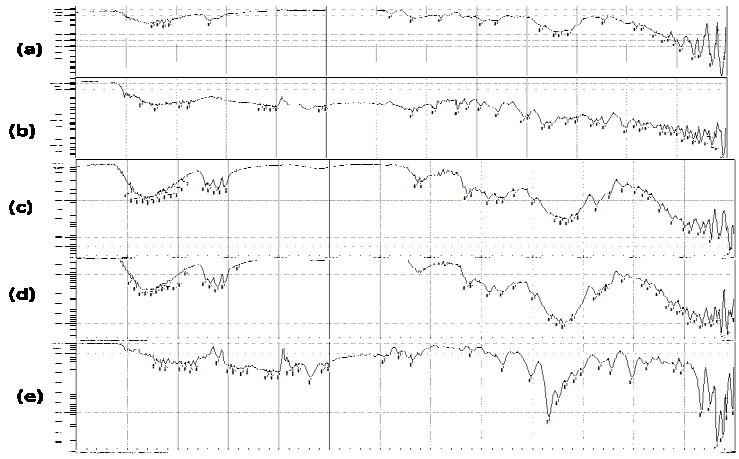

Figure 1
FTIR spectra of (a) pure drug (b) Pure chitosan (c) pure HPMC 4km (d) HPMC and chitosan (e) Drug+HPMC+chitosan

CONCLUSION

The aim of this study was to prepare bilayered multipolymeric films comprising a hydrophilic drug, risedronate sodium using interpolymer complexation of two different polymers in various blends. The physicochemical data, mucoadhesion and drug release data obtained in this study, confirmed the potential of this bilayered multipolymeric film as a potential candidate for sustained buccal delivery of bisphosphonates

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