ABSTRACT

Mucoadhesive polymers that bind to the gastric mucin or epithelial cell surface are useful in drug delivery for the purpose of increasing the intimacy and duration of contact of drug with the absorbing membrane. Several synthetic polymers are in use for this purpose. Since the biodegradability of the synthetic polymers are questionable, in this investigation an oral mucoadhesive controlled delivery system has been developed for terbutaline sulphate (TS) using natural mucoadhesive materials extracted from the edible fruits like *Zizyphus mauritiana* (ZM) and *Aegle marmelos* (Linn.) Cor. (AM) that have better mucoadhesive property than synthetic polymer hydroxypropylmethylcellulose K4M (HPMC K4M). The *in vitro* adhesive and mucoadhesive strength of mucoadhesive materials extracted from the fruits of ZM and AM were evaluated and compared with HPMCK4M using both Share Stress and Wilhelmy Plate. Different formulations of oral mucoadhesive coated TS tablets were prepared using these natural materials and compared with tablets prepared with HPMCK4M and hardness, thickness, friability, weight variation and drug content of tablets were tested. The *in vitro* release of TS was studied in buffer pH 7.2 at 37°C± 0.5°C. Tablets were orally administered to rabbits and blood plasma concentration of TS was determined using HPLC. It was found that mucoadhesive materials extracted from the fruits of ZM and AM exhibited better adhesiveness and mucoadhesiveness as compared with the HPMC-K4M. The *in vitro* study of TS exhibited showed greater drug release profile for tablets prepared with natural materials than synthetic polymers and confirmed with *in vivo* study. *In vitro* and *in vivo* correlation showed the same release profile.

**Key words:** Terbutaline Sulphate, natural mucoadhesive materials, HPMC-K4M
INTRODUCTION

Mucoadhesion, or the attachment of a natural or synthetic polymer to a biological substrate, is a practical method of drug immobilization or localization and an important new aspect of controlled drug delivery (1). While the subject of mucoadhesion is not new, there has been increased interest in recent years in using mucoadhesive polymers for drug delivery (2-3). Substantial effort has recently been focused on placing a drug or a formulation in a particular region of the body for extended periods of time (4). This is needed not only for targeting of drugs but also to better control of systemic drug delivery. Drugs that are absorbed through the mucosal lining of tissues can enter directly into the blood stream and not be inactivated by enzymatic degradation in the gastrointestinal tract (5). Several polymeric bioadhesive drug delivery systems have been fabricated and studied in the past. Different types of bioadhesive synthetic polymers such as acrylic-based hyrogels (6) including carbopol 934, carbopol 937 and hydroxypropylmethylcellulose are used to prepare oral mucoadhesive tablets (7). However, the adhesiveness and drug delivery capabilities of these devices can continue to be improved, as presently known bioadhesive materials, and more bioadhesive materials are discovered (8-19).

Since the biodegradability of the synthetic polymers is questionable, some natural mucoadhesive materials extracted from edible fruits and vegetables having good mucoadhesive properties are used for this purpose (20). Terbutaline Sulphate (TS) is widely used as an effective broncho-dilator in the management of asthma (21). This is used as prophylactic drug as well as to prevent acute exacerbations of asthma. During acute attack of asthma it becomes difficult for a patient to take oral medications repeatedly. Hence, it is rational to administer terbutaline sulphate (TS) in a sustained release dosage form, which will minimize repeated administration of the drug.

The objective of the present study was (a) to prepare mucoadhesive controlled release TS tablets using natural mucoadhesive materials including ZM and AM, and synthetic polymer HPMCK4M, (b) to examine the in vitro release characteristics of TS from formulated tablets, (c) to examine the in vivo drug absorption characteristics of TS in rabbit blood plasma from formulated tablets and (c) to make a correlation between in vitro release characteristics of TS and in vivo absorption characteristics of TS in rabbit blood plasma.

MATERIALS AND METHODS

MATERIALS

Terbutaline sulphate (TS), metaproterenol hemisulphate and HPMCK4M were obtained as gift samples from M/S Union Drugs Ltd, Kolkata, India. Acetone GR was procured from M/S Loba Chemicals, Mumbai, India. Dihydrogen potassium phosphate LR was purchased from Process Chemical Ltd, Kolkata, India. Monobasic potassium phosphate LR, absolute ethanol and pancreatin were procured from E. Marck (India) Ltd, Mumbai. Terbutaline sulphate RS was collected from Central Drug Laboratory, Kolkata, India. The fruits of Zizyphus mauritiana (ZM) and Aegle marmelos (Linn.) Cor. (AM) were purchased from local market of India.

Extraction materials such as solid phase extraction columns were 3 ml polypropylene columns packed with 200 mg of C18 bonded phase from J.T. Baker (Phillipsburg, NJ, U.S.A.). Reagent grade...
monobasic and dibasic sodium phosphates were from Mallinckrodt Specialty Chemicals Co. (Chesterfield, MO, U.S.A.). Molecular biology grade ammonium chloride and Sigma Ultra grade reduced glutathione were from Sigma Chemical Co. Syringe filters were 4 mm diameter, 0.2 μm porosity nylon membrane units from Alltech Associates (Deerfield, IL, U.S.A.). Mobile phase materials such as monobasic potassium phosphate and anhydrous dibasic sodium phosphate were reagent grade from Mallinckrodt. The remaining mobile phase reagents were of HPLC grade and obtained from commercial sources. The 47 diameter, 0.2 μm porosity polyvinylidene difluoride (PVDF) filtration membranes were from Gelman Sciences (Ann Arbor, MI, U.S.A.).

METHODS

Extraction of mucoadhesive agents from ZM and AM:

The mucilage from above materials was extracted a modified method of Rao et al (22). In this method, 250 gm edible fruits of ZM and AM were soaked in 1000 ml of double distilled water and boiled for 5 hrs in a water bath until a slurry was formed. The slurry was cooled and kept in refrigerator overnight so that most of the undissolved portion was settled down. The clear solution was decanted off and centrifuged at 500 rpm 20 min. The supernatant was concentrated at 60°C on a water bath until the volume reduced to one third of its original volume. Solution was cooled down to the room temperature and was poured into thrice the volume of acetone by continuous stirring. The precipitate was washed repeatedly with acetone and dried at 50°C under vacuum. The dried material was powdered and kept in a desiccator.

Shear Stress Method:

Two smooth, polished plexi glass blocks were selected; one block was fixed with adhesive ‘Araldite’ on a glass plate, which fixed on leveled table. To the upper block a thread was tied and the thread was passed down through a pulley. At the end of the thread a beaker was fixed. The length of the thread from pulley to beaker was 7 cms. The weight of the beaker was counteracted. The volume of 0.75% w/v solution of natural mucoadhesive materials extracted from the fruits of ZM, AM and HPMCK4M were prepared using purified water I.P. as solvent. A fixed volume (0.5 ml) of 0.75% w/v solution of HPMCK4M and natural bioadhesive material solutions of ZM and AM were kept on the centre of the fixed block with a pipette, and then second block was placed on the first block and pressed by applying 100 gm of weight, so that the drop of synthetic polymer and natural bioadhesive material solutions spreads as a uniform film in between the two blocks. After keeping it for a fixed time intervals of 5, 10, 15, and 20 min, purified water was added into the beaker gradually, the weight of purified water just sufficient to pull the upper block or to make it slide down from the base block was recorded. This weight was considered as the adhesion strength, i.e. shear stress required to measure the adhesion. Before every experimentation care was taken so that no air bubble form in between the two blocks, which may give erratic results, and the distance from pulley to glass block was always same in all observations (22).

Wilhelmy Plate Method:

Mucoadhesiveness of natural materials and HPMCK4M were determined by a modified method of Wilhelmy Plate. In this method small glass plates were coated uniformly by HPMCK4M and natural bioadhesive material solutions and dried at 60°C. The prepared coated plates were immersed in U.S.P. simulated intestinal fluid (pH 6.0) for 5, 10, 15, and 20
Preparation of mucoadhesive tablets:

Mucoadhesive coated tablets each containing 7.5 mg of TS were prepared by conventional wet granulation method employing part of the mucoadhesive materials as filler and part of the natural mucoadhesive materials and HPMCK4M as binding agent as per the formulae given in Table 1. A blend of all ingredients was granulated with water. The wet masses were passed through 12-mesh sieve and the resulting granules were dried at 60°C for 24 h. The dried granules were passed through 18-mesh sieve. After blending with talc and magnesium stearate in a laboratory cube blender for 10 min, they were compressed into 100 mg tablets to a harness of 4-5 kg/cm² on a single punch tablet machine. All the prepared tablets were coated with 1% w/v aqueous solution of natural mucoadhesive materials and HPMCK4M and then evaluated for hardness, friability, average weight and disintegration time (23).

Identification and Estimation of TS Tablets:

The identification test and assay of TS were performed as per the procedure of Indian Pharmacopoeia. A quantity of the powdered tablets equivalent to 20 mg of TS was shaken with 50 ml of 0.1M sodium hydroxide for 10 minutes, and diluted to 100 ml with 0.1M sodium hydroxide and it was filtered. Then 20 ml of the filtrate was diluted to 50 ml with 0.1M sodium hydroxide. The light absorption in the range 200 to 400nm of the resulting solution exhibits a maxima only at about 276 nm. To determine the percentage purity of TS, twenty tablets were powdered in a glass mortar. The powder equivalent to 5 mg of terbutaline sulphate was taken in a 50 ml volumetric flask. 30 ml of 0.01 (M) hydrochloric acid was added and stirred for 10 minutes and then it was filtered. The first 5 ml of the filtrate was rejected. To 5 ml of the filtrate 35 ml buffer (phosphate buffer at pH 7.4) solution was added. Then 1.0 ml freshly prepared 2.0% w/v solution of 4-aminonitropryine and 1.0 ml freshly prepared 8.0% w/v solution of potassium ferricyanide were added to the solution with vigorous stirring. Then sufficient buffer solution was added to produce 50 ml. Exactly 75 seconds after the addition of the potassium ferricyanide solution, the absorbance of resultant solution was measured at 550 nm using distilled water as blank. The percentage of terbutaline sulphate was determined against 0.01% w/v solution of terbutaline sulphate R.S. as standard solution. Jasco double beam UV-VIS Spectrophotometer (Model, V-530) was used for these purpose (24).

In-vitro Drug release study:

Release of TS from the mucoadhesive coated tablets was studied in phosphate buffer of pH 7.2 (900 ml) as prescribed in the dissolution rate test of TS tablets in USP XXIV (Method A) using USP Apparatus II by the rotation of the paddle at 100 rpm. Samples were withdrawn through a filter (0.45µm) at different time intervals, suitably diluted and assayed for TS at 276 nm. Drug release experiments were conducted in triplicate (25).

In-vivo Drug absorption study:

Apparatus

The mobile phase was pumped through the system by a reciprocating piston pump (Model LC 10-AD, Shimadzu Scientific Instruments, Colombia, MD, U.S.A.). Samples were injected using a variable

The prepacked 5 μm C₁₈ guard column was from Alletch Associates. The analytical column was a 150 x 4.6 mm Dynamax column from Rinin Instrument Co. (Woburn, MA, U.S.A.) packed with 5 μm, 100 angstrom pore size, Microsorb silica C₁₈ stationary phase. Analytes were detected using a Coulochem II amperometric detector with a Model 5011 high sensitivity flow cell and a Model 5020 guard cell, all from ESA Inc. (Bedford, MA, U.S.A.). The detector signal was processed on a Shimadzu model CR 501 computing integrator.

Chromatographic Conditions

The mobile phase was 25 mM phosphate buffer, pH 7.4 : methanol (77:23, vol/vol), with 2 mM 1-octanesulfonic acid. It was filtered, degassed by sonication and pumped through the system at a flow rate of 0.7 ml/min, at room temperature. The electrochemical detector guard cell was set at +700 mV potential. The analytical cell screen electrode was set at +450 mV and the analytical electrode were set at +700 mV potential. The signal filter was set to 0.2 seconds. These potential were based on recommendations from an application note from the detector manufacturer and experiments our laboratory showing terbutaline begins to oxidize at a potential of about +450 mV. This low potential was used for the analytical cell screen electrode to improve sensitivity, realizing this would also cause a loss of selectivity. Detector response peaked at about +1000 mV, but running at this potential caused rapid loss of response, due to fouling of the electrode by oxidizable materials from the sample. The +700 mV was selected as the potential for the analytical electrode to minimize fouling of the electrode and also to minimize the size (and thus interference) of the glutathione peak. Running at +700 mV required periodic storing of the column and rinsing the system with a mobile phase containing 0.9 M acetic acid to remove materials absorbed to the electrodes.

Extraction Procedure

Six health male albino rabbits weighing between 2.5-3.0 kg were fasted overnight. The oral TS tablets (7.5 mg) were administered to rabbits. At determination time intervals, 1 ml blood samples were withdrawn from the marginal ear vein. One milliliters of plasma were added to a culture tube. 20 μl of a 1 ng/μl solution of metaproterenol (internal standard) in methanol and 1 ml of 10 mM sodium phosphate buffer (pH 7.5) were then added, and the samples mixed. Solid phase extraction columns were preconditioned with 2 X 3 ml of ethanol, followed by 2 X 3 ml water. Plasma samples were then passed through columns. They were next to rinsed with 2 X 3 ml of water. Receiver tubes containing 50 μl of 50 mM glutathione were then placed inside the vacuum manifold. The drugs were eluted from the columns with 1 ml of 95:5 (vol/vol) ethanol: 50 mM ammonium chloride buffer, pH 8.5. The samples were dried under nitrogen in a water bath at 30 °C, reconstituted with 200 μl of mobile phase, vortex-mixed, and transferred to 1.5 ml microcentrifuge tubes. The tubes were centrifuged at 13,400 x g for 2 minutes. Each sample was then passed through a syringe filter into a conical polypropylene autosampler vial. The auto sampler was programmed to inject 170 μl of each sample. The Institutional Ethics Committee has approved and given the permission to conduct the in vivo study using healthy rabbits (26).

RESULTS AND DISCUSSIONS

Natural mucoadhesive material obtained from the fruits of AM (27-30) and ZM (31-32) are reported to be nontoxic. The results obtained from Shear Stress and Wilhelmy Plate method are presented in Figure 1 and 2. From these figures it is confirmed that the mucoadhesive materials extracted from the fruits of ZM and AM showed better adhesive and
mucoadhesive property than the synthetic polymer HPMCCK4M. The adhesive and mucoadhesive strength of this synthetic polymer and natural mucoadhesive materials was increased with time and it was maximum in case of material extracted from the fruits of ZM and minimum in case of HPMCCK4M.

Figure 1: Results of adhesiveness extracted from ZM, AM and HPMCCK4M* by Shear Stress Method.

*Weight required was average of six determination (±SD). 0.75% w/v solution of synthetic polymer and mucoadhesive materials in purified water I.P. was used.

Figure 2: Results of mucoadhesiveness extracted from ZM, AM and HPMCCK4M* by Wilhelmy Plate Method.

*Weight required was average of six determination (±SD). 0.75% w/v solution of synthetic polymer and mucoadhesive materials in purified water I.P. was used.
Table 1. Formulations of Coated Tablets of TS.

<table>
<thead>
<tr>
<th>No.</th>
<th>Ingredient</th>
<th>Formulation (mg/tablet)</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>TS</td>
<td></td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>2.</td>
<td>Extract of ZM</td>
<td></td>
<td>7.5</td>
<td>-</td>
<td>-</td>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Extract of AM</td>
<td></td>
<td>-</td>
<td>7.5</td>
<td>-</td>
<td>-</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>H.P.M.C. K4M</td>
<td></td>
<td>-</td>
<td>-</td>
<td>7.5</td>
<td>-</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td>5.</td>
<td>Dibasic calcium phosphate</td>
<td></td>
<td>83</td>
<td>83</td>
<td>83</td>
<td>75.5</td>
<td>75.5</td>
<td>75.5</td>
</tr>
<tr>
<td>6.</td>
<td>Magnesium stearate</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>7.</td>
<td>Talc</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 3: Release Profiles of Mucoadhesive Tablets of TS*.

*Average of the six dimensions (±SD). Only batches F1 and F2 release drug up to 12h. In case of other batches total amount of drug was released before 12h.
Figure 4: Mean plasma concentration of TS (mcg/ml) of different batches*.

*Average of the six dimensions (±SD) were used.

Figure 5: Relationship between percent TS released in-vitro and absorbed
in-vivo for batch F1*.

*Average of the six dimensions (±SD) were use
The desired sustained release rate of TS for all the batches followed zero order kinetic after a lag time of 1.0 h and up to 95.5% was released and gave slow release over a period of 12h. for the tablets of TS prepared by natural mucoadhesive materials obtained from the fruits of ZM and AM in 1:1 drug-polymer ratio (batch F1 and F2) (Figure 3). This extension of release time is greater than the tablets prepared by HPMCK4M in the same drug-polymer ratio (batch F3). Tablets prepared by the natural mucoadhesive materials in 1:2 drug : mucoadhesive materials ratio (batch F4 and F5) showed extended release over a period of 11 h. Total about 95% drug was released at that period and this extension of release time is also greater than the tablets prepared by HPMCK4M (10h) in the same drug-polymer ratio (batch F6). Plasma TS concentrations and standard deviations achieved following oral administration of the different batches are shown graphically in Figure 4. Formulated mucoadhesive tablets prepared by natural materials were compared to a formulation prepared by HPMCK4 in order to determine their relative availability and mucoadhesive characteristics. From these results it was confirmed that batches F1 and F2 which were prepared by natural materials and where drug-mucoadhesive material ratio is 1:1 exhibited a smooth and extended absorption phase upto 12 hours. But other batches which were prepared either by synthetic polymer HPMCK4M (batch F3 and F6) or natural materials but the amount of natural material was more than batches F1 and F2, did not show the same extended drug release property as compare to batches F1 and F2. It is also confirmed that if TS mucoadhesive tablets were prepared by natural materials extracted from ZM and AM in 1:1 drug: mucoadhesive material ratio shows the desired mucoadhesive property by in-vivo experiment. A direct correlation between the percent drug released and percent drug absorbed of batch F1 and F2 are plotted in Figure 5 and 6 (33). From these figures it is...
confirmed that a good correlation exists between \textit{in-vitro} drug release and \textit{in-vivo} drug absorption of two batches. These two batches have a much slower but continuous absorption as compared to other batches.

CONCLUSION

In conclusion, the results of the present study indicated that the formulation F1 which was prepared from the mucoadhesive materials extracted from the edible fruits of ZM and used in 1:1 drug:material ratio and the formulation F2 which was prepared from the mucoadhesive materials extracted from the edible fruits of AM and used in 1:1 drug:material ratio have shown promising results (release about 95.5% drug in 12 h) with reasonably good mucoadhesive properties of natural materials.
REFERENCES


AUTHOR AFFILIATIONS:
Department of Pharmaceutics,
Himalayan Pharmacy Institute,
Majhitar, Sikkim, INDIA

ADDRESS FOR COMMUNICATION:
Department of Pharmaceutics,
Himalayan Pharmacy Institute,
Majhitar, Sikkim, INDIA
Email: amitoli@rediffmail.com