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Oriental Pharmacy and Experimental Medicine 2007 **7(1)**, 51-64

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In vitro and *in vivo* studies on theophylline mucoadhesive drug delivery system

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SUMMARY

Mucus is an aqueous gel complex with a constitution of about 95% water, high molecular weight glycoprotein (mucin), lipid, salts etc. Mucus appears to represent a significant barrier to the absorption of some compounds. Natural mucoadhesive agent was isolated and purified from the aqueous extract of the seeds of prosopis pallida (PP). Formulated tablet with the isolated material by wet granulation method. Some natural edible substances are in consideration for candidates as mucoadhesive agents to claim more effective controlled drug delivery as an alternative to the currently used synthetic mucoadhesive polymers. Subjected the materials obtained from natural source i.e. PP and standard synthetic substance, sodium carboxymethyl cellulose for evaluation of mucoadhesive property by various *in vitro* and *in vivo* methods. Through standard dissolution test and a model developed with rabbit, evaluated *in vitro* controlled release and bioadhesive property of theophylline formulation. Mucoadhesive agent obtained from PP showed good mucoadhesive potential in the demonstrated *in vitro* and *in vivo* models. The results suggest that the mucoadhesive agent showed controlled release properties by their application, substantially. In order to assess the gastrointestinal transit time *in vivo*, a radio opaque X-ray study performed in healthy rabbit testing the same controlled release formulation with and without bioadhesive polymer. Plasma levels of theophylline determined by the HPLC method and those allowed correlations to the *in vitro* mucoadhesive study results. Better correlation found between the results in different models. PP may acts as a better natural mucoadhesive agent in the extended drug delivery system.

Key words: Mucoadhesion; Detachment force; Prosopis pallida; Controlled drug delivery; *In vitro* and *in vivo* models

INTRODUCTION

Oral delivery is the preferred route of administration and a problem frequently encountered with controlled release is the inability to increase residence time of the dosage form. Among various researchers, one of the most promising strategies seems to be the incorporation of bioadhesive polymers into the formulation (Gupta *et al.*, 1990). The scope and aims of mucoadhesive research have broadened to include not only the search for good adhesive candidates but also the evaluation of physiological features of mucoadhesive polymers (Lehr, 1996). In the field of local drug delivery (nasal, intra oral, ocular, vaginal and rectal) mucoadhesive polymers have proven to be highly useful. Adhesion can be defined as the bond produced by contact between a pressure sensitive adhesive and a surface (Jime´nez-Castellanos *et al.*, 1993). The American Society of Testing Materials (ASTM,

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1984) has defined it as the stage in which interfacial forces, which may consist of valence forces, interlocking action or both, hold two surfaces together. Good (1976) defines bioadhesion to be the phenomenon in which two materials, at least one being of biological in nature, are held together for extended periods of time by interfacial forces. It also defined as the ability of material (synthetic or biological) to adhere to a biological tissue for extended period of time (Peppas and Buri, 1985; Kamath and Park, 1994).

Mucosal adhesives or mucoadhesives are natural agents or synthetic polymers, which interact with the mucus layer covering the mucosal epithelial surface and mucin molecules constituting a major part of mucus (Ahuja *et al.*, 1997). The exact mechanism of the mucoadhesion is not well known but may be assumed that the agents with more hydrogen bond forming hydrophilic functional groups such as -OH, -COOH, $-SO_3H$ and $-NH_2$ appears to play a major role in wet adhesion (Chen and Cyr, 1970; Gandhi and Robinson, 1988; Lehr *et al.*, 1992). A satisfactory bioadhesive bond forms between a polymer carrier and the mucus of the stomach, intestine, buccal, vaginal and rectal area, if strong interaction exists between two surfaces. Bioadhesive bonds may be physical or mechanical bonds and secondary chemical bonds (polar forces, Van der Waals forces, and hydrogen bonding) shown to be desirable for the development of bioadhesive strength. To date however, putative formulations have largely been subject to *in vitro* evaluation (Guney *et al.*, 1984; Mikos and Peppas, 1986) and comparatively little *in vivo* data is available (Sanctus *et al.*, 1997; Iscan *et al.*, 1998).

Bioadhesive controlled release formulations prepared with carbomer and cutina (hydrogenated castor oil) shown encouraging results towards colon delivery of drug effectively (Sanctus *et al.*, 1997). Sanctus *et al.* (1996) prepared microgranules (125 - 400 mm) with controlled release properties. δ-scintigraphy has been used extensively as a non-invasive technique for *in vivo* evaluation of oral dosage forms (Wilson and Washington, 1988; Digenis and Sandefer, 1991; Wilding *et al.*, 1991). It is useful to assess the performance, localization of drug release, to characterize variation in gastrointestinal (GI) transit and also to understand the mechanism of food effect (Davis *et al.*, 1984; Davis *et al.*, 1986; Reilly *et al.*, 1987; Sangekar *et al.*, 1987).

Generally, the mucoadhesive formulations made by using mixtures of bioadhesive polymers and the drug or by coating tablets and other dosage forms with the bioadhesive polymers when dissolved in organic solvents (Park and Robinson, 1984; Ch'ng *et al.*, 1985; Nagai and Machida, 1985). The current report reviews the mucoadhesive characteristics of substance derived from natural source in the demonstrated *in vitro* methods to substantiate the ability of natural substance, towards the mucoadhesive drug delivery in *in vivo* aspects.

MATERIALS AND METHODS

Materials

The seeds isolated from the pods of prosopis pallida (PP) were used for this piece of study. Procured theophylline, sodium carboxymethyl cellulose (SCMC) and 8-chlorotheophylline, as a gift sample from Universal aids. Other chemicals were reagent grade and used without further purification.

Isolation of natural agent for mucoadhesive study

Washed seeds obtained from the pods of PP were further soaked in water for 24 h and boiled with water for 4 h using glass containers to prevent the darkening that takes place in metallic containers. The gel like swollen tegmen (the inner covering layer of a seed), separated manually and soaked in glass in an aqueous solution of 0.1% w/v sodium metabisulphite for 24 h. At the end of this period, the material was homogenized. The highly viscous material obtained was passed through a muslin cloth to remove any gritty particles and the filtrate was added with thrice the volume of acetone as per the method adopted by Kulkarini *et al.* (2002). Thus obtained precipitate was filtered

and dried under reduced pressure and pulverized using a mortar and pestle and then passed through a 150 μm sieve.

In vitro evaluation methods

Robinson's method: Method reported earlier (Park and Robinson, 1985) utilizes animal tissue in the evaluation of mucoadhesion of polymers. Fixed fresh section of tissue isolated from fundus portion of goat intestine on a glass vial, facing mucosal side out and set in simulated gastric fluid (pH 1.2) without pepsin. Kept another portion of mucus side exposed tissue over a rubber stopper and secured with an aluminium cap. The mucoadhesive agent was spread on the exposed mucus layer uniformly in second case, and kept in contact with the former tissue and was then connected with a pan by which the weight can be raised. At specific intervals, applied weight and measured the force required to detach as an adhesive strength.

Wilhelmy's method: Method reported by Smart *et al.* (1984) was followed with little modification. Coated mucoadhesive agents on a small glass plates (2 × 5 cm) and was kept in a gel, (material obtained from fresh goat intestine by scraping and diluting with equal volume of water and after centrifugation the gel remained in middle portion) at predetermined intervals. Nylon thread attached at one end of the glass plate and passed over a pulley. At the end of the nylon thread, given provision to raise the weight. Added weight at specified intervals, to detach the coated glass plates from gel and measured the force required to detach as an adhesive strength.

***In vitro* dissolution study:** The *in vitro* dissolution test performed according to USP XXIII paddle method at 37 ± 1°C and 50 rpm. Used dissolution mediums, USP XXIII hydrochloric acid buffer pH 1.2 and phosphate buffer pH 7.4. Samples were withdrawn at an interval of every half an hour and the content of theophylline was measured by using UV spectrometry at 274 nm as per the earlier methods described for theophylline estimation (Yasunori *et al.*, 2003).

Data analysis

All the results expressed are the mean of three to six experiments.

Preparation of dosage forms

Wet granulation (massing and screening) method was used. Mixed the isolated dry extract (40 g) and diluent (dicalcium phosphate, 60 g), in a mortar for 5 min. Added disintegrant (starch, 5 g) and continued mixing for another 5 min. The liquid binder (2.5 and 5% *m/m* starch mucilage) added to the powder mix in 2 ml portions. The moistened mass was forced through a 1,000 m sieve, dried at 60°C for 1 h to give a moisture content of 4 - 6%, determined on an Ultra X moisture balance (August Gronert Co., Germany). The granules again passed through a 1,000 μm screen to break up agglomerates. To prepare the dosage forms, used barium sulphate and theophylline (50 mg/tablet) in appropriate quantity.

In vivo evaluation method

Oral administration of Radio opaque tablet study: The potential of the mucoadhesive oral tablets to deliver conventional drugs like theophylline to the systemic circulation in a sustained formulation was evaluated by conducting an experiment that include administration of the dosage from with a radio opaque substance and subsequently locating the administered dosage form by means of X-ray studies. Wilding *et al.* (1991) performed a δ-scintigraphy study which radiolabeled the delivery system and correlated position in the GI tract. As our study will provide the same data that is simple to perform and can be done in any laboratory, we used barium sulphate as a radio opaque substance. The aim of this experiment was to put a figure on the *in vivo* mucoadhesive capacity (bioadhesive strength) of theophylline oral tablets prepared with PP, administered to GI intestinal mucosa of rabbit. Rabbit's GI tract was utilized as a model since the tract provided a flat and uniform platform for the exposure to the dosage form with the physiological

conditions as that of the human being. The tablet was administered in oral route by taking utmost care not to chew by the animal while administration. Given water to the animal *ad libitum* and set free in the accommodated ambience. The tablet was then monitored by means of X-ray photographs at 1 h of the administration of tablet and subsequently at 3, 5, 7, 9 and 12 h.

HPLC estimation of theophylline in blood plasma (Chromatographic Conditions): Concentrations of theophylline and 8-chlorotheophylline were determined by HPLC, which consists of a model LC-10AT*vp* HPLC pump (Shimadzu, Kyoto, Japan), and a model SPD-2A UV detector (Shimadzu) set at 274 nm (extinction). A Lumina column packed with ODS ($C_{18,}$) 25.0 × 0.46 cm I.D. (Phenomonex) was used at room temperature. The mobile phase was 20% methanol in 20 mM potassium phosphate (monobasic) buffer and the mixture, adjusted to pH 5.6 with 8% phosphoric acid. The flow-rate was 1.0 ml/min and the UV detector at 274 nm was used, absorbance at 2 AUFS and the pressure at 170 - 225 pounds. pH of the HPLC mobile phase adjusted with a pH meter. Ultrasonic cleaners used for the cleaning of mobile phase for the dissolved gases and related substances.

Preparation of standard solutions

A stock standard solution was prepared by dissolving 10 mg of theophylline in 10 ml of deionized water. This stock solution (50 μg per 50 μl) serially diluted with deionized water to prepare 10, 5, 2.5, 1.25, 0.25 and 0.05 μg per 50 μl of theophylline.

Preparation of plasma standards

Duplicate plasma standards were prepared by spiking control plasma (0.2 ml) with an adequate volume (10 - 100 μl) of the standard solutions to produce 50, 25, 12.5, 2.5, 0.5, 0.1, and 0.05 μg/ml.

Extraction method

0.2 ml of plasma was added with 0.1 ml of the internal standard (1 mg per 100 ml of 8-chlorotheophylline in water) and one drop of 1 M hydrochloric acid to adjust the mixture to pH 2 - 3. After vortexing, the samples extracted with 3 ml of 10% isopropyl alcohol in chloroform by rotormixing for 20 min and centrifuged. The upper layers carefully removed with a pipette and discarded. Back extracted approximately 2.5 ml of each of the organic layer with 0.2 ml of 0.1 M sodium hydroxide by rotormixing for 20 min. After centrifugation, withdrawn the basic aqueous layer and 20 μl of each were analyzed by HPLC as described.

Pharmacokinetic Analysis

Pharmacokinetic parameters such as maximum serum concentration (C_{max}) and time of its occurrence (T_{max}) were calculated directly from the individual serum concentration time profile. The other pharmacokinetic parameters like biological half-life, mean residence time (MRT), elimination constant (Kel) and area under curve ($AUC_{0-\mu}$) were calculated by linear trapezoidal rule. Evaluated pharmacokinetic parameters statistically by student's *t*-test.

RESULTS AND DISCUSSION

Several approaches been tried to prolong gastric residence, one of which is the use of oral bioadhesive formulations (Longer and Robinson, 1986). When bioadhesives come in contact with an aqueous medium, they swell and form a gel (Gu *et al.*, 1988). The rate and extent of water uptake by a polymer been reported to be an important factor in determination of its relative bioadhesive strength. Uptake of water results in relaxation of the originally stretched entangled or twisted polymer chains, resulting in exposure of all polymer bioadhesive sites for bonding. The faster this phenomenon occurs, the rapid the polymer adheres to its substrate (Mikos and Peppas, 1986; Duchene *et al.*, 1988).

PP would consider as a candidate for mucoadhesive application. Adhesive strength of standard polymer SCMC used to compare PP extract. Adhesive

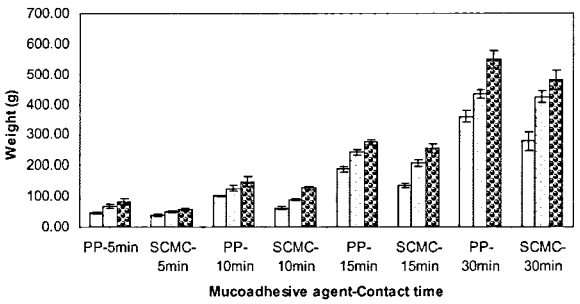

Fig. 1. Results of bioadhesive qualities of natural and synthetic agents by shear stress method at different concentrations and time (n = 3, $P < 0.05$).

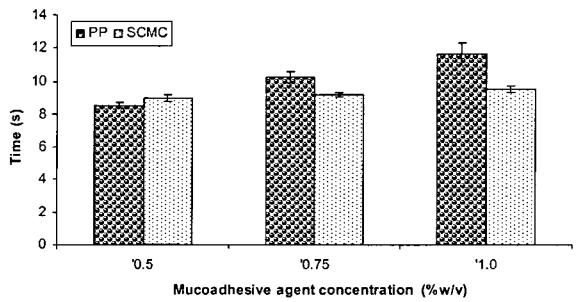

Fig. 2. Results of bioadhesive qualities of natural and synthetic agents by falling sphere method at different concentrations and time (n = 3, $P < 0.05$).

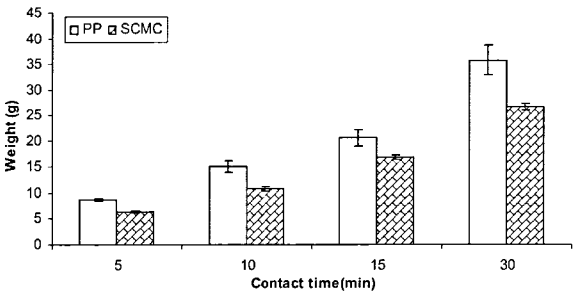

Fig. 3. Results of bioadhesive qualities of natural and synthetic agents by Robinson's method at different concentrations and time (n = 3, $P < 0.05$).

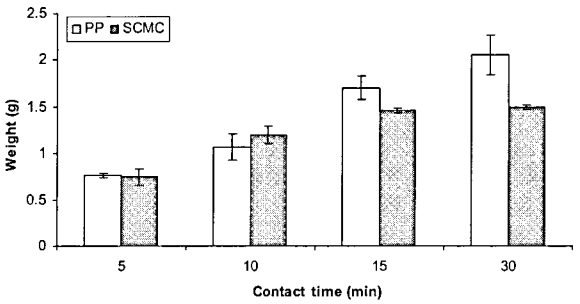

Fig. 4. Results of bioadhesive qualities of natural and synthetic agents by Wilhelmy's method at different concentrations and time (n = 3, $P < 0.05$).

strength of PP extract (0.5%, 0.75% and 1%), as shown in Figs. 1 - 4 found that the adhesiveness increased when the contact time increases. The same relationship was observed when percentage of concentrations as well. The results obtained for standard polymer SCMC is in agreement with the previous published data by Banerjee and Perumal (2004) on mucoadhesion. It was also reported that SCMC is an excellent adhesive (Smart *et al.*, 1984). The detachment forces of mucoadhesive materials by Robinson's method was measured as described earlier (Park and Robinson, 1985). The force required for the detachment of mucoadhesive materials, as shown in Fig. 3, found similar pattern when compared to standard polymer SCMC in various concentrations. The behavior proportionately increased on increase of concentration. The results obtained were in accordance with shear stress method and Wilhelmy's method.

In Fig. 2, the time profiles obtained for the mucoadhesive agent and standard polymer for falling ball experimentations were illustrated. We found that PP extract demonstrated good mucoadhesion while comparing with SCMC. These results are in agreement with shear stress method, Wilhelmy's method and Robinson's method that are indicators of polymer mucoadhesion. Evaluated mucoadhesive property of the extract obtained from the natural source by shear stress method (Duchene *et al.*, 1988), Wilhelmy's method (Smart *et al.*, 1984), falling ball method, Robinson's method (Park and Robinson, 1985). By these methods, it found that the extract possessed better mucoadhesive properties.

Shown the results in Figs. 1 - 4 and appraised the measurement of mucoadhesive strength based on various parameters, which are the indicators of mucoadhesion and are unique in nature. Though the exact method to measure the mucoadhesive

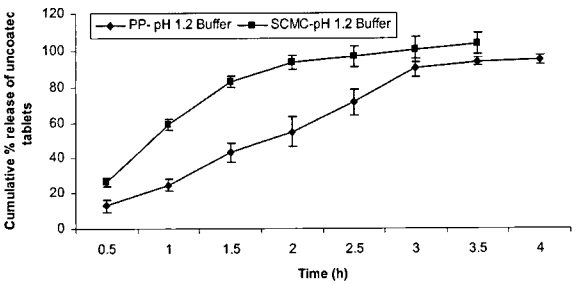

Fig. 5. Dissolution profile of uncoated theophylline tablets in pH 1.2 hydrochloric acid buffer (n = 3, $P < 0.05$).

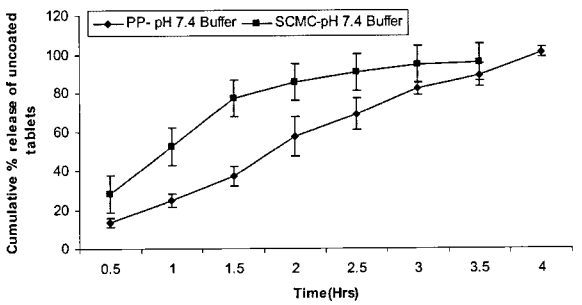

Fig. 6. Dissolution profile of uncoated theophylline tablets in pH 7.4 phosphate buffer (n = 3, $P < 0.05$).

strength in a single experiment is not possible, the conducted study will definitely provide an appraisal of mucoadhesive agents for their efficiency in delivering a controlled release formulation targeted to GI tract by means of mucoadhesion.

In vitro dissolution studies

Drug dissolution testing of pharmaceutical products is a procedure used to evaluate drug release characteristics of solid oral products such as tablets and capsules. The rationale behind conducting dissolution testing is that if a drug is to be absorbed from the GI tract, it usually has to dissolve. Therefore, for a drug to be absorbed, it has to release from the product and dissolved in the GI fluid. Thus, a dissolution test is an established analytical test to assess the qualities of a drug product, based on its rate and extent of dissolution, i.e., release characteristics. Therefore, at least in principle, a drug dissolution test, be considered as a surrogate marker of bioavailability of drugs in humans as well as in animals (Saeed *et al.*, 2003). The main objective of making the formulation was to have a release in the range of 35 - 60% by 2 h, 55 - 80% by 4 h, 70 - 95% by 8 h and for the 12 h sustained release preparation according to USP XXII specification. In order to attain the objective, coated theophylline tablets with mucoadhesive agents as described. Subjected coated and uncoated theophylline tablets for dissolution studies in buffer mediums of hydrochloric acid buffer pH 1.2 and phosphate buffer pH 7.4.

The uncoated mucoadhesive formulation containing SCMC was found to release about 90% of theophylline within 2 h in pH 1.2 HCl solution. The ionized carboxylic acid groups may be responsible for the rapid dissolution of the formulations containing SCMC and this is in correlation with the similar view reported (Hussain *et al.*, 1994).

The coated theophylline tablets containing PP extract sustained the release of drug in pH 1.2 HCl solution for 9 - 10 h. The theophylline tablets coated with PP registered a sustained release of 8.5 h in pH 7.4 phosphate medium (Figs. 7 - 8). The wash off was relatively rapid in phosphate buffer than in acid buffer. The coating of the natural mucoadhesive agent increased considerably the dissolution time in overall, of the coated tablets. The increased release rate in alkaline medium may relate partially to the drug solubility and it may consider that the release in both the medium be controlled by diffusion and

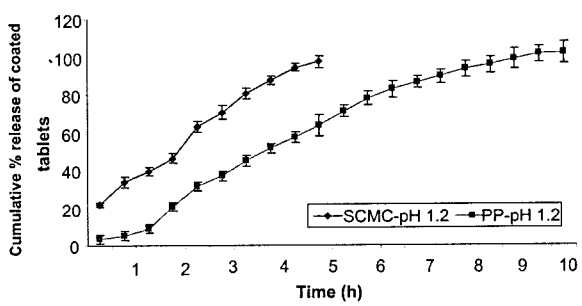

Fig. 7. Dissolution profile of coated theophylline tablets in pH 1.2 hydrochloric acid buffer (n = 3, $P < 0.05$).

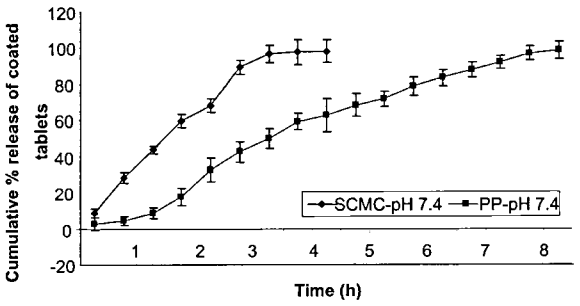

Fig. 8. Dissolution profile of coated theophylline tablets in pH 7.4 phosphate buffer (n = 3, $P < 0.05$).

erosion of the coated tablet (Takka *et al.*, 2001).

We observed the tablets containing mucoadhesive agents were having better sustained release pattern of drug, since they form a jelly like viscous mass when they are in contact with the liquid or mucus environment. The increase of viscosity may attribute to the strong hydrogen bonding between the carboxyl groups and hydroxyl groups in the natural mucoadhesive agent or in the polymer, leading to strong cross linking between two materials (Walker and Wells, 1982; Takka *et al.*, 2001). Hence the contact of theophylline and natural mucoadhesive agent results in increase of viscosity of the gel layer, which retards the drug diffusion from the tablet. The reduction in release rate may probably relate partially to the formation of a complex between cationic parts of the drug and anionic parts of the mucoadhesive agent (Takka *et al.*, 2001). Dabbagh *et al.* (1999) reported a similar report.

Addition of anionic polymers to the nonionic polymer containing dosage form can modify the release rate of weakly basic drugs. Formation of a complex between the drug and anionic polymer may affect the mucoadhesive complex formation, thus leading to fast delivery of drugs (Takka *et al.*, 2001). Thus, the dissolution may controlled by a combination of interaction between drug and mucoadhesive agent and it influences the dissolution through erosion.

The residence time in the stomach expected to be high, since the coated mucoadhesive dosage form stayed in the stomach for long time on the conducted X-ray study. This is the expected and good properties of a mucoadhesive dosage form, which would stay in the stomach for a much longer time to enable the dosage form to release the expected drug to the expected time and to the target. In our study, the concentration of theophylline in blood plasma was determined after administered to rabbit. Clinically, the dose of theophylline for treating and or managing asthma and the nocturnal attacks requires high frequency of dosing in conventional therapy and which may cause many adverse effects. Mucoadhesive drug delivery study conducted may provide therapeutic concentration at a much lower dose, which may significantly reduce the adverse effects as well as frequent dosing.

Considering the fact that in the *in vitro* release test, about 40% of theophylline released in 1.5 h (Fig. 5), we assume it to be the burst release of theophylline-mucoadhesive agent complex when exposed to aqueous medium. We thought it would be due to excess hydration and formation of water diffusible gel, which can not hold theophylline in its molecular complex. From the result of the *in vivo* clearance, we could deduce that, with the increase of mucoadhesive agent concentration on the outer surface of the dosage form, it could forms a strong gel that disallows fragmentation of the dosage form and ultimately the drug release get delayed. A small increase in the rate of drug release may attribute to the hydrophilic properties of the mucoadhesive agent which may have acted as wetting/disintegrating agents. The observed release profiles are typical to that of controlled release matrix system where release is faster in the first phase of hours with a subsequent slower rate of release at later time points. The *in vitro* mucoadhesion of formulations using mucoadhesive agent and standard polymer SCMC represented in Figs. 1 - 4. Santus *et al.* (1997) reported in their study that polycarbophil, carbomer, and SCMC exhibited the best *in vitro* adhesive characteristics. Hydroxypropylmethyl cellulose

alone had poor adhesive properties, but when used in combination with carbomer, the overall adhesion was increased (Santus *et al.*, 1997). These results are in line of our study and the selection of the standard polymer SCMC may rational to this part of study.

***In vivo* evaluation method**

Oral administration of radio opaque tablet study: The experiment was to put a figure on the *in vivo* mucoadhesive capacity (bioadhesive strength) of PP coated theophylline oral tablets by administering to rabbit. Observed a good correlation in our experiment for the mucoadhesive agent coated barium sulphate tablet and SCMC coated tablet. After an interval of 1 h, both the tablets were residing at the body of the stomach and the tablets were intact (Fig. 9). After 3 h, SCMC tablet started to disintegrate as seen in the X-ray photograph that the tablet is bulged. This may attribute to the absorption of water. But the PP coated tablets were intact up to 3 h. At 5th h the SCMC tablet was scrambled and the PP tablet was bulged. However the tablet was resided in the body portion of the stomach itself. Gradually the tablets disintegrated and the residence time of the tablets increased to a greater extent. The extension of gastric residence time may be due to the formation of link or bonds between mucin and the polar groups present in the mucoadhesive agents. GI transit of formulation demonstrated that the onset of drug absorption coincided with the pattern of the radio opaque material in the GI tract.

HPLC method: The coefficient of variation (CV)

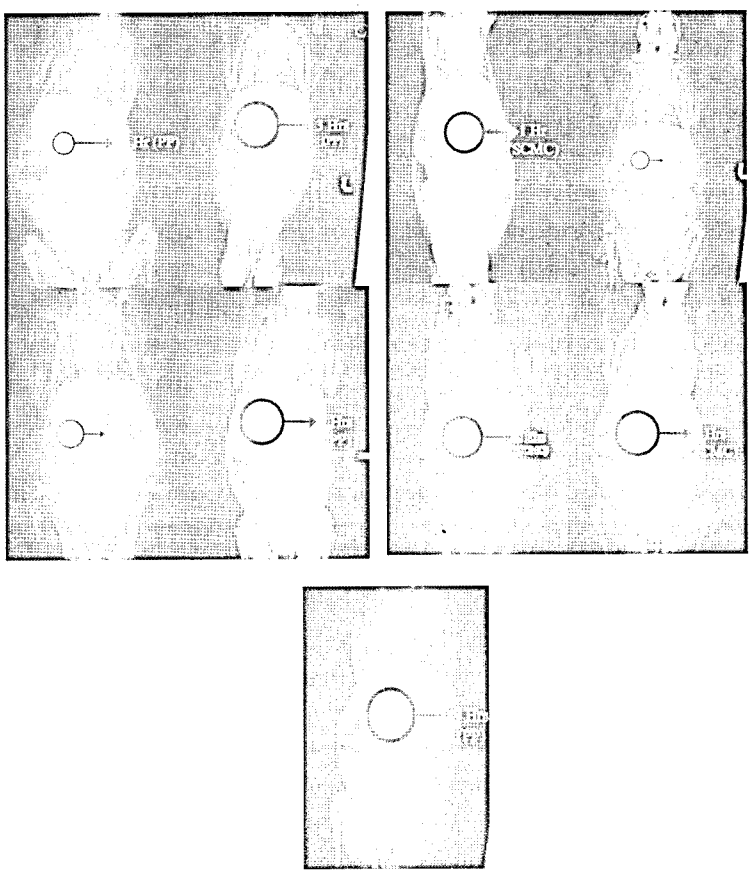

Fig. 9. X-ray Photographs of rabbit administered with mucoadhesive formulations.

Table 1. Pharmacokinetic results of prosopis pallida coated tablets

Parameters	Mean	SD	CV (%)
Cmax	9.236667	0.443195	4.798219
Tmax	4.923	0.816497	20.41241
Kel	0.192338	0.009314	4.842593
t1/2	3.484857	0.277914	7.974901
AUC0-t	68.87867	3.929437	5.704869
AUC0-inf	80.76218	5.184863	6.419915
AUMC0-t	369.421	26.93455	7.291018
AUMC0-inf	458.6901	43.79863	9.548633
MRT	5.358434	0.097013	1.810472

for each standard curve ranged from 0.5 to 10.2% and the squared correlation coefficient was over 0.990.

Theophylline serum concentration profiles

Shown the pharmacokinetic parameters in Tables 1 and 2 and the theophylline curves represented in Fig. 10. Theophylline AUC, C_{max} and T_{max} were not statistically different between treatments, however, the onset of absorption shown to be statistically later for the bioadhesive formulation. Mean theophylline AUC's of SCMC had a reduced AUC considerably following oral administration of the PP bioadhesive formulation.

Fig. 10 shows mean theophylline serum profiles. Initial review of the individual curves indicated that the onset of action for the bioadhesive formulation containing PP tend to be later than the formulation that does not contain the mucoadhesive agent. In other words, C_{max} attained earlier by the formulation that does not contain mucoadhesive agent. This is due to uncontrolled release type just like a conventional dosage form. Several of the individual curves also indicated a plateau region where the rate of theophylline input was approximately equal to theophylline elimination.

Plasma concentration-time profiles of theophylline after oral administration shown in Fig. 10. Pharmacokinetic parameters summarized in Tables 1 and 2. If the availability of theophylline at

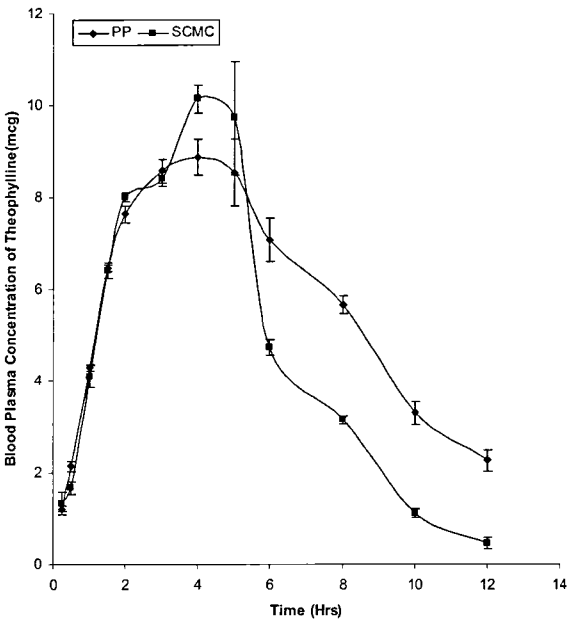

Fig. 10. Plasma theophylline concentration profile for the mucoadhesive agents (n = 3, $P < 0.05$).

Table 2. Pharmacokinetic results of SCMC coated tablets

Parameters	Mean	SD	CV (%)
Cmax	10.85033	0.27149	2.502136
Tmax	4.066667	0.471405	10.10153
Kel	0.425928	0.027558	6.470127
t1/2	1.712223	0.265474	15.50465
AUC0-t	56.52208	1.420755	2.513627
AUC0-inf	57.6214	1.850693	3.211816
AUMC0-t	254.2097	5.775578	2.271974
AUMC0-inf	257.4588	6.798072	2.640451
MRT	4.498079	0.049852	1.108293

the early hours of administration is low, it may be a good symptom that the dosage form is more suitable for colon-targeted so as to avoid enterohepatic circulation. On the other hand, theophylline appeared in plasma after around a 1 - 2 h lag time and its 't' was 9 - 10 h, showing the time max for theophylline to be suitable for controlled release due to the presence of mucoadhesive agent. Using the pharmacokinetic parameters (Tables 1 and 2), plasma concentration-time profiles was predicted for theophylline.

There were several kinds of theories that might explain the mechanism of mucoadhesiveness between adhesive materials and mucin, including electric, adsorption (van der waals, hydrogen bonds), wetting, diffusion and fracture theories, etc (Chickering *et al.*, 1995; Mortazovi, 1995; Nikolaos and Jennifer, 1996; Khalid and Ch'ng, 1998; Dobrozsi *et al.*, 1999; Burjak *et al.*, 2001).

Mean-while, lots of *in vitro* and *in vivo* tests were designed to evaluate the mucoadhesiveness, such as the rinsing method used by Ranga Rao *et al.* (1989) measurement of detachment force, the everted sac technique, novel theoretical approach *in vitro* and GI transit using radio-opaque microspheres, δ-scintigraphic technique, the microspheres retarding at rats GI, isolated internal loop in rats etc (Lehr *et al.*, 1990; Richerdson *et al.*, 1996; Chickering III *et al.*, 1997; Carreno-Go'mez *et al.*, 1999; Santos *et al.*, 1999; Riley *et al.*, 2001; Jaspreet *et al.*, 2003; Miyazaki *et al.*, 2003). Synthetic polymer, which is widely used for research as well as formulations (for example Carbopol) deserves contrary reports for its mucoadhesive characteristics. Some researchers have reported that it has good mucosal adhesive properties. Nagahara *et al.* (1998) prepared microspheres and the *in vivo* mucoadhesiveness test showed that the microspheres could reside in a rat's stomach for a longer period. But in the study of Cuna *et al.* (2001) carbopol did not help to prolong the residence time of the amoxicillin-resin complexes. The report of Akiyama *et al.* (1995) says that adhesive force between the polymer and mucous layer depended on the distribution state of carbopol in the microspheres, e.g., as a coat layer or being dispersed in the microspheres. Both *in vitro* and *in vivo* tests showed that the dispersed one could adhere to the mucosa more strongly than the coat layer can.

The *in vitro* and *in vivo* tests also showed that the formulation containing mucoadhesive agent had better adhesive effects when compared with those formulation without mucoadhesive agent. From the result of the *in vitro* release test, we observed about 90% of theophylline was released in the pH 1.0 HCl solution within 3.5 h (Fig. 2). While *in vivo* evaluation of mucoadhesiveness showed that 50% of theophylline still remained in stomach 4 h after administration, which might infer that theophylline could release almost completely when the formulation is resided in the stomach for an extended period of time.

In case of theophylline tablets coated with PP, the C_{max} attained at 4 h and the plasma concentration time curve interpolation shows a concentration over 8.87 μg/ml. The formulations appear to maintain the serum concentration at a therapeutically effective concentration level for a period up to 9 - 11 h, as evident from the low elimination rate of drug and prolonged half-life of that formulation (Fig. 9).

Pharmacokinetic parameters like $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$, T_{max} and C_{max} of PP extract was significantly higher when compared with the tablets of SCMC. The MRT values of PP extract found highly significant when compared with the tablets SCMC (Tables 1 and 2).

The therapeutic efficacy of this mucoadhesive formulation was significantly higher than the standard. This may suggest that these formulations sustain the release over a prolonged period by virtue of their higher degree of mucoadhesive characteristics.

The C_{max} in case of SCMC formulation appeared at 3 - 4 h whereas the same was at 5 - 6 h in case of PP. The early occurrence of C_{max} in the former case could be due to the release of maximum fraction of drug from the formulation during the transit though stomach by simple disintegration of the dosage form. There may be very less releasable fraction of drug remaining in the theophylline tablets coated with SCMC after 3 h, hence the disposition appear to be rapid. Whereas, in theophylline tablets coated with PP the maintenance of the steady state serum concentration indicates that the formulations were sustaining the release by virtue of their mucoadhesiveness (Fig. 9). These above findings are in line of the *in vitro* dissolution studies and the findings already reported in

respect of the pharmacokinetic parameters of the standards undertaken in this study (Varshosaz *et al.*, 2000). It may be required to note that a great variability in some pharmacokinetic parameters found between four sustained release theophylline preparations orally administered to dogs (Kortiz *et al.*, 1986). The theophylline tablets coated with SCMC chosen as standard for comparison.

The formulations resulted in comparable pharmacokinetic (PK) parameters. There was no significant difference between the PK parameters of the standard formulations. The inter-individual (within group) variations were also negligible. The minimum effective serum concentration attained at 3 - 4 h followed by elimination of the drug up to 11 h. The serum concentration of the drug seems to have reduced to sub-therapeutic concentrations after 9 h (Fig. 9). Moreover, human patient compliance seems to improve with the use of sustained release formulations. However, clinically important pharmacokinetic differences exist between the marketed products (Hendeles and Weinberger, 1986).

In conclusion, mucoadhesive formulation prepared in this study could stay in the GI tract for a longer period of time and could keep the entrapped theophylline in gastric surrounding.

CONCLUSION

Mucoadhesion is a channel that has a great potential for pharmaceutical controlled release dosage forms design and patient compliance. Polymers are playing a vital role in the process of mucoadhesion. The development of mucoadhesive dosage forms with natural mucoadhesive agent depends on the availability of agents with expected adhesiveness in mucosal area, stability and non toxicity. The natural mucoadhesive agent studied in our laboratory obtained from PP is stable and edible. Better correlation found between the results of the detachment force measurement method using different models and different *in vivo* studies. PP found to have mucoadhesiveness in *in vitro* and in *in vivo* models and it will acts as a better mucoadhesive agent in the extended drug delivery system designing through mucoadhesion to combat the crisis of nocturnal asthmatic attacks that too with lesser adverse effects during the treatment regime and also with more patient compliance.

ACKNOWLEDGEMENTS

Author P Perumal acknowledges the support rendered by N Sendamaraai, Sceretary and Correspondent, JKK Nataraja educational Institutions, during the research work.

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