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Research Article

Characterization of Soy Polysaccharide and Its *In Vitro* and *In Vivo* Evaluation for Application in Colon Drug Delivery

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Abstract. The objective of the present investigation was to establish potential of commercially available soy polysaccharide (Emcosoy®) for colon drug delivery. The soy polysaccharide–ethyl cellulose films were fabricated and characterized. The effect of the pectinase enzyme on the tensile strength and surface morphology of the film was evaluated. The permeation of chlorpheniramine maleate (CPM), a model hydrophilic drug from pectinase enzyme treated and untreated films was measured in pH 7.4 buffer. The soy polysaccharide–ethyl cellulose films were also incubated with *Lactobacillus* sp. culture for a specific duration, and effect on the CPM permeation was evaluated. The CPM capsules were coated with the soy polysaccharide–ethyl cellulose mixture, and Eudragit S100 was applied as a secondary coat. The coated CPM capsules were radiolabelled, and their *in vivo* transit was evaluated in human volunteers on oral administration. The pectinase enzyme had a significant influence on the tensile strength and surface morphology of the soy polysaccharide–ethyl cellulose films. The permeability of pectinase enzyme-treated and *Lactobacillus* sp.-treated films was significantly higher than that of untreated films. The CPM capsules were coated with the soy polysaccharide–ethyl cellulose mixture and Eudragit S100 and were successfully radiolabelled by a simple method. Gamma scintigraphic studies in human volunteers showed that the radiolabelled capsules maintained integrity for at least 9 h after oral administration. Thus, the soy polysaccharide has a potential in colon drug delivery.

KEY WORDS: capsules; colon targeting; Eudragit S100; pectinase enzyme; soy polysaccharide.

INTRODUCTION

Targeting of drug to the specific organ offers advantages such as maximum availability of the drug at the site of action, possibility of dose reduction, and reduction in the untoward off-target effects of the drug. Targeting of the drug to colon is considered to be advantageous in the treatment of various disorders such as colon cancer, ulcerative colitis, and inflammatory bowel disease or Chron's disease (1–3). However, due to distal location of the colon, the colon targeted drug delivery system should overcome physiological barriers such as pH and enzymatic conditions in upper gastrointestinal tract (GIT) and absorptive mechanisms in the GIT (1–3). To achieve colon targeted delivery via oral route, various approaches such as use of time-dependant systems (4), pressure-dependant systems (3), and microbially activated systems (5) have been designed.

The microbially activated systems make use of colonic microbial flora for the site-specific release of the drug. The colonic microbial flora mainly consists of anaerobic bacteria, e.g., *Bacteroides*, *Bifidobacteria*, *Eubacteria*, *Clostridia*, *Enterococci,* and *Enterobacteria*. These microbial species secrete a variety of enzymes such as β-glucuronidase, β-xylosidase, α-arabinosidase, β-galactosidase, azo reductase, nitro reductase, deaminase, and urea hydroxylase (5). Due to presence of these colon-specific enzymes, microbially activated systems are dependent on the use of prodrugs (6) [which can be specifically degraded by the aforementioned enzymes] and/or use of polysaccharides (5,7–9) [which are substrate for the enzymes secreted by colonic microflora]. Various natural gums such as xanthan gum, guar gum, and natural polysaccharides such as pectin, chitosan, and amylose have been employed for the development of colon targeted delivery systems (5,7–9). These natural polysaccharides have been used either alone or in combination with semi-synthetic cellulosics (hydroxypropylmethyl cellulose and ethyl cellulose) and acrylate polymers (Eudragits) to obtain colon targeted delivery systems (10–12). Furthermore, various dosage forms such as compression coated tablets (13), film-coated capsules (14), film-coated pellets (15), and hydrogels (16) have also been evaluated to achieve colon targeted delivery using natural polysaccharides.

Soy polysaccharide, which is sourced from dehulled and defatted soybean flakes, is soft white to light–tan fibrous powder and does not contain starch or sugar. It has 75% dietary fiber with the main components including five types of higher polysaccharides, viz, cellulose, hemicellulose, pectin, gum, and mucilage (17). The soy polysaccharide has been used in the dietary supplements due to high fiber content, and few

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studies also explored the nutritional benefits of the soy polysaccharides (18,19). The commercial version of the soy polysaccharide (Emcosoy®) is being employed as a superdisintegrant in compressed tablets (17). However, until today, there are no reports on the potential of the soy polysaccharide in the colon targeted drug delivery systems. The present investigation was focused on the evaluation of the soy polysaccharide for the fabrication of the colon targeted delivery systems. In the present investigation, soy polysaccharide was characterized, and films containing a mixture of soy polysaccharide and ethyl cellulose were evaluated for the potential in colon-specific drug delivery. The soy polysaccharide–ethyl cellulose mixture was film-coated on the capsules (containing chlorpheniramine maleate as a model drug), and effect of the coating on the drug release was evaluated. Finally, the *in vivo* proof to demonstrate potential of soy polysaccharide–ethyl cellulose film-coated capsules in colon drug delivery was established.

MATERIALS AND METHODS

Materials

Chlorpheniramine maleate (CPM) was received as a gift from Nivedita Chemicals, Mumbai, India. Ethyl cellulose 45 cps (Colorcon, Mumbai, India), Eudragit S100 (Degussa India Ltd., Mumbai, India), Emcosoy® (Soy polysaccharide ; JRS Pharma Delhi, India), empty hard gelatin capsules (ACG Worldwide, Mumbai, India), and triethyl citrate (Unichem Laboratories, Mumbai, India) were obtained as a gift samples. Fluid thioglycolate medium dry powder (Hi Media, Mumbai, India), pectinase enzyme (Sigma Chemicals, Mumbai, India), lyophilized spores of lactobacilli (Laff, manufactured by Pharma Med, India), methylene chloride, and isopropyl alcohol and talc (AR grade; s.d. fine chemicals, Mumbai, India) were purchased for the study. All the materials were used as received.

Characterization of Soy Polysaccharide

The soy polysaccharide (powder) was observed under polarized microscope in dry and wet states. The particle size distribution was evaluated by Malvern Mastersizer 2000 (Malvern Instruments, USA).

Preparation of the Soy Polysaccharide–Ethyl Cellulose Films

The composition of the soy polysaccharide–ethyl cellulose films has been shown in the Table I. Briefly, triethyl citrate, soy polysaccharide, and ethyl cellulose were added to isopropyl alcohol (4 mL) under stirring. Methylene chloride (6 mL) was added to the above dispersion slowly, and the mixture was stirred for 1 h. A thin polythene sheet was cut in size of 12×18 in. and was stuck on wooden board with the help of tapes. The soy polysaccharide–ethyl cellulose dispersion was sprayed onto a thin polythene sheet. The spraying parameters have been given in the Table II.

Table I. Composition Employed for the Preparation of Soy Polysaccharide–Ethyl Cellulose Films

Ingredients	Quantity
Ethyl cellulose 45 cps	10 g
Triethyl citrate	1 g
Soy polysaccharide	1 g
Isopropyl alcohol	40 mL
Methylene chloride	60 mL

Evaluation of Tensile Strength of the Films

The tensile strength of the films of uniform thickness was measured using tensile tester (Zwick 2014, Switzerland). Samples with a length of 150 mm and a width of 10 mm were cut from the film. The measuring speed used was 5 mm/min, and the initial gauge length was 40 mm. All the measurements were repeated for five times.

Evaluation of the Effect of the Pectinase Enzyme Treatment on the Various Properties of the Soy Polysaccharide–Ethyl Cellulose Films

Treatment of Soy Polysaccharide–Ethyl Cellulose Films with the Pectinase Enzyme

The soy polysaccharide–ethyl cellulose films were stored in the 2% pectinase enzyme solution at 37°C for 24 h. The films were washed thoroughly with distilled water.

Effect of the Pectinase Enzyme Treatment on the Tensile Strength of the Soy Polysaccharide–Ethyl Cellulose Films

The tensile strength of the pectinase enzyme-treated films was measured as per the procedure described in the earlier section.

Scanning Electron Microscopy (SEM) of the Soy Polysaccharide-Ethyl Cellulose Films Before and After Pectinase Enzyme Treatment

SEM studies were carried to understand effect of pectinase enzyme treatment on surface of soy polysaccharide–ethyl cellulose films. The soy polysaccharide–ethyl cellulose films which did not receive pectinase enzyme treatment were considered as control films. The control films and pectinase enzyme-treated films were mounted on a sample holder of

Table II. Spraying Conditions for Preparation of Soy Polysaccharide–Ethyl Cellulose Films

Parameter	Condition
Nozzle diameter	1 mm
Spray pressure	0.2 Kg/cm^2
Amount	10 mL
Distance of spraying	150 mm approx.

the JSM-840 A scanning electron microscope (JEOL Ltd, Japan). The films were coated with gold palladium for 300 s under an argon atmosphere. The surface characteristics of the films were evaluated by SEM at 10 kV and magnification of 500X.

Effect of the Pectinase Enzyme Treatment on the Permeability of the Soy Polysaccharide–Ethyl Cellulose Films

For this experiment, the pectinase enzyme-treated films were prepared as per the procedure described in the earlier section. The soy polysaccharide–ethyl cellulose films which were not treated with pectinase enzyme were used as control films. The effect of the pectinase enzyme treatment on the permeability of the soy polysaccharide–ethyl cellulose films was evaluated by measuring permeability of CPM, a model hydrophilic drug through the pectinase enzyme-treated films and control films. The *in vitro* permeation of CPM through films was evaluated by using Franz-type diffusion cells. The pectinase enzyme-treated films and control films were mounted on the Franz diffusion cells. The Franz diffusion cells used for the experiment were fabricated locally. The specifications were:

1. Height of donor compartment, 5.18±0.22 cm
2. Angle between receptor cell and side arm, 133.3±2.58
3. Internal diameter of receptor cell, 1.85±0.08 cm
4. Internal diameter of donor cell, 1.94±0.16 cm, and
5. Volume of receptor cell, 18.75±0.87 mL

The Franz diffusion cells were validated as per reported method (20).

The receptor compartment of Franz diffusion cells was filled with phosphate buffer (pH 7.4). The donor compartment of the Franz diffusion cell was filled with 5 mL of CPM solution (concentration, 1 mg/mL) in phosphate buffer (pH 7.4). The medium in receptor compartment was stirred continuously with the help of magnetic needle and maintained at 37±1°C with a circulating water bath. Aliquots (1 mL) were periodically withdrawn from the receptor compartment and replaced with the same volume of fresh buffer. The aliquots were centrifuged at 5,000 rpm for 20 min in order to remove all the contaminants, and the concentration of CPM permeated in the receptor medium was analyzed at 262 nm by UV spectrophotometer V 530 (Jasco, Japan). The total amount of CPM permeated as a function of time was evaluated for all the films. All the experiments were performed in triplicate, and mean±SD for each time point was calculated. The *in vitro* permeation of CPM from pectinase-treated films and control films was evaluated by Student's *t* test. The differences were considered significant at $p<0.05$.

Influence of Bacterial Culture on the Permeability of the Soy Polysaccharide–Ethyl Cellulose Films

Preparation of Lactobacilli Culture

Briefly, 2.975 g of fluid thioglycolate medium dry powder was accurately weighed and dissolved in 100 mL of distilled water with boiling. The clear solution obtained was sterilized by autoclaving at 121°C, 15 lb pressure for 15 min. The medium was cooled to 35°C and transferred to pre-sterilized test tubes. To these test tubes, 0.50 g of lyophilized spores of *Lactobacillus sp.* (concentration, two billion spores per 2 g) were added, and the test tubes were anaerobically stored at 37°C for 24 h. This was treated as a primary culture.

Exposure of Films to Lactobacilli Culture

Lactobacillus sp. was selected to study the degradation of the soy polysaccharide–ethyl cellulose films. The soy polysaccharide–ethyl cellulose films were fabricated and were incubated for 4 and 7 days at 37°C anaerobically in the VPI diluent fluid medium inoculated with *Lactobacillus* culture (21,22). These films were labeled as 'treated films-4 days' and 'treated films-7 days,' respectively. The soy polysaccharide–ethyl cellulose films incubated in media without *Lactobacilllus* microorganisms in identical conditions were labeled as 'control films-4 days' and 'control films-7days,' respectively. After the incubation period, all the films were rinsed thoroughly with distilled water.

In Vitro Permeation of CPM Through Treated Films and Control Films

The treated films-4 days, treated films-7 days, control films-4 days, and control films-7days were mounted on the Franz diffusion cells. The *in vitro* permeation of the CPM through all the films was studied. The procedure for evaluating the *in vitro* permeation of the CPM through films was same as described in the earlier section. The total amount of CPM permeated as a function of time was evaluated for all the films. All the experiments were performed in triplicate, and mean± SD for each time point was calculated. The *in vitro* permeation of CPM from treated films and control films was evaluated by Student's *t* test. The differences were considered significant at $p<0.05$.

Preparation of CPM Capsules and Coating of CPM Capsules

Hard gelatin capsules (size 2) were manually filled with a premix containing CPM, lactose, and Aerosil. The CPM capsules complied with all the standard requirements for the capsules such as drug content, uniformity of content, and uniformity of weight. The CPM capsules were coated with the coating dispersion containing soy polysaccharide–ethyl cellulose mixture (Table I) in fluid bed coater (Umang Pharma Tech, Mumbai, India). The batch size was 20 capsules. Coating conditions were as follows: inlet temperature, 38°C; bed temperature, 33°C; blower rpm, 1,600; fluidization pressure, 0.8–1 kg/cm^2; nozzle air pressure, 3 kg/cm^2. The coating was continued until the weight gain of 6% *w/w*. The soy polysaccharide–ethyl cellulose-coated CPM capsules were further coated with Eudragit S100 using fluid bed coater. The composition of the Eudragit S100 coating solution is given in the Table III. The coating parameters were kept constant. The Eudragit S100 coating was continued till weight gain of 6% *w/w*.

SEM of the Coated CPM Capsule

The transverse section of the coated CPM capsule was taken using a very sharp blade, and the sample was prepared

Table III. Composition of the Eudragit S100 Coating Solution

Ingredients	Amount
Eudragit S 100	6 g
Talc	0.75 g
Triethyl citrate	1.20 g
Isopropyl alcohol	30 mL
Methanol	70 mL

for SEM as per the procedure described in the earlier section. The section was evaluated for presence of coating by SEM.

In Vitro Dissolution of the CPM-Coated Capsules

The *in vitro* release of CPM from the coated capsules was evaluated by dissolution testing using the USP Dissolution Tester, Apparatus I (rotating basket) maintained at 37.0± 0.5°C and operated at the speed of 100 rpm. The *in vitro* release of CPM was evaluated in 500 mL of pH 1.2 buffer for the first 2 h. After 2h, the dissolution medium was replaced with pH 7.4 buffer, and the release of CPM was evaluated. The aliquots (1 mL) were taken after every 1 h and were replaced with fresh dissolution medium. After 6 h, liquid pectinase enzyme (1.66 mL; activity, 26,000 polygalacturonase activity mL^{-1}) was introduced in the dissolution medium of half of the capsule samples. The *in vitro* release of CPM was evaluated by analyzing the concentration of CPM in the aliquots at 262 nm by UV spectrophotometer. All the experiments were performed in triplicate.

Radiolabelling of the Coated CPM Capsules

Coated CPM capsules were prepared as per the method described earlier. Radiolabelling of the coated CPM capsules was performed as per the method described by Ofori-Kwakye *et al*., with suitable modifications (23). Briefly, coated capsule was punctured using 26-guage needle to create a small hole of diameter 0.1 mm. The concentrated stock solution of ^{99m}Tc-labelled diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) of known radioactivity was introduced into the capsule with the help of insulin syringe. A 8% *w/w* ethyl cellulose (100 cp) solution was prepared in methylene chloride. The ethyl cellulose solution was applied on the capsule with the help of brush to seal the hole in the capsules. The capsules were air-dried for 30 min. The radiolabelling was validated by introducing radiolabelled capsules in a glass beaker containing pH 1.2 buffer (200 mL). The contents of beaker were stirred slowly using magnetic needle for 2 h. After 2 h, the dissolution medium was replaced with pH 7.4 buffer, and the stirring was continued for 3 h. The aliquots were withdrawn every 1 h, and the radioactivity of the aliquots was measured on the gamma scintillation counter (Gamma Spectrometer, Electrical Corporation of India, India).

Gamma Scintigraphy Studies in Humans

Three male healthy volunteers (with mean age 22 years ranging from 21 to 25 years; mean weight 65 kg ranging from 55 to 75 kg) participated in the study after an overnight fasting. The protocol of the study was approved by the Human Ethics Committee of the Radiation Medicine Center, Tata Cancer Hospital, Mumbai, India, and each subject provided written informed consent for the study. A radiolabelled capsule containing ^{99m}Tc was administered to each volunteer with 250 mL of water at approximately 10.00 AM. All the volunteers fasted for 3 h after the administration of the capsule after which a standard lunch was provided. An evening meal was also provided at 10 h postdose. The volunteers remained moderately active throughout the study. The imaging of the capsules was done at 4, 6, 7, 8, 9, and 24 h after the administration of the capsule. At the time of imaging, the volunteers lay on the bed equipped with the gamma camera (Unit: Gantry, Model: Infinia, Hawkeye GP-3, General Electric Medical Systems USA), and the images were captured.

RESULTS

Characterization of Soy Polysaccharide and Evaluation of Films

The particle size ($D_{10\%}$, $D_{50\%}$, and $D_{90\%}$) of the soy polysaccharide was evaluated by Malvern particle analyzer. The $D_{10\%}$ was 3.304±0.057 μ, $D_{50\%}$ was 10.593±0.321 μ, and $D_{90\%}$ was found to be 38.527±1.985 μ. The photomicrographs of dry soy polysaccharide powder and hydrated soy polysaccharide powder at 40X magnification are shown in Fig. 1. The ethyl cellulose–soy polysaccharide films could be successfully fabricated. The tensile strength of the ethyl cellulose–soy polysaccharide films was 3.23±0.51 N/mm^2.

Evaluation of the Effect of the Pectinase Enzyme Treatment on the Various Properties of the Soy Polysaccharide–Ethyl Cellulose Films

The effect of pectinase enzyme treatment on the various properties of the ethyl cellulose–soy polysaccharide films was evaluated. The tensile strength of pectinase enzyme-treated ethyl cellulose–soy polysaccharide films was 3.01±0.30 N/mm^2. SEM of the ethyl cellulose–soy polysaccharide film has been shown in Fig. 2a whereas SEM of the pectinase enzyme-treated ethyl cellulose–soy polysaccharide film has been shown in Fig. 2b. The *in vitro* permeation profile of the CPM through pectinase enzyme-treated and pectinase enzyme-untreated ethyl cellulose–soy polysaccharide films has been shown in Fig. 3. At the end of 8 h, the *in vitro* permeation of the CPM through pectinase enzyme-treated ethyl cellulose–soy polysaccharide films was 44.46%±5.28 whereas *in vitro* permeation of CPM through pectinase enzyme-untreated cellulose–soy polysaccharide films was 19.96%±5.61 (Fig. 3).

Influence of Bacterial Culture on the Permeability of the Soy Polysaccharide–Ethyl Cellulose Films

The *in vitro* permeation profiles of the CPM through bacterial culture (*Lactobacillus* sp.)-treated and bacterial culture-untreated ethyl cellulose–soy polysaccharide films have been shown in Figs. 4 and 5. The percent CPM permeation through bacterial culture untreated ethyl cellulose–soy

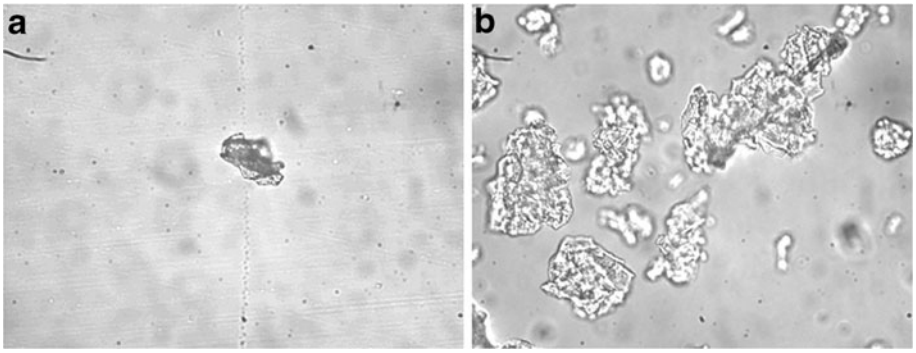

Fig. 1. Photomicrograph of **a** dry soy polysaccharide powder and **b** hydrated soy polysaccharide powder at 40X magnification

polysaccharide films, viz., 'control films-4 days' and 'control films-7 days' was 10.39±0.44% and 11.03±2.95%, respectively, at the end of 8 h whereas percent CPM permeation through bacterial culture treated ethyl cellulose–soy polysaccharide films, viz., 'treated films-4 days' and 'treated films-7 days' was 21.15±3.21% and 33.15±4.28%, respectively, at the end of 8 h (Figs. 4 and 5). The percent CPM permeation through 'treated films' was significantly higher than that of control films ($p<0.05$).

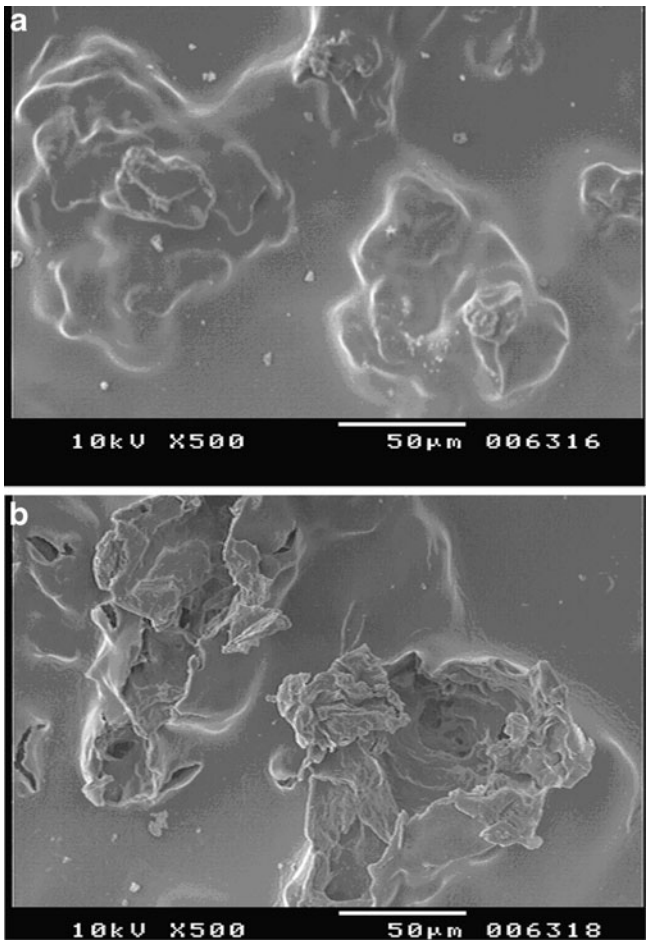

Fig. 2. SEM of soy polysaccharide–ethyl cellulose films **a** without treatment of pectinase enzyme and **b** with treatment of pectinase enzyme

Coating of CPM Capsules and SEM of Coated CPM Capsules

The CPM filled capsules were prepared and were successfully coated with the ethyl cellulose–soy polysaccharide mixture. Eudragit S100 was applied as a secondary coat. The SEM of the transverse section of the coated CPM capsule is shown in Fig. 6. The SEM clearly showed the presence of additional coat around the capsule shell but could not distinctly show two different coats of soy polysaccharide–ethyl cellulose and Eudragit S100.

In Vitro Dissolution of the CPM-Coated Capsules

The *in vitro* release profile of the CPM from the coated capsules is shown in Fig. 7. The coated CPM capsules did not show any CPM release in the pH 1.2 buffer whereas CPM was released from the capsules in the pH 7.4 buffer. The introduction of the liquid pectinase enzyme in the dissolution medium had influence on the CPM release from the coated CPM capsules. At the end of 10 h, coated CPM capsules showed 62.62±2.13% CPM release in medium devoid of pectinase enzyme whereas pectinase enzyme-treated coated CPM capsules showed 77.01±0.83% CPM release in medium containing pectinase enzyme (Fig. 7).

Radiolabelling of the Coated CPM Capsules

The coated CPM capsules were radiolabelled with ^{99m}Tc-DTPA using a procedure reported by Ofori-Kwakye *et al.*, with suitable modifications (23). The radiolabelling procedure involved creation of tiny hole in the capsule shell to introduce ^{99m}Tc-DTPA solution followed by resealing with the ethyl cellulose solution. The *in vitro* release studies in pH 1.2 buffer (duration, 2 h) and pH 7.4 buffer (duration, 3 h) were carried out to detect leaching of the radioactive material from the capsule. The capsules did not show any leaching of the radioactive material in pH 1.2 buffer whereas pH 7.4 buffer resulted in release of radioactive agent from the capsule shell (~30% radioactive material was released at the end of 3 h). This study indicated that the radioactive material would not be released into the gastrointestinal tract.

Gamma Scintigraphy Studies in Humans

The gamma scintigraphy studies in humans were carried out to obtain *in vivo* proof of concept for soy polysaccharide coating. The gamma scintigraphic imaging of the volunteers was

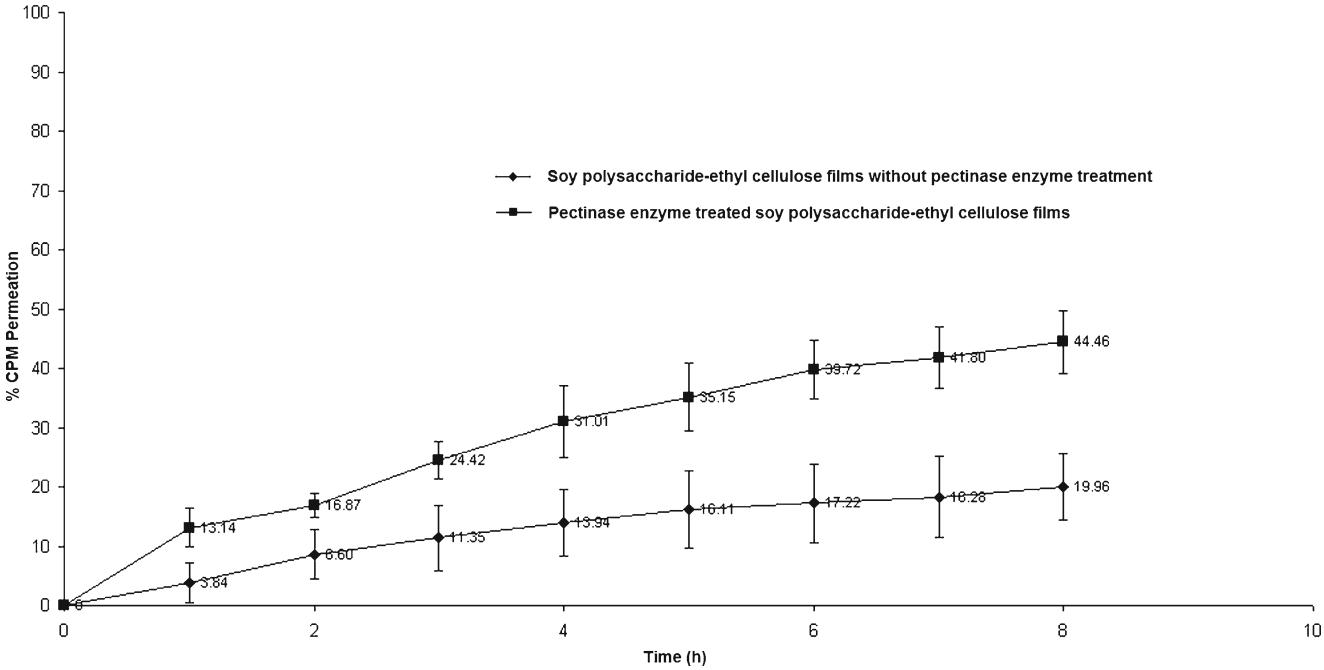

Fig. 3. *In vitro* permeation of CPM through pectinase enzyme-treated and pectinase enzyme-untreated soy polysaccharide–ethyl cellulose films (n=3)

performed after 4, 6, 7, 8, 9, and 24 h after the administration of the radiolabelled capsule. The gamma scintigraphic studies showed that coated CPM capsules were intact for 9 h in all the human volunteers and scintigraphic images taken at the end of 24 h showed 90% to complete release of the radioactive material from the capsules. The representative gamma scintigraphic images from one volunteer have been shown in Fig. 8.

DISCUSSION

Polysaccharides have a long history of use in the colon targeted drug delivery. Polysaccharides such as pectin, various types of starches, and gums are commonly used for the fabrication of colon targeted delivery systems. In the present investigation, the potential of soy polysaccharide for colon drug delivery was evaluated. The commercially available soy polysaccharide (Emcosoy®) is a mixture of the polysaccharides such as pectin, cellulose, and hemicellulose. Since pectin (one of the components of soy polysaccharide) has been widely used for colon targeted delivery systems, it was anticipated that soy polysaccharide could also have potential in colon drug delivery. As no characterization data are available for Emcosoy®, it was important to characterize the polysaccharide for morphology and feasibility of film formation. Optical

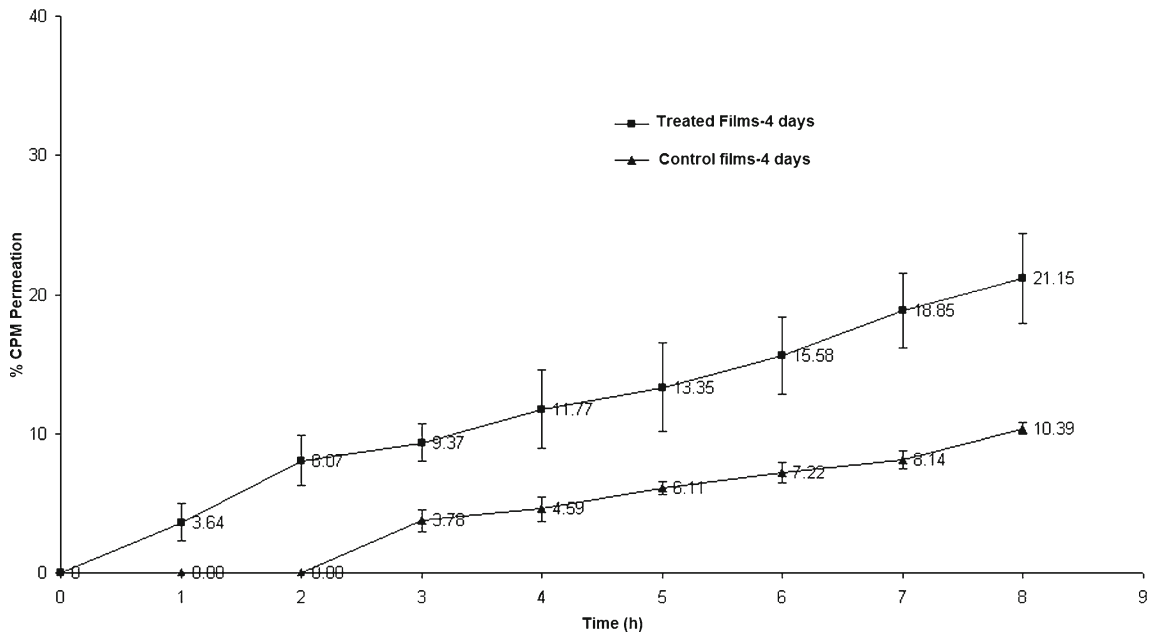

Fig. 4. *In vitro* permeation of CPM through control films-4 days and treated films-4 days (n=3)

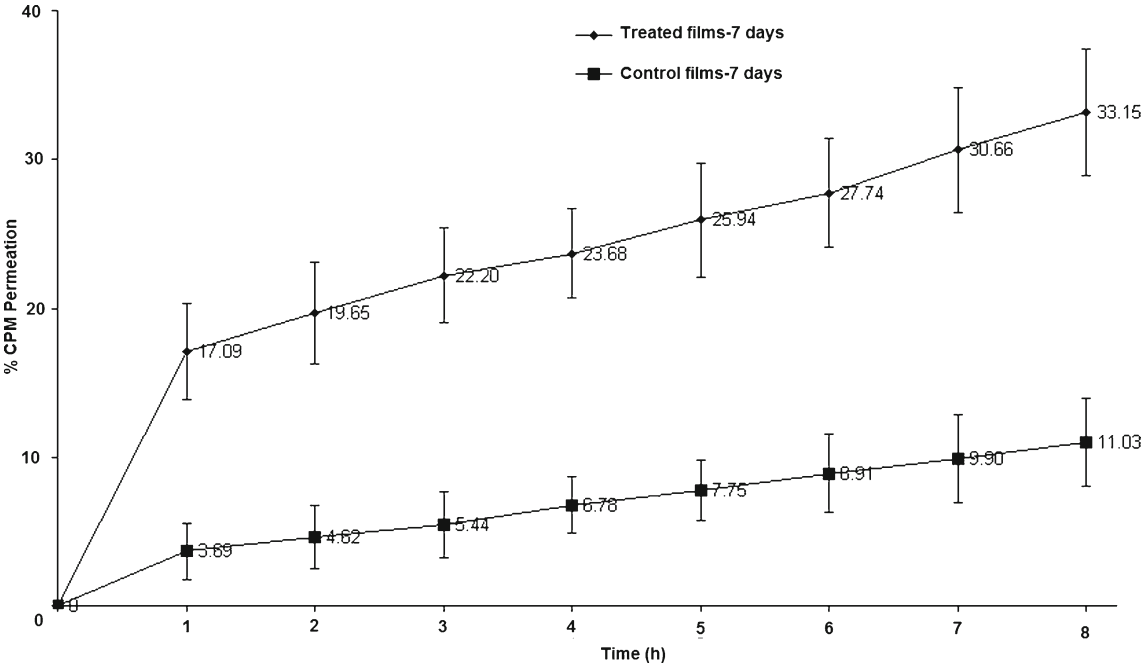

Fig. 5. *In vitro* permeation of CPM through control films-7 days and treated films-7 days ($n=3$). Control films-4 days: soy polysaccharide–ethyl cellulose films incubated for 4 days in the VPI diluent fluid medium without *Lactobacillus sp*.; treated films-4 days: soy polysaccharide–ethyl cellulose films incubated for 4 days in the VPI diluent fluid medium inoculated with *Lactobacillus sp*

microscopy revealed crystalline nature of the dry soy polysaccharide (Fig. 1a) whereas hydrated soy polysaccharide showed transparent swollen particles suggesting the swelling of gums and mucilage present in the soy polysaccharide (Fig. 1b). The soy polysaccharide alone could not form acceptable films for coating (data not shown). It has been reported that colon targeted delivery systems based on polysaccharides (pectin or starch derivatives) employ a mixture of polysaccharide and ethyl cellulose (a water-insoluble polymer with excellent film forming capability) (24,25). The use of ethyl cellulose was anticipated to augment film formation. Furthermore, presence of ethyl cellulose in the film coating also reduces swelling of the polysaccharide in the film coating which helps in reducing the drug leaching in the stomach and small intestine. Thus, feasibility of forming soy polysaccharide–ethyl cellulose blends was evaluated. It was possible to obtain films of uni-

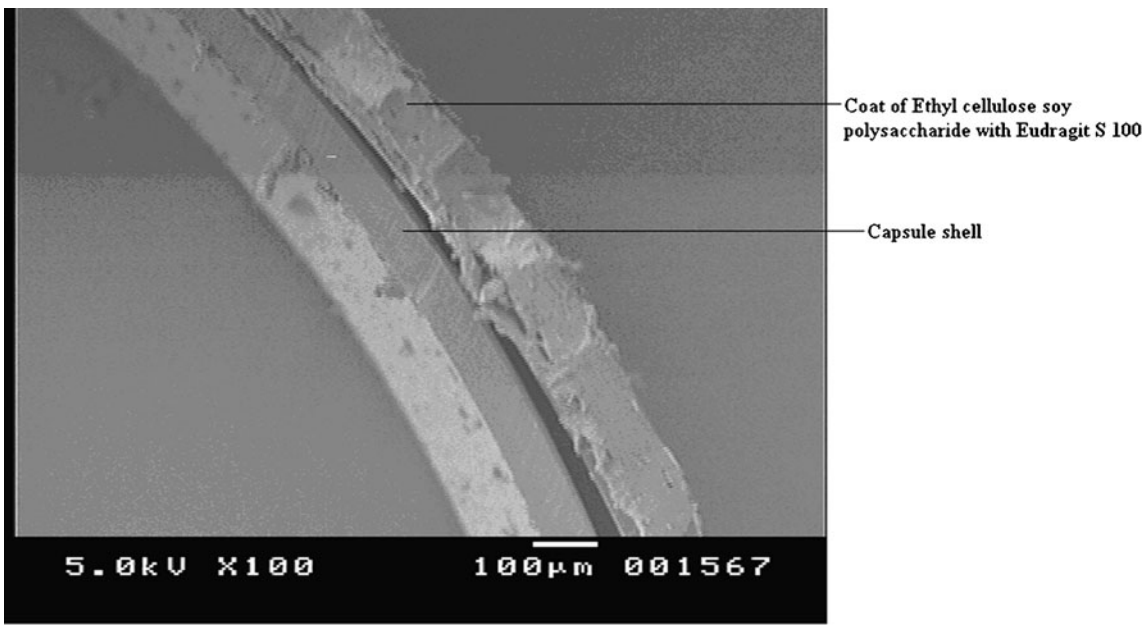

Fig. 6. SEM of transverse section of the coated CPM capsules. Control films-7 days: soy polysaccharide–ethyl cellulose films incubated for 7 days in the VPI diluent fluid medium without *Lactobacillus sp*.; treated films-7 days: soy polysaccharide–ethyl cellulose films incubated for 7 days in the VPI diluent fluid medium inoculated with *Lactobacillus sp*

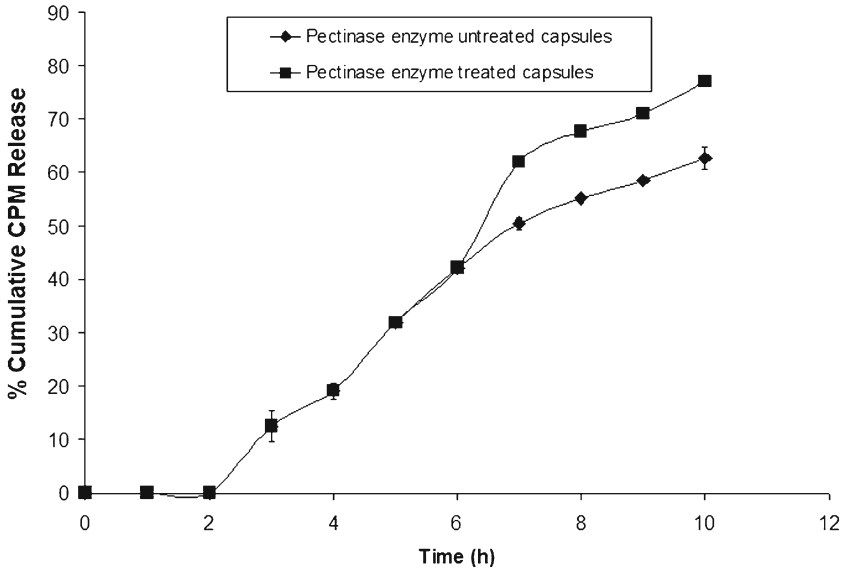

Fig. 7. *In vitro* release profile of CPM through coated CPM capsules ($n=3$)

form thickness using soy polysaccharide–ethyl cellulose mixture. The soy polysaccharide–ethyl cellulose films with 20% of the soy polysaccharide could be successfully fabricated. It has been reported in the literature that incorporation of polysaccharides such as pectin in the ethyl cellulose films significantly influences various film properties such as water vapor transmission and swelling index (24). Furthermore, Karrout *et al.* observed that the energy required for breaking the ethyl cellulose–polysaccharide film decreases with the increase in the polysaccharide content in the films (25). In view of this, we evaluated the effect of incorporation of soy polysaccharide on the tensile strength of the ethyl cellulose films. The tensile strength of the plain ethyl cellulose films (prepared as per Table I excluding soy polysaccharide) was 9.25 ±3.33 N/mm^2 whereas soy polysaccharide–ethyl cellulose films had tensile strength of 3.23±0.51 N/mm^2. The incorporation of the soy polysaccharide significantly altered the film properties. The reduction in the tensile strength could be attributed to the alteration of ethyl cellulose film integrity due to presence of soy polysaccharide particles. The treatment of soy polysaccharide–ethyl cellulose films with pectinase resulted in reduction of the tensile strength which could be due to degradation of the pectin present in the film matrix. The SEM of the pectinase enzyme-treated films showed film defects such as perforations (Fig. 2b) which could be due to pectinase-mediated degradation of the polysaccharide components whereas the pectinase-untreated soy polysaccharide–ethyl cellulose films did not show such surface imperfections (Fig. 2a).

To further evaluate the effect of the pectinase enzyme treatment on the films, the permeation of a model drug chlorpheniramine maleate (CPM) through films was studied. The CPM was selected as a model drug due to its good water solubility and permeability which would preclude any constraints with respect to maintaining sink conditions. The *in vitro* CPM permeation studies clearly showed that the percent CPM permeation through pectinase enzyme-treated films is significantly higher than that of pectinase enzyme-untreated films ($p<0.05$). Thus, *in vitro* CPM permeation studies corroborated the results obtained with tensile strength and SEM studies. In order to enable colonic drug delivery, soy polysaccharide should be degraded in the colon by colonic microflora. It is well known that *Lactobacillus* sp. are present in colonic microflora (22), and they secrete pectinolytic enzymes (26). Hence, the soy polysaccharide–ethyl cellulose films were stored in the *Lactobacillus* sp. culture for 4 and 7 days, and the permeation of CPM through the control and bacterial culture-treated films was studied. Interestingly, the bacterial culture treated soy polysaccharide–ethyl cellulose films showed significantly higher permeation of the CPM as compared with the bacterial culture-untreated (control) films. It is true that the exposure of the films to bacterial culture for 4 and 7 days does not mimic real-time situation. However, the colon has several microorganisms that can augment degradation of the soy polysaccharide, and our *in vivo* gamma scintigraphic studies corroborate this statement. As only one bacterial species (*Lactobacillus* sp.) was selected for *in vitro* studies, we had to increase the contact period in order to evaluate possibility of degradation of soy polysaccharide by bacteria. In order to obviate possibility of hydrolytic degradation of the polysaccharides, control films without bacterial culture were also prepared and used for permeation studies. In short, this study clearly indicated potential of soy polysaccharide in the colon drug delivery.

The soy polysaccharide–ethyl cellulose mixture was coated onto CPM capsules using fluid bed coater, and the capsules were further coated with Eudragit S100, a pH sensitive polymer soluble at pH 7.4. The coating of the capsules with Eudragit S100 was important to prevent the drug release in the upper gastrointestinal tract after *in vivo* administration. The use of Eudragit S100 as a protective coating has been reported for tablets containing polysaccharide core (13,27,28). Furthermore, it has been shown that tablets coated with a mixture of Eudragit S100 and polysaccharide have better chances of accurate colonic drug delivery due to dual trigger (29). Hence, the coating of capsules with Eudragit S100 was considered to be advantageous. Due to presence of the Eudragit S100 coating, the coated CPM capsule did not show any CPM release in the pH 1.2 buffer whereas when the

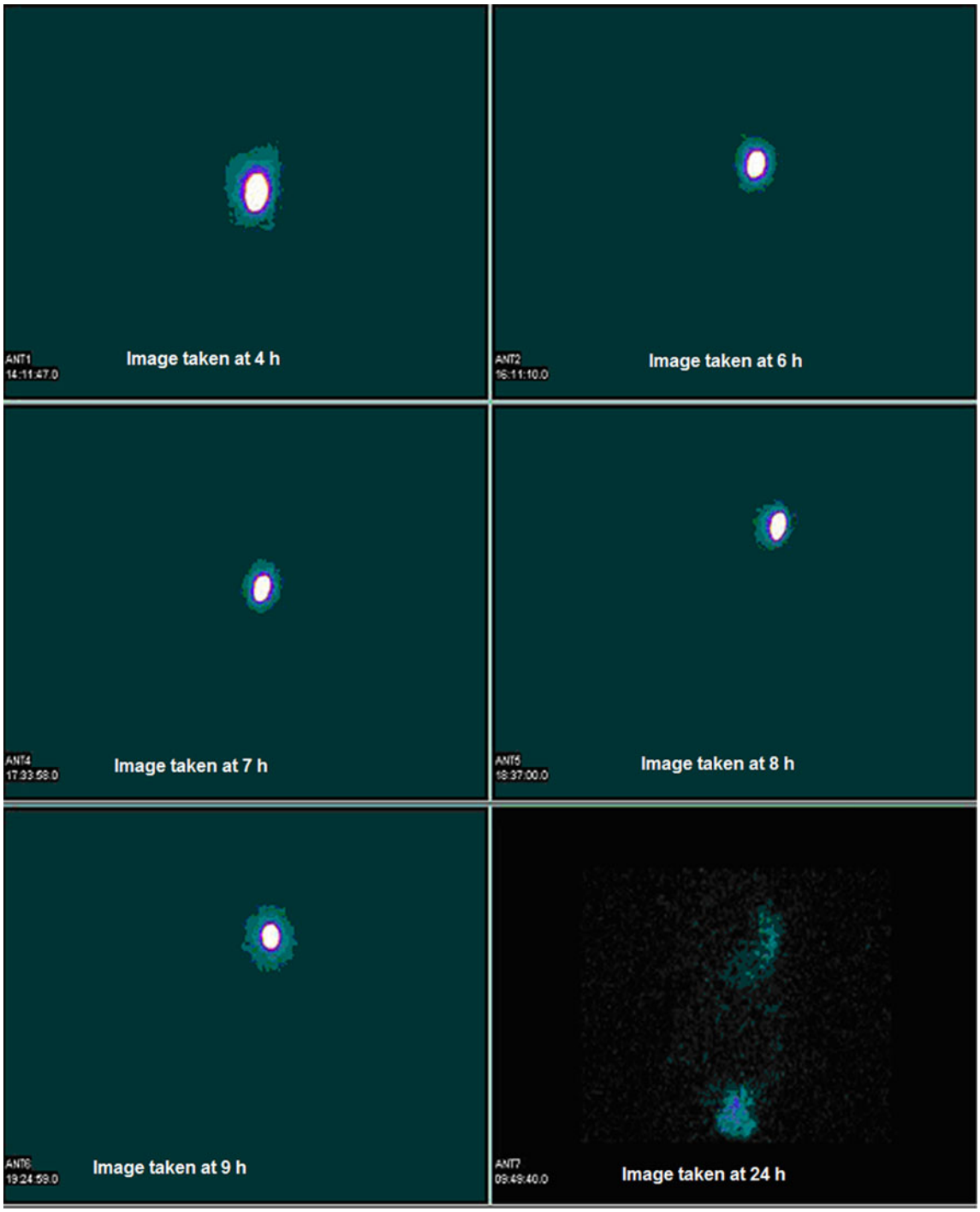

Fig. 8. Representative gamma scintigraphic images of the radiolabelled CPM capsules coated with soy-polysaccharide–ethyl cellulose and Eudragit S100 on oral administration

dissolution medium was replaced with pH 7.4, the CPM release was observed due to dissolution of the Eudragit S100 coat. The addition of the pectinase enzyme to the dissolution medium showed greater CPM release from the capsules due to degradation of the polysaccharide matrix by pectinase enzyme. The radiolabelling of the coated CPM capsules was achieved by a simple method, and the efficiency of the method was validated. The radiolabelled capsules were administered to the human volunteers to establish the *in vivo* proof of concept. The gamma scintigraphic studies indicated that the radiolabelled capsules were almost intact for 9 h indicating the potential of soy polysaccharide-coated capsules in the colon drug delivery. Interestingly, the gamma scintigraphic images taken at 24 h after administration showed presence of radioactivity in bladder and a part of colon (Fig. 8) indicating complete disintegration of the soy polysaccharide matrix *in vivo*.

There are very limited investigations in the literature that report use of coated capsules for the colon targeting. Han et al. have shown that capsules composed of Eudragit S100,

Eudragit RSPO, guar gum, and hydroxypropylmethyl cellulose start releasing the contents in the colon after 7.5 h (30). Schellekens *et al*. have reported that capsules coated with Eudragit S100 and superdisintegrant can give a pulse time of around 5 h (31). It is also noteworthy that tablets coated with Eudragit S100 and polysaccharide show a median lag time of 7.2 h (29) whereas tablets coated with pectin–chitosan–HPMC films released 20–30% drug in 7 h in the human subjects (23). Interestingly, the capsules described in the present investigation were almost intact for 9 h. This could be due to the presence of the double coating of soy polysaccharide–ethyl cellulose and Eudragit S100. Since Eudragit S100 and soy polysaccharide–ethyl cellulose coated capsules showed tendency for higher lag time, they might find applications in the pulsatile release systems for nocturnal asthma or angina pectoris.

CONCLUSION

The soy polysaccharide can form uniform films with ethyl cellulose which could be successfully used for coating of capsules. The soy polysaccharide degrades in the presence of pectinase enzyme and colonic microflora such as *Lactobacillus sp*. The soy polysaccharide–ethyl cellulose mixture coating can be useful for the colon drug delivery as evidenced by the gamma scintigraphic studies in human volunteers.

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