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Research paper

Preparation and characterization of free mixed-film of pectin/chitosan/Eudragit® RS intended for sigmoidal drug delivery

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Abstract

Polyelectrolyte complex (PEC) film between pectin as an anionic polyelectrolyte and chitosan as a cationic species was prepared by blending two polymer solutions at weight ratio of 2:1 and then solvent casting method. Besides pectin/chitosan PEC film, Eudragit® RS, pectin/Eudragit® RS and pectin/chitosan/Eudragit® RS films were also prepared by aforementioned method. In mixed-film formulations, a fixed weight ratio of 1:5 of pectin or pectin/chitosan complex to Eudragit® RS was used. Characterizations of pectin/chitosan interaction in solution were investigated by turbidity and viscosity measurement and in the solid state by Fourier transform infrared (FTIR) spectroscopy, wide angle X-ray diffraction (WAXRD) and thermogravimetric analysis (TGA). It was observed that the swelling profile of pectin/chitosan film was pH-dependent and its swelling ratio in phosphate buffer solution (PBS) pH 7.4 was about 2.5-fold higher than that of PBS pH 6.0. Formulation containing only pectin/chitosan could not protect free film from high swelling in the aqueous media, therefore, Eudragit® RS as a water-insoluble polymer must be included in the mixed-film. The formation of PEC between pectin and chitosan resulted in a decrease in the crystallinity and thermal stability caused by the interactions between polyions. Drug permeation or diffusion studies were carried out using Plexiglas diffusion cell consisting of donor and acceptor compartments. Theophylline was selected as a model drug to measure permeability coefficient. Drug permeation through pectin/chitosan/Eudragit® RS showed a sigmoidal pattern; whereas drug diffusion through pectin/Eudragit® RS and Eudragit® RS films followed a linear characteristic. The drug permeation through the ternary mixed-film showed a burst release upon exposure to PBS pH 6.0. This mixed-film formulation showed the potential for sigmoidal drug delivery with an initial, controllable slow release followed by a burst release immediately after the change in pH. The burst drug permeation might possibly be due to change in film's porosity.

Keywords: Chitosan; Eudragit® RS; Mixed-film; Pectin; Polyelectrolyte complex; Sigmoidal drug delivery

1. Introduction

The use of mixed-films in drug delivery is for optimizing the physicochemical and permeability properties of the resultant films [1].

It is well known that the release characteristics of film-coated controlled release formulations are strongly dependent on the permeability of the film [2]. Aulton [3] suggested that evaluation of free films as a means to predict the properties of applied films will be experimentally economical and statistically efficient. Variables such as the substrate to which the film is initially applied and the application technique must be considered. In general, a free film may be prepared by casting or by spraying the polymer

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solution onto an inert substrate to mimic a spray-coating process.

Pectin is a natural, non-toxic and anionic polysaccharide extracted from cell walls of most plants. Pectin consists mainly of linearly connected α-(1-4)-D-galacturonic acid residues partially esterified with methanol. The degree of methoxylation (DM) is used to classify the pectins as high methoxyl pectins (DM > 50) and low methoxyl pectins (DM < 50) [4,5].

Chitosan is a linear cationic polysaccharide prepared from chitin, found in shells of shrimps, lobsters and crabs. Chitosan consists of *N*-acetyl glucosamine and is classified according to the degree of deacetylation (DDA) [6].

Combinations of pectin and chitosan form a polyelectrolyte complex (PEC) at pH values in the range of 3–6 [7,8]. In addition to the formation of a PEC, pectin and chitosan also interact by hydrogen bonding at low pH values (pH < 2). At these pH values, pectin will be unionized and the importance of electrostatic interactions is suppressed, and an interaction between pectin and chitosan will probably take place via hydrogen bonding [9].

Blends of the aforementioned polymers have been studied as excipients in colon drug delivery systems for example in press-coated tablets [10], hydrogel beads [11] and as film coating in combination with HPMC [12] and without HPMC [13].

The major problem encountered with the native degradable polysaccharides is their high solubility in aqueous media. Consequently, film coatings consisting of these polymers alone or even combination of them will be unable to prevent the release of drugs during the transit through the stomach and the small intestine. However, the incorporation of hydrophilic degradable polysaccharides in water-insoluble film-forming polymers, such as cellulosic or acrylic polymers, could provide a promising alternative.

Semdé and co-workers investigated the suitability of free mixed-films, prepared from water-insoluble polymers, i.e. ethylcellulose and acrylic polymers (Eudragit® RS30D and Eudragit® NE30D) containing pectin or calcium pectinate for colonic drug delivery. They concluded that the association of pectin with Eudragit® RS30D was likely to give a suitable colonic coating material for colon-specific drug delivery [14].

In other investigations [1,8,15], the PEC between pectin and chitosan was formed *in situ* (via exposure of pectin and chitosan physical mixture to the simulated gastrointestinal medium). While, for fabrication of mixed film in the current work, firstly, pectin/chitosan PEC was prepared and then it was added to the Eudragit® RS30D.

The aim of the current study was to prepare and characterize some physicochemical properties of pectin/chitosan/Eudragit® RS, pectin/Eudragit® RS and pectin/chitosan mixed-films. Turbidity and viscosity measurement, Fourier transform infrared (FTIR) and ^{1}H NMR spectroscopy, Wide angle X-ray diffraction (WAXRD), thermogravimetric analysis (TGA), scanning electron microscopy (SEM) as well as the drug diffusion through the mixed-films have been carried out for this purpose. The background for this study was the intended use of the mixed-film as a coating material for sigmoidal drug delivery. Fig. 1 shows the molecular structures of the pectin, chitosan and Eudragit® RS.

2. Materials and methods

2.1. Materials

Pectin (from citrus fruit) and chitosan (viscosity of 1% w/v in acetic acid solution (1% v/v), 34 mPas) were employed as ingredients for PEC and mixed-film formulation (Sigma–Aldrich, Dorset, UK). Eudragit® RS30D was kindly donated by Röhm Pharma GmbH (Darmstadt, Germany) and used as another ingredient for mixed-film fabrication. Eudragit® RS30D is a 30% w/w aqueous latex of poly (ethylacrylate methylmethacrylate trimethylammonioethyl methacrylate chloride). Theophylline anhydrous purchased from BASF (Aktiengesellschaft, Germany) was used as a model drug. Pectinex® Ultra SP-L (pectinolytic enzymes, extracted from *Aspergillus niger*) was supplied a Novo Nordisk Ferment (Dittingen, Switzerland). Other chemicals employed in this work included acetic acid, hydrochloric acid, sodium hydroxide and triethyl citrate (Merck, Darmstadt, Germany). Distilled water was freshly prepared.

2.2. Methods

2.2.1. Determination of the degree of esterification of pectin

The methoxy and galacturonic acid content of pectin was determined by the titrimetric method of Food Chemical Codex (FCC) IV [16] and US Pharmacopeia (USP) XXVI [17] with slight modification. Dried sample of pectin (500 mg) was transferred to a 250-mL flask, moistened with 2 mL of ethanol and dissolved in 100 mL of carbon dioxide–free water. After the sample was completely dissolved, five drops of phenolphthalein were added, the sample was titrated with 0.5 M sodium hydroxide and the result was recorded as the *initial titer*. Then, 10 mL of 0.5 M sodium hydroxide was added, the sample was shaken vigorously, and allowed to stand for 15 min; 10 mL of 0.5 M hydrochloric acid was added and the sample was shaken until the pink color disappeared. Phenolphthalein (five drops) was added and the solution was titrated with 0.5 M sodium hydroxide to a faint pink color that persisted after vigorous shaking (end-point). This volume of titration was recorded as the *saponification titer* (the final titer). Each milliliter of 0.5 M sodium hydroxide used in the *saponification titer* and in the total titration (the *initial titer* added to the *saponification titer*) was equivalent to 15.52 mg of methoxy and 97.07 mg of galacturonic acid, respectively. The degree of methoxylation (DM) or degree of esterification (DE) of pectin was calculated from the following formula:

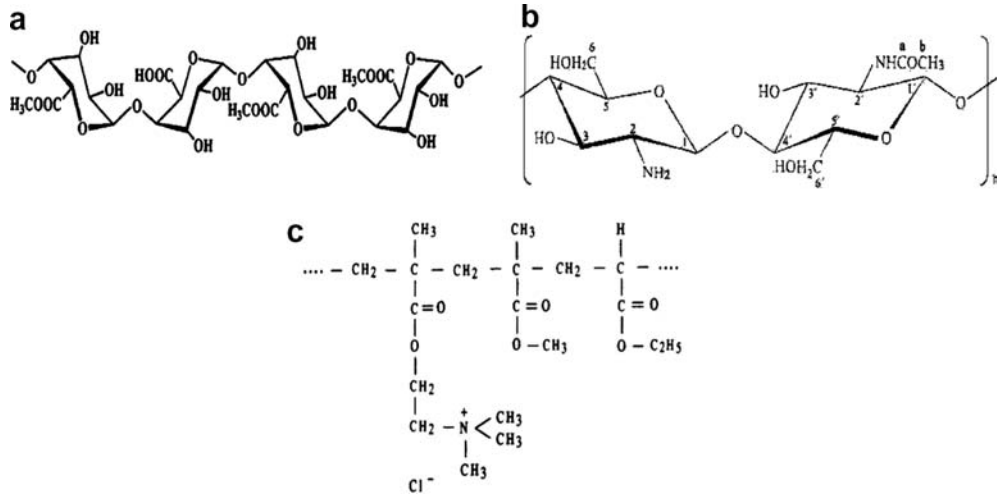

Fig. 1. Schematic representation of (a) pectin, (b) chitosan and (c) Eudragit® RS structures.

$$\%\mathrm{DE} = [\text{the final titer}/(\text{the initial titer} + \text{the final titer})] \times 100 \quad (1)$$

2.2.2. Intrinsic viscosity and estimation of molecular weight of pectin

Intrinsic viscosity measurement of pectin solutions (0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 mg/mL) was performed with a Cannon-Fenske capillary viscometer (Cannon Instrument Co., PA, USA) at 25 ± 0.1 °C (three determinations per concentration). The solvent was 1% w/w of sodium hexametaphosphate $(NaPO_3)_6$ pH 4.5. Intrinsic viscosity ([η], mL/g) was estimated by fitting Martin's equation to the data as follows:

$$\log \eta_{sp}/C = \log[\eta] + K[\eta]C \quad (2)$$

In Eq. (2), C represents the polymer concentration in g/mL and K is a constant.

Mean molecular weights, M, were then estimated from the [η] values using the Mark–Houwink relationship:

$$[\eta] = KM^{\alpha} \quad (3)$$

Anger and Berth [18] have calculated $K = 9.55 \times 10^{-2}$ (mL/g) and $\alpha = 0.73$ for this type of pectin and we used above values for K and α.

2.2.3. Determination of the degree of deacetylation of chitosan

Chitosan was dissolved in D_2O/DCl (100:1) solution and the ^{1}H NMR spectrum (500 MHz) was obtained (DRX 500 model, Bruker, Switzerland). The degree of deacetylation (DDA) of chitosan was determined by the following equation [19]:

$$\mathrm{DDA}(\%) = \left(\frac{\text{H1-D}}{\text{H1-D} + \text{H-Ac}/3}\right) \times 100 \quad (4)$$

where H1-D and H-Ac are the integrals of the peak of anomeric proton H1 of deacetylated monomer and of the peak of the three protons of acetyl or acetamido group, respectively.

2.2.4. Estimation of molecular weight of chitosan

Resembling the above explanations for pectin (Section 2.2.2), the intrinsic viscosity ([η], mL/g) of chitosan solutions (0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 mg/mL) was measured with a Cannon-Fenske capillary viscometer in 0.2 M CH_3COOH/0.1 M CH_3COONa at 30 ± 0.1 °C (three determinations per concentration). According to the experiments of Wang and colleagues, the values obtained for K and α were not constant if the DDA of chitosan changed [20]. They found $K = 1.424 \times 10^{-3}$ (mL/g) and $\alpha = 0.96$ for chitosan with 84% of DDA. Thus, in this study, the stated values for K and α were used.

2.2.5. Viscosity and turbidity measurements in pectin/chitosan complexation

Appropriate volumes of aqueous pectin solutions in the concentration range of 0.0125–0.05% (w/w) were added to conical flasks, followed by aqueous chitosan solutions in 0.1 M acetic acid, in the same concentration range of pectin, to give a total volume of 100 mL and a specified pectin/chitosan weight ratio. A minimum of eight solutions were prepared at pH values of 1.5 (hydrochloric acid), 3.8 and 5.4 (acetate buffer). The flasks were shaken in a water bath at 37 °C for 24 h. The presence of a gel-like precipitate was observed in some of the flasks. The contents of each flask were filtered under vacuum through filter paper (Whatman, GF/D grade) and the specific viscosities (η_{sp}) of the resultant supernatant determined by a calibrated, Cannon-Fenske capillary viscometer. The specific viscosity was determined according to the following equation:

$$\eta_{sp} = \eta/\eta_0 - 1 \quad (5)$$

where η is the viscosity of sample and η_0 is the viscosity of hydration medium.

Moreover, the formation of the polyelectrolyte complex through ionic interaction between pectin and chitosan at various weight ratios and pH values was also investigated by turbidity method. In this method, the transmittance of dispersions was measured at a wavelength of 600 nm (where no absorption due to the polymers took place) by using a spectrophotometer (UV-1601, Shimadzu, Japan).

2.2.6. Preparation of mixed-films

2.2.6.1. Formation of pectin/chitosan PEC and mixed-polymeric dispersions. Chitosan and pectin were dissolved separately in 0.1 M acetic acid and distilled water, respectively. The chitosan solution was then added to the pectin aqueous solution slowly. After all chitosan was added, the pH of the blend was adjusted to 5.4 with either 0.1 M HCl or 0.1 M NaOH solutions and allowed to react one hour under mechanical stirring. Afterward, the PEC formed between pectin and chitosan was filtered and the gel filtrate was washed with 0.1 M acetic acid to remove free chitosan and washed again with warm water to eliminate free pectin. According to Ref. [21] along with slight modification, pectin/chitosan complex was dried at 50–60 °C and subsequently dissolved in 10% v/v formic acid. Then, the appropriate amount of the pectin/chitosan or pectin solution was added to the Eudragit® RS30D that was previously mixed for 2 h with triethyl citrate (TEC) as a plasticizer (10% w/w, related to the dry solid content of Eudragit® RS30D). Mixed-polymeric dispersions were prepared according to the formulas given in Table 1. According to our previous study, the maximum yield of PEC formation between pectin and chitosan at pH 5.4 was acquired in pectin/chitosan ratio of 2/1 [22]. Therefore, in the present study, whenever pectin–chitosan complex will be cited, it denotes the above ratio. In all mixed-film formulations (Table 1), a fixed weight ratio of 1:5 of pectin or pectin–chitosan to Eudragit® RS was used.

2.2.6.2. Preparation of mixed-films from mixed-polymeric dispersions. Mixed-polymeric dispersions were cast using a self-constructed automatic film application apparatus. The dispersions (12 mg/cm^2) were cast on the surface of exactly horizontal aligned Teflon® coated glass plates of 180×350 mm. Glass plates were placed on a planar aluminium plate, which was kept at a constant temperature of 30 °C controlled by a mobile electronic temperature control unit (Kassel, Germany). The polymeric dispersions (Table 1) were filled into a film applicator made of aluminium with a gap clearance of 0.8×150 mm. The film applicator moved at a constant speed of 2.5 mm/s on the upper surface of the Teflon® coated glass plates. The mixed-polymeric dispersions spread onto the glass plate and the film formation process started. Warming up the dispersion to 50 °C resulted in enhancing its spreading property on the Teflon® coated glass plates, due to the decreased hydrogen bond forces at increased temperatures. To complete this process and for tempering purposes, free films were cured for 24 h at 45 °C. The resulted mixed-films were allowed to cool and stored in a desiccator at room temperature until use. The film thickness was measured at six different places by using a micrometer (Neuss, Germany). The film formation, homogeneity of content and the appearance of the film surface were examined by visual observation, and also by scanning electron microscopy (Section 2.2.13).

2.2.7. Swelling studies on mixed-films

Mixed-film samples (1×2 cm) were cut from the bulk film. Samples with thicknesses in the range of 80–120 μm were tested by placing in phosphate buffer solution (PBS) pH 6.0 or pH 7.4 at 37 ± 0.5 °C. The sample weights were accurately determined (±0.0001 g) at 10, 30, 60, 90, 120, 150 and 180 min after carefully blotting dry each sample. Swelling was expressed as a swelling ratio (SR):

$$\mathrm{SR} = [(W_t - W_0)/W_0] \times 100 \quad (6)$$

where W_0 is weight of dry sample (g) and W_t is weight of sample at time t (g). Swelling tests were separately carried out in simulated small intestinal medium (PBS pH 7.4) and simulated colonic fluid (PBS pH 6.0 included pectinex® Ultra SP-L). All experiments were carried out in triplicate.

2.2.8. Fourier transform infrared (FTIR) spectroscopy

About 2% (w/w) of pectin, chitosan, Eudragit® RS, pectin/chitosan complex and pectin/chitosan/Eudragit® RS, with respect to the potassium bromide (KBr) disc, was mixed with dry KBr (FTIR grade, Aldrich, Germany). The mixture was ground into a fine powder using an agate mortar before compressing into a disc. Each disc was

Table 1
Composition details for mixed-film formulations

Ingredients	Pectin/chitosan/Eudragit RS film	Pectin/Eudragit RS film	Eudragit RS film	Pectin/chitosan film
Eudragit® RS30D (g)	10	10	12	–
Eudragit® RS (g, in dry solid content)	3	3	3.6	–
Pectin (g)	NA[a]	0.6	–	NA
Pectin/chitosan complex[b] (g)	0.6	–	–	3.6
Triethyl citrate (g)	0.3	0.3	0.3	0.3

[a] Not applicable: pectin alone was not incorporated in pectin/chitosan/Eudragit RS and pectin/chitosan films.
[b] Weight ratio of pectin/chitosan = 2/1.

scanned at a resolution of 4 cm^{-1} over a wavenumber region of 400–4000 cm^{-1} using a FTIR spectrometer (Avatar, Nicolet Magna-IR™ 550 spectrometer, USA) coupled to a personal computer with Omnic analysis software. The characteristic peaks of IR transmission spectra were recorded.

2.2.9. Wide angle X-ray diffraction (WAXRD)

The wide angle X-ray diffraction patterns of the pectin, chitosan, Eudragit® RS, pectin/chitosan complex and pectin/chitosan/Eudragit® RS were measured by an X-ray diffractometer (X′Pert model, Philips, The Netherlands). X-ray diffraction was performed on the samples by exposing them to CuKα_1 radiation (40.0 kV, 30.0 mA) and scanned from $2\theta = 3°$ to 80° at a step size of 0.02° and step time of 0.5 s.

2.2.10. Thermogravimetric analysis (TGA)

Thermogravimetric analysis of pectin, chitosan, Eudragit® RS, pectin/chitosan complex and pectin/chitosan/Eudragit® RS was performed using a thermogravimetric analyzer (TGA 951, TA Instruments, USA).

TGA was performed with a 7–10 mg sample in aluminium pans under a dynamic nitrogen atmosphere. The experiments were run at a heating rate of 10 °C/min.

2.2.11. Diffusion and permeability studies on mixed-films

Drug (theophylline) permeation tests were conducted in horizontal side-by-side Plexiglas diffusion cells consisting of donor and acceptor compartments. Film samples measuring (circle, diameter of 2.95 cm) were cut with a scalpel and the film thickness measured at six different places with a micrometer. Samples with mean thickness values in the range of 80–120 μm were selected and mounted between the donor and acceptor compartments. The volume of each compartment was 75 mL.

Theophylline (0.15% w/w) was used as the permeant (donor) in medium solutions of PBS pH 7.4 and/or PBS pH 6.0 including Pectinex® Ultra SP-L (0.4% v/v). These solutions were chosen, as they are likely to be encountered in the small intestine and colon, respectively. To achieve the sink conditions for permeability experiments, nearly saturated concentration of drug was used in the donor compartment. The acceptor cells contained blank phosphate buffer solutions. The diffusion cell was placed in a water bath maintained at 37 ± 0.5 °C and each compartment was stirred continuously with a magnetic stirrer. At predetermined time intervals, 2 mL of medium from acceptor cell was sampled and replaced by an equal volume of fresh medium for a period of 4 h. The drug content was assayed spectrophotometrically at 271.7 nm (UV-1601, Shimadzu, Japan). Each permeation experiment was repeated three times and the cumulative amount of drug permeated and corrected for the acceptor sample replacement was plotted against time.

The permeability coefficients (P) of the various mixed film formulations were calculated by using Eq. (7) [23,24].

$$P = K_{app}H/A \tag{7}$$

where K_{app} is the rate of drug diffusion, which is obtained from the slope of the linear drug permeation profiles, H is the film thickness (cm) and A is the surface area of the film (cm^2). The permeability coefficients (μg/min/cm) can be regarded as being obtained under pseudo-steady state conditions, as the membrane will be changing by dissolution and swelling during the experiment.

2.2.12. Statistical analysis

All of the experiments were done in triplicate. One-way analysis of variance was performed to assess the significance of the differences among data. Tukey–Kramer post-test was used to compare the means of different treatment data. Results with $p < 0.05$ were considered statistically significant.

2.2.13. Microscopic study

Surface morphology of pectin/chitosan, Eudragit® RS and pectin/chitosan/Eudragit® RS films was studied under a scanning electron microscope (SEM) (XL30 model, Philips, The Netherlands). The films were attached to the slab surfaces with double-sided adhesive tapes and then coated with gold to a thickness of approximately 30 nm under vacuum to make the films conductive.

3. Results and discussion

3.1. Pectin specifications

According to titrimetric method (Section 2.2.1), the methoxy and galacturonic acid content of pectin was obtained 8.9% and 88.2%, respectively. The degree of methoxylation or esterification of pectin, which was calculated from Eq. (1), was 63.2%. Therefore, this type of pectin is classified as high methoxylated (HM) pectin.

The results from capillary viscometry study of pectin in $(NaPO_3)_6$ showed that the intrinsic viscosity ($[\eta]$) and molecular weight of pectin were 261 mL/g and 5×10^4 Da, respectively.

3.2. Chitosan specifications

The degree of deacetylation (DDA) is one of the most important properties of chitosan because of its influence on the physicochemical properties of this polymer and its applications. In the current work, ^{1}H NMR was used for the DDA determination of chitosan. The ^{1}H NMR spectrum of chitosan is shown in Fig. 2. The signal centered at $\delta \approx 1.7$ ppm corresponds to the hydrogens of the methyl moieties belonging to the acetamido groups (H-Ac). The signal observed at $\delta \approx 2.8$ ppm corresponds to the hydrogen bonded to the C_2 glucosamine ring of deacetylated monomer (Fig. 1b), while the signals between 3.1 and 3.8 ppm correspond to hydrogens bonded to the carbon atoms C_3, C_4, C_5 and C_6 of the glucopyranose that are

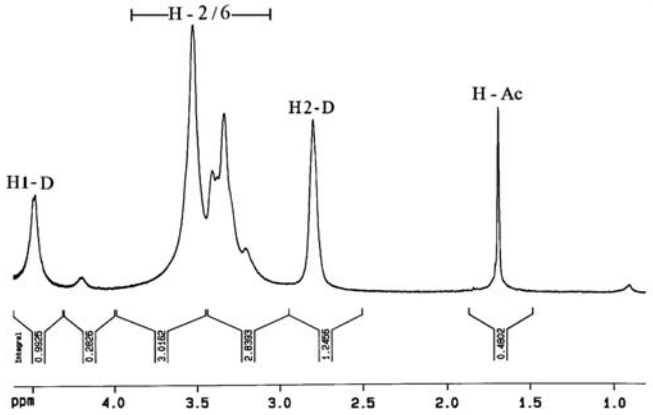

Fig. 2. ^{1}H NMR spectrum of chitosan sample in D_2O/DCl (100:1).

overlapped. The hydrogen bonded to the anomeric carbon (C_1) of deacetylated monomer (H1-D) gives rise to the signals in the range of $4.4 < \delta < 4.7$ ppm. According to Fig. 2 and Eq. (4), the value of 86.11% was estimated for DDA of chitosan.

Viscosity measurement of chitosan in 0.2 M CH_3COOH/0.1 M CH_3COONa showed that the intrinsic viscosity ([η]) and molecular weight of chitosan were 115.6 mL/g and 1.3×10^5 Da, respectively.

3.3. Viscosity and turbidity analysis in mixture of pectin and chitosan

The formation of PEC between pectin and chitosan leads to the production of a precipitate and a concomitant reduction in the specific viscosity (η_{sp}) of the resultant supernatant. Optimum conditions for PEC formation thus occur at the minimum viscosity of the supernatant. On either side of this optimum, the viscosity will increase to that of the original pectin or chitosan solution. Fig. 3 shows the relationship between specific viscosity of the supernatant and pectin concentration. The minimum occurs at different concentrations depending on the pH of the reaction. Both pectin and chitosan solutions alone show η_{sp} proportional to concentration. Similarly, at a pH of 1.5, no PEC is formed and there is a linear relationship between η_{sp} and the percent pectin in the reaction mixture.

PECs are formed by the electrostatic combination of the protonated amine group on the chitosan molecule and the negatively charged carboxylate group on the polyanion molecule, e.g. pectin [25,26]. In order to produce the optimum amount of PEC, increasing amounts of pectin are required as the pH is lowered because a lower percentage of the carboxylic acid groups will be ionized. At the pH values considered, a $pK_a = 6.3$ [27] for chitosan would mean that it had an extensive degree of ionization, even at the highest pH (5.4). At the extremely low pH where hydrochloric acid (pH 1.5) is the solvent, there is insufficient ionization of the pectin to form the PEC at any ratio. Therefore, as shown in Fig. 3, η_{sp} varies linearly with percent pectin in the reaction mixture, indicating a lack of non-ionic interaction between the two polymers. These results are similar to those of Chavasit and co-workers who noted that no insoluble complex was detected between chitosan and polyacrylic acid at pH values below 2.0 [28].

PEC formation was also investigated using turbidity measurement. Fig. 4 shows turbidimetric titration curves of pectin by solutions of chitosan with different concentrations of both components at different pH values. The appearance of turbidity is caused by the formation of a low solubility product of hydrophobic nature with further aggregation of polymeric chains. The point of maximum turbidity (minimum transmittance) corresponds to the ratio in which the components form the PEC and depends on the pH of the medium. The results obtained from turbidity measurement were fitted well to the viscosity analysis. As a result, the optimum pectin/chitosan ratio for PEC formation lay somewhere between 2/1 and 3/1 for pH 5.4 and 3/1 for pH 3.8.

By mixing chitosan and pectin solutions under acidic condition (pH 1.5), it is possible to obtain a homogeneous solution without any ionic crosslinking (PEC) between them. The PEC is then obtained by adjusting the pH to 3.8 or 5.4 by addition of 0.1 M NaOH solution. Polyelec-

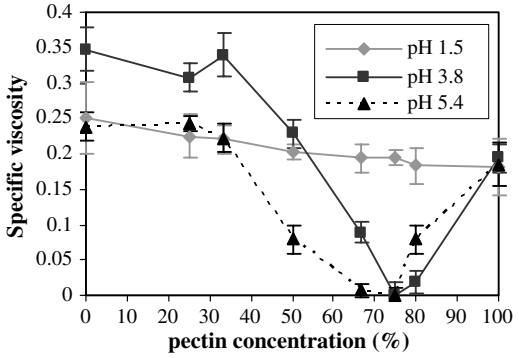

Fig. 3. Specific viscosity of the supernatant versus pectin concentration (%) in the mixture of pectin and chitosan at different pH values (mean ± SD, $n = 3$).

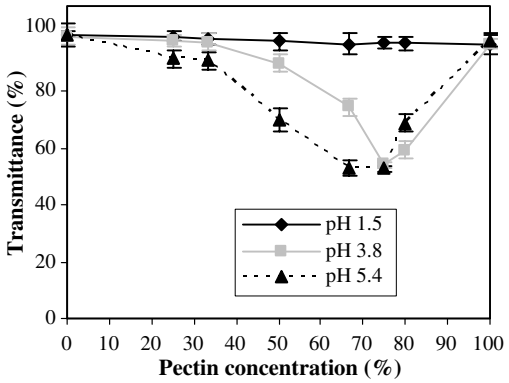

Fig. 4. Transmittance change versus pectin concentration (%) in the mixture of pectin and chitosan at different pH values (mean ± SD, $n = 3$).

Table 2
Composition of polyelectrolyte complexes of pectin and chitosan at pH 5.4

PEC samples	Pectin/chitosan weight ratio (%) w/w	Product yield (%)
PEC 1	33.3:66.7	5.2
PEC 2	50.0:50.0	28.1
PEC 3	66.7:33.3	70.9
PEC 4	75.0:25.0	66.3
PEC 5	80.0:20.0	27.8

trolyte complex reaction between pectin (P—COOH) and chitosan (C—NH_2) can be represented schematically as follows:

$$P—COOH + C—NH_3^+ \rightleftarrows P—COO^{-+}NH_3 - C + H^+$$

The extent of above reaction is dependent on the pH of the medium. Table 2 shows the product yield of PEC samples at pH 5.4 when the pectin/chitosan ratio increases. The yield was calculated as a percentage of initial polysaccharide content. The optimum weight ratio was taken at the point where a maximal yield of solid complex was obtained. The maximum yield was obtained when the ratio of pectin/chitosan was 66.7:33.3 (% w/w).

3.4. Swelling studies on films

Fig. 5a shows the swelling profile of pectin/chitosan film. This PEC film is hydrophilic and swells considerably in phosphate buffer solutions, reaching a maximum swelling ratio after 60 min. This process shows pH-dependent pattern and the swelling ratio at pH 7.4 is about 2.5 times higher than that pH 6.0. At pH 6.0, in viewpoint of pK_a of pectin and chitosan (4.0 and 6.3, respectively), over 99% of pectin will be in its ionized form, i.e. COO^- and chitosan will exist as both NH_3^+ and NH_2. Within the PECs, there also exists the possibility of intramolecular H-bonding between the OH or $COOCH_3$ groups within the network. However, at pH 7.4, the amine group of chitosan will be partially unionized. Therefore, at this pH, the PEC network will be looser as a result of suboptimal $NH_3^{+-}OOC$ ionic interaction. In other words, stronger ionic interaction may result in a tightening of the PEC network leading to a reduced swelling capacity at pH 6.0 as compared with pH 7.4.

As it can be clearly seen from Fig. 5b, inclusion of Eudragit® RS to pectin/chitosan film reduces the swelling ratio. However, the swellability profiles of pectin/chitosan/Eudragit® RS at two different pH values have similar characters as compared with pectin/chitosan film. During swelling experiments, films containing pectin/chitosan had been highly expanded in size (especially at pH 7.4); whereas films with Eudragit® RS did not show so much expansion. According to the specification of Röhm Pharma Co., Eudragit® RS is recommended as a pH-independent carrier and this can be obviously observed in Fig 5b, i.e. no significant difference ($p > 0.05$) in Eudragit® RS swelling ratio at pH 6.0 and 7.4.

3.5. Fourier transform infrared (FTIR) spectroscopy

The infrared spectra of the mixed polymers and their components are depicted in Fig. 6.

In the FTIR spectrum of pectin (Fig. 6a), bands related to C=O stretching of the ester and carboxyl group could be observed at 1751.92 and 1613.66 cm^{-1}, respectively [29]. The C=O stretching vibration (amide I) at 1642.42 $cm^{-1}$and the NH bending (amide II) band at 1619 cm^{-1} region were observed in the IR spectrum of chitosan [30]. The spectrum of pectin/chitosan complex indicated the main changes in the range of 1800–1600 cm^{-1}, evidence of the interaction of the amino and carboxyl groups. In the spectrum of pectin and chitosan physical mixture at 2:1 ratio, two relatively broad bands in the region of 1800–1600 cm^{-1} resulted and it might be attributed to bands overlapping. The intensity of the

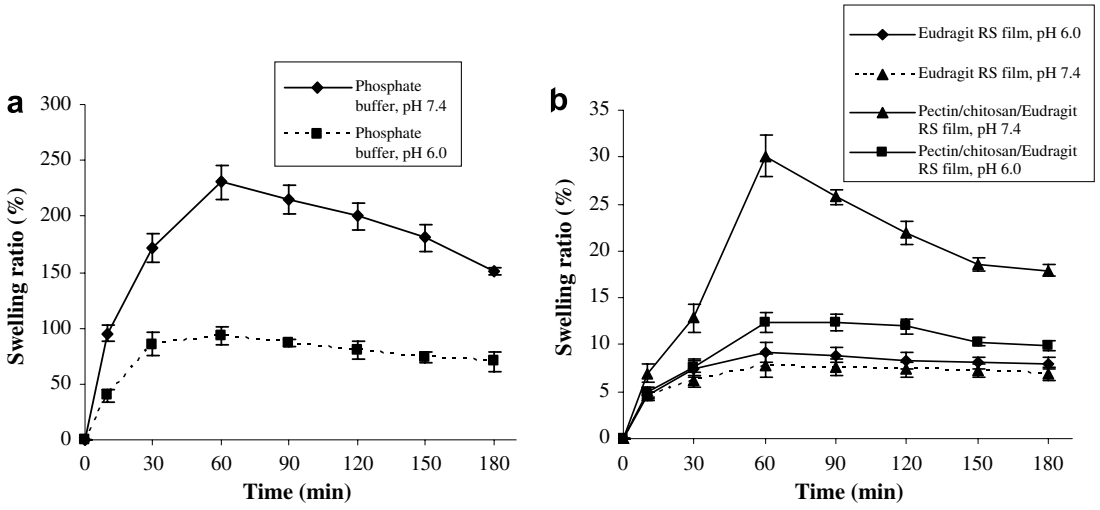

Fig. 5. Swelling ratio (%) versus time (min) for (a) pectin/chitosan film, (b) Eudragit® RS and pectin/chitosan/Eudragit® RS films; immersed in different phosphate buffer solutions at 37 ± 0.5 °C (mean ± SD, $n = 3$).

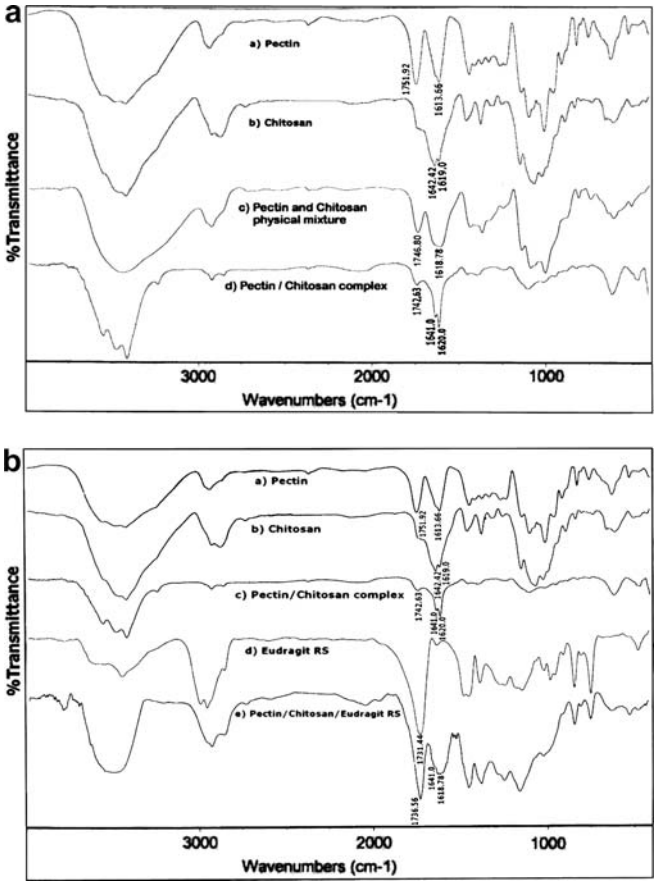

Fig. 6. FTIR spectra of (a) pectin, chitosan, their physical mixture and polyelectrolyte complex, (b) pectin/chitosan/Eudragit® RS blend and its components.

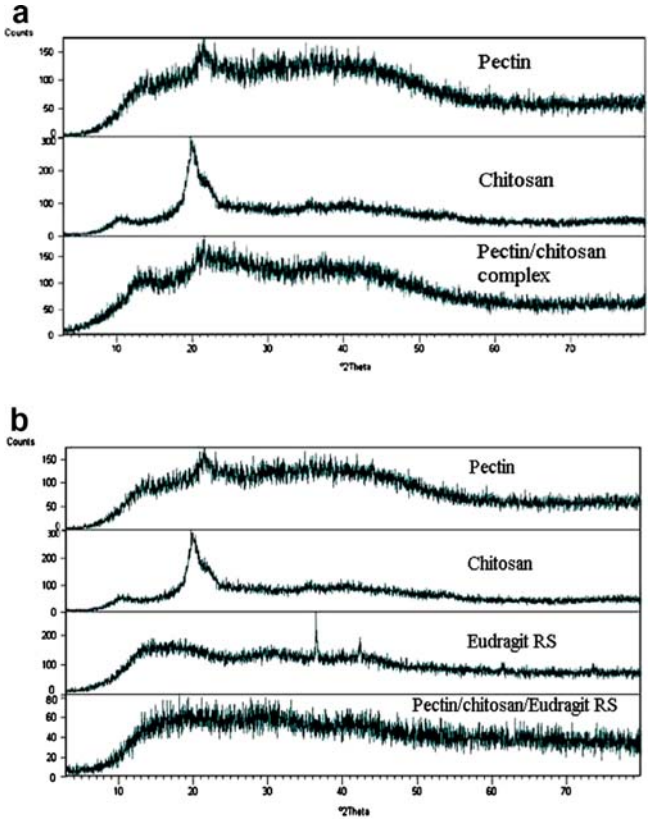

Fig. 7. X-ray diffraction patterns of (a) pectin/chitosan complex and its components, (b) pectin/chitosan/Eudragit® RS blend and its components.

esterified carboxyl group at 1751.92 cm^{-1}, which is typical of pectin, decreased and shifted to a lower frequency, i.e. 1742.63 cm^{-1} following pectin/chitosan complexation. A strong peak at 1620 cm^{-1} (asymmetric stretching vibration of carboxylate) appeared and indicated the formation of interchain or intermolecular ionic salt bonds, i.e. PEC between amino groups of chitosan and carboxyl groups of pectin [31]. In other words, it was assumed that pectin/chitosan complex formation proceeded at the expense of electrostatic interaction between the positively charged amino groups on C_2 of the chitosan pyranose ring and the negatively charged carboxyl groups on C_5 of the pectin pyranose ring and led to formation of a crosslinked polyelectrolyte complex.

In Fig. 6b, Eudragit® RS showed a strong and sharp characteristic band of C=O ester vibration at 1731.44 cm^{-1}. The pectin/chitosan/Eudragit® RS showed identical IR spectra, indicating no significant interactions between the pectin/chitosan and Eudragit® RS.

3.6. Wide angle X-ray diffraction

Powder X-ray diffraction patterns of pectin, chitosan and pectin/chitosan complex are demonstrated in Fig. 7a. Pectin showed a diffraction peak at 21.3° (2θ). The diffractogram of pure pectin showed that it was mostly amorphous. The diffraction peaks at approximately $2\theta = 11°$ and $2\theta = 20°$ were observed for chitosan. It can be noted that the chitosan powder was of semicrystalline state. In the PEC between pectin and chitosan, the characteristic peaks of chitosan disappeared and the halo diffraction pattern was observed, indicating an amorphous state. This can be explained by the strong interactions like hydrogen bonds and ionic interaction between pectin and chitosan, which has destroyed the close packing of the chitosan molecules for the formation of regular crystallites. The diffractogram of pure Eudragit® RS is shown in Fig. 7b. Eudragit® RS showed two diffraction peaks ($2\theta = 36.4°$, 42.3°), confirming the presence of crystalline domains in its structure. This result was not in agreement with another study [32]. In that study, it was reported that Eudragit® RS was an amorphous polymer and did not show any peak in its diffraction pattern. However, it would seem that the crystallinity percent of Eudragit® RS was negligible. Diffractogram of pectin/chitosan/Eudragit® RS was similar to that of pectin/chitosan and it would seem that its amorphous characteristic was more considerable than that of pectin/chitosan.

3.7. Thermogravimetric analysis

Fig. 8 shows the results of thermogravimetric analysis (TGA) of pectin, chitosan, Eudragit® RS, pectin and chito-

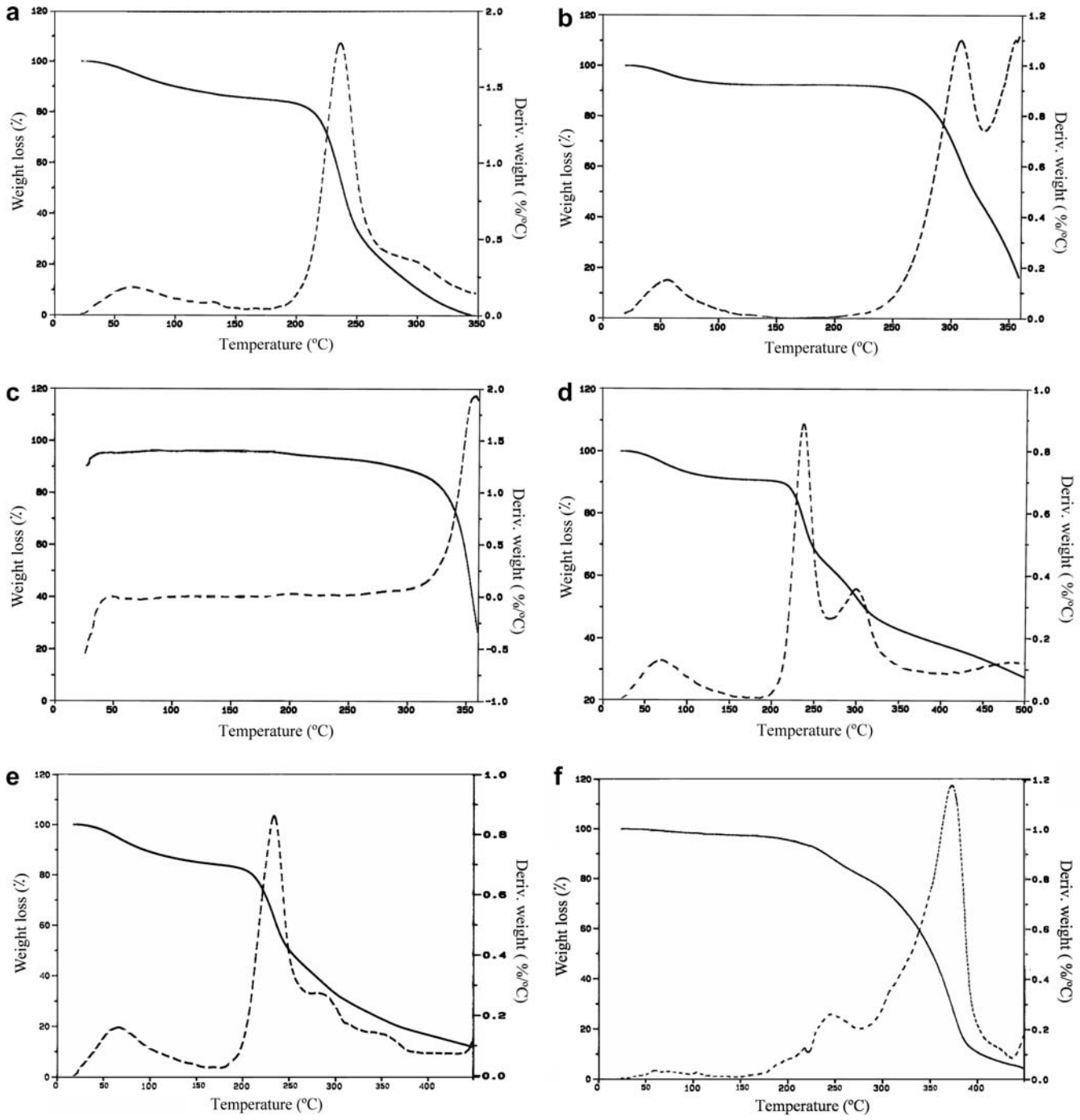

Fig. 8. Thermogravimetric analysis of (a) pectin, (b) chitosan, (c) Eudragit® RS, (d) pectin and chitosan physical mixture, (e) pectin/chitosan complex and (f) pectin/chitosan/Eudragit® RS blend.

san physical mixture, pectin/chitosan complex and pectin/chitosan/Eudragit® RS blend. The pure pectin thermal degradation consisted of two stages and the first thermal event was a weight loss of up to 10% in the range of 50–100 °C, which was related to the evaporation of water presented in the sample. The second thermal event started at 200 °C with the maximum at 234 °C and it was related to depolymerisation of pectin chains. The pure chitosan also showed the first thermal event centered at 56.7 °C, which was related to the evaporation of unbound water. The second thermal event for chitosan was observed at about 200 °C with the maximal rate at 308 °C. In the case of Eudragit® RS, no thermal event was observed up to 300 °C and the first thermal event occurred at maximal rate of 354 °C. In the pectin and chitosan mixture, the first thermal event was similar to both of them and there were two peaks in the second thermal event as a function of derivative weight loss, i.e. 240 and 300 °C that could be related to

pectin and chitosan, respectively. The degradation of the pectin/chitosan complex showed only event characteristic for the pectin. The absence of the event typical for chitosan at about 300 °C in this complex indicated on some interactions between the polymers and might be considered as a proof of their miscibility and complexation. Moreover, a weak thermal event at about 350 °C could be observed in the thermogram of pectin/chitosan complex, which was not observed in the physical mixture of the two polymers. The degradation profile of the blend containing pectin/chitosan/Eudragit® RS consisted of the events typical for both pectin and Eudragit® RS. This behavior could be observed for degradation of polymeric mixtures and indicated on their immiscibility in the ternary blend.

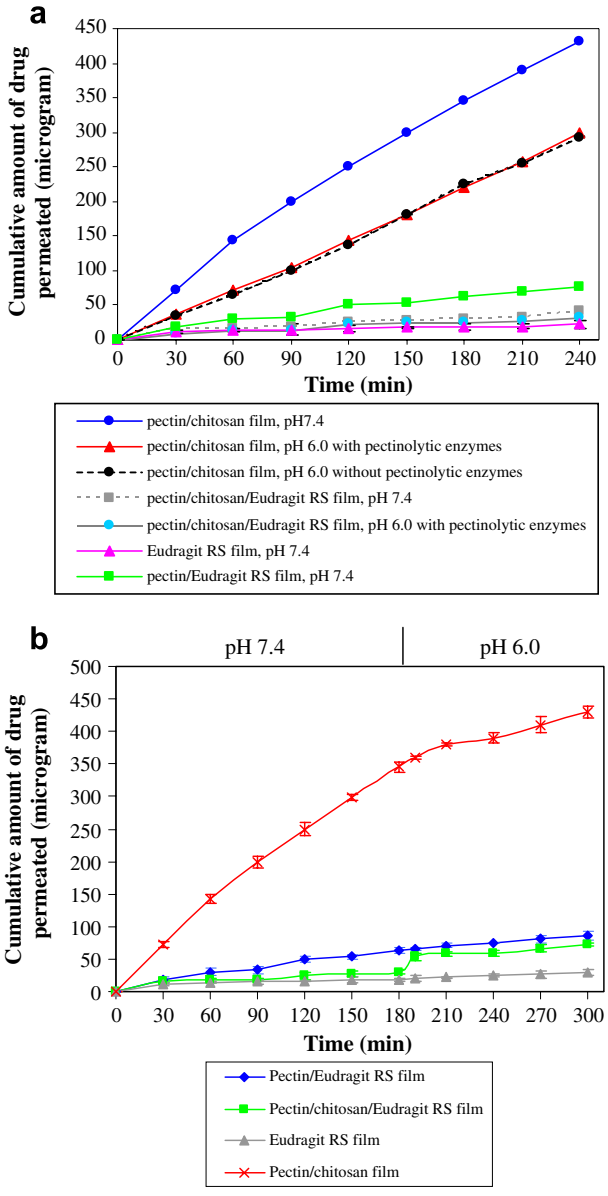

Fig. 9. Permeation of drug through mixed-films in (a) phosphate buffer solutions pH 7.4 or pH 6.0 (with or without pectinolytic enzymes), (b) phosphate buffer solution pH 7.4 and then pH 6.0 at 37 ± 0.5 °C; in the upper part, each point represents the mean value of data ($n = 3$) and in the lower one, each bar diagram signifies mean ± SD ($n = 3$).

3.8. Diffusion or permeability studies on mixed-films

In this investigation, theophylline was chosen as a model basic drug ($pK_a = 8.77$ [33]), so that variation in drug permeation would not be due to ionization but be attributed to the physicochemical properties of the films and the changes they undergo in the diffusion media. The influence of mixed-film composition on the cumulative amount of drug permeated within 4 h at different media, i.e. PBS pH 7.4 and PBS pH 6.0 (with or without pectinolytic enzymes), is shown in Fig. 9a. The rate of drug permeation through the pectin/chitosan film at pH 7.4 was higher than that of pH 6.0 and this was well correlated to the swelling study at those pH values (Section 2.2.7). The decrease in the permeability of pectin/chitosan film with the inclusion of Eudragit® RS might be due to the hydrophobic and low water permeability of Eudragit® RS. In Fig. 9a, it can be observed that Eudragit® RS film depicted the lowest drug permeability as compared with the other mixed-films. According to Fig. 9a and Table 3, the difference in drug permeability at PBS pH 6.0 was not significant ($p > 0.05$) for the pectin/chitosan film in the presence or absence of pectinolytic enzymes. Our experiment showed that the pectinolytic enzymes did not influence significantly on the film's permeability.

In Fig. 9b, the drug permeation through the films at PBS pH 7.4 (simulated small intestinal fluid) for the first 180 min and then 60 min at PBS pH 6.0 with Pectinex® Ultra SP-L (simulated colonic medium) is demonstrated. Pectin/chitosan/Eudragit® RS showed a sigmoidal drug permeation pattern; whereas drug permeation through pectin/Eudragit® RS and Eudragit® RS films followed a relatively linear characteristic. In the case of pectin/chitosan film, a decrease in diffusion rate could be seen in the PBS pH 6.0 as compared with PBS pH 7.4. These results can be described by the following explanations.

Generally, drug can be permeated through polymeric membranes (films) by two mechanisms: the *pore* mechanism and the *partition* mechanism [34,35]. In the pore mechanism, drugs permeate the polymeric membrane by diffusion through pores within the membrane at a rate controlled mainly by the pore size of the membrane and the molecular

Table 3
The influence of film composition on the drug permeability coefficient (mean ± SD, $n = 3$)

Film	Permeability coefficient (P) × 10^4 (μg/min/cm)
Pectin/chitosan film, in PBS pH 7.4	26.18 ± 0.58
Pectin/chitosan film, in PBS pH 6.0 with pectinolytic enzymes	15.31 ± 0.19
Pectin/chitosan film, in PBS pH 6.0 without pectinolytic enzymes	15.29 ± 0.08
Pectin/Eudragit® RS film, in PBS pH 7.4	7.12 ± 0.14
Pectin/chitosan/Eudragit® RS film, in PBS pH 7.4	1.97 ± 0.22
Pectin/chitosan/Eudragit® RS film, in PBS pH 6.0 with pectinolytic enzymes	1.67 ± 0.16
Eudragit® RS film, in PBS pH 7.4	1.08 ± 0.13

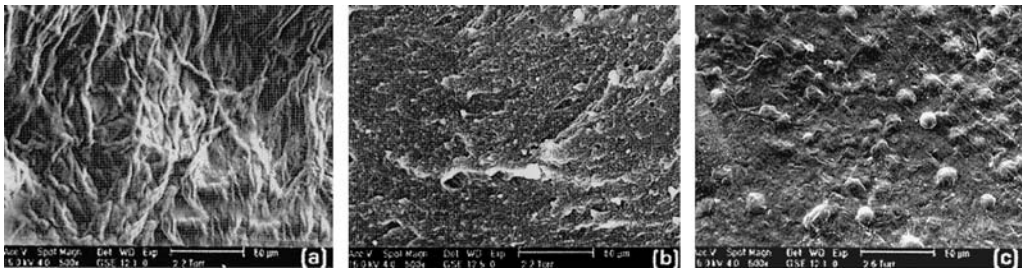

Fig. 10. SEM micrographs of the surface of pectin/chitosan film (a), Eudragit® RS film (b) and pectin/chitosan/Eudragit® RS film (c).

volume of the drug. The partition mechanism involves drug dissolution in the polymer structure followed by drug diffusion along and between the polymer segments that make up the membrane structure. In practice, drug permeation probably occurs by both mechanisms but one is more likely to predominate [34]. The pectin/chitosan moiety in the ternary blend is hydrophilic and as mentioned previously, its swelling ratio at pH 7.4 is higher than that at pH 6.0. However, according to Fig. 5b, the swellability profile of Eudragit® RS is pH-independent. Exposure of pectin/chitosan to PBS pH 7.4 results in swelling and after medium exchange, shrinkage of pectin/chitosan can occurr. In the simulated colonic medium, the void spaces between pectin/chitosan and Eudragit® RS chains and subsequently the film's porosity will increase and therefore, a burst (sudden) drug permeation could be observed. In other words, at pH 7.4, a fairly "tight" network is formed, while at pH 6.0 a more "open" network is expected with a larger average distance between polymeric chains. In conclusion, ternary mixed-film showed initial burst drug permeation in the colonic medium and might be attributed to an increase in the film's porosity at pH decrease from 7.4 to 6.0. After burst release, a steady state in the drug diffusion was observed. Permanently increased porosity should lead to increase diffusion throughout the colonic medium, while the observed reduction in diffusion rate down to what was observed prior to the change in pH requires a subsequent rearrangement of the polymer network.

Shrinkage (deswelling) of pectin/chitosan might be the reason for decreasing of diffusion rate in the PBS pH 6.0 compared to PBS pH 7.4.

According to the following equation [36], a higher porosity leads to an increase in the diffusion coefficient, which results in a higher drug permeability and a faster permeation rate.

$$D = D_w e/\tau \quad (8)$$

where D is the diffusion coefficient of the drug, D_w represents the diffusion coefficient in the medium, e is the porosity factor and τ is the tortuosity factor. Therefore, changes in the film's porosity during contact to PBS, pH 6.0, would alter the diffusion coefficient, resulting in an increase in drug permeation rate.

Table 3 shows the effect of mixed-film and medium composition on the drug permeability coefficient. As shown in Table 3, drug permeability coefficient related to pectin/Eudragit® RS was higher than that of pectin/chitosan/Eudragit® RS ($p < 0.05$). The higher water-solubility of pectin compared with that of pectin/chitosan might be the reason for obtained result. On the other hand, dissolution mechanism was compatible with channel formation through the pectin/Eudragit® RS film by pectin dissolution. Free film containing Eudragit® RS had the lowest permeability to drug in all the formulations ($p < 0.01$). Lower permeability at pH 6.0 than that at pH 7.4 was also observed for pectin/chitosan films and it was in agreement with the swelling results (Fig. 9 and Table 3).

3.9. Films' morphology

Fig. 10 shows the SEM micrographs of the pectin/chitosan, Eudragit® RS and pectin/chitosan/Eudragit® RS films. It can be seen from Fig. 10b, the Eudragit® RS film has relatively homogeneous and smooth morphology. SEM of ternary mixed-film (Fig. 10c) showed particle aggregation and had rough surface due to PEC formation between pectin and chitosan. It can be also resulted that the blend of pectin/chitosan with Eudragit® RS showed some immiscibility.

4. Conclusions

Films of the polyelectrolyte complex between pectin and chitosan, pectin/Eudragit® RS, Eudragit® RS and pectin/chitosan/Eudragit® RS were prepared by casting/solvent evaporation method. With theophylline as a model drug, we studied the films' structures and characterizations, especially its potential capacity in drug delivery system. The results of the present investigation verified the formation of polyelectrolyte complex (PEC) between pectin and chitosan at pH values in the vicinity of the pK_a interval of the two polymers. The optimal weight ratio of pectin to chitosan for PEC formation was 66.7:33.3% w/w, which produced highest product yield. Pectin/chitosan film was hydrophilic and swelled in PBS. Its swelling process was pH-dependent. The results showed that pure pectin/chitosan film exhibited high swelling ratio and its swellability tended to decrease with the addition of Eudragit® RS. In other words, formulation containing only pectin/chitosan was unable to protect premature swelling and drug release

in simulated small intestine medium, therefore, Eudragit® RS as a water-insoluble polymer must be included in the mixed-film formulation. The examination of the FTIR and TGA properties of the pectin/chitosan/Eudragit® RS film revealed that the pectin/chitosan and Eudragit® RS were not miscible in the ternary blend. All mixed-films were in amorphous form.

This study confirmed the potential of pectin/chitosan/Eudragit® RS as a mixed-film blend, capable of achieving sigmoidal drug delivery. Contrary to our previous study [22], pectinolytic enzymes could not influence significantly on drug diffusion through this system. However, it would seem that the mechanism of sigmoidal drug permeation through that mixed-film was due to the pH-dependent swelling of pectin/chitosan.

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