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Preparation, characterization and properties of partially hydrolyzed ethylene vinyl acetate copolymer films for controlled drug release

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ABSTRACT

In this study, partially hydrolyzed ethylene vinyl acetate (EVA) copolymers with three hydrolysis degrees (12.2%, 32.6% and 46.9%) were obtained by alkaline hydrolysis of EVA copolymer, characterized by Fourier-transform infrared spectroscopy (FTIR), ^{1}H NMR and gel permeation chromatography (GPC). Paclitaxel-loaded and drug-free films based on the partially hydrolyzed EVA copolymers were fabricated. The swelling behaviors, crystallinities, mechanical properties of the fabricated films were investigated, and the effects of hydrolysis degree, film thickness and drug loading dose on *in vitro* drug release from the films were also investigated. *In vitro* swelling study showed that the swelling of partially hydrolyzed EVA films was greater than the EVA film and the film with higher hydrolysis degree swelled more intensively. X-ray diffraction (XRD) results exhibited that the crystallinity of the polymer increased with increasing hydrolysis degree. In paclitaxel-loaded EVA film, a part of paclitaxel was in crystalline form; while in paclitaxel-loaded partially hydrolyzed EVA films, paclitaxel was distributed in amorphous form or molecularly dispersed. In the *in vitro* drug release test, the film with higher hydrolysis degree and smaller thickness released paclitaxel more quickly. With higher drug loading dose, the drug release rate was larger. The partially hydrolyzed EVA films were applied for drug delivery systems for the first time, and demonstrated to have great capability of controlling drug release thanks to the adjustable hydrolysis degree.

1. Introduction

Although the systemic route of drug administration is available, it has intractable drawbacks due to the safety, systemic toxicity and dose-limited efficacy (Huang et al., 2007). Consequently, more and more attention has been paid to the local drug delivery during the past few decades. With the development of polymer material science, various polymeric devices have been investigated to achieve the local delivery of drugs (Cheng et al., 2010; Dorta et al., 2002; Li et al., 2010).

Ethylene vinyl acetate (EVA) copolymer, a flexible, heat-processable, stable and inexpensive material (Miyazaki et al., 1982), is widely studied for broad applications, such as hot melt adhesive (Park et al., 2006), modifiers of mortars and concretes (Mansur et al., 2009), wire and cable sheathing (Bahattab et al., 2010) and medical catheters (Ringrose and Kronfli, 2000). On account of its good biocompatibility, EVA has been utilized to prepare various local drug delivery systems in order to achieve sustained and controlled drug release. Studies showed that drug-loaded EVA rods provided stable drug levels for 6 months and presented no adverse effects (Costantini et al., 2004). EVA films were also used as matrix systems for the delivery of different drugs (Guo et al., 2007b; Tallury et al., 2007) and proteins (Amsden and Cheng, 1995).

As is well known, controlling of drug release is the major challenge in local drug delivery. Previous studies have shown that the release of drugs from EVA films can be regulated by changing the vinyl acetate content (VAc) and drug loading dose (Arnold et al., 2008; Cho et al., 2005; Guo et al., 2007a; Kim and Shin, 2004; Shin and Lee, 2002). Furthermore, adding PEG to EVA films (Fishbein et al., 2001) and coating EVA films with polymer materials (Lesser et al., 1996; Tallury et al., 2007) can effectively increase and decrease the rate of drug release, respectively. However, slow release of drug is always encountered in EVA-based matrix systems, which may not achieve the therapeutic threshold in practical use, thus more effective strategies should be adopted to facilitate the release of drug.

Hydrolysis of EVA can change the acetate groups on polyethylene backbone into hydroxyl groups (Fig. 1) and enhance the hydrophilicity of the polymer. Ethylene vinyl alcohol (EVAl)

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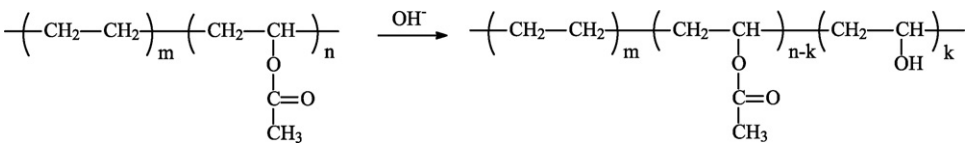

Fig. 1. The hydrolysis of EVA.

copolymer, a polymer consists of hydrophobic ethylene units and hydrophilic vinyl alcohol units, can be obtained by complete hydrolysis of EVA. With good biocompatibility, EVAl has been studied as a matrix material of drug delivery systems over the past few decades (Arnold et al., 2008; Coluccio et al., 2005; Lai et al., 2006; Seki et al., 1989; Shieh et al., 2002; Xu and He, 1998, 2000; Yao et al., 2002). Nevertheless, the methods for controlling drug release from drug-loaded EVAl films, such as changing drug loading dose and coating the films with different polymers, cannot allow sufficient variability of drug release.

It was reported that partially hydrolyzed EVA films presented different gas and liquid water permeability when the hydrolysis degree changed (Hirata et al., 2005). The hydroxyl group content is one of the main factors which affect the permeability of partially hydrolyzed EVA films. It can be expected that the water uptake and resulting drug release from drug-loaded partially hydrolyzed EVA films can probably be well regulated by controlling the hydrolysis degree and the resultant hydrophilic hydroxyl group content. Accordingly, compared to EVA and EVAl (completely hydrolyzed EVA), partially hydrolyzed EVA has a greater capability in controlling drug release. However, to our knowledge, few works have reported drug delivery systems based on partially hydrolyzed EVA.

In this study, we prepared and characterized the partially hydrolyzed EVA films. *In vitro* swelling behaviors of the films with various hydrolysis degrees were presented. Moreover, the films were loaded with antineoplastic paclitaxel to test the drug loading performance and to investigate the influence of hydrolysis degree on *in vitro* drug release behavior. The effects of drug loading dose and film thickness on drug release from films were also investigated.

2. Materials and methods

2.1. Materials

Ethylene vinyl acetate (EVA) copolymer (with VAc of 42% and melt index of 60 g/min) was purchased from Shanghai Research Institute of Chemical Industry (Shanghai, China), and paclitaxel was from Xi'an Haoxuan Biological Technology Co. Ltd. (Xi'an, China). All other chemicals were of analytical grade and used as received.

2.2. Hydrolysis of EVA

Hydrolysis of EVA was carried out as reported by Hirata et al. (2005) and Tambe et al. (2008). In a 250 mL three-necked round bottom flask with a reflux condenser, 25 g EVA was added and dissolved in 125 mL tetrahydrofuran, then 40 mL alcoholic NaOH solution (0.5 mol/L) was added at 30 °C. After a predetermined reaction time (i.e., 1, 3 and 5 h), the reaction medium was neutralized by 7.5 mL HCl solution (1 mol/L). The reaction solution was then poured into 2 L pure water to obtain precipitates. The precipitated polymers were cut into pieces and washed repeatedly by pure water to remove inorganic salts, then vacuum dried at 36 °C till constant weight.

2.3. Determination of hydrolysis degree

The hydrolysis degree was determined as reported by Tambe et al. (2008). In a 150 mL round bottom flask with a reflux condenser at 80 °C, 1.0 g hydrolyzed product or EVA (blank test) was dissolved in 25 mL toluene, then 5 mL mixture of acetic anhydride and pyridine (7:3 (v/v)) was added and the reaction was continued for 24 h. After that, 5 mL distill water was added and the reaction was stopped after 10 min. The condenser was washed with 10 mL *n*-butanol when the reaction mixture cooled down. Using phenolphthalein as the indicator, unreacted acetic anhydride was titrated with standard alcoholic KOH solution (0.5 mol/L) in order to determine the hydrolysis degree. The hydrolysis degree was calculated as follows:

$$\text{Hydrolysis degree (\%)} = \frac{(B - S) \times 0.5 \times 10^{-3}}{(VAc \times 1)/86} \times 100 \quad (1)$$

where B is the volume of KOH (mL) consumed in blank test and S is the volume of KOH (mL) consumed for hydrolyzed product.

2.4. Preparation of polymer films

1.2 g EVA or partially hydrolyzed EVA was dissolved in 12 mL tetrahydrofuran in a glass sample bottle, then the polymer solution was poured onto a glass plate. Afterwards, the plate was put in the fume hood for about 18 h in order to evaporate off the solvent. The obtained films were vacuum dried at 36 °C till constant weight. The thickness of polymer films was measured with a thickness gauge.

2.5. Characterization

2.5.1. Fourier-transform infrared spectroscopy (FTIR)

FTIR spectra of the polymer samples were recorded in an attenuated total relflection (ATR) using a Varian 640-IR Spectrophotometer in the range of 400–4000 cm^{-1} with a 4 cm^{-1} resolution and 32 scans per spectrum.

2.5.2. ^1H NMR measurement

The ^1H NMR spectra of the samples were dissolved in $CDCl_3$ with 0.03% (v/v) tetramethylsilane (TMS) as internal standard and recorded by a Varian Mercury plus 400 spectrometer.

2.5.3. Gel permeation chromatography (GPC)

Different molecular weights of EVA and partially hydrolyzed EVA were determined by gel permeation chromatography (GPC) using a Waters HPLC system equipped with a model 2414 refractive index detector, a model 1525 binary HPLC pump and a series of Styragel® columns (HR3 and HR4) at 40 °C. As an eluent, tetrahydrofuran was used at a flow rate of 1.0 mL/min. The GPC system was calibrated with polystyrene standards.

2.5.4. Melting range and solubility

Melting ranges of EVA and partially hydrolyzed EVA were determined using a micro melting point instrument (SGW X-4, SPSIC, Shanghai, China). The solubility of polymers in different solvents at 37 °C was determined as follows: 0.2 g EVA or partially hydrolyzed EVA was added to 2 mL solvent in a hermetic glass bottle. After incubation for 12 h at 37 °C, the dissolution condition was examined macroscopically. For the films those did not dissolve completely,

another 18 mL solvent was added to each bottle followed by continued incubation for 24 h, then the dissolution condition was examined again.

2.6. In vitro swelling studies

The tested films were weighed to obtain the dry weight (W_0), and then incubated in 50 mL pure water at 37 °C. At different time intervals, films were taken out and immediately weighed to get the wet weight (W_t) after surface water was removed with filter paper. The water uptake was calculated as follows:

$$\text{Water uptake}\ (\%) = \frac{(W_t - W_0)}{W_0} \times 100 \quad (2)$$

where W_0 is the dry weight of the film before incubation and W_t is the wet weight of the film at each time point.

2.7. HPLC determination of paclitaxel

Paclitaxel concentration was analyzed by a HPLC system (LC-10Advp, Shimazu, Japan) equipped with a Diamonsil™ C_{18} column (5 μm particle size, 250 mm × 4.6 mm) at the wavelength of 227 nm. The mobile phase consisted of a mixture of HPLC grade methanol and deionized water (75:25 (v/v)), and the flow rate was 1.0 mL/min.

2.8. Paclitaxel loading and measurement of drug uniformity

Different amounts of paclitaxel and polymer were dissolved in tetrahydrofuran in order to obtain paclitaxel-loaded films with different compositions and thicknesses. Films were prepared using the solution casting method as mentioned above. The paclitaxel-loaded films obtained were vacuum dried at 36 °C till constant weight.

Drug uniformity of the paclitaxel-loaded films was measured as below: the films were cut into discs 1.2 cm in diameter. Each film was accurately weighed, then incubated in 20 mL methanol in a hermetic glass bottle at 37 °C for about 6 h to extract the whole paclitaxel-loaded in the film. The concentrations of paclitaxel in the methanol solutions were assessed by HPLC.

2.9. In vitro drug release studies

Paclitaxel-loaded films were cut into discs 1.2 cm in diameter. Each disc was placed in a polyethylene tube containing 15 mL of PBS pH 7.4 with 1% Tween. The tubes were placed in a shaking water bath at 37 °C with a shaking speed of 75 rpm. At different time points, the release medium was completely withdrawn for HPLC determination and replaced with fresh PBS.

2.10. X-ray diffraction (XRD)

XRD analyses for the polymer films and paclitaxel powder were carried out on an X-ray diffractometer (D/max 2200, Rigaku, Japan) equipped with a Cu Kα radiation source (40 kV, 20 mA), and the XRD traces were recorded in a 2θ range of 2° and 40° at a rate of 5°/min.

2.11. Mechanical tests

Mechanical properties of the films were measured at 25 °C in unidirectional tension at a rate of 20 mm/min, using a universal testing machine (T1-FR020 A50, Zwick, Germany). The tensile strength was defined as the maximum strength in the stress–strain curve, the maximal strain as the breaking strain, and Young's modulus as the slope of the stress–strain curve in the elastic (linear) region.

Table 1
Effect of reaction time on hydrolysis degree and melting ranges of EVA and partially hydrolyzed EVA ($n = 3$; Mean ± S.D.).

Polymer	Reaction time (h)	Hydrolysis degree (%)	Melting range (°C)
EVA	0	0	60–76
E1	1	12.2 ± 5.0	82–96
E3	3	32.6 ± 2.7	84–95
E5	5	49.6 ± 5.0	81–97

2.12. Scanning electron microscopy (SEM)

A JSM-7401F scanning electron microscope (JEOL, Tokyo, Japan) was used to image the samples. Prior to imaging the film samples were placed on a metal sample holder, and then sputter coated at 20 mA for 30 s, using an Emitech K-575 Sputter Coater with a gold–palladium target. Images were obtained at 20 mA current and 1 kV accelerating voltage.

3. Results and discussion

3.1. Hydrolysis of EVA

With three different hydrolysis degree of 12.2%, 32.6% and 46.9%, respectively, partially hydrolyzed EVA (E1, E3 and E5) were obtained (Table 1). The hydrolysis degree obviously increased when reaction time extended. It indicates that the hydrolysis of EVA, which leads to the change of acetate groups into hydroxyl groups, is a time dependent reaction. However, it was found that once the reaction time was longer than 5 h, the hydrolysis degree maintained at about 50% and did not increase anymore (data not shown). The cause might be that the hydrolysis reaction of EVA had already reached the balance at 5 h.

3.2. Characterization

3.2.1. Fourier-transform infrared spectroscopy (FTIR)

Fig. 2 shows the FTIR spectra of EVA and E5. In the spectrum of E5, the peak related to acetate group at 1735 cm^{-1} was much less obvious than that in EVA spectrum. Meanwhile, in contrast with the spectrum of EVA which showed no peak around 3300 cm^{-1}, in the

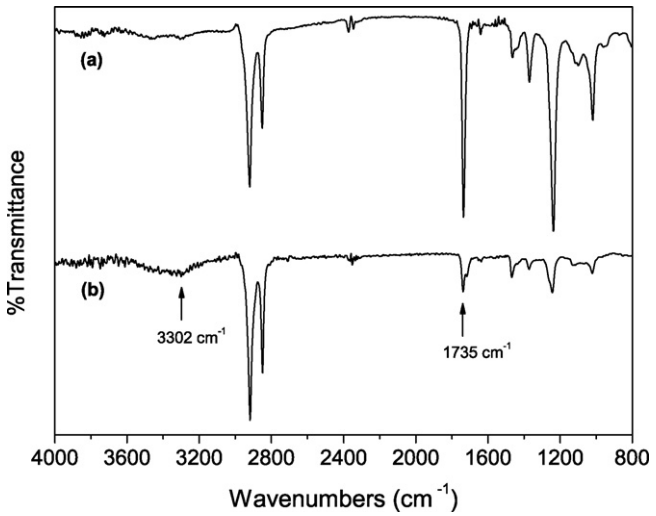

Fig. 2. ATR-FTIR spectra of (a) EVA and (b) E5.

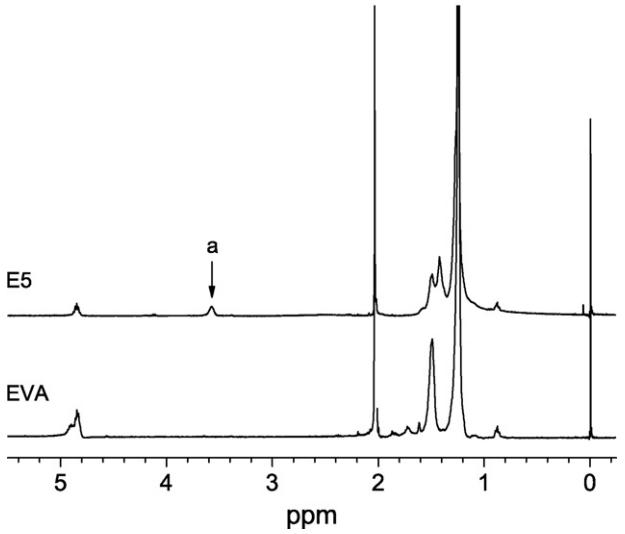

Fig. 3. ^{1}H NMR spectra of EVA and E5.

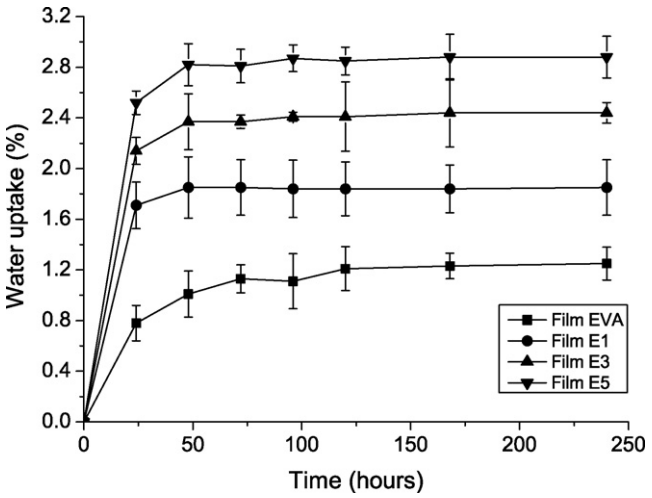

Fig. 4. The water uptake of Films EVA, E1, E3 and E5 in pure water at 37 °C. (n = 3; Mean ± S.D.).

spectrum of E5 there displayed the peak related to hydroxyl groups at about 3302 cm^{-1}. In addition, as the hydrolysis degree increased, the intensity of hydroxyl group peak increased and the intensity of acetate group peak decreased (data not shown). The results indicate that the partially hydrolyzed EVA was successfully prepared, and some of the acetate groups were successfully changed into hydroxyl groups. The peak related to hydroxyl groups in E5 spectrum is broad, which is probably attributed to the hydrogen bonding (Tambe et al., 2008). Moreover, the weak peak of acetate groups can be still observed in Fig. 2b, and this is due to the incomplete hydrolysis of acetate groups (Tambe et al., 2008; Yin et al., 2006).

3.2.2. ^{1}H NMR measurement

The ^{1}H NMR spectra of EVA and E5 are shown in Fig. 3. At δ = 2.03 and 4.84, EVA presented characteristic peaks due to the methyl and methyne protons of the vinyl acetate units, respectively. Compared to EVA, E5 displayed an additional peak at δ = 3.57 (peak a). The peak can be probably attributed to the protons of hydroxyl groups. These results confirmed that a part of the acetate groups were changed into hydroxyl groups after the hydrolysis reaction.

3.2.3. Gel permeation chromatography (GPC)

Molecular weights of EVA and partially hydrolyzed EVA were determined by GPC. It was apparent that with the increase of hydrolysis degree, both the number-average molecular weight (M_n) and the weight-average molecular weight (M_w) slightly decreased (Table 2). The slight decrease of molecular weights was caused by the hydrolysis of acetate groups and the resulting loss of acetyl groups. The results in turn indicate that the backbone chain was not broken and still maintained its integrality.

3.2.4. Melting range and solubility

It was found that there existed differences in melting range and solubility between EVA and partially hydrolyzed EVA, due to the hydrolysis of acetate groups into hydroxyl groups. Table 1 lists the melting ranges of EVA, E1, E3 and E5. The melting range of E1, E2 and E3 (approximately 81–97 °C) was much larger than that of EVA (about 60–76 °C). On the other aspect, the solubility of EVA, E1, E3 and E5 varied in different solvents at 37 °C (Table 3). All of the polymers were soluble in tetrahydrofuran and $CHCl_3$ but indissolvable in CH_3OH, CH_3CH_2OH and acetone. However, the solubility of EVA and partially hydrolyzed EVA in CH_2Cl_2 was different. EVA could easily dissolve in CH_2Cl_2, but E1, E2 and E3 were indissolvable.

Table 2
Different molecular weight numbers of EVA and partially hydrolyzed EVA.

Sample	M_w	M_n	M_w/M_n
EVA	97,100	66,000	1.4
E1	90,800	62,600	1.5
E3	85,100	59,500	1.4
E5	80,700	55,800	1.4

3.3. Properties of the drug-free and drug-loaded films

3.3.1. In vitro swelling behavior

The compositions of the EVA and partially hydrolyzed EVA films with different drug loading doses and thickness were listed in Table 4. Since the hydrolysis of acetate groups into hydroxyl groups, the hydrophilicity of the polymer may change. The *in vitro* swelling properties of Films EVA, E1, E3 and E5 were investigated. As shown in Fig. 4, the water uptake percentages of all the polymer films increased quickly in the first 24 h and slightly in the next 24 h, then maintained relatively constant. The swelling degrees of Films E1, E3 and E5 were greater than Film EVA. It suggests that the hydrophilicity of E1, E3 and E5 was much better than EVA. The good hydrophilicity of partially hydrolyzed EVA is due to the existence of hydrophilic hydroxyl groups which could bind with water molecules via hydrogen bonding. In Fig. 4, it is also obvious that the polymer with higher hydrolysis degree swelled more intensively, which could be attributed to the larger number of hydrophilic hydroxyl groups.

3.3.2. X-ray diffraction (XRD)

The X-ray spectra of Films EVA, E1, E3 and E5 are displayed in Fig. 5a. It was obvious that the peak of Film EVA was broad while the peaks of partially hydrolyzed EVA films were sharper. The broad diffractogram of Film EVA suggested the presence of amorphous structure. The sharper peaks of partially hydrolyzed EVA films indicated the higher crystalline nature, which was probably caused by the intermolecular hydrogen bonds between hydroxyl groups. Moreover, the peak areas under the XRD curves of Films EVA, E1, E3 and E5 with the same thickness (240 μm) were 8491.7, 8648.6, 8822.1 and 9243.0, respectively. With the increase of hydrolysis degree, the peak area increased. It indicates that the crystallinity increased when the number of hydroxyl groups increased. The

Table 3
The solubility of EVA and partially hydrolyzed EVA in different solvents.

Polymer	CH_2Cl_2	Tetrahydrofuran	$CHCl_3$	CH_3OH	CH_3CH_2OH	Acetone
EVA	+	+	+	–	–	–
E1	–	+	+	–	–	–
E3	–	+	+	–	–	–
E5	–	+	+	–	–	–

+: 0.2 g polymer can easily dissolve in 2 mL solvent at 37 °C.
–: 0.2 g polymers were indissolvable in 20 mL solvent at 37 °C.

Table 4
Compositions and thicknesses of the tested films ($n = 4$; Mean ± S.D.).

Film	Polymer	Polymer content (%)	Paclitaxel loading (%)	Thickness (μm)
Film EVA	EVA	100	–	240 ± 4
Film E1	E1	100	–	240 ± 9
Film E3	E3	100	–	240 ± 11
Film E5	E5	100	–	240 ± 10
Film EVA-P20%	EVA	80	20	240 ± 8
Film E1-P20%	E1	80	20	240 ± 7
Film E3-P20%	E3	80	20	240 ± 11
Film E5-P20%	E5	80	20	240 ± 19
Film E5-P20%-T100	E5	80	20	100 ± 3
Film E5-P20%-T360	E5	80	20	360 ± 23
Film E5-P10%	E5	90	10	240 ± 22

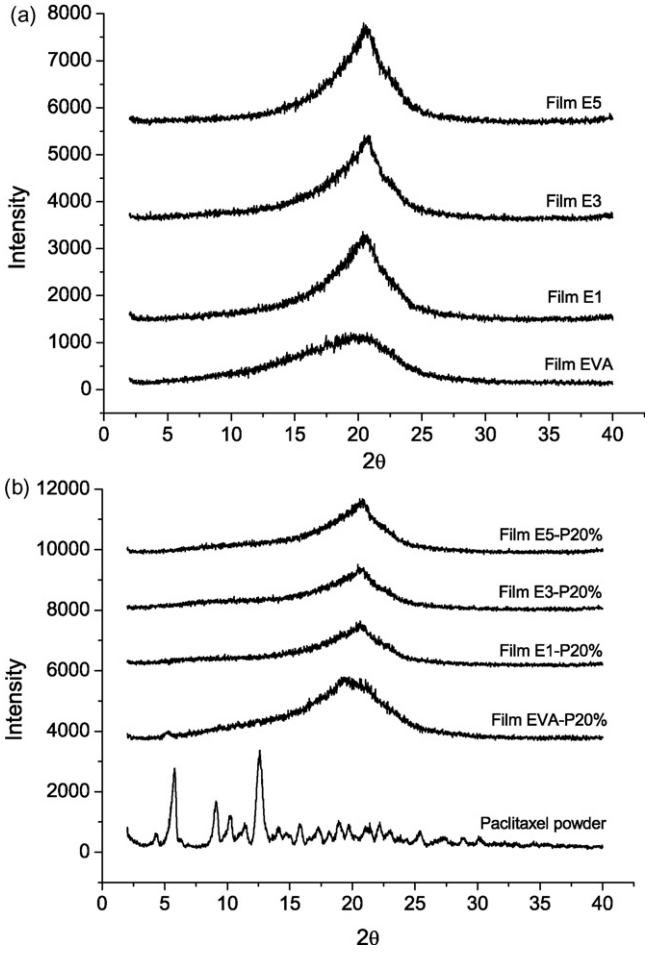

Fig. 5. Wide-angle X-ray diffraction diagrams of (a) Films EVA, E1, E3 and E5 as well as (b) paclitaxel powder, Films EVA-P20%, E1-P20%, E3-P20% and E5-P20%.

intermolecular hydrogen bonding between hydroxyl groups might enhance the crystallinity.

In order to analyze the crystallinity of paclitaxel in different films, paclitaxel powder and paclitaxel-loaded polymer films were examined by X-ray diffraction (Fig. 5b). Paclitaxel powder exhibited intense diffraction peaks at 2θ value of 5.80°, 9.08°, 12.60°, etc., indicating the crystalline nature of paclitaxel particles. However, Film EVA-P20% showed weak peaks at 2θ value of 5.80° and 12.60°. Moreover, the broad peak of Film EVA-P20% at about 19.34° was much sharper than Film EVA. The results imply that a part of paclitaxel was in crystalline form in paclitaxel-loaded Film EVA. Conversely, paclitaxel-loaded Films E1, E3 and E5 showed no crystalline peaks of paclitaxel, suggesting paclitaxel was molecularly dispersed in partially hydrolyzed EVA films or distributed in an amorphous form.

3.3.3. Mechanical properties

In order to investigate the influence of hydrolysis degree and drug loading dose on mechanical properties, elastic modulus, maximum tensile strength and elongation of the films were determined (Table 5). The elastic modulus and maximum tensile strength of partially hydrolyzed EVA films were larger than those of Film EVA. Moreover, as the hydrolysis degree increased, the elastic modulus and maximum tensile strength increased. The increase of maximum tensile strength might be caused by the intermolecular hydrogen bonds between hydroxyl groups. On the contrary, the elongation decreased when the hydrolysis degree increased. After the loading of paclitaxel and as the drug loading dose increased, the elongation of E5 films increased, but elastic modulus and maximum tensile strength decreased (Table 5). The results indicated that the incorporated paclitaxel could interfere with the intermolecular interaction among the polymer molecules.

3.3.4. Paclitaxel uniformity of drug-loaded films

To confirm that paclitaxel uniformly dispersed in drug-loaded films, drug uniformity of Films EVA and E5 with the theoretical drug loading dose of 20% was studied. The determined paclitaxel loading doses of Films EVA-P20% and E5-P20% were 18.2 ± 0.6% and 17.9 ± 0.2% within the film, 19.0 ± 1.4% and 18.0 ± 0.2% between films, respectively ($n = 4$; Mean ± S.D.). Be comparatively close to the theoretical value, the determined paclitaxel loading dose values showed low standard deviation values. It indicates that paclitaxel well dispersed in drug-loaded films.

Table 5
Mechanical properties of films with various compositions ($n = 4$; Mean ± S.D.).

Film	Elastic modulus (MPa)	Maximum tensile strength (MPa)	Elongation (%)
Film EVA	1.6 ± 0.6	19.8 ± 1.0	833.3 ± 48.7
Film E1	267.9 ± 26.5	20.6 ± 0.4	663.3 ± 2.4
Film E3	408.7 ± 48.9	22.5 ± 0.5	646.7 ± 44.3
Film E5	534.4 ± 35.7	23.3 ± 0.5	566.7 ± 34.3
Film E5-P10%	859.4 ± 42.1	17.1 ± 0.4	466.7 ± 5.8
Film E5-P20%	1239.0 ± 32.9	8.4 ± 0.5	72.7 ± 3.4

3.4. In vitro drug release

Fig. 6a shows the cumulative release profiles of Films EVA, E1, E3 and E5 containing 20% paclitaxel. All films released paclitaxel rapidly at the beginning then slowly during the later period. During the first 23 h, the cumulative percentages of paclitaxel released from Films EVA-P20%, E1-P20%, E3-P20% and E5-P20% were 4.9 ± 0.4%, 7.6 ± 0.4%, 8.2 ± 0.6% and 10.1 ± 0.2%, respectively. The burst release might be contributed by the immediate release of paclitaxel on or near the film surface after the films were incubated in the release medium. The release rate of paclitaxel from partially hydrolyzed EVA films was remarkably higher than that from Film EVA. Furthermore, the paclitaxel release rate increased with the increasing hydrolysis degree of EVA. The faster drug release rate of drug-loaded film with higher hydrolysis degree was probably ascribed to its better hydrophilicity and swelling properties. When the hydrophilicity and swelling extent of polymer is higher, the rate of dissolution and subsequent diffusion of paclitaxel out of the film was faster.

The release profiles of paclitaxel from drug-loaded Film E5 with the same drug loading dose of 20% but different thicknesses of 100 μm, 240 μm and 360 μm was shown in Fig. 6b. It is obvious that paclitaxel was released faster when the film was thinner. The cause might be that water was easier to permeate into the thinner film and paclitaxel in the thinner film was easier to diffuse into the release medium by virtue of the shorter diffusion path.

The effect of drug loading dose on paclitaxel release from drug-loaded Film E5 was also investigated. As shown in Fig. 6c, the cumulative amount of drug released from the Film E5-P20% with higher drug loading dose (20%) was larger than Film E5-P10% with lower drug loading dose (10%). It might be attributed to that the polymer matrix which played the role of retarder in the release process, was comparatively less in the film with higher drug loading dose. Moreover, in the film with higher drug loading dose, a larger number of caverns can be generated by the initial larger amount of paclitaxel released, which in turn facilitates the later drug release.

From the *in vitro* drug release results, it is apparent that paclitaxel release was affected by the hydrolysis degree of polymers, thickness of film and drug loading dose. With higher hydrolysis degree and smaller thickness, the film released paclitaxel faster. With higher drug loading dose, the drug release rate was larger. Hence the paclitaxel release can be well controlled through regulating these factors in order to achieve a desirable drug release behavior.

The kinetics of paclitaxel release from the films was clarified by fitting the release data to different mathematic models which have been widely used to interpret drug release mechanism. Table 6 shows that the Higuchi equation, which deals with the diffusional kinetics of drug from planar system, fitted the release data best during the first 9 days. This indicates that the paclitaxel release rate during the first 9 days was time dependent. During the later release phase, the release profile was linear, suggesting that the later drug release was in a zero-order pattern.

3.5. The surface morphologies of films

The surface morphologies of films before and after drug release are shown in Fig. 7. Drug-free Films EVA and E5 both presented smooth surface with no micropores (Fig. 7a and d). However, after the loading of paclitaxel, Films EVA and E5 displayed distinct surface appearances. The surface of Film E5-P20% presented a smooth appearance (Fig. 7e). But there existed irregularly shaped flakes on the surface of paclitaxel-loaded Film EVA (Fig. 7b). Interestingly, these flakes disappeared after drug release for 22 days (Fig. 7c). Thus, the flakes on drug-loaded Film EVA before drug release were considered to be the paclitaxel located on the surface of film. The results exactly correspond with the XRD results, which show that Film EVA-P20% (with drug flakes on the surface) presented weak paclitaxel peaks at 2θ value of 5.80° and 12.60° in Fig. 5b. The burst release (Fig. 6a) of Film EVA-P20% was probably due to the quick release of these drug flakes. In contrast, the surface of Film E5-P20% presented no flakes, indicating that paclitaxel was well entrapped in the matrix of Film E5-P20%.

After drug release for 22 days, not only did the paclitaxel flakes disappear but also some small pores appeared on the surface of Film EVA-P20% (Fig. 7c). These pores were attributed to the release of paclitaxel particles in the superficial zone of the film. In comparison with Film EVA-P20%, the surface of drug-loaded Film E5 after drug release for 22 days was much rougher and there existed more small pores on it. This may be attributed to the larger amount of paclitaxel released and slight erosion of the surface of hydrophilic Film E5.

Table 6
Fitting results of the drug release data by Higuchi and zero-order models.

Film	Higuchi model ($M_t/M_\infty = kt^{0.5}$)[a] (0–9 days)		Zero-order ($M_t/M_\infty = kt$)[a] (9–22 days)	
	k	r	k	r
Film EVA-P20%	0.008	0.989	1.00×10^{-4}	0.999
Film E1-P20%	0.014	0.987	8.00×10^{-5}	0.984
Film E3-P20%	0.015	0.985	8.00×10^{-5}	0.984
Film E5-P20%	0.020	0.984	1.00×10^{-4}	0.997
Film E5-P20%-T100	0.029	0.980	2.00×10^{-4}	0.996
Film E5-P20%-T360	0.012	0.984	4.00×10^{-4}	0.981
Film E5-P10%	0.026	0.989	7.00×10^{-5}	0.984

[a] M_t/M_∞, fractional drug release; t, the release time; k, a constant of the drug–polymer system; r, correlation coefficient.

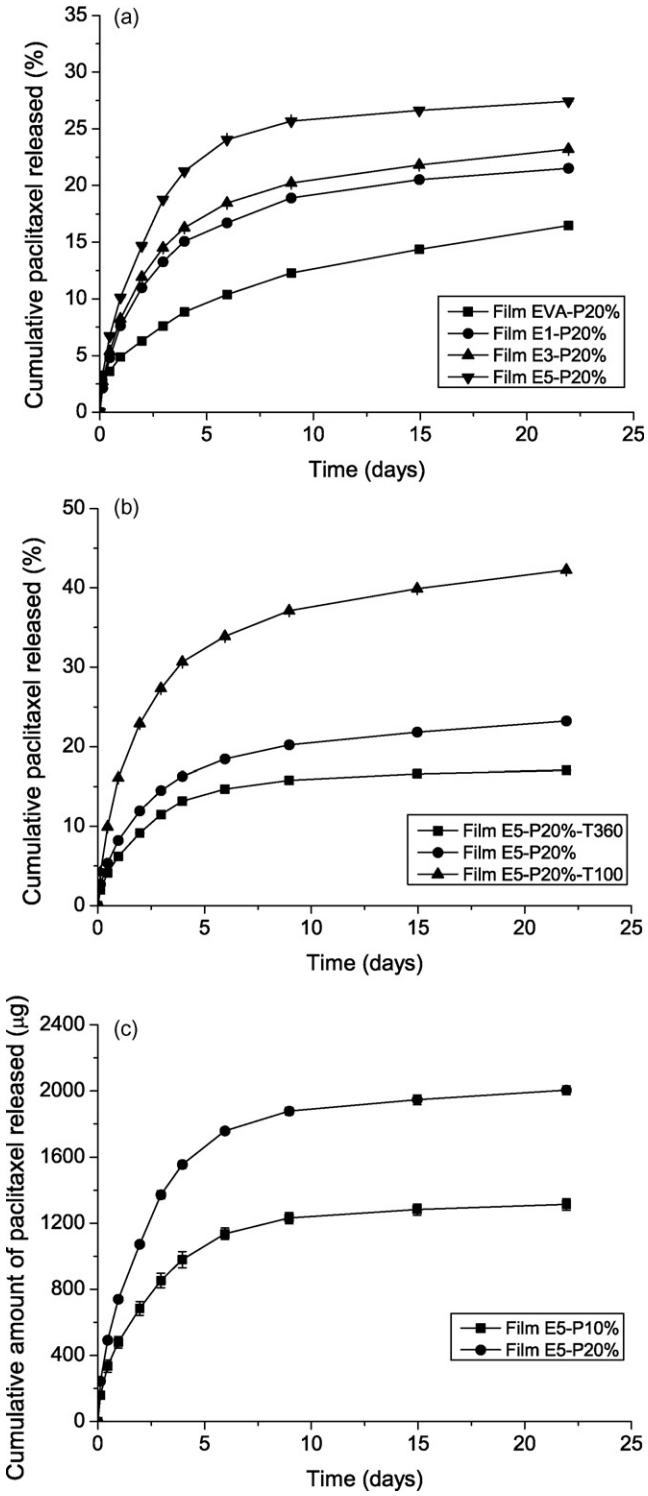

Fig. 6. Cumulative release of paclitaxel from films in PBS pH 7.4 with 1% Tween at 37 °C. ($n = 4$; Mean ± S.D.). Cumulative percent of paclitaxel released from (a) Films EVA-P20%, E1-P20%, E3-P20% and E5-P20% as well as (b) Films E5-P20%, E5-P20%-T100 and E5-P20%-T360. (c) Cumulative amount of paclitaxel released from Films E5-P10% and E5-P20%.

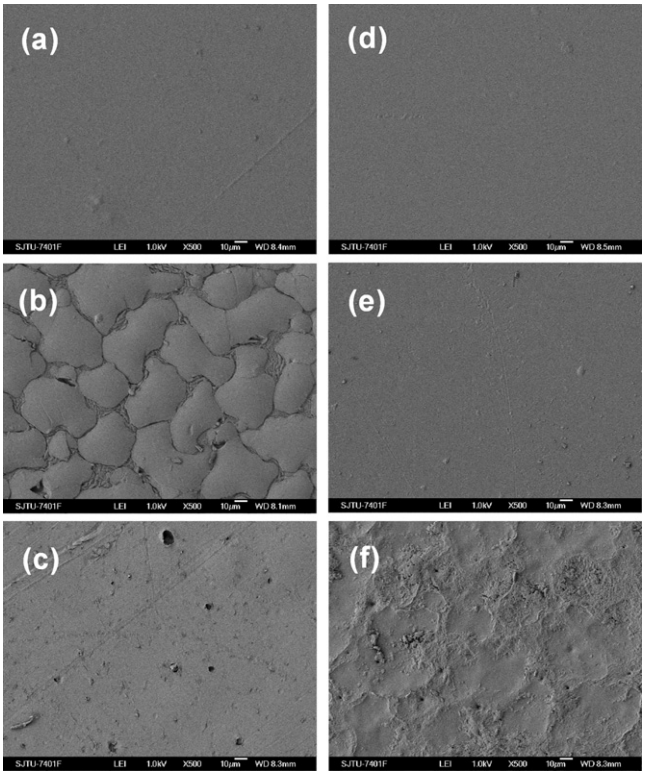

Fig. 7. SEM images of (a) Film EVA, (b) Film EVA-P20%, (c) Film EVA-P20% at 22 days after drug release, (d) Film E5, (e) Film E5-P20%, and (f) Film E5-P20% at 22 days after drug release.

4. Conclusions

Paclitaxel-loaded films based on partially hydrolyzed EVA with different hydrolysis degrees were successfully fabricated. Partially hydrolyzed EVA films swelled more intensively than the EVA film and the swelling degree of polymer was greater when the hydrolysis degree was higher. Paclitaxel can be molecularly dispersed or distributed in an amorphous form in drug-loaded partially hydrolyzed EVA films. The *in vitro* release of paclitaxel from partially hydrolyzed EVA films was remarkably faster as compared with that from EVA film, and it underwent a typical first quick and a later constant-rate release. The drug release profile could be regulated by changing hydrolysis degree, the thickness of films and drug loading dose. With higher hydrolysis degree and smaller thickness, the film could release paclitaxel more quickly; while with a higher drug loading dose, a larger amount of drug can be released during a certain incubation time. Based on the performances, partially hydrolyzed EVA films could be a promising drug-delivery vehicle with high adjustability of drug release for long-term treatment.

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