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Chemical modification of zein by bifunctional polycaprolactone (PCL)

Qiangxian Wu, Tomoyuki Yoshino, Hiroshi Sakabe, Hongkang Zhang, Seiichiro Isobe*

Food Engineering Division, National Food Research Institute, 2-1-12 Kannondai, Tsukuba, Ibaraki 305-8642, Japan

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Abstract

Prepolymer was synthesized by use of PCL and hexamethylene diisocyanate (HDI), and then used to prepare modified zein-based polymers (ZPs). Solid-state ^{13}C NMR results showed that at least four amino acids (Glu, Gln, Tyr and His) reacted with the prepolymer, and urea–urethane links were prominent. Thermal analysis indicated that micro-phase separation formed between zein matrix and PCL–HDI (PCLH) component in ZPs. With the increasing PCLH content, the melting point of PCL in ZP decreased, and the T_g of zein reduced due to plasticizer role of PCLH. The breaking elongation of modified zein containing 10% PCLH content, increased about 15 times while its strength at break only reduced by about 2 times than that of commercial zein. In addition, with the increasing PCLH content, the flexibility of modified zein sheet improved dramatically with negligible reduction in strength. This indicates that PCL was an elastic fraction in ZPs. Therefore, it is an effective way to improve the mechanical properties of zein by modification with PCL, showing a potential in the field of biodegradable polymers.

Keywords: Zein; Polycaprolactone; Modification

1. Introduction

Protein is one of the most important renewable resources and can be used as biodegradable material [1]. In the last decade, much attention has been paid to soy protein isolate (SPI) [2], zein [3,4], wheat gluten [5], myofibrilar protein [6], and so on.

Zein, an alcohol-soluble protein extracted from corn or corn gluten meal, has attracted attentions due to its hydrophobic property. Commercial α-zein has two components with molecular weights of 24,000 and 22,000, respectively [4]. Each component is made up of several hundreds of amino acid residuals, and has a similar structure with nine antiparellel helices clustered within a distorted cylinder. Hydrophilic residuals (Gln, Glu, Asn, Ser etc.) distribute along the cylinder surface, which contributes to the water sensitivity of zein [7]. In the early 20th century, zein was used to prepare thermoplastic [8]. Recently, more and more attempts have been conducted to produce zein-based biodegradable materials [9,10]. There exist some problems for zein-based materials, such as, their high cost, sensitivity to moisture, and weak mechanical properties [3]. For dried zein cast film, it is too fragile and rigid to be used for packaging; it is weak in strength if pure zein film takes up water. In order to overcome some of these drawbacks, some plasticizers having high boiling point temperature were used to improve the flexibility of zein. The plasticizers include glycerol [11], polyol [12,13] and fatty acid [14]. However, these hydrophilic plasticizers are ineffective at low humidity since their plasticizing efficiency for proteins depends upon water action, and these plasticizers themselves can easily absorb moisture and make the plasticized materials unstable in high moisture atmosphere.

Chemical modification is another way to enhance the mechanical properties of zein. Sturken [15] and Lee et al. [16] used formaldehyde and silicate to modify zein, respectively. The modified zein had high strength but low toughness. Especially in dry state, the modify zein was brittle. In fact, improvement of the toughness at dry state and water resistance at wet state are the two most important factors for zein in practice. To improve the water resistance of zein, one method is to modify hydrophilic side groups such as $-NH_2$, $-OH$, $-COOH$ and $-SH$, by hydrophobic groups or polymer segments. Modified zein may show novel characters in both mechanical properties and water resistance. Zein can dissolve in nonaqueous solvents, for example, N, *N*-Dimethylformamide (DMF), which makes

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* Corresponding author. Tel.: +81-29-838-8029; fax: +81-29-838-8122. *E-mail address:* seiichi@nfri.affrc.go.jp (S. Isobe).

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zein easier to be chemically modified in solution than other proteins. The other works about modified zein have not been adequately reported.

Polycaprolactone (PCL) is one of the few synthetic polymers that are biodegradable [17,18]. PCL has been used to modify starch [5,19] and SPI [20], due to its hydrophobicity. Starch-*g*-PCL was synthesized in dimethyl sulfoxide solvent, the copolymer showed good thermal stability [19] and effective compatibility [5]. Zhong blended SPI and PCL (50/50) by weight with addition of 1–5% methylene diphenyl diisocyanate. The compression-molded sheets showed high compatibility and good toughness [20]. PCL-grafted starch was synthesized by ring-opening polymerization in presence of granular or plasticized starch and a good interfacial was formed between PCL and starch [21]. The reaction ratio of PCL was from 33 to 55% as reported by Choi et al. [22], should be further enhanced. PCL based copolymer can be used as a good compatibilizer due to its excellent miscibility with a variety of other polymers [23]. However, the amount of PCL should be low to develop economically biodegradable materials due to the high cost of PCL.

In this study, the aim was to improve the toughness of zein material by using low quantity of PCL. PCL/hexamethylene diisocyanate (HDI) prepolymer was synthesized to prepare modified zein-based polymers (ZPs). The synthesizing conditions, structure, thermal properties, and mechanical properties of modified zein were investigated.

2. Experimental

2.1. Materials

α-zein (SHOWA ZEIN™) containing 96.3% protein, and 3.4% water, supplied by Showa Sangyo Co., Ltd in Japan, was vacuum-dried at 105 °C for 48 h before use. PCL diol ($M_w = 2000$, CAPA® 2200) was supplied by Solvay Interox Ltd, Cheshire, UK. The hydroxyl value is 56 mg KOH/g, and the water content is less than 0.02%. HDI (Wako first Grade), Tin (II) 2-ethylhexanoate, and DMF (dehydrated for organic synthesis) were purchased from Wako Pure Chemical Ind., Ltd. Dibutyl L-tartrate (DBT, 98%) was from Aldrich (USA).

2.2. Measurement of NCO content

Peak-height method was used to measure the isocyanate group (NCO) content in prepolymer by use of fourier transform infrared spectroscopy (FTIR). PCL blends with different HDI concentration were prepared in melting state at 65 °C in 5 ml glass tubes, then cooled quickly and were recorded with a FTIR spectrometer (Nicolet Avatar 320) using an attenuated total reflectance (ATR) cell at room temperature. ATR method instead of transmission method was used owing to that the former was convenient and quick for testing solid or semi-solid samples. The resolution and scanning time were 2 cm^{-1} and 32 times, respectively. The height ratio of peak at 2272 cm^{-1} to the one at 1724 cm^{-1} (R_{ir}) was obtained to normalize the isocyanate groups content in the blends, where the absorption peak at 2272 cm^{-1} resulted from the isocyanate groups, and at 1724 cm^{-1} mainly from C=O in PCL. The R_{ir} was defined as follows:

$$R_{ir} = H_{2272}/H_{1724} \tag{1}$$

where H_{2272} and H_{1724} correspond to the peak height at 2272 cm^{-1} and peak height at 1724 cm^{-1}, respectively. Three duplications were done for each sample. A standard curve for height ratio of peak to NCO content was plotted and used to predict NCO content in prepolymer. The NCO content was calculated using the following equation:

$$\text{NCO content (\%)} = W_{HDI} \times 0.4996 \times 100/W_T \tag{2}$$

where W_{HDI} is the weight of HDI, W_T is the total weight of HDI and PCL, and the NCO content in HDI is 49.96%.

2.3. Synthesis of NCO-terminated PCL prepolymer

NCO-terminated PCL prepolymer was prepared in bulk. The ratio of NCO/OH in this case was 2.0. PCL diol (100.0 g) and HDI (16.0 ml) were added into a 250 ml four-necked flask fitted with a thermometer, a stirrer, an inlet and an outlet of dry nitrogen, and stirred at 60 °C for 10 min to melt the PCL diol. The stirring speed was 300 rpm. After 110 μl (0.12 wt%) of Tin (II) 2-ethylhexanoate, as a catalyst, was introduced into the flask under dry nitrogen with a syringe, reaction was carried out at 60 °C for 50 min, then at 90 °C until the NCO groups content reached about 3.6%, which was determined by FTIR at regular intervals. The synthesized PCL prepolymer was cooled in a silical gel desiccator and stored at −30 °C in a refrigerator.

2.4. Preparation of ZPs

PCL prepolymer (4.5 g) and DMF (10 ml) were placed in a 500 ml three-necked flask under nitrogen atmosphere, stirred at 70 °C for 5 min to dissolve prepolymer into DMF, and cooled to 0 °C. Zein (10.5 g) dissolved in DMF (140 ml) was added into the flask. Reactions were subsequently performed with stirring speed of 300 rpm at 0 °C for 30 min, at 60 °C for 30 min and at 90 °C for 3 h. Finally, 5 ml of ethanol was added to the flask to terminate possible residual NCO group. White precipitate was obtained after the clear and yellow solution was precipitated into 3 l distilled water (pH = 4.9), then filtered and washed with water two times. The precipitate was milled in ice/water mixture by use of a sample mill (WB-1, from Osaka Chemical Co., Ltd in Japan), then filtered and washed with water two times again. After vacuum freeze-dried for 24 h, the precipitate was milled to pass through a 600 μm aperture sieve. The unreacted PCL was removed out of a filter thimble by Soxlet extraction with toluene for 24 h. The PCL reaction ratio was calculated by the follow equation:

$$\text{Reaction ratio of PCLH (wt\%)} = (1 - W_L/W_T) \times 100 \quad (3)$$

where W_L and W_T correspond to the weight loss of sample and the total sample weight before extraction, respectively. Three duplications were done. The extracted powder sample was vacuum-dried at 133 Pa and 45 °C for 24 h, then coded as ZP30, which means modified zein containing 30 wt% PCL in feed. ZPs with different PCL content were prepared by changing the ratio of zein to PCL, and coded as ZP10, ZP20, ZP40, and ZP50, respectively. All samples of ZP10, ZP20, ZP30, ZP40, and ZP50 were coded as ZP10-50. As controls, samples with no PCL (coded as CZ) and with no zein (coded as PCLHE) were also prepared under the above synthesizing procedure, respectively. CZ means commercial zein, and PCLHE, shorten from PCL–HDI–ethanol, is a derivative from prepolymer and ethanol. All samples were stored in a silica gel desiccator for following tests. The procedure of synthesizing ZPs is shown in Scheme 1. The PCL–HDI component in ZPs was coded as PCLH, whose structure is shown in Scheme 1.

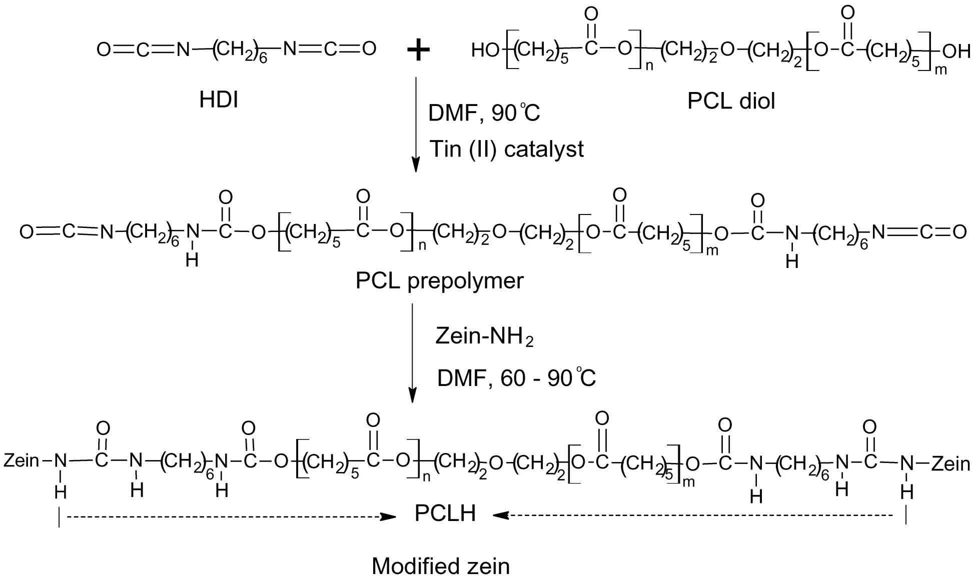
2.5. Preparation of sheets by compression-molding

The powder samples were compression-molded according to our previous procedure [24]. CZ, ZP10, ZP20, ZP30, ZP40, and ZP50 were mixed with DBT by a constant ratio of 1:0.5 by weight, respectively. PCLHE is a kind of wax-like material and impossible to be compression-molded for tensile test. The mixture was stirred in a 50 ml beaker using a glass bar for 10 min, and then milled in the above sample mill at 25000 rpm for 30 s. Finally, premix was sealed and equilibrated in an oven at 60 °C for 12 h to obtain a homogeneous mixture. Each sheet was prepared according to the followed procedure: premix (2.5 g) was placed into a mold (110 × 110 × 0.20 mm^3) and covered with aluminum papers at both sides. The mold was placed between the two steel blocks of a hot press (Mini Test Press, Toyoseiki, Japan), whose temperature was controlled to be 155 °C, and then was compression-molded at 17 kN for 1 min. After the premix was pre-heated, the pressure was quickly increased from 17 to 100 kN. Ten minutes later, the mold was cooled to below 50 °C by water-cooling system at a rate of 40 °C/min. A slight yellow and transparent sheet was released from the mold, and stored in a desiccator. A series of sheets were prepared and coded as ZPS10, ZPS20, ZPS30, ZPS40, and ZPS50 from ZP10–ZP50, respectively. Sheet from CZ was coded as CZS.

2.6. Characterization

The FTIR spectra of samples were recorded with the above FTIR spectrometer under the similar conditions.

Vacuum-dried sample (1.3 mg/ml) was dissolved in sodium dodecyl sulphate (SDS)/sample buffer solution with 1% 2-mercaptoethanol for sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) experiment. Both Sample solution and standard protein solution (Protein Marker 7701S, Biolabs Inc. New England) were heated at 100 °C for 5 min. The sample solution (10 μl) was applied to a slab polyacrylamide gel contained with 10% resolving gel and 3% stacking gel. Electrophoresis was done at 10 mA current for 5–7 h to determine the molecular weight and composition of CZ. By use of a densitometer (CS-9300PC, Shimadzu), unmodified zein content in ZPs was qualitatively evaluated on the base of the relative intensity of stained bands in the images of electrophoresis [25]. The reaction ratio of zein was calculated with the



Scheme 1.

following equation:

$$\text{Reaction ratio of zein (wt\%)} = (1 - W_U/W_T) \times 100 \qquad (4)$$

where W_U and W_T are the unmodified zein content and total zein content, respectively.

Solid-state ^{13}C NMR experiment was carried out at ambient temperature on a NMR spectrometer (Bruker AVANCE-600) at the resonance frequency of 150.92 MHz. The NMR spectra were obtained with cross polarization technique, using magic angle sample spinning and high-power decoupling (CPMAS). A 6 μs π/2 pulse was used with a 1 ms contact time, 10000 scans, 4 s of recycle time and a 60 kHz decoupling bandwidth. The ^{13}C chemical shift scale was set with glycine as a solid external reference standard. The samples were packed into zirconia rotors and spun at 8 kHz.

Differential scanning calorimetry (DSC) analysis was performed with a thermal analyzer (DSC220C, Seiko Instrument, Japan) under nitrogen atmosphere. Samples were packed down and sealed into aluminum pan with lid. Indium was used for temperature and heat capacity calibration of the instrument. The glass transition temperatures (T_g, the midpoint of glass transition), the change in heat capacity (ΔC_p), and melting temperature (T_m, the maximum of melting peak) were determined by heating samples (10 mg) from 32 to 140 °C at a rate of 10 °C/min, followed by cooling down to −120 °C at a rate of 30 °C/min, and then rescanned at a rate of 10 °C/min to 210 °C. All samples were analyzed in triplicates.

Powder sample was molded into a disk (5 mm diameter and 1.5 mm height) in a home-made mold by use of the above Mini Test Press. Compression-molding pressure (8 kN) was kept for 1 min at 25 °C. The disk was vacuum-dried at 133 Pa and 45 °C for 36 h, heated to 90 °C for 3 h to melt crystals, and then quenched in liquid nitrogen. Finally, disk was vacuum-dried at 25 °C for 12 h to remove possible moisture, and stored in a desiccator containing P_2O_5 for a week. Thermal properties of disks were analyzed on a dynamic load thermomechanical analyzer (DTMA) (TMA/SS150C, Seiko Instrument Inc., Japan) in compression mode under nitrogen atmosphere. Indium and Tin were used to calibrate the temperature scale of furnace. A sinusoidal stress (offset, −20 g; amplitude, 10 g; frequency, 0.1 Hz) was applied on sample to produce a strain. Scanning was performed from −95 to 230 °C at a scanning rate of 2 °C/min. Linear thermal expansion rate (R_{exp}), measured by probe, was evaluated by the following equation:

$$R_{exp}\ (\%) = (H_T - H_{-95})/H_{25} \times 100 \qquad (5)$$

where H_T, H_{-95}, and H_{25} are the height of disk at a given temperature, at −95 °C, and at 25 °C, respectively. Samples were tested in triplicates. The curves of loss tangent (tan δ) to temperature, and R_{exp} to temperature were plotted.

Samples were annealed at 90 °C for 2 h, and then stored in a desiccator for 10 days before using for X-ray diffraction studies. At 28.5 °C, wide-angle X-ray diffraction patterns (WXRDPs) were recorded with an X-ray diffractometer (Shimadzu XRD-6000, Japan) and with Cu Kα radiation ($\lambda = 1.5405 \times 10^{-10}$ m) at 40 kV and 30 mA in the range of $2\theta = 5$–$40°$. A typical pattern was selected from three replications for analysis.

2.7. Measurement of mechanical properties

The mechanical properties of the sheets were measured using an Instron tensile tester (Instron 5542, USA) according to ASTM D882-81 with some modification. Sheets with dimensions of 110 × 10 × (0.1–0.4) mm^3 were stored in a silica gel desiccator, in which relative humidity (RH) was about 0%, for 26 days. The testing temperature and RH was 25.5 °C and 70%, respectively. Stress-strain curves, strength at break and breaking elongation were recorded at 25 mm/min of tensile speed. At least five replications were used in each treatment.

3. Results and discussion

3.1. Preparation and structure of ZPs

As shown in Scheme 1, the content of C=O groups in PCL soft segments was almost constant during modification. Therefore, the height ratio of peak at 2272 cm^{-1} to the one at 1724 cm^{-1} (R_{ir}) can be used to quantitatively determine NCO content in prepolymer. NCO content was often analyzed by the method of dibutylamine back titration [26], but this method is labor-intensive according to our previous work [27]. Peak-height method is a convenient way to analyze the group content in polymer. For example, butene content in ethylene–butene copolymer can be measured by FTIR [28]. The standard curve to determine the NCO content is shown in Fig. 1. A good linearity can be observed on this curve. As we calculated, the primary NCO content at the start of polymerization was 7.2%, and the final NCO content when total PCL consumed was 3.6%. Hence, it is effective to measure the NCO content in prepolymer because the NCO content range in the curve was from 1.3 to

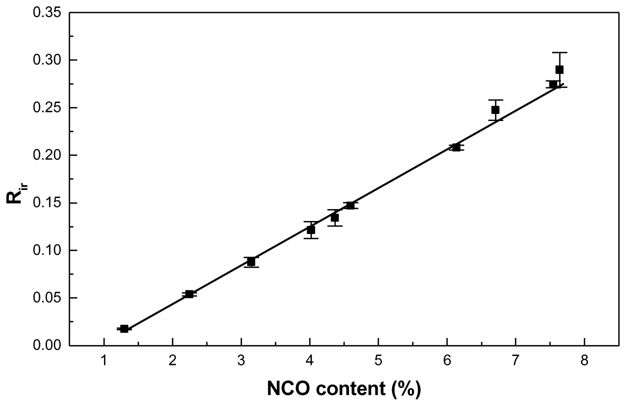


Fig. 1. FTIR calibration curve to determine the NCO content in prepolymer.

7.6%. By use of this standard curve, the NCO content in prepolymer at a given time could be measured easily, which is shown in Fig. 2. Without any catalyst, NCO content kept as high as 5.8% after 420 min of reaction, indicating that NCO and OH groups reacted slowly. When the catalyst concentration increased to 0.03%, NCO content decreased quickly from 7.2 to 5.0% within the first 60 min, and kept constant during the later 290 min (above data not shown in Fig. 2). Once 0.12% of the catalyst was used, the NCO content decreased sharply to about 3.9% within 20 min as shown in Fig. 2, which resulted from the effect of Tin (II) 2-ethylhexanoate. After 2 h, at the second stage, almost all hydroxyl groups of PCL were reacted with NCO groups. If the reaction continued after the second stage, the residual NCO would react with the urethane groups on molecular chains of prepolymer, which made cross-linking occurred in the third stage [29]. In fact, in our experiment gel-like material was easy to obtain in the third stage. Therefore, temperature should be decreased from 90 °C to room temperature to stop the reaction after the NCO content was 3.6%. The prepolymer, prepared for this paper, contained 3.4% of NCO groups. NCO content decreased very slowly for prepolymer stored at −30 °C, about a decrease of 3.3% within 13 days, compared with the primary value. Hence, it is effective to store PCL prepolymer at low temperature to decrease the activity of NCO groups. During the preparation of ZPs, FTIR was used to detect the content of residual NCO groups in DMF solution before ethanol was dropped to terminate the NCO groups, and no any absorption at 2272 cm^{-1} was observed. This implied that the content of residual NCO groups was too low to be detected. The reaction ratios of PCLH are shown in Table 1. In order to verify the correction of reaction ratio, the experiments were repeated three times along with controls. One control experiment was to extract CZ and another was to extract residual PCL by Soxlet extraction. The weight loss of CZ and PCLHE were 0.5 ± 2.1%, and 98.4 ± 0.7%, respectively. This indicated that in CZ there was no compound that was soluble in toluene, and PCLHE could be extracted almost completely. Therefore, it is effective to extract residual PCL residuals out of ZPs. The average reaction ratio of PCLH for ZP10-50 is 96.9%, higher than the reaction ratio (89%) of starch-*g*-PCL reported by Tang et al. [5], which suggested that almost all of prepolymer had reacted onto the molecular chains of zein. According to the structure of zein [7], there exists more than one NH_2 groups on a zein molecular chain, which makes cross-linking reaction possible. It is important to control reaction time, temperature and the concentration of solution to avoid the formation of gel.

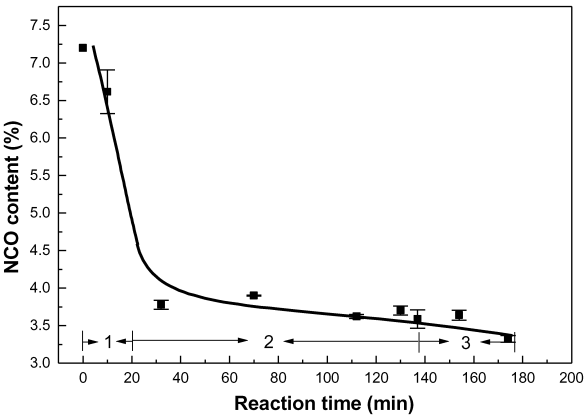


Fig. 2. Effect of reaction time on the NCO content in prepolymer.

The electrophoresis pattern of CZ, ZP10-50 is shown in Fig. 3. CZ had two bands with molecular weight of 2.33×10^4 and 2.12×10^4, which is similar with that the report from Paulis, whose results showed two components in α-zein [30]. Electrophoresis patterns showed that there existed a part of zein in ZPs. The unmodified zein content was calculated by use of densitometry method and shown in Table 1. The average value of unmodified zein content for ZP20-50 was only 3.3 wt%, implying that the polymers could be considered as a pure modified zein.

FTIR spectra of CZ, ZPs, PCLHE, prepolymer and PCL diol are displayed in Fig. 4. Compared with the spectrum of PCL diol, the spectrum of prepolymer had a new peak at 1528.7 cm^{-1}, which was a mixed contribution of the N–H in-plane bending and the C–N stretching of urethane groups [31]. This peak was also observed in the spectrum of PCLHE, suggesting a formation of new urethane groups to PCLHE and prepolymer. The peak at 2272 cm^{-1} for prepolymer was attributed to the absorption of NCO groups. The band at 1733 cm^{-1} was assigned to the mixed absorption of carbonyl group of PCL and carbonyl of urethane group in ZPs. With the increasing PCLH content, the intensity of absorption at 1733 cm^{-1} increased, which was in agreement with the trend of reaction ratio of PCLH in Table 1, suggesting a successful modification. The absorption band of carbonyl of PCLH in ZPs (1733 cm^{-1}) was upshifted about 8 wavenumbers than that in PCLHE

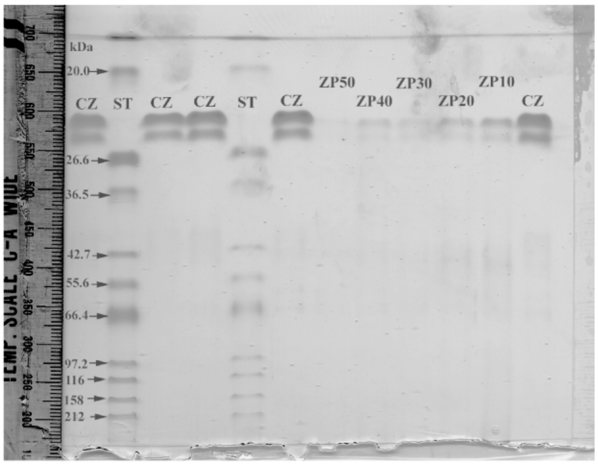


Fig. 3. Electrophoresis patterns of CZ, ZP10-50, and standard proteins. ST represents standard proteins whose molecular weights were marked on this pattern.

Table 1
Reaction ratio and compositions of ZPs

Sample	PCLH component			Zein matrix		
	PCLH in feed (wt%)	PCLH in polymer (wt%)[a]	Reaction ratio (%)[a]	Total zein in polymer (wt%)	Unmodified zein (wt%)[b]	Reaction ratio (%)[b]
ZP10	10	10	100.4 ± 3.9	90	9.3	90.0
ZP20	20	19.7	98.6 ± 4.8	80	5.4	93.3
ZP30	30	27.7	92.4 ± 2.2	70	2.9	95.9
ZP40	40	39.0	97.5 ± 1.2	60	2.8	95.3
ZP50	50	47.8	95.6 ± 1.0	50	1.9	96.2

[a] Measured by Soxlet extraction.
[b] Measured by SDS-PAGE [25].

(1724.6 cm^{-1}), which indicates that the structure of PCL was changed dramatically.

Owing to that part of ZP50 was insolvable, resulting from cross-linking, solid samples were used for NMR analysis. The solid ^{13}C NMR spectra of CZ, ZP50 and PCLHE are shown in Fig. 5. On the basis of previous works in ^{13}C NMR analysis of PCL [32] and polyurethane [33], we assigned the chemical shifts of carbons of PCLHE as shown in Fig. 5 (PCLHE). Compared with the spectrum of PCL reported by Wang et al. [32], a new sharp peak for PCLHE occurred at 65.2 ppm, corresponding to the resonance from terminal ethylene carbon, which implied a formation of aliphatic alcohol–urethane linkages. But the peak intensity decreased dramatically in the spectrum of ZP50, suggesting that aliphatic alcohol–urethane linkages did not dominate the linkages between zein molecules and PCLH component, and further indicating that aliphatic alcohol in zein side groups had no priority to link with NCO groups in prepolymer. Zein had a complex composition that resulted in a complex solution ^{13}C NMR spectrum [34]. In this study, CZ also showed an unresolved spectrum in most regions. For CZ, the resonance signals at 172, 100–140, 45–70, and 15–45 ppm were assigned to the carbonyls from peptide groups and side chains, those from aromatic residuals, the α carbons, and those from aliphatic side chains, respectively [35]. In zein, Glu, Gln, Tyr, His, Arg, Asp, Asn, Thr, Ser, and Cys are the possible amino acids containing active side groups, which had potential to react with NCO groups in PCL prepolymer. Only the side groups from Glu, Gln, Tyr and His exhibited relatively resolved peaks in Fig. 5. Their structure and chemical shifts are summarized in Table 2. The chemical shift peaks of terminal carbons from Arg, Asp, Asn, Thr, Ser, and Cys occurred at 159.4 [34], 177.1 [36], 173.0 [36], 68.2 [36], 64.7 [34], 26–30 ppm [37], respectively, were difficult to be observed in this figure because of considerable overlap of peaks. The peak of –SH group in Cys occurred from 26 to 30 ppm [37] but was

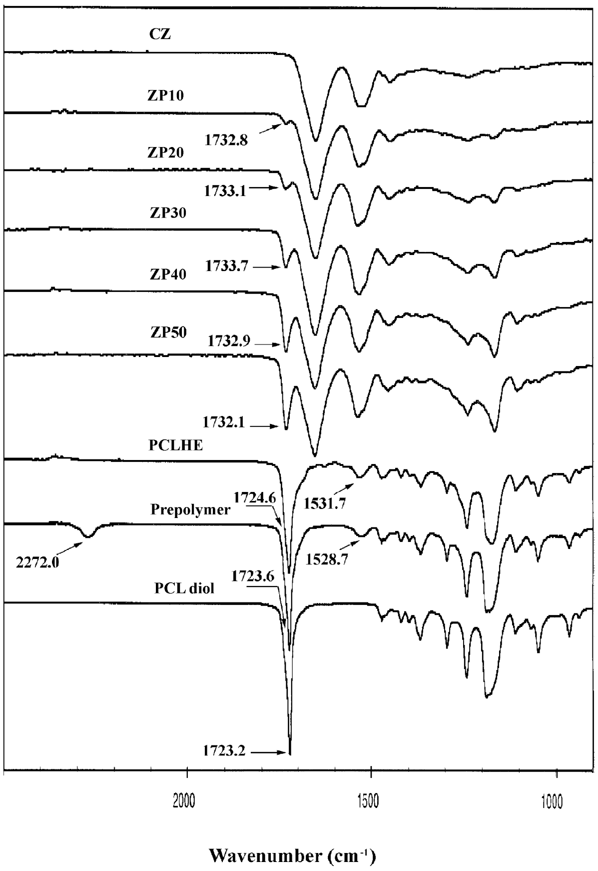
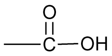
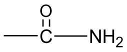
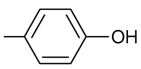
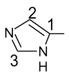


Fig. 4. FTIR spectra of CZ, ZPs, PCLHE, prepolymer and PCL diol.

Table 2
^{13}C chemical shifts of the active side groups in CZ, which could form resolved peaks in Fig. 5

Name	Structure of active group	Chemical shift (ppm)
Glu	 $-\overset{\text{O}}{\overset{\|}{\text{C}}}-\text{OH}$	181.5
Gln	 $-\overset{\text{O}}{\overset{\|}{\text{C}}}-\text{NH}_2$	180.5
Tyr		Cζ 154.8 Cδ 131.5 Cε 115.8
His		C2/C3 134

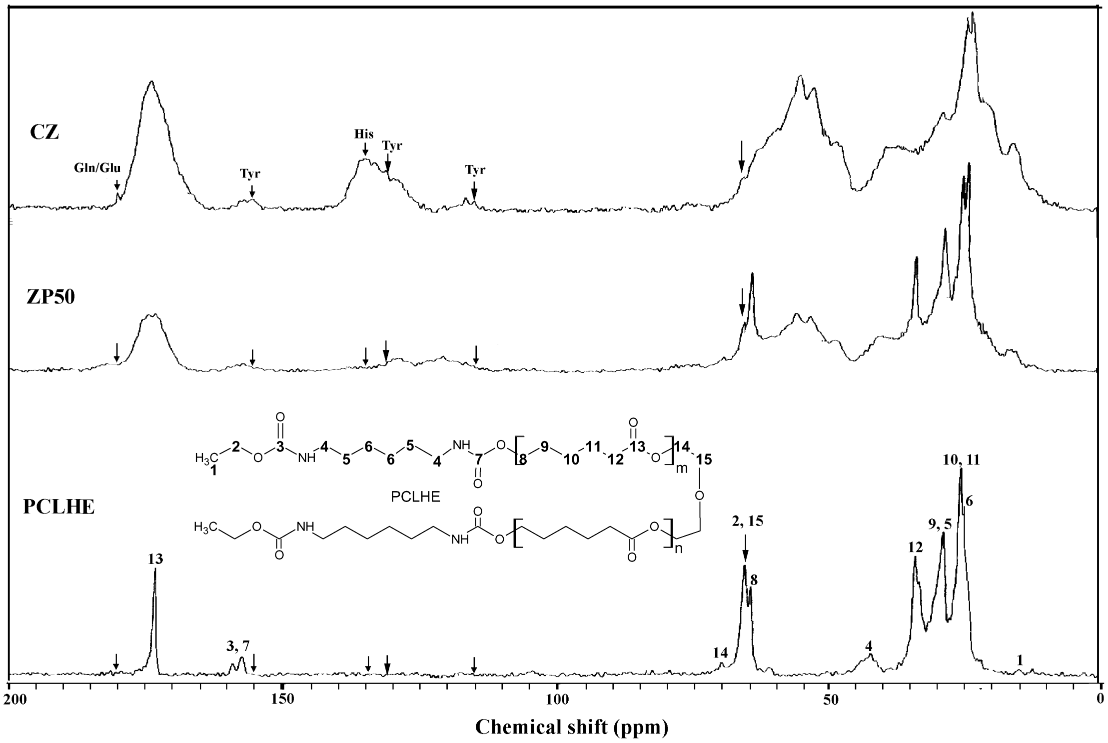


Fig. 5. Solid ^{13}C NMR spectra of CZ, ZP50 and PCLHE.

unresolved due to the overlap of peaks in this region. The peaks in the aromatic region (100–140 ppm) of CZ, which were assigned to only three amino acids: Phe, His and Tyr [34], became obviously weak in ZP50 spectrum. This indicated that both His and Tyr were modified on the consideration of no active group existing in Phe. The other two carbons (Cε and Cζ) of Tyr (Table 2) showed less intensity peaks in ZP50 spectrum than in CZ spectrum, which further inferred that phenolic hydroxyl groups of Tyr also could react with NCO groups. A little peak at about 181 ppm (Fig. 5, CZ), which was assigned to the C-terminal carbonyl of Glu and Gln, disappeared in the spectrum of ZP50, implying that –COOH group of Glu and $-NH_2$ group of Gln had almost completely reacted with NCO group of prepolymer. Therefore, urea–urethane links was prominent in total ureathane links because NH_2 groups in Gln were the main parts in total active side groups [38]. According the above NMR analysis, at least four amino acids including Glu, Gln, Tyr and His were modified by PCL prepolymer, implying a complex cross-linking reaction. Even we increased the scanning times of NMR, and changed the spin speed from 8 to 6 and 4 kHz, respectively, well-resolved spectra could not be obtained.

3.2. Thermal properties

DSC thermograms for CZ, ZPs, and PCLHE are exhibited in Fig. 6, and the related results are shown in Table 3. The ΔH_m for PCL diol ($M_w = 2000$) at the second scanning was 71 J g^{-1} [39], and decreased with the incorporation of hard domains [40]. HDI hard domains restricted the mobility of PCL segments, and hindered the ordering (crystallization) of PCL segments. Therefore, it is significant that the ΔH_m value of PCLHE containing HDI hard domains decreased to 46.2 J g^{-1} (Table 3). The degree of crystallinity of PCLHE was calculated as 32.5 ± 0.8% on the base of the ΔH_m value of completely crystalline PCL (142 J g^{-1}) [41]. Endothermic peaks were observed on the DSC thermograms for ZPs and PCLHE, which was attributed to the melting of PCL crystal. The corresponding ΔH_m values in ZPs increased with an increase of PCLH content (Table 3). The melting point of PCL decreased from 57 to 33 °C with an increase of zein content (Table 3). This is due to the 'diluent effect' of PCLH, which is associated with the mole fraction of crystallizable component in ZPs

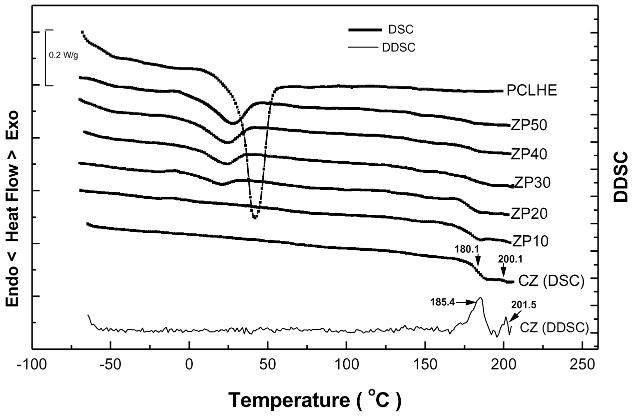


Fig. 6. DSC thermograms for CZ, ZPs and PCLHE.

Table 3
DSC results of CZ, ZPs and PCLHE

Material	Glass transition of zein matrix				Melting peak of PCL segment			
	T_{go}[a] (°C)	T_{gm}[b] (°C)	T_{ge}[c] (°C)	ΔC_P (J g^{-1} K^{-1})	T_{mo} (°C)	T_{mm} (°C)	T_{me} (°C)	ΔH_m (J g^{-1})
CZ	180.1 ± 0.3	182.7 ± 0.4	185.6 ± 0.7	0.43 ± 0.03	–	–	–	–
ZP10	174.5 ± 0.6	177.1 ± 1.2	179.6 ± 1.9	0.29 ± 0.03	–	–	–	–
ZP20	175.1 ± 0.2	177.7 ± 0.1	180.4 ± 0.2	0.21 ± 0.02	−1.6 ± 3.0	21.5 ± 0.8	33.2 ± 0.5	3.1 ± 0.4
ZP30	175.5 ± 1.2	179.6 ± 0.8	183.5 ± 0.8	0.13 ± 0.01	0.5 ± 2.3	24.1 ± 0.4	39.0 ± 0.7	5.2 ± 0.2
ZP40	175.4 ± 1.9	179.0 ± 0.9	182.6 ± 1.2	0.14 ± 0.02	−2.1 ± 0.5	24.9 ± 0.3	41.2 ± 0.3	9.3 ± 0.0
ZP50	174.8 ± 3.3	178.1 ± 1.8	181.8 ± 1.9	0.09 ± 0.02	0.6 ± 0.9	27.9 ± 0.1	44.7 ± 0.6	11.8 ± 0.5
PCLHE	–	–	–	–	30.3 ± 0.3	42.1 ± 0.4	57.1 ± 0.4	46.2 ± 1.1

[a] o, expressed as the onset temperature.

[b] m, the midpoint temperature.

[c] e, the end point temperature.

[42]. T_g and ΔC_p of PCL in PCLHE were −53.4 ± 0.9 °C and 0.26 ± 0.03 J g^{-1}k^{-1}, respectively. However, ΔC_p data of PCL were not available in ZPs due to their weakness in the change of heat capacity. The ΔC_p value of commercial zein was 0.43 J g^{-1}k^{-1}, which is in agreement with that for commercial zein (0.415 J g^{-1}k^{-1}) at the second scanning, reported by Di Gioia et al. [43]. With the increasing PCLH content in ZPs, zein content decreased, which leaded to the reduction of ΔC_p value of zein as shown in Table 3. The T_g of zein in ZPs decreased nonmonotonously with the increasing PCLH content. But according to the Gordon–Taylor equation [44], the decreasing trend should be monotonous. This may result from that weak glass transitions of zein component in ZP30-50 made their T_gs ill-defined. For CZ, a small transition at 200.1 °C was observed from DSC curve, and a small peak at 201.5 °C from the derivated DSC curve of CZ (Fig. 6). Two major fractions of soy protein isolates (7S and 11S fractions) had different but close glass transitions [45]. The situation is similar to zein, implying that the smaller transition and the bigger transition maybe distributed to the two subfractions (Z22 and Z24) of zein as shown in Fig. 3. Z22 should show a more obvious transition than that of Z24, owing to that Z22 was richer than Z24 in CZ (Fig. 3). Therefore, we considered that the bigger transition at 180.1 °C to Z22 fraction and the smaller transition at 200.1 °C to Z24 fraction of zein, respectively. Of course, a direct evidence is to separate the two fractions and study their T_gs, respectively.

DTMA was further used to determine the T_g of ZPs because it is sensitive to glass transition of polymer than DSC [46]. The tan δ curves can be seen in Fig. 7, and the results of DTMA are shown in Table 4. A big relaxation peak for CZ was observed at 180.4 °C, which was associated with glass transition of zein. The height of this peak reduced gradually due to the reduction of zein content in ZPs. This is in agreement with that of ΔC_p value at glass transition, which decreased while zein content reduced (Table 3). Generally, the tan δ peak height is proportional to the volume fraction of the material undergoing the transition, and the change in ΔC_p is related to the weight fraction of the polymer participating in glass transition [47]. Table 4 clearly shown that T(tan δ) reduced slowly from 180 to 171 °C with the increasing PCLH content, indicating that it is certain for T_g of zein in ZPs to decreased monotonously while the amount of PCLH raised. Similar trends can be seen on the $T(E')$ and $T(E'')$. But this trend could not be observed in DSC data (Table 3), which supports the above assumption that weak glass transition made the T_g of zein in ZPs ill-defined by use of DSC. The reduction of T_g in ZPs originated from the plasticizer role of PCLH, a common phenomenon in polymer that can be quantitatively described [44]. We also found that the size of sample made an obvious effect on DTMA data. In order to describe this kind of effect, the height of disk-like CZ sample was changed from original 1.5 to 4.4 mm but the diameter was kept constant to 5 mm, and four duplications was conducted. It was found that the T(δ) increased from 180.4 ± 0.8 to 189.4 ± 1.4 °C, owing to the greater heat resistance of sample with increasing height. The related curve was not shown. In Fig. 7, a small tan δ peak at 205.6 °C can be seen for CZ, and reduced with the reduction of zein content in ZPs. This transition can be observed clearly in the curve of linear expansion rate (Fig. 8). This small transition was also

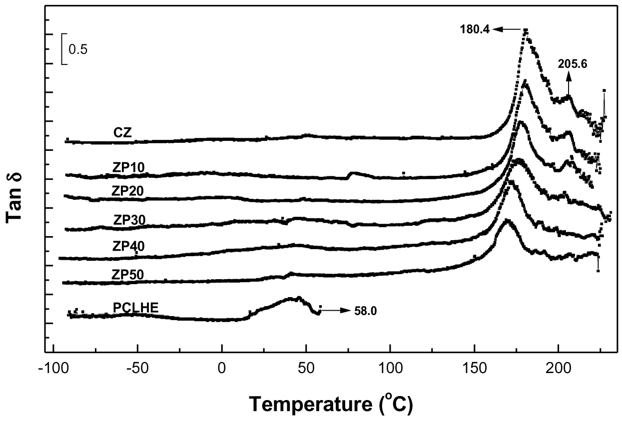


Fig. 7. Effect of PCLH content on the loss tangent (tan δ) of CZ, ZPs and PCLHE.

Table 4
Experimental results of DTMA

Material	$T(E')$ (°C)[a]	$T(E'')$ (°C)	$T(\tan\delta)$ (°C)	$T(R_{\mathrm{exp}}\ 2)$ (°C)	$T(R_{\mathrm{exp}}\ 1)$ (°C)
CZ	161.9 ± 1.4	174.6 ± 2.0	180.4 ± 0.8	183.4 ± 0.6	210.7 ± 0.9
ZP10	160.0 ± 4.8	171.3 ± 3.5	180.7 ± 2.9	183.4 ± 2.5	206.3 ± 4.6
ZP20	160.0 ± 7.2	171.3 ± 3.4	179.5 ± 3.2	180.1 ± 4.0	210.5 ± 2.1
ZP30	155.3 ± 2.4	166.2 ± 3.0	177.7 ± 2.4	182.2 ± 1.5	221.9 ± 1.3
ZP40	152.6 ± 3.2	160.8 ± 1.6	171.5 ± 2.1	174.8 ± 0.3	220.7 ± 0.4
ZP50	150.1 ± 1.0	159.1 ± 1.6	171.2 ± 2.3	176.6 ± 4.0	220.9 ± 0.2

[a] $T(E')$ represents the onset temperature in storage modulus drop. $T(E'')$, $T(\tan\delta)$, $T(R_{\mathrm{exp}}\ 2)$ and $T(R_{\mathrm{exp}}\ 1)$ are the peak temperatures of loss modulus, loss tangent, two linear expansion rates, respectively.

found in DSC curve (Fig. 6). This evidence and the above DSC data make us further believe that this small transition was a glass transition of one compound in zein. But this transition has not been well reported before.

The existence of PCL crystal (Fig. 6) and no dramatic decrease in T_g of zein matrix implied that zein matrix and PCLH component were in micro-phase separation. However, the decreases of T_m of PCL crystal (Table 3) and of T_g of zein matrix (Table 4) indicated a partial miscibility between zein matrix and PCLH component. Therefore, ZPs were heterogeneous in which zein matrix and PCLH component were partial miscible.

WXRDPs of CZ, ZP30, ZP50, and PCLHE are shown in Fig. 9. PCLHE showed two sharps peaks and a shoulder peak at $2\theta = 24.5°$, 22.2°, and 20.4°, respectively. The results are in agreement with the report of Bogdanov et al. [39]. However, ZP30 and ZP50 were amorphous. The temperature in X-ray diffraction experiment was 28.5 °C, higher than the T_ms of ZP30, ZP50 and lower than that of PCLHE (Table 3), which leaded to the melting of PCL crystal in ZP30 and ZP50, and crystallization peak for PCLHE.

3.3. Mechanical properties

Typical stress/strain curves of CZS and ZPS10-50 are shown in Fig. 10. Final water content in zein material should be considered because the mechanical properties of zein are significantly affected by water. In this study, all sheets were stored in a silica gel desiccator for 26 days to measure the mechanical properties of these modified zein materials at dry state, because it is easy to evaluate the fragility of zein at dry state. The error bars of strength at break and breaking elongation are also displayed in Fig. 10. CZS exhibited a brittle failure with no yield point on its stress/strain curve, and showed high strength (24.5 MPa) but low breaking elongation (4.3%). Santosa et al. reported that zein sheet plasticized by oleic acid (0.5 g/1 g zein), stored at 50% RH for 48 h in a desiccator, showed 9.4 MPa of tensile strength and 5.9% of breaking elongation [48]. In addition, after stored at 52% RH for 24 h, casting film of plasticized zein had tensile strength ranged from 5 to 24 MPa, and low breaking elongation (1–8%) [49]. All these data indicated that plasticized zein material in low RH trends to become brittle, and is limited in practice. However, if zein was modified with 10% PCLH (Fig. 10, ZPS10), the breaking elongation increased to 66%, about 15 times than that of commercial zein, and strength at break only decreased about 2 times compared with that of commercial zein. The stress/strain curve of ZPS10 also showed a yield point, suggesting a ductile failure. The toughness of zein modified by a little amount of PCLH, improved quickly, resulting from the flexibility and elasticity of PCL. With the increasing PCLH content, the breaking elongation increased dramatically and strength at yield point decreased drastically, but the

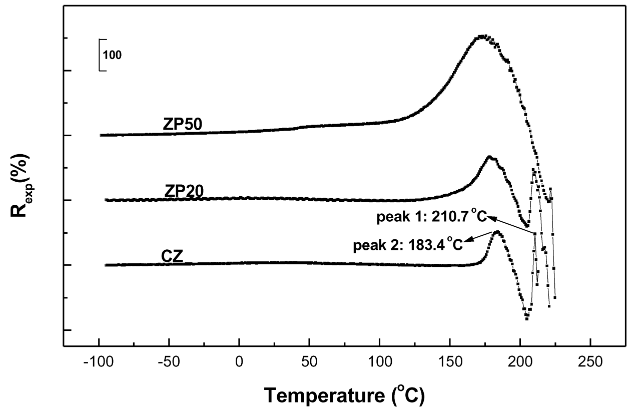


Fig. 8. Curves of linear expansion rate to temperature of CZ, ZP20 and ZP50.

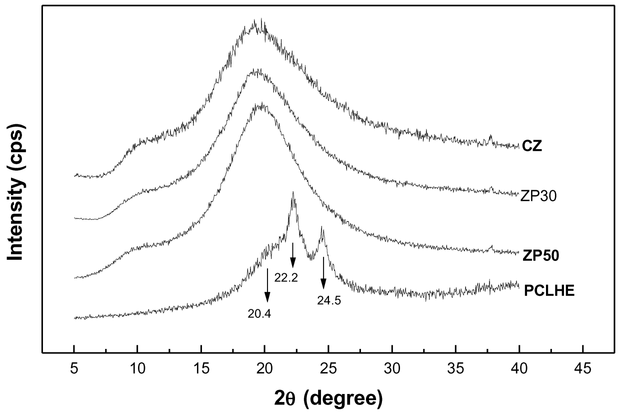


Fig. 9. Wide-angle X-ray diffraction patterns of CZ, ZP30, ZP50 and PCLHE.

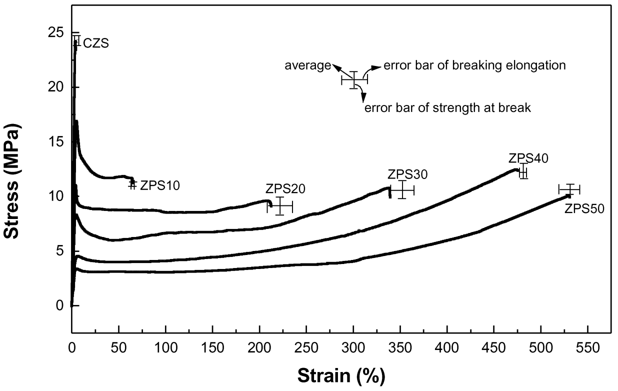


Fig. 10. Stress and strain curves of CZS, ZPS10-50, stored in a silica gel desiccator for 26 days before test. Notes: ZP10 powder was plasticized and compression-molded into ZPS10 sheet; the '*S*' in ZPS10 represents sheet.

strengths at break constantly ranged from 9.3 to 11.9 MPa, indicating that under the condition of almost constant strength, PCLH component can significantly enhance the elasticity of zein-based material. For ZPS50, the strength at break was 10.7 MPa, only a decrease of 0.6 MPa compared with that of ZPS10, but the breaking elongation was as high as 522%. Zein component provided strength and PCL component provided flexibility, which is similar with the known polystyrene–polybutadiene–polystyrene (SBS) resin in which polystyrene improves the strength of SBS and polybutadiene enhances the elasticity of SBS. The data about mechanical properties of PCLHE was not available because it was a wax-like material and could not be used for stress/strain test. The relationship between the structure and properties of the sheets will be studied furthermore in our later work.

4. Conclusions

Zein-based polymers with various PCLH content were synthesized. The average reaction ratio of PCLH was as high as 97.5%. FTIR results showed that the PCLH content in polymer increased with the increasing PCLH content in feed, indicating a successful modification. At least four amino acids (Glu, Gln, Tyr and His) reacted with prepolymer, and urea–urethane links were prominent in ureathane groups. Thermal analysis implied that ZPs were micro-heterogeneous due to the formation of micro-phase separation. The T_g of modified zein diminished with the increasing PCLH content as observed from DTMA analysis, but similar result could not be obtained from DSC analysis due to errors resulted from the weakness of ΔC_p. The melting point decreased with the increasing PCLH content. A novel small glass transition for zein was found at about 201 °C, which can be observed on DSC curve, and was further proved by DTMA results. After 10% PCLH was crosslinked with zein, the breaking elongation of zein sheet increased about 15 times, and strength at break reduced only by about 2 times, compared with commercial zein. Furthermore, with the increasing PCLH content, the flexibility of modified zein sheet improved dramatically but the strength remained roughly constant. Therefore, it is very effective to improve the toughness of zein by the modification.

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