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Solvent mediated microstructures and release behavior of insulin from pH-sensitive nanoparticles

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ABSTRACT

The insulin loaded nanoparticles composed of poly (lactic-*co*-glycolic acid) (PLGA) and hydroxypropyl methylcellulose phthalate (HP55) were prepared via the emulsions solvent diffusion method with two different solvents, namely, DMSO and acetone/water. The microstructures of the nanoparticles were studied by the solubility parameters theory, DSC, FTIR, and the nitrogen adsorption technique. Phase-separated PLGA domains were observed from the nanoparticles prepared with both types of solvents. Mesopores were observed from the nanoparticles prepared with DMSO as the solvent and almost did not exist with acetone/water. An in vitro drug release study showed that the pH-sensitivity of nanoparticles was not only attributed to the pH-dependent dissolubility of HP55 but also to the internal microstructure. The formation of mesopores accelerated the release of insulin, leading to no obvious pH-sensitivity of the nanoparticles prepared with DMSO. However, for the nanoparticles prepared with acetone/water, the release of insulin was pH-dependent. The results demonstrated that solvents played an important role in affecting the microstructures of nanoparticles, which influenced markedly the insulin release behavior.

1. Introduction

pH-sensitive nanoparticles for oral delivery of insulin has been the subject of research with a view to enhancing the bioavailability and reducing the pain of repeated injection compared with standard daily administration [1–6]. The ideal pH-sensitive nanoparticles must be capable of reducing release of insulin in acidic environment of the stomach and promoting in near neutral conditions of the small intestine. The release ability of nanoparticles could be improved by adjusting the composition of pH-sensitive polymer such as poly-γ-glutamic acid, hypromellose phthalate, polymethacrylic acid, alginate and dextran sulfate, etc. [1,6–8]. Moreover, it has been long thought that the burst release was attributed to insulin bound onto the surface of nanoparticles [9,10]. However, the underling mechanism that control the pH-sensitive release of insulin have not been further studied, especially the microstructures in the nanoparticles.

It has been investigated that thin layer of polymers can enable fast penetration of drug through the continuous water-filled channels [11]. With introducing a compact structure into polymeric particles, the cumulative amounts of released drug were significantly reduced in medium condition [7]. In addition, different degree of phase separation from the blended polymers depending on thermodynamic properties of the chosen polymers leads to the variation of their hydration, degradation and drug release behavior [12,13]. Importantly, porosity of polymer particles could influence the rate of polymer swelling and dissolution as well as the corresponding rate of drug release [14–16]. Hence, the release of drug from polymeric particles is closely connected with the internal microstructures of particles.

The factors such as formulation [14], polymer architecture [16,17], and preparation conditions [18–20] could influence the microstructures of polymeric particles. It is well known that polymeric particle preparation by emulsion–solvent evaporation/diffusion method is a complex process in which the organic solvent may generate some pores in the particles during its evaporation/diffusion [21,22]. This may lead to changes in the microstructure of particles significantly enough to affect their physical properties, especially the release kinetics. It has been demonstrated that solvent evaporation/diffusion could induce polymer phase separation which might produce hierarchically porous particles [23–25]. Based on the different characteristics of the solvents used in the preparation of polymeric particles, drug delivery systems exhibit different porous microstructures and release behavior [14]. Thus, it will be meaningful to study the solvent mediated microstructures and release behavior of drug from polymeric nanoparticles.

Insulin loaded pH-sensitive nanoparticles generally involve three components with distinct properties, i.e., hydrophobic polymer, pH-sensitive polymer, and hydrophilic drug. The compatibility among these components may play an important role in the successful fabrication and performance of pH-sensitive nanoparticles.

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An in-depth investigation of the compatibility could be conducted to allow the rational understanding of the effect of solvents on the microstructure of pH-sensitive nanoparticles. The internal microstructure of nanoparticles, in particular the distribution of insulin within the solid matrix, may be a key contributor to the drug release behavior. In this work, insulin loaded nanoparticles composed of poly (lactic-*co*-glycolic acid) (PLGA) and hydroxypropyl methylcellulose phthalate (HP55) were prepared via the emulsions solvent diffusion method with two solvents, namely, DMSO and acetone/water. The study about solvent mediated microstructure will help elucidate the underlying mechanisms of the drug loading and release behavior of pH-sensitive nanoparticles at different pH conditions.

2. Experiment

2.1. Materials

Pure crystalline porcine insulin (27 IU/mg) was purchased from Xuzhou Wanbang Bio-Chemical Co. Ltd, (No. 0312A02, Jiangsu, China). Poly (lactide-*co*-glycolide) (PLGA 50/50, *M*w ≈ 20,000) was acquired from Shandong Medical Instrument Institute. Polyvinyl alcohol (PVA, *M*w = 31,000–50,000) and hydroxypropyl methylcellulose phthalate (HP55, *M*w ≈ 45,000) were purchased from Acros Organics (New Jersey, USA). All other reagents and solvents used were analytical grade. Distilled and deionized water (Mili-Q water systems, Bedford, USA) was used for preparation of all sample solutions.

2.2. Preparation of insulin loaded pH-sensitive nanoparticles

PLGA/HP55 nanoparticles were prepared by the emulsion solvent diffusion method. Two solvents used in this study were dimethylsulfoxide (DMSO) and acetone/water (7/1, v/v). Briefly, PLGA (50 mg), HP55 (50 mg) and insulin (5 mg) were dissolved completely in the solvent (3.5 Ml) to form the PLGA/HP55/insulin solution. The solution was injected to the 40 mL of PVA solution (1.0%, w/v) with the needle under moderate magnetic stirring. The nanoparticles were allowed to harden at the room temperature for 1 h using a magnetic stirrer. Then the entire dispersed system was centrifuged and resuspended in distilled water to wash out the free insulin and PVA. This process was then repeated, and the insulin loaded PLGA/HP55 nanoparticles were freeze-dried overnight.

2.3. Characterization of nanoparticles

The size of nanoparticles was determined by photon correlation spectroscopy (PCS) at 25 °C with a detection angle of 90° by using a Malvern Zetasizer II (Malvern Instruments, UK). Measurements were made on aqueous dilute nanoparticles suspension.

The morphological examination of the insulin loaded nanoparticles at slightly basic condition was performed by transmission electron microscopy (TEM, Hitachi JEM-100CXII, Japan) following negative staining with sodium phosphotungstate solution (2%, w/v). Simply, the lyophilized nanoparticles were resuspended in water, and then ultrasonically dispersed for 1 h to separate the dissolved HP55 from undissolved PLGA particles in pH 7.4 condition. An aqueous droplet of both the sample and a phosphotungstate solution were placed on a 300 mesh copper grid with a carbon-coated Formvar membrane. After one minute, the excess of fluid was removed using filter paper and the sample was air-dried before examination by TEM.

The drug encapsulation efficiency and loading capacity were calculated according to formula [26]:

$$\text{Encapsulation efficiency}\,(\%) = \frac{(M_{\text{total}} - V_{\text{supernatant}} \times C_{\text{supernatant}})}{M_{\text{total}}} \times 100\% \quad (1)$$

$$\text{Loading capacity}\,(\%) = \frac{(M_{\text{total}} - V_{\text{supernatant}} \times C_{\text{supernatant}})}{W_{\text{nanoparticle}}} \times 100\% \quad (2)$$

where M_{total} is initial amount of insulin (mg), $V_{\text{supernatant}}$ is the volume of supernatant (mL), $C_{\text{supernatant}}$ is the concentration of insulin in supernatant (mg/mL), $W_{\text{nanoparticle}}$ is the weight of nanoparticles. The concentration of insulin was determined by reverse phase HPLC method (Agilent 1200, ZORBAX 300 SB-C18 column 150 mm × 4.6 mm, 5 μm, USA) [27].

2.4. Solvent–polymer interaction parameter

The solvent–polymer interaction parameter (χ) can be related to the solubility parameters via [28]:

$$\chi = \frac{V_{\text{solvent}}}{RT}(\delta_{\text{solvent}} - \delta_{\text{polymer}})^2 \quad (3)$$

where V_{solvent} is the molar volume of solvent and R is the gas constant. The solubility parameter (δ) was obtained by Hansen's approach [29], which used partial solubility parameters to calculate the total solubility parameters as:

$$\delta = (\delta_{\text{d}}^2 + \delta_{\text{p}}^2 + \delta_{\text{h}}^2)^{1/2} \quad (4)$$

The partial solubility parameters for polymers were calculated by the group contribution method using the following three equations [30]:

$$\delta_{\text{d}} = \frac{\sum F_{\text{di}}}{V} \quad (5)$$

$$\delta_{\text{P}} = \frac{\sqrt{\sum F_{\text{pi}}^2}}{V} \quad (6)$$

$$\delta_{\text{h}} = \frac{\sqrt{\sum E_{\text{hi}}}}{V} \quad (7)$$

where F_{di}, F_{pi} and E_{hi} refer to the specific functional group contributions respectively: van der Waals dispersion forces (F_{di}), dipole–dipole interactions (F_{pi}), and hydrogen bonding (E_{hi}), which were obtained by the Hoftyzer-Van Krevelen's method [31]. The total molar volume (V) of various polymer repeat units was obtained by the Fedors method [32]. We divided PLGA and HP55 molecules into small chemical groups and used their F_{di}, F_{pi} and E_{hi} values to calculate the partial and total solubility parameters.

2.5. DSC analysis

Glass transition temperatures (T_g) of the polymers, insulin and insulin loaded nanoparticles were measured with a differential scanning calorimeter (DSC, NETZSCH STA449C, Germany). Sample was prepared by carefully weighing 7–8 mg of the nanoparticles or insulin into an aluminum oxide pan and then hermetically sealed. The pans were then heated from 35 °C to 250 °C at a rate of 5 °C/min under a constant flow of nitrogen gas. Calibration of the system was performed using indium and zinc standards.

Table 1
Calculated solubility parameters of solvents and polymers used in the preparation of nanoparticles.

Parameter	Water $(MPa)^{1/2}$	Acetone $(MPa)^{1/2}$	DMSO $(MPa)^{1/2}$	PLGA $(MPa)^{1/2}$	HP55 $(MPa)^{1/2}$
$\delta_d = \sum F_{di}/V$	12.3	15.5	18.4	18.3	19.8
$\delta_p = (\sum F_{Pi}{}^2)^{1/2}/V$	31.3	10.4	16.4	8.2	7.87
$\delta_h = (\sum E_{hi}/V)^{1/2}$	34.2	6.9	10.2	12.9	14.3
$\delta_t = (\delta_d{}^2 + \delta_p{}^2 + \delta_h{}^2)^{1/2}$	47.9	19.9	26.6	23.8	25.7

2.6. FTIR spectroscopy

The sample mixed with KBr was pressed to a disk, and was scanned in the range from 400 to 4000 cm^{-1} on a Fourier transform infrared spectrometer (FTIR, NICOLET 380, USA). FTIR spectra were obtained at a resolution of 4 cm^{-1} with a minimum of 256 scan per spectrum. All measurements were taken at room temperature, and all spectra were baseline corrected and normalized.

2.7. Porosity measurements

Nitrogen adsorption experiments and porosity measurements were performed using a surface area and porosity analyzer (TristarII 3020, Micromeritics, USA) as described by Sant et al. [16]. Weighed amounts of the nanoparticles were placed in the glass cells, and degassed at 30 °C for 72 h before analysis. The samples (sealed glass tube was used in cell to minimize the dead space) were immersed in liquid nitrogen at −196 °C. The high-resolution nitrogen adsorption isotherms were obtained from the volume of nitrogen (cc/g) adsorbed onto the surfaces of the nanoparticles as a function of relative pressure.

2.8. Viscometry measurements

The viscometry measurements were performed in a suspended-level ubbelohde glass capillary viscometer with capillary diameter 0.442 mm at 20 °C. Flow time was determined by the serial dilution technique. Five dilutions were made for each blend composition of PLGA and HP55 polymer. The specific viscosities η_{sp} at different concentrations were calculated from the flow time. Intrinsic viscosity $[\eta]$ and interaction constant k' were determined by linear regression analysis from the plot of η_{sp}/c vs. polymer concentration c according to Eq. (8) [33].

$$\frac{\eta_{sp}}{c} = [\eta] + k'[\eta]^2 c \tag{8}$$

2.9. In vitro release

5 mg of the nanoparticles were dispersed in 5 mL simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.4), respectively, and shaken at 50 r/min at 37.8 °C using a constant-temperature shaker (SHA-B, Guohua Co. Ltd., China). At specified time intervals (0, 30, 60, 120 and 240 min), supernatant were collected by centrifugation. The concentrations of insulin in the supernatant were determined by HPLC, and total amount of insulin released from the nanoparticles was calculated. The each experiment was carried out in triplicate.

3. Results and discussion

3.1. Particle size distribution and encapsulation efficiency (EE)

The insulin loaded PLGA/HP55 nanoparticles prepared with DMSO and acetone/water (7/1, v/v) were found to be monodispersed, having diameters of 250–280 nm as determined by PCS (Fig. S1 in the Supporting Information). The size of nanoparticles prepared with DMSO is slightly smaller than with acetone/water. The insulin encapsulation efficiencies in nanoparticles prepared with DMSO and acetone/water (7/1, v/v) were 87.8% and 85.4%, respectively. The blend of PLGA and HP55 showed a good EE of insulin, which could be attributed to the enhanced interaction between the insulin and polymers, thus reducing premature diffusion of insulin into the external aqueous phase during solidification of the nanoparticles.

Supplementary material related to this article found, in the online version, at doi:10.1016/j.colsurfb.2012.01.029.

3.2. Compatibility of the components in the insulin loaded nanoparticles

The compatibility between every two components affects the microstructures of drug loaded nanoparticles, which further influences the drug release behavior. In this section, different techniques, including the group contribution method, DSC, and FTIR, would be used to study the compatibility and the phase separation of components.

The compatibility depends strongly on the solubility parameters (δ), which are widely used to predict the miscibility of polymer blends. According to the solubility parameter theory, a similar δ value indicates similar solubility properties of polymers, and a higher δ value indicates the polymer is more hydrophilic [34]. The δ values calculated with group contribution method were listed in Table 1. The solubility parameters of PLGA, HP55, acetone, DMSO, and water are 23.8, 25.7, 19.9, 26.6, and 47.9 $(MPa)^{1/2}$, respectively. The PLGA and HP55 could be easily dissolved in acetone and DMSO according to their δ values, which also observed in the experiment. However, the δ values of PLGA and HP55 are much lower than that of water, indicating that the two polymers are hydrophobic. Moreover, PLGA is more hydrophobic than HP55, because the δ value of PLGA is lower than that of HP55. This different hydrophobicity of PLGA and HP55 could induce their phase separation in the solvent. When the water was introduced to the polymer blends, PLGA molecules would aggregate first due to the stronger repulsion between PLGA and water molecules. This phase separation may help for analyzing the microstructures of polymer blend nanoparticles, which would be further discussed in the following sections.

DSC was carried out to further study the phase separation of PLGA and HP55 and the results are shown in Fig. 1. Insulin, PLGA, and HP55 exhibit endothermic peaks around 65 °C, 56 °C and 168 °C, which correspond to their glass transition temperatures (T_g), respectively. These similar peaks are in agreement with the finding reported in the literatures [24,35,36]. When insulin was introduced to the polymer blend nanoparticles prepared respectively with acetone/water and DMSO, the endothermic peak of PLGA was also observed at around 56 °C. Generally, solitary T_g value for the polymer blend, between the T_g values of the used pure components, is regarded as an evidence of polymer miscibility [37]. The observation of this peak of PLGA indicated that most PLGA molecules could not be miscible well with HP55 and insulin molecules. This provided an evidence of phase separation of PLGA from other components. However, the endothermic peak of HP55 could not be observed from the DSC profiles of drug loaded nanoparticles, suggesting that HP55 has a stronger interaction with insulin compared with PLGA. Meanwhile, the endothermic peak of

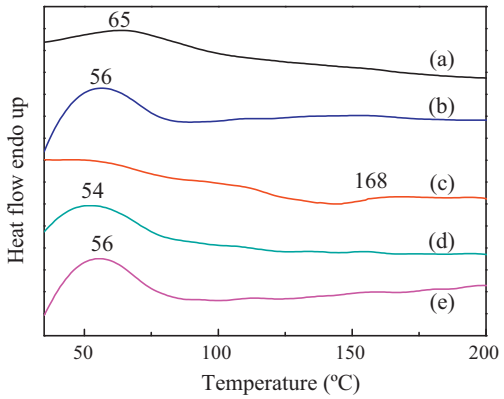

Fig. 1. Thermograms of pure insulin (a), PLGA (b), HP55 (c), and insulin loaded PLGA/HP55 nanoparticles prepared with acetone/water (d) and DMSO (e).

insulin could not be also observed from the DSC profiles of drug loaded nanoparticles. Broadening of the glass transition is generally observed in miscible blends and is attributed to both concentration fluctuations and differences in component mobility [38,39]. In these cases, it is likely that the insulin domains were more finely dispersed in the continuous HP55 phase as a result of the drug-polymer interaction, which would be further confirmed by FTIR.

FTIR spectroscopy, a very useful and convenient technique in research of polymer blends, can provide some information on inter-polymer interaction [40]. The FTIR spectra of pure insulin, PLGA, HP55, and insulin loaded nanoparticles were examined and shown in Fig. 2. The spectrum of pure insulin shows two shoulders of bands in the amide I (1655 cm^{-1}) and amide II (1539 cm^{-1}) (Fig. 2a), which are characteristic of protein spectra. The spectrum of PLGA shows bands for carbonyl groups at 1759 cm^{-1} (Fig. 2b) and HP55 for carboxylic groups at 1735 cm^{-1} (Fig. 2c). However, for the insulin loaded PLGA/HP55 nanoparticles prepared with acetone/water and

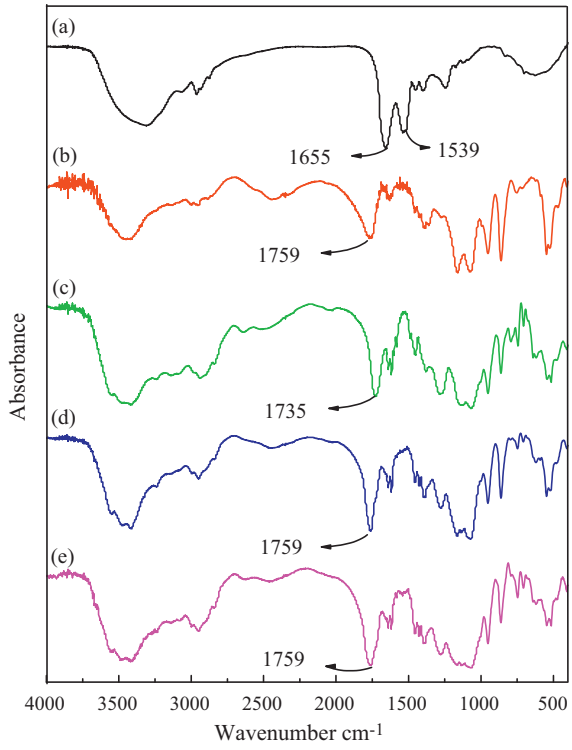

Fig. 2. FTIR spectra of pure insulin (a), PLGA (b), HP55(c), and insulin loaded PLGA/HP55 nanoparticles prepared with acetone/water (d) and DMSO (e).

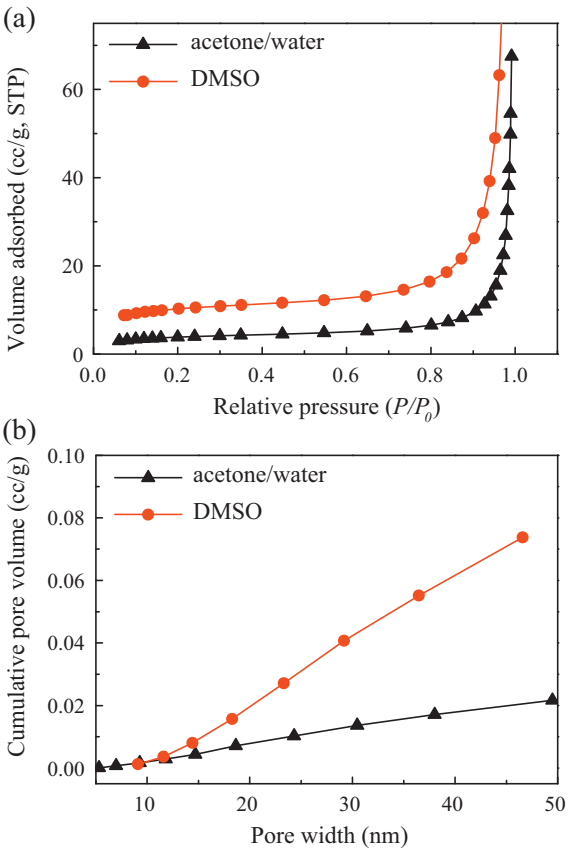

Fig. 3. Nitrogen adsorption isotherms (a) and mesoporosity (b) of the PLGA/HP55 nanoparticles prepared with acetone/water and DMSO.

DMSO, respectively, the two peaks of amino groups of insulin (i.e. 1539 cm^{-1} and 1655 cm^{-1}) and the carboxylic absorption of HP55 around 1735 cm^{-1} disappeared, while no shift could be observed for the peak of carbonyl groups of PLGA at 1759 cm^{-1} (Fig. 2d and e). These changes could be attributed to the strong interactions via hydrogen bonding between the carboxylic groups in phthalate groups of HP55 and amide groups in insulin, while the interaction between insulin and PLGA was weak [41]. Due to the interpolymer interaction of hydrogen bonding, HP55 has been found to be miscible or partially miscible with some polymer counterparts [36]. Therefore, the formation of hydrogen bonding enhanced the compatibility between insulin and HP55 molecules, leading to the main distribution of insulin in HP55 matrix during the preparation of insulin loaded PLGA/HP55 nanoparticles.

3.3. *The effect of solvents on the porosity of insulin loaded nanoparticles*

In order to investigate the microstructures of the nanoparticles, pore size distributions (PSD) of insulin loaded nanoparticles were measured using nitrogen adsorption porosimetry, as shown in Fig. 3. The cumulative pore volume was calculated according to the Barret–Joyner–Halenda (BJH) method, which is based on the Kelvin equation and corrected for multiplayer adsorption, including calculations of the PSD over the mesopore and part of the macropore range [42,43]. The nanoparticles prepared with DMSO showed higher nitrogen uptake compared to those prepared with acetone/water (Fig. 3a), indicating that the mesopore volumes for the nanoparticles prepared with DMSO were higher than those with acetone/water. A comparison of cumulative mesopore volumes for different nanoparticles was shown in Fig. 3b. It can be seen that

the mesopore volumes for nanoparticles prepared with different solvents were also in the order of DMSO > acetone/water. This difference could be attributed to the interactions between polymers and different solvents, which would be further discussed in the following content.

The difference of interactions between solvents and polymers may affect the microstructures and physical properties of the resulting nanoparticles [44]. The value of solvent–polymer interaction parameter (χ) shows the degree of affinity between solvent and polymer. As $\chi_{solvent-polymer}$ increases, the degree of affinity between polymer and solvent decreases [45]. The $\chi_{acetone-HP55}$ value calculated from Eq. (3) is 1.10 and $\chi_{DMSO-HP55}$ is only about 0.03, indicating that the affinity between DMSO and HP55 is stronger than that between acetone and HP55, while the difference between $\chi_{acetone-PLGA}$ (0.49) and $\chi_{DMSO-PLGA}$ (0.26) is not obvious. According to the literature's report [46], a stronger affinity between solvent and polymer could lead to more solvent remaining in the supersaturated polymer region, resulting in a higher viscosity of the solution. The experimental results (Fig. 4a) showed the same trend that the viscosity of the PLGA/HP55/DMSO solution is higher than the PLGA/HP55/acetone/water solution at the same weight fraction for HP55. The increase of intrinsic viscosity with the increase of HP55 weight fraction was consequence of the higher molecular weight of HP55 compare to PLGA. Therefore, the motion of solvent DMSO toward the water is hampered by a greater viscosity of PLGA/HP55/DMSO solution. In addition, the interaction constant (k') shows the interactions of polymer chains in a solvent. As k' value increases, the interaction decreases [47]. According to the values of k' (Fig. 4b), the interaction between PLGA and HP55 was weaker in the DMSO than in acetone/water. When PLGA/HP55/DMSO solution was introduced to the aqueous solution, only a little amount of DMSO diffused into the water in a short time and larger amount of DMSO stayed in the HP55/PLGA precipitate, leading to a swollen state. With the freeze-drying process of the nanoparticles, mesopores formed gradually [48]. In contrast, the lower intrinsic viscosity of PLGA/HP55/acetone solution and higher polymer interaction constants could contribute to the formation of nanoparticles with more compact and less porous structure. The mesopores may accelerate the release of insulin from the nanoparticles, which may also influence the pH-sensitivity of nanoparticles.

3.4. Microstructural analysis of the insulin loaded nanoparticles

Based on the mentioned studies of the compatibility and mesoporosity of the nanoparticles, the potential formation processes of insulin loaded nanoparticles with different microstructures were proposed, as shown in Fig. 5. In the preparation of insulin loaded nanoparticles with emulsion solvent diffusion method, PLGA and HP55 were dissolved in the solvents (DMSO or acetone/water).

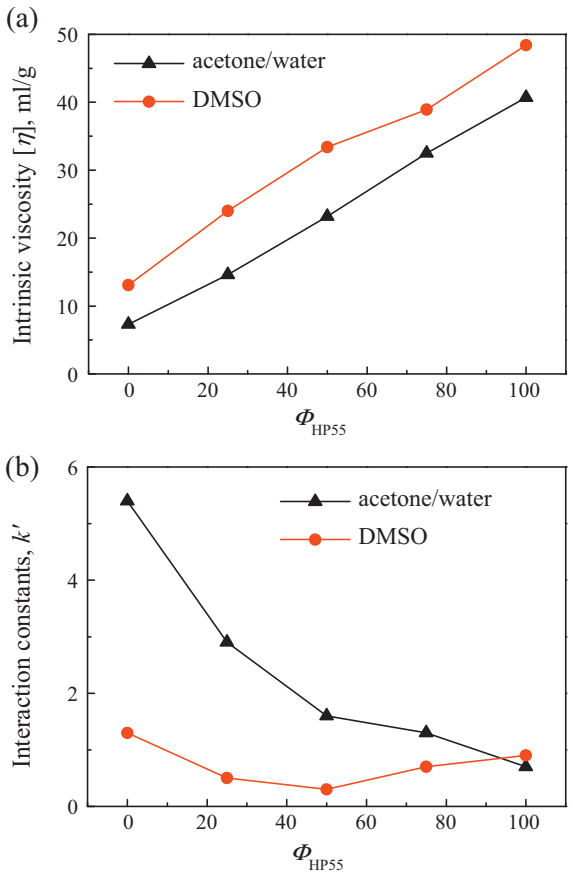

Fig. 4. Variations of intrinsic viscosity of solutions (a) and interaction constant k' (b) as a function of weight fraction Φ for HP55 in PLGA/HP55 blend using acetone/water and DMSO as solvent.

When the resulted solution was mixed with a large amount of water, the PLGA and HP55 precipitate and aggregate due to their hydrophobic nature, and PLGA molecules distribute inside the core of aggregation and are surrounded by HP55, as a result of the stronger repulsion between PLGA and water than that between HP55 and water. Insulin molecules distribute mainly in the HP55 phase, which has been attributed to the stronger interaction between insulin and HP55 confirmed by the DSC and FTIR results above.

For the both drug loaded nanoparticles prepared with DMSO or acetone/water solvents, there are no obvious differences for distributions of each component. The main difference is the porosity of the nanoparticles. For the nanoparticles prepared with DMSO, a large amount of DMSO molecules cannot diffuse into the external

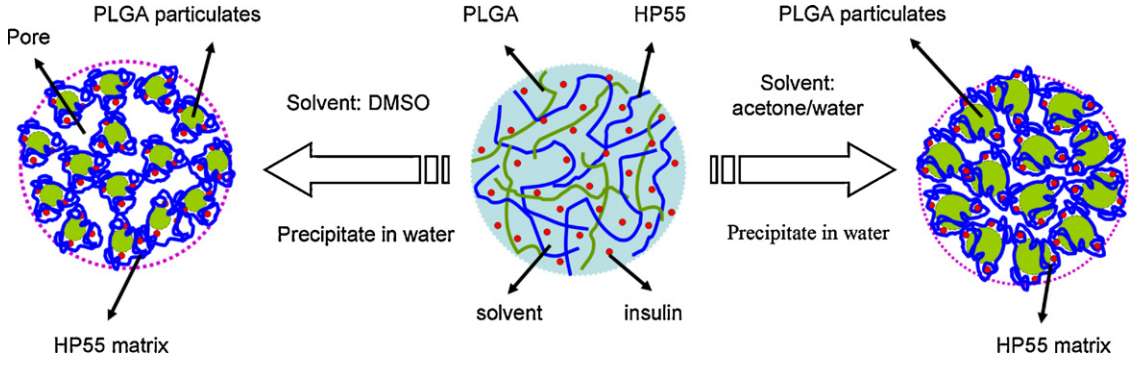

Fig. 5. Schematic of phase-separated (in water) PLGA/HP55 nanoparticles.

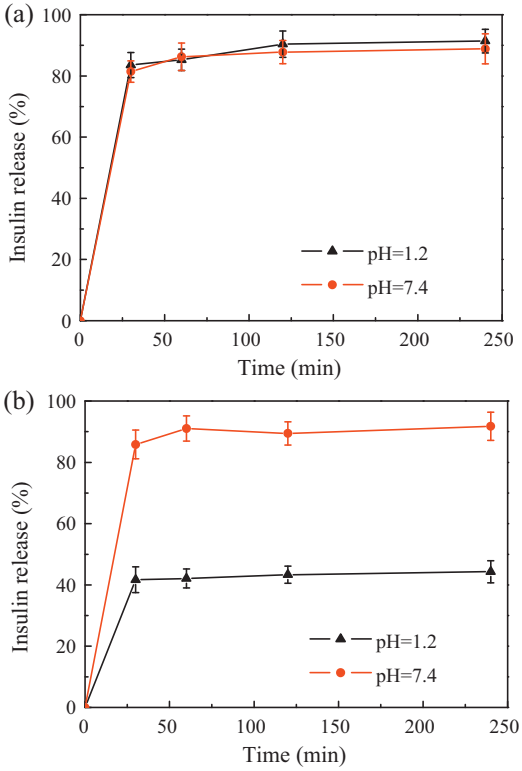

Fig. 6. In vitro release profiles of insulin from nanoparticles prepared with DMSO (a) and acetone/water (b) at pH 1.2 and 7.4.

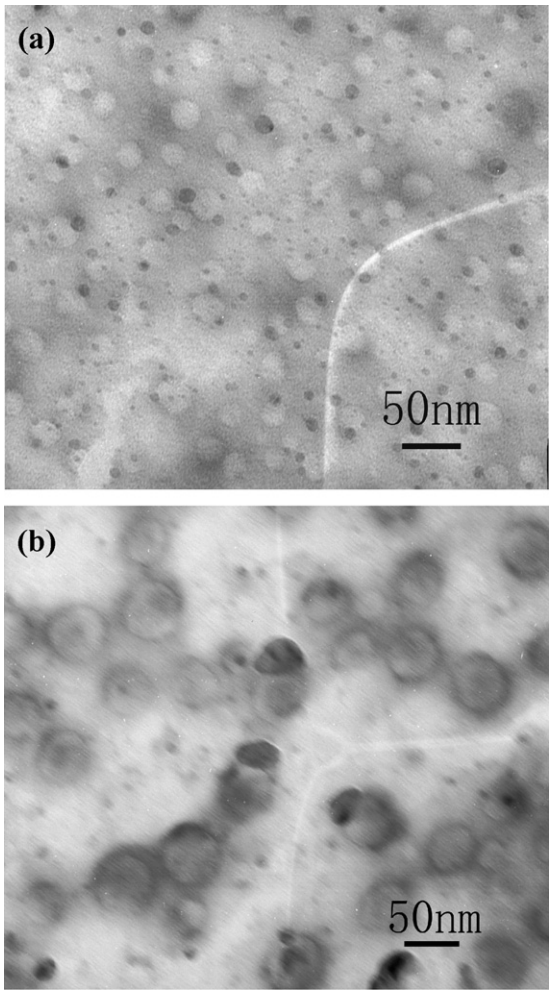

Fig. 7. TEM images of nanoparticles prepared with DMSO (a) and acetone/water (b) immersed in pH 7.4 medium for 1 h.

water phase at the early stage of nanoparticles formation due to the high viscosity of solution and the strong interaction between DMSO and HP55. It would take a long time for water molecules to replace DMSO molecules inside the nanoparticles in the solvent diffusion process, resulting in a swollen structure of the nanoparticles. So the mesopores could form when the water molecules were removed during the freeze-drying of nanoparticles. For the nanoparticles prepared with acetone/water, the acetone molecules can easily diffuse into the external water phase in a short time due to the low viscosity of solution and the weak interaction between acetone and HP55, and the nanoparticles form rapidly in a short time. Thus, the amount of water remained inside the nanoparticles is much less than that of nanoparticles prepared with DMSO. Few water molecules inside the nanoparticles would reduce the formation of mesopores in the nanoparticles. The structural changes of pore channels could influence drug permeability [49], and the change of pore size would affect the diffusion of drug [50]. For the insulin loaded nanoparticles prepared with DMSO, insulin molecules may be easily released through the large mesopores. This may reduce the pH-sensitivity of nanoparticles. However, for the insulin loaded nanoparticles prepared with acetone/water, with only a small amount of mesopores, HP55 must dissolve first, and then insulin molecules released from the nanoparticles. The microstructure would play an essential role for the release of insulin from the nanoparticles.

3.5. The effect of microstructure on release behavior of insulin from the insulin loaded nanoparticles

Insulin loaded PLGA/HP55 nanoparticles were prepared using the emulsion solvent diffusion method. In vitro insulin releases of the nanoparticles were performed respectively under gastric environment (pH 1.2) and intestinal conditions (pH 7.4), and the results are shown in Fig. 6. For the nanoparticles prepared with DMSO (Fig. 6a), there are no obvious differences for the insulin release behavior at pH 1.2 and 7.4, indicating that the pH-sensitivity of HP55 does not play a role in the release of insulin. As discussed above, mesopores could be observed in the nanoparticles prepared with DMSO. The presence of pores in drug delivery systems does not only increase the mobility of the involved diffusing species (drug molecules, acids and bases), but it fundamentally alters the contributions of the involved physicochemical processes to the overall control of drug release [15,51]. The formation of mesopores provided the diffuse channels, which could accelerate the release of insulin from the nanoparticles, leading to no obvious pH-sensitivity of nanoparticles. For the nanoparticles prepared with acetone/water (Fig. 6b), the release rate of insulin from the nanoparticles is markedly pH-dependent. At pH 1.2, only 40% insulin could be released, while at a higher pH value of 7.4, about 90% insulin could be released from the nanoparticles. The mesopores in the nanoparticles prepared with acetone/water are much less than that prepared with DMSO. In general, the greater the mesopore volume, the larger the amount of drug release from the mesoporous controlled delivery systems [52]. At pH 1.2, the hydrophobic nanoparticles with small mesoporous volume could reduce the contact of water and insulin molecules, and prevent the release of insulin. At pH 7.4, HP55 can easily dissolved in water, facilitating the release of insulin molecules which mainly distribute in the HP55 phase. Thus, not only the pH-sensitivity of

the polymer HP55, but also the mesoporosity has some effects on the pH-sensitive release of insulin from the nanoparticles.

We further investigated the effect of mesopores on the dissolution of nanoparticles, by immersing of the two kinds of nanoparticles at pH 7.4 medium for 1 h and morphology measured by TEM (Fig. 7.). The residual particle size after dissolving at pH 7.4 medium was found to be about 50 nm for nanoparticles prepared with acetone/water, compared to about 20 nm for nanoparticles prepared with DMSO. These decreases in nanoparticles size may be due to the dissolution of pH-sensitive polymer at basic medium [53]. However, the presence of mesopores in nanoparticles could accelerate dissolution of HP55, probably leading to shift of the size of residual particles toward lower values. The phenomena have to be carefully taken into account when developing and optimizing this type of pH-sensitive protein delivery systems.

4. Conclusion

In this work, pH-sensitive nanoparticles of PLGA/HP55 were prepared using emulsion solvent diffusion method with two different solvents of DMSO and acetone/water. The compatibility of components was studied via solubility parameters calculated by the group contribution method. No obvious differences were observed with the phase separation microstructures of nanoparticles prepared with both types of solvents. The results were further confirmed by DSC and FTIR results. However, a large amount of mesopores was observed from the insulin loaded nanoparticles prepared with DMSO. The formation of mesopores accelerated the release of insulin, leading to no obvious pH-sensitivity of nanoparticles. In contrast, few mesopores form in the nanoparticles prepared with acetone/water, where the release of insulin from the nanoparticles mainly depends on the pH-dependent solubility of HP55. Thus, the solvent mediated microstructure of nanoparticles is an important factor influencing the insulin release from nanoparticles.

Acknowledgements

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