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Keywords:	chitosan, molecular dynamics, poly(vinyl pyrrolidone), DRIFTS, 13C CP/MAS NMR

Molecular modeling simulation and experimental measurements to characterize chitosan and poly(vinyl pyrrolidone) blend interactions

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Abstract

Blends of chitosan and poly(vinyl pyrrolidone) (PVP) have a high potential for use in various biomedical applications, and in advanced drug delivery systems. Recently, the physical and chemical properties of these blends have been extensively characterized. However, the molecular interaction between these two polymers is not fully understood. In this study, the intermolecular interaction between chitosan and PVP was experimentally investigated using ^{13}C cross-polarization magic angle-spinning nuclear magnetic resonance (C^{13} CP/MAS NMR) and diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS). According to these experimental results, the interaction between the polymers takes place through the carbonyl group

of PVP and either the OH-C6, OH-C3 or NH-C2 of chitosan. In an attempt to identify the interacting groups of these polymers, molecular modeling simulation was performed. Molecular simulation was able to clarify that the hydrogen atom of OH-C6 of chitosan was the most favorable site to form hydrogen bonding with the oxygen atom of C=O of PVP, followed by that of OH-C3, whereas that of NH-C2 was the weakest proton donor group. The nitrogen atom of PVP was not involved in the intermolecular interaction between these polymers. Furthermore, the interactions between these polymers are higher when PVP concentrations are lower and interactions decrease with increasing amounts of PVP.

Keywords: chitosan; poly(vinyl pyrrolidone) (PVP); molecular dynamics; DRIFTS; ^{13}C CP/MAS NMR.

1. Introduction

Polymer blends have received a great deal of attention in recent years, since blending is a simple and effective method to develop new materials with specific properties that cannot be achieved by the individual polymer. Chitosan, (1-4)-2-amino-2-deoxy-β-D-glucan, is a natural polymer obtained by alkaline deacetylation of chitin, (1-4)-2-acetamido-2-deoxy-β-D-glucan. The molecule contains amino and hydroxyl groups on its backbone which can serve as proton donors/proton acceptors in hydrogen bonding interaction between chitosan molecules, or between chitosan and other polymers. Chitosan has been widely investigated for its potential use in industrial, medical and pharmaceutical applications. In addition to the use of chitosan as a single polymer, it is also often blended with various other hydrophilic polymers.[1,2] Chitosan and poly(vinyl pyrrolidone) (PVP) blends have been prepared and investigated for their potential use as controlled release drug delivery systems, and for enhancing mucoadhesive properties.[3,4] Both chitosan and PVP (Figure 1) are biocompatible and nontoxic and they demonstrate interesting biological properties.[5-7]

Generally, the important factor that determines the properties of a blend is the compatibility/miscibility of polymer pairs. The chemical structures of the polymeric components have a significant effect on the interactions between the polymers resulting in miscibility of the polymer blend. For chitosan and PVP blends, both the hydroxyl and the amine functional groups of chitosan have the potential to interact with the amide groups of PVP via hydrogen bonding. Various methods including viscosity measurement,[7-9] differential scanning calorimetry (DSC)[10] and Fourier transform infrared (FTIR) spectroscopy[8] have been previously used to explore the interactions between these two polymers. Nevertheless, most of these experimental studies have not been able to reveal which of the two hydroxyl groups (i.e. at C6 and

at C3) and/or amine group at C2 of chitosan are involved in the interaction in these blends.

Molecular modeling has been effectively used to study the interaction between two different species based on the calculation of radial distribution function (RDF).[11,12] In the present study, molecular dynamics simulations and RDF calculations have been performed with the aim to deduce the specific groups of chitosan that are responsible for intermolecular interaction with PVP. In addition to this computational method, the interactions between these two polymers have also been evaluated using ^{13}C solid-state nuclear magnetic resonance (NMR) and diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS).

2. Experimental

Chitosan, middle viscosity with a degree of deacetylation of 75-85%, was obtained from Fluka. PVP K-90 (Kollidon 90), with an average molecular weight ($\overline{M}_w$) of 1,100,000, was kindly supplied by BASF Thailand. All other reagents were of analytical grade.

2.1 Preparation of the blend

Chitosan solution was prepared by dissolving 1.5031 g of chitosan in 50 mL (0.05 M) HCl at ambient temperature with constant stirring overnight. PVP (1.5043 g) was dissolved in water purified by reversed osmosis. The PVP solution was mixed with the chitosan solution in a 1:1 volume ratio. The solution of this blend was lyophilized to obtain the solid blend.

2.2 DRIFTS Spectra Collection

The samples were placed in a micro sample cup for Perkin-Elmer Spectrum One FTIR diffuse reflectance accessory using the supplied sample cup holder. The FTIR measurements were performed on a Perkin-Elmer Spectrum One and a Perkin-Elmer DRIFTS accessory. The spectra were recorded from 4400 to 450 cm^{-1} by averaging 64 scans at 4 cm^{-1} resolution. All reflectance spectra were converted to Kubleka-Munk (KM) unit by the use of Perkin-Elmer Spectrum for Windows version 5.02 software package.

2.3 ^{13}C solid-state nuclear magnetic resonance (NMR)

The NMR experiments were performed on a Bruker Avance 300 NMR spectrometer operating at 75.51 MHz for ^{13}C using standard 4 mm cross-polarization magic angle-spinning probes (CP/MAS). The samples were spun at the magic angle at a rate of 10620 Hz with a total number scans of 10000. A ^{13}C contact time of 5.0 ms was used and a recycle delay between scans for all the samples was 3 s. The ^{13}C chemical shifts were referenced with respect to tetramethylsilane (= 0 ppm) using solid adamantine as a secondary standard. There were no spinning side band (SSB) interferences due to the fact that the samples were spun at 10 KHz. The deconvolution of NMR spectra was performed using GRAMS/AI7 by fitting the spectra with a Gaussian function.

2.4 Computational method

Simulations of chitosan and PVP blend were performed using Materials Studio 4.2 (Accelrys, Inc.) on a dual core Pentium-based computer. A COMPASS (condensed-phase optimized molecular potentials for atomistic simulation studies)

force field was employed for all calculations. This force field has been widely used to optimize and predict the conformation and thermophysical condensed phase properties of a broad range of molecules and polymers.[13]

Polymer assemblies containing 3 chains of PVP (20 monomer units) in its atatic stereochemical structure and 3 chains of chitosan (10 monomer units) were generated for the simulation of a 50/50 composition of the blend as previously conducted.[14] For the simulation of PVP/chitosan 20/80, 33/67, 67/33 and 80/20 blends, 3 chains of each polymer were also employed but the numbers of monomer units were 20/40, 20/20, 40/10 and 80/10, respectively. Each generated polymer chain was minimized using the Discover module and the blend systems were built inside a box with periodic boundary conditions constructed using the amorphous cell module of the Materials Studio. The density of the blend system was estimated from densities of the pure polymers, i.e. 1.04 g/cm^3 for PVP and 0.67 g/cm^3 for chitosan.[14] Thus, the simulation cell densities for PVP/chitosan 20/80, 33/67, 50/50, 67/33 and 80/20 were 0.744, 0.792, 0.855, 0.917 and 0.966 g/cm^3, respectively. The polymer assemblies were energy minimized using the steepest descent method followed by the conjugate gradient method with a convergence level of 0.01 kcal/mol/Å. Group-based cutoff of 12.5 Å and a switching function with the spline and buffer widths of 3 and 1 Å, respectively, were applied to evaluate nonbonded interactions. Molecular dynamics simulations were performed in the NVT ensemble (constant particle numbers, volume and temperature) at 298 K with a time step of 1 fs. Molecular dynamics simulations were run for 1500 ps. Subsequently, the calculation of radial distribution function (RDF), g(r), was carried out of the trajectory files of simulations where the dynamic shows a stable performance.

3. Results and discussion

The ^{13}C-NMR chemical shifts or line shapes of carbon resonance in the cross-polarization/magic angle spinning (CP/MAS) spectra can provide information about the chemical environment of the carbon nucleus; therefore, their changes can indicate the intermolecular interactions between the blend components. The ^{13}C NMR spectra of PVP, chitosan and the 50/50 PVP/chitosan blend are shown in Figure 2. According to these ^{13}C-NMR spectra, the chemical shift of the PVP carbonyl carbon is observed at 176.23 ppm. This carbonyl carbon peak of the blend appears almost in the same chemical shift. However, its shape is broader and asymmetric compared to that of the pure PVP. Chen et al[15] have resolved ^{13}C CP/MAS NMR spectra at the carbonyl region to identify the crystalline and amorphous fractions of materials. In the present study, the carbonyl carbon peak of PVP in the blend was resolved into two Gaussian peaks using a curve-fitting procedure (inset, Figure 2). PVP is an amorphous material. According to X-ray diffraction result, the PVP/chitosan blend is also amorphous (data not shown). Hence, these two resolved peaks will not specify whether the form of PVP present is amorphous or crystalline. Multipeak curve fitting of NMR spectra has been previously signified as the occurrence of different interactions or environments of a specific group.[16,17] Thus, in this study, the downfield shift of one peak may possibly result from the intermolecular hydrogen bonding between the carbonyl group of PVP and the hydrogen atom of OH-C6, OH-C3 or NH-C2 group of chitosan. The other peak may be the unreacted carbonyl carbon. As shown in Figure 2, the chemical shift of C2 and C6 of chitosan appears at 57.65 ppm and that of carbon atom C3 displays at 71.69 ppm which correspond to the previous assignment.[18] The upfield shifts of 0.70 (from 57.65 to 56.95 ppm) and 0.39 ppm (from 71.69 to 71.30 ppm) were observed for C2,6 and C3, respectively, of the

blend. As previously observed, the upfield shift of a carbon atom is attributed to the formation of hydrogen bonds of a connected proton donor group.[19] In the present study, the upfield shifts may therefore result from the intermolecular bonding between the proton donors of the OH groups at C3 and/or C6 as well as the NH group at C2 with C=O of PVP.

In addition to ^{13}C-CP/MAS NMR, DRIFTS was employed to investigate the interactions between these two polymers. Figure 3 shows DRIFTS spectra of PVP, chitosan and the 50/50 PVP/chitosan blend. The spectrum of pure chitosan shows an amino band at 1637 cm^{-1}. In addition, chitosan displays a broad peak at around 3400 cm^{-1} resulting from the N-H and O-H vibrations. The amide carbonyl stretching of PVP shows a prominent peak at 1685 cm^{-1}. In the blend, this peak is shifted to the lower wavenumber at 1669 cm^{-1} indicating the incidence of interaction between PVP and chitosan. This interaction is attributed to the hydrogen bonds formed between the proton acceptor C=O of the PVP and the proton donor groups, such as OH-C6, OH-C3 and NH-C2 groups, of chitosan. Due to the overlapping band of OH and NH functions of chitosan, it is somewhat difficult to indicate which group is the proton donor in this system.

Molecular dynamics simulations and subsequent RDF calculations were performed to investigate the specific proton donor group of chitosan that interacts with the proton acceptor group of PVP. The RDF, also referred to as pair-correlation function, demonstrates the average density of atoms at a distance from a specified atom. The RDF analyses were performed in the interval of simulations where the simulation shows a stable behavior, i.e. from 800 to 1500 ps as illustrated in Figure 4. Figure 5 displays the intermolecular RDFs for the 50/50 PVP/chitosan blend. As shown in Figure 5a, a pronounced peak at 1.75 Å with the g(r) function of 4.76

corresponds to the hydrogen bonding between the hydrogen atoms of OH-C6 of chitosan and the oxygen atoms of C=O of PVP. Meanwhile the g(r) function of about 2.81 at 1.75 Å was observed for hydrogen atoms of OH-C3 chitosan and oxygen atoms of C=O of PVP. This indicates that hydrogen atoms of OH-C6 form stronger hydrogen bonds with C=O of PVP than hydrogen atoms of OH-C3. This is possible due to the free rotation of OH-C6 compared to OH-C3 and consequently there is more accessibility to interact with oxygen atoms of PVP. This computational model is the first study that distinguishes the proton donor capacity between OH-C6 and OH-C3 of chitosan on interacting with other polymers.

The shift of the peak to 2.25 Å with the lower g(r) of 1.41 is related to the hydrogen atoms of NH-C2 of chitosan and the oxygen atoms of C=O of PVP. Therefore, the intermolecular hydrogen bond involving the amino group as the proton donor is significantly weaker than that of the hydroxyl groups of chitosan. This may be due to the fact that an N-H bond is less polar than an O-H bond. Thus the N-H···O=C hydrogen bond is weaker than the O-H···O=C counterpart. Figure 5b displays the RDF calculations for the nitrogen atom of PVP and the proton donors groups of chitosan. It can be observed that the amide nitrogen of PVP is less likely to function as a proton acceptor. Generally, nonbonding electron pairs of the amide nitrogen will delocalize to the carbonyl group. Therefore, in this case, when the proton donors of chitosan interact with the amide function of PVP, they will form a bond at the oxygen atom in preference to the nitrogen atom.

As proton acceptors, the oxygen atoms of the hydroxymethyl groups were previously reported to be more reactive than the nitrogen atoms of the amino groups of chitosan in forming hydrogen bonds with proton donors of polyvinyl alcohol (PVA) or poly(2-hydroxyethyl methacrylate) (P2HEM) at some ratios of these

polymer blends.[11,20] However, with the higher amounts of PVA and P2HEM, the interactions with the amino groups of chitosan increase in intensity. Although, in the present study, proton donors and acceptors are reversed compared to the above-mentioned investigations, it was of interest to determine the interacting groups at different compositions of our polymer blend. Therefore, molecular dynamic simulations of PVP/chitosan at compositions of 20/80, 33/67, 67/33 and 80/20 were performed and the RDFs for these blends were presented in Figures 6 and 7. The same results were obtained as the above-mentioned RDF for the 50/50 PVP/chitosan blend, at either lower or higher amounts of PVP, hydrogen atoms of NH-C2 of chitosan function less as proton donors compared to those of OH-C6 and OH-C3. In the previous study, it was also observed that the interactions between hydrogen atoms of NH-C2 of chitosan and proton acceptor groups of P2HEM at various compositions of these blends were lower than those of the OH groups.[11]

Additionally, at lower amounts of PVP, the g(r) functions observed for the oxygen atom of C=O of PVP and the hydrogen atoms of OH-C6 and OH-C3 of chitosan are 6.29 and 2.52, respectively, for the 33/67 PVP/chitosan blend (Figure 6a), and those of 5.76 and 1.70, respectively, for the 20/80 PVP/chitosan blend (Figure 6b). Furthermore, for higher proportions of PVP, the g(r) functions for the oxygen atom of C=O of PVP and the hydrogen atoms of OH-C6 and OH-C3 of chitosan are 3.75 and 2.58, respectively, for the 67/33 PVP/chitosan blend (Figure 7a) and those of 3.12 and 2.17, respectively, for the 80/20 PVP/chitosan blend (Figure 7b). This indicates that the intermolecular hydrogen bonding between these two polymers is higher with lower amounts of PVP in the blends, and lower with the higher proportions of PVP (Figures 6 and 7, respectively). According to the previous investigations[4,8] of various compositions of these two polymers blends, it

demonstrates that these blends are miscible with only one glass transition temperature (Tg) was detected for each blend. In addition, the DSC thermograms of PVP/chitosan blends from both studies show positive deviations of Tgs from the linearity in the blends with lower PVP proportions whereas Tgs are lower in the blends with high amounts of PVP. Generally, the Tgs of polymer blends will be in the linear line which ranges between the initial Tg of each polymer depending on the relative amount of each polymer in the blend. However, if the two polymers bind more strongly to each other than to themselves, the Tg will be higher than expected (positive deviation from linearity). If the two polymers bind less strongly with each other than with themselves, the Tgs of the blends are lower than expected (negative deviation). Our dynamic simulations results are consistent with these findings[4,8] in terms of positive/negative deviations related to the forces of intermolecular interactions between the polymers at lower/higher compositions of the PVP.

4. Conclusion

^{13}C CP/MAS NMR and DRIFTS spectra demonstrate that the C=O group of PVP interacts with chitosan in the blending of these two polymers. Considering the chemical structures of these molecules, the hydrogen atoms of OH-C6, OH-C3 and NH-C2 of chitosan can possibly function as proton donors in these intermolecular hydrogen bonding interactions between PVP and chitosan. However, these experimental results have not been able to identify with certainty the specific groups of proton donors. Molecular modeling simulation was then employed to identify the proton donor group of chitosan. The computational results are consistent with the experimental findings that intermolecular interactions take place via the C=O of PVP. Additionally, the molecular simulations verify that the hydroxyl groups of chitosan are the more favorable sites for hydrogen bond formations than the NH-C2 group.

The hydrogen atom of OH-C3 is less favorable than that of OH-C6 as a proton donor group. Besides the oxygen atom of PVP, the nitrogen atom is another possible proton acceptor site. According to the computational modeling results, however, this nitrogen atom does not serve as a proton acceptor in these systems. Furthermore, the computational results agree with the previous studies in relating the force of interaction between the polymers to the compositions of the polymers, i.e. there is higher interactions with the lower amounts of PVP in the blend, and lower interactions with the increasing amounts of PVP in the blend.

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Figure Captions

Figure 1. Structures of chitosan and poly(vinyl pyrrolidone)

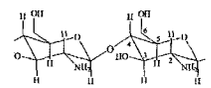
Figure 2 ^{13}C CP/MAS NMR spectra of chitosan, PVP and 50/50 PVP/chitosan blend; inset: the resolved spectrum of C=O band of the 50/50 PVP/chitosan blend.

Figure 3 DRIFTS spectra of chitosan, PVP and 50/50 PVP/chitosan blend.

Figure 4 Radial distribution functions for the 50/50 PVP/chitosan blend representing hydrogen atom of OH-C3, OH-C6 and NH-C2 of chitosan relative to the distance of a) the oxygen atom of C=O of PVP and b) the nitrogen atom of PVP.

Figure 5 Radial distribution functions for the 33/67 PVP/chitosan blend representing hydrogen atom of OH-C3, OH-C6 and NH-C2 of chitosan relative to the distance of the oxygen atom of C=O of PVP.

Figure 6 Radial distribution functions for the 67/33 PVP/chitosan blend representing hydrogen atom of OH-C3, OH-C6 and NH-C2 of chitosan relative to the distance of the oxygen atom of C=O of PVP.



Chitosan

Poly(vinyl pyrrolidone)

Figure 1

Figure 1. Structures of chitosan and poly(vinyl pyrrolidone)
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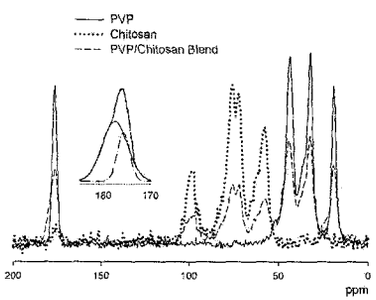

Figure 2

13C CP/MAS NMR spectra of chitosan, PVP and 50/50 PVP/chitosan blend; inset: the resolved spectrum of C=O band of the 50/50 PVP/chitosan blend.
215x279mm (300 x 300 DPI)

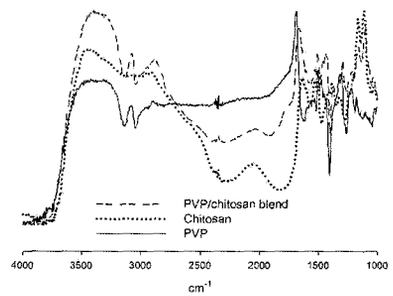

Figure 3

DRIFR spectra of chitosan, PVP and 50/50 PVP/chitosan blend.
215x279mm (300 x 300 DPI)

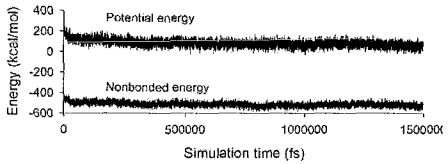

Figure 4

Potential and nonbonded energy vs simulation time of 1500 ps.
215x279mm (300 x 300 DPI)

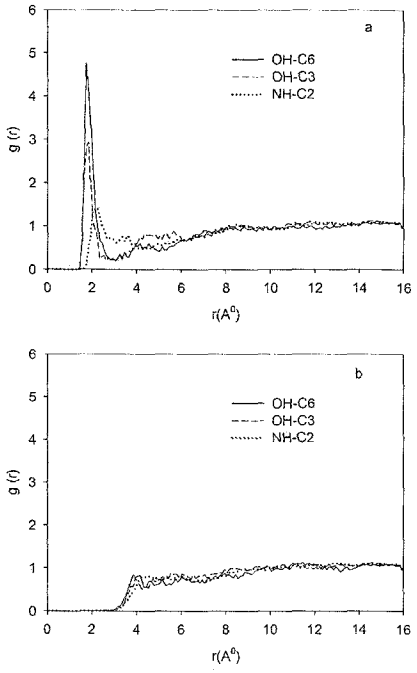

Figure 5

Radial distribution functions for the 50/50 PVP/chitosan blend representing hydrogen atom of OH-C3, OH-C6 and NH-C2 of chitosan relative to the distance of a) the oxygen atom of C=O of PVP and b) the nitrogen atom of PVP.
215x279mm (300 x 300 DPI)

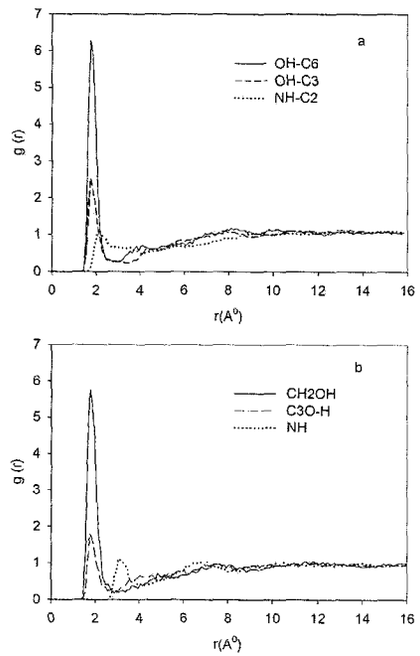

Figure 6

Radial distribution functions for a) 33/67 and b) 20/80 PVP/chitosan blend representing hydrogen atom of OH-C3, OH-C6 and NH-C2 of chitosan relative to the distance of the oxygen atom of C=O of PVP.
215x279mm (300 x 300 DPI)

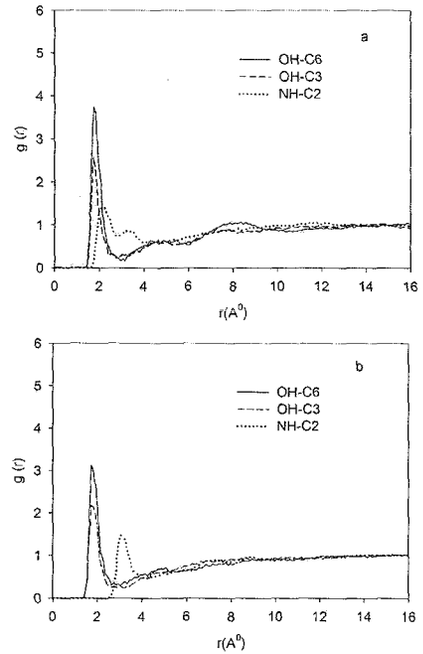

Figure 7

Radial distribution functions for a) 67/33 and b) 80/20 PVP/chitosan blend representing hydrogen atom of OH-C3, OH-C6 and NH-C2 of chitosan relative to the distance of the oxygen atom of C=O of PVP.
215x279mm (300 x 300 DPI)