

# Development of High-Strength Soy Protein Adhesives Modified with Sodium Montmorillonite Clay

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Received: 22 September 2015 / Revised: 10 August 2016 / Accepted: 18 August 2016 / Published online: 15 September 2016  
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**Abstract** This study investigated the high strength of a soy protein adhesive system with good flowability at high protein concentration. Sodium montmorillonite (Na MMT), the most widely used silicate clay, was incorporated into viscous, cohesive soy protein adhesives at concentrations ranging from 1 to 11 % (dry basis, w/w). Hydroxyethyl cellulose was used as a suspension agent to stabilize the soy protein and nano clay to be the dispersion system. The interaction between soy protein and Na MMT was characterized by XRD, FTIR, Zeta potential and DSC. Results indicated that soy protein molecules were adsorbed on the surface of the interlayer of Na MMT through hydrogen bonding and electrostatic interaction. The soy protein/Na MMT adhesives had the intercalation structure with Na MMT contents ranging from 1 to 11 %. Adhesion strength, specifically wet adhesion strength, of soy protein adhesives at isoelectric point (*pI*) was significantly improved by the addition of Na MMT. It is believed that the physical cross-linking reactions between soy protein and Na MMT mainly contribute to the improved adhesion performance of soy protein adhesives. Wet adhesion strength increased from 2.9 MPa of control soy protein adhesive to 4.3 MPa at 8 % Na MMT. An increase of pH beyond *pI* value resulted in decreased adhesion strength due to increased surface charges of soy protein and slightly reduced affinity of soy protein on the nano clay surface.

**Keywords** Soy protein adhesive · Sodium montmorillonite · XRD · FTIR · Water resistance

## Introduction

Soy-based adhesives made from soybean seed, which is abundant and renewable, have shown great potential as alternatives to petroleum based adhesives. However, very few soybean adhesives are applied commercially in the wood industry due to their limitations (i.e. moderate to low dry strength, moderate to low water resistance, low solid content) [1, 2]. Previous efforts improved adhesion performance of soy protein by altering protein molecular structure or conformation through physical modifications and cross-linking agents [3–6]. Nanoparticle-reinforced adhesives have recently attracted research attention. Previous studies have shown that mechanical properties of adhesives were significantly improved with the addition of nano scale filler due to the large surface area of the filler and its ability to interlock mechanically with polymer [7, 8]. Liu *et al.* [9] reported that the wettability, affinity, adhesion strength, and water resistance of soy protein/CaCO<sub>3</sub> glue was significantly improved compared to the pure soy protein adhesive. The environmentally friendly nanocomposite shows promise as a wood adhesive, and provides new opportunities for the wood composite industry.

Sodium montmorillonite (Na MMT), the most widely used type of silicate clay in polymer nanocomposites, is naturally abundant, nontoxic, inexpensive, and chemically and thermally stable [10]. Due to its unique nano-scale layered structure and high aspect ratio (the ratio of its longer side to its shorter side), it is used extensively as nano-filler and reinforcing material to prepare nanocomposites [11]. A clay nanocomposite system is produced through two

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interactions that significantly influence composite properties: intercalation and exfoliation. In an intercalation structure, polymer chains are intercalated between silicate layers; the polymer and inorganic layers alternate, resulting in a well-ordered multilayer morphology. An exfoliated, or delaminated, structure is obtained when the silicate layers are completely and uniformly dispersed in the polymer matrix [12].

Nano-layered silicates have been reported to potentially improve adhesive performance with polyurethane, polyvinyl acetate, poly (ethylene glycol) acrylate, and starch because of the high aspect ratio and the presence of unsaturated chemical bonds on the nano surfaces [13–16]. Many studies have characterized the molecular interaction and structure of soy protein/clay composites in plastic and film applications with the improved mechanical and barrier properties [10, 17, 18], but only few works have focused on adhesion properties of soy protein affected by nano clay. Zhang *et al.* [1, 19] reported slightly negative effects of MMT on the adhesion strength of soy protein at 3 % loading level. In their study, the nano-scale MMT platelets mostly likely blocked some active groups of soy protein via intercalation/exfoliation, which effectively retarded the cross-linking reactions between protein and cross-linkers such as polyisocyanate, methylene diphenyl diisocyanate, and glyoxal. They also assumed that the nano-scale blocking could reduce polar groups of the cross-linked soy protein adhesive on the wood substrate, thereby decreasing adhesion strength. However, in preliminary experiments, our lab-developed soy protein/Na MMT adhesives with pH close to protein isoelectric point (*pI*) demonstrated significantly improved adhesion performance, especially water resistance. Therefore, we assumed that the soy protein/nano clay adhesives with the dense cross-linking structure favoring adhesion strength improvement might overcome the negative effects of Na MMT on adhesion properties of soy protein at *pI*.

The objective of this study was to develop an innovative soy protein/clay system with strong adhesion and good flowability at high protein content. Sodium bisulfite ( $\text{NaHSO}_3$ )-modified soy protein adhesive (SP) with pH 4.3 was used as the starting material [20], and MMT was incorporated into the soy protein system with loading levels ranging from 1 to 11 % (protein content basis). Hydroxyethyl cellulose (HEC) was used as suspension agent to stabilize the soy protein and nano clay in the dispersion system. Interaction between soy protein and clay was characterized in terms of X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and Zeta potential. Adhesion strength and water resistance of soy protein/clay adhesives were characterized in terms of dry and wet strength.

## Materials and Methods

### Materials

Defatted soy flour (Cargill, Cedar Rapids, IA, USA) was used as the starting material. The soy flour contained approximately 50 % protein and 10 % moisture and a protein dispersibility index (PDI) of 90.  $\text{NaHSO}_3$  was obtained from Fisher Scientific (Fair Lawn, NJ, USA), while Na MMT was purchased from Sigma Aldrich (St. Louis, MO, USA). Natrosol 250 HHXR (HEC) was provided by Ashland (Covington, KY, USA). Cherry wood veneers with dimensions of  $50 \times 127 \times 5$  mm (width  $\times$  length  $\times$  thickness) were provided by Veneer One (Oceanside, NY, USA).

### Adhesive Preparation and Modification

SPA was prepared based on our previous research [20]. The aqueous protein extract was prepared by mixing defatted soy flour in water at 6.25 % solid content and pH 9.5.  $\text{NaHSO}_3$  was added to the slurry at 6 g/L on the basis of water volume, and the slurry was stirred for 2 h at room temperature. The insoluble carbohydrate was removed from the soy protein by centrifuging at  $10,000 \times g$ . The pH of the supernatant was adjusted to 4.1 with 2 N HCl, centrifuged at  $10,000 \times g$ , and the precipitate with 35 % solid content was the viscous, cohesive soy protein base.

Twenty percent HEC solution was added slowly to the soy protein base (wet basis) during mixing. The concentration of HEC solution was 0.5 % (0.28 % HEC, dry protein basis). Then 1 % urea (dry protein basis) was used to partly denature the highly aggregated soy protein sample and the mixture was stirred for 2 h. The resulting homogeneous soy protein dispersion with smooth protein texture was used as the control soy protein adhesive, and coded as SP. Various amounts of Na MMT powder were then added to the SP dispersion at concentration of 1, 3, 5, 8, and 11 % (dry basis). Mixture was stirred for 2 h to form SP/Na MMT adhesives. Those samples were coded as 1, 3, 5, 8, and 11 % Na MMT. The ratio of HEC, urea, Na MMT and soy protein (dry basis) in the mixture is expressed as 0.28/1/8/100 using 8 % Na MMT as an example. Two replicates of each soy protein adhesive treatment were prepared for plywood production (five panels for each batch of protein adhesive), and all the other physicochemical analyses were conducted on one of the batches.

### X-ray Diffraction

XRD patterns of SP/Na MMT adhesives were obtained on a Bruker D8 X-ray diffractometer with  $\text{Cu K}\alpha$  radiation according the method described by Zhang *et al.* [19].

Diffraction data were collected in the range of  $2\theta = 2^\circ\text{--}12^\circ$  with a step interval of  $0.02^\circ$ , accelerating voltage of 40 kV, and current of 30 mA. All measurements were done in duplicate and the average was reported.

### Fourier Transform Infrared Spectroscopy

FTIR spectroscopic data were collected in the region of  $800\text{--}4000\text{ cm}^{-1}$  using a PerkinElmer Spectrum 100 FTIR spectrometer (Waltham, MA, USA). Based on the method described by Qi *et al.* [3], SP/Na MMT adhesives were freeze dried and ground for FTIR analysis. Each disk was scanned 16 times at a resolution of  $2\text{ cm}^{-1}$ ; and transmission spectra were recorded.

### Zeta Potential

Zeta potential of SP with various levels of MMT was measured on a ZetaPALS Zeta Potential Analyzer (Brookhaven Instruments Corporation, Holtsville, NY, USA) using hydrodynamic light scattering and laser Doppler electrophoresis according to the method described by Chen and Zhang [12]. Protein adhesive suspensions with varying amounts of MMT content were prepared with solid content of 0.1 % for each sample. For each measurement, the instrument tested five runs each comprising of ten cycles and the reported result was the average value from the five runs. (“Runs” mean that the instrument measured the sample  $X$  times, while a cycle is defined as the collection of data from the sample during the application of an electric field once in the positive and once in the negative direction). Triplicate measurements were conducted for each sample.

### Differential Scanning Calorimetry

Thermal denaturation properties of soy protein in SP/Na MMT adhesives were evaluated by a differential scanning calorimeter (DSC) (Q200, TA instrument, Schaumburg, IL, USA) calibrated with indium and zinc according to the method described by Qi *et al.* [3]. Approximately 20 mg of wet SP/Na MMT sample were hermetically sealed in Tzero aluminum hermetic pans. Each sample was held at  $20^\circ\text{C}$  for 1 min and then scanned from  $20$  to  $130^\circ\text{C}$  at a heating rate of  $10^\circ\text{C}/\text{min}$ . Universal Analysis 2000 software was used to calculate peak temperatures and denaturation enthalpies from thermograms. All measurements were done in duplicate and the average was reported.

### Thermogravimetric Analysis

Thermal degradation patterns of SP/Na MMT adhesives were studied using a TGA (TGA 7, Perkin-Elmer, Norwalk,

CT, USA) in a nitrogen atmosphere. A sample of 15 mg of the freeze dried and ground powder was weighed into a platinum cup and scanned from  $20$  to  $700^\circ\text{C}$  at a heating rate of  $10^\circ\text{C}/\text{min}$  following the method described by Chen and Zhang [12]. There were two replicates for each measurement.

### Apparent Viscosity

Apparent viscosity measurements of SP/Na MMT adhesive samples were performed using a Bohlin CVOR 150 rheometer (Malvern Instruments, Southborough, MA, USA) with a parallel plate (PP20, 20 mm plate diameter and 500  $\mu\text{m}$  gap) according to the method described by Liu *et al.* [21]. The adhesives were well mixed immediately before placing in the rheometer and measured at shear rate of  $25\text{ s}^{-1}$  for 2 min after the plate closure; 15 data points were collected and the average value was reported as apparent viscosity for each measurement. The testing temperature was  $23^\circ\text{C}$ . A thin layer of silicone oil was spread over the circumference of the sample to prevent sample dehydration during the test. There were two replicates for each measurement.

### Plywood Preparation

Cherry wood veneers with dimensions of  $50 \times 127 \times 5\text{ mm}$  (width  $\times$  length  $\times$  thickness) were preconditioned in a chamber (Electro-Tech Systems, Inc., Glenside, PA, USA) for 7 days at  $23^\circ\text{C}$  and 50 % relative humidity. The adhesives were brushed onto one end of a piece of cherry wood with dimensions of  $127 \times 20\text{ mm}$  (length  $\times$  width) until the entire area was completely wet with adhesive. Approximately 0.6 g of adhesive was brushed on each piece (wet basis). Two brushed wood pieces were assembled immediately and conditioned for 15 min at room temperature. The assembled wood specimens were pressed with a hot press (Model 3890 Auto M; Carver, Inc., Wabash, IN, USA) at 1.4 MPa and  $170^\circ\text{C}$  for 10 min, in order to compare the adhesion data under the same press conditions described previously by Qi *et al.* [20]. Based on our plywood preparation procedure for two-layer cherry wood, when using hot press condition of  $130^\circ\text{C}$ , dry strength of soy protein adhesive always had 100 % wood failure (even with 5 min curing), while wet strength was low and had no wood failure. Therefore, in order to emphasize the wet adhesion strength, we increased the press temperature ( $170^\circ\text{C}$ ) and time (10 min) to allow us to differentiate the variations between adhesion modifications. There were five replicates of plywood panels for each adhesive samples. Each panel was cut into five pieces, producing a total of 25 small pieces for the adhesion strength test for each adhesive samples.

## Shear Strength Measurement

The five wood assemblies were conditioned at 23 °C and 50 % relative humidity for 48 h, and each one cut into five pieces with dimensions of 80 × 20 mm (glued area of 20 × 20 mm). Typically, 13 specimens were used for wet testing and twelve for dry testing. The cut wood specimens were conditioned for another 4 days before measurements were taken. The dry strength of wood specimens was tested with an Instron Tester (Model 4465, Canton, MA, USA) according to ASTM Standard Method D2339-98 [22] at a crosshead speed of 1.6 mm/min. Shear adhesion strength at maximum load was recorded. Reported values were the average of five specimen measurements. Wet strength of the wood assemblies was measured following ASTM Standard Methods D1183-96 and D1151-00 [23, 24]. The preconditioned specimens were soaked in tap water at 23 °C for 48 h, and wet strength was tested immediately after soaking.

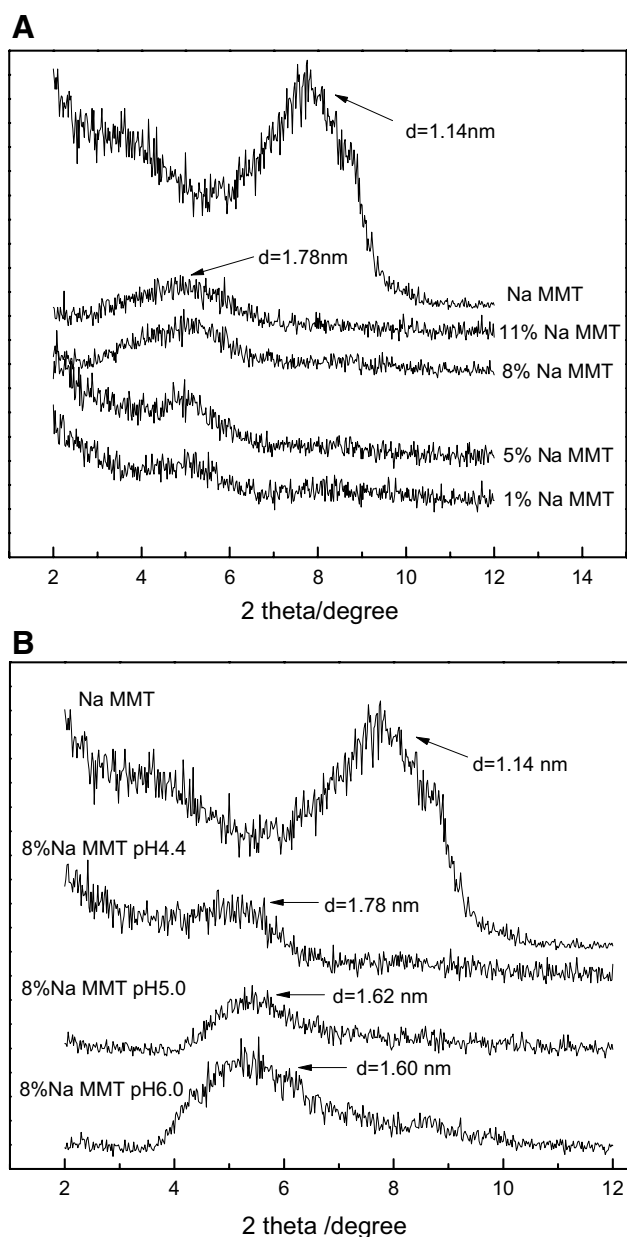
## Experimental Design and Statistical Analysis

The experimental design involved measurement of adhesion strength (dry and wet) and physicochemical properties (XRD, FTIR, Zeta potential, thermal properties, apparent viscosity) of soy protein adhesive as a function of Na MMT concentration. Two replicates of each soy protein adhesive treatment were prepared for plywood production (five panels for each batch of protein adhesive), and all the other physicochemical analyses were conducted on one of the batches. Reported values corresponded to group means with their respective standard error. The data from experiments were analyzed through analysis of variance (ANOVA) and least significant difference (LSD) at the 0.05 level according to procedures in the SAS statistical software package (SAS Institute 2005, Cary, NC, USA).

## Results and Discussion

### X-ray Diffraction Results

XRD patterns of pristine Na MMT and SP/Na MMT adhesives are shown in Fig. 1a. Na MMT exhibited a strong XRD diffraction peak at  $2\theta$  of  $7.7^\circ$  with a d-spacing value of 1.14 nm. SP did not show any peaks in the studied  $2\theta$  range from 2 to 12, indicating that there is no crystallized protein structure (results not shown). As shown in Fig. 1a, basal peaks of Na MMT in sample SP/Na MMT adhesives remarkably shifted to the lower angle of  $4.9^\circ$ , corresponding to the d-spacing of 1.78 nm. Peak intensity was attenuated with decreasing levels of Na MMT. Results indicated that the interlayer basal spacing of clay was enlarged as



**Fig. 1** a XRD patterns of SP/Na MMT adhesives with various Na MMT contents; b XRD patterns of SP/Na MMT adhesives with various pH values

a result of protein chain penetration into the clay gallery (Urea and HEC had no effects on the clay interlayer basal spacing during the mixing at room temperature, result not shown). The intercalation structure of the adhesives was formed at loading levels between 1 and 11 % Na MMT. Dispersion of clay inside the protein matrix was attributed to interactions between the charged silicate groups and polar peptides in proteins. In addition, the pre-modification of  $\text{NaHSO}_3$  (reducing agent) and urea (denaturant) could make the protein molecules lie along the clay surface as a

linear strand [25] and less steric hindrance would also be expected. This might help the protein affinity on the clay surface, making the protein/nano adhesives more stable.

The XRD pattern of SP/Na MMT adhesives as affected by pH shown in Fig. 1b demonstrated that the basal peak of Na MMT shifted to a higher angle as pH of the SP/Na MMT adhesive increased beyond the *pI*. At pH 6.0, the  $2\theta$  peak was located at  $5.5^\circ$  with *d* spacing 1.60 nm, compared to  $5.0^\circ$  (1.76 nm) at *pI* of 4.5. The result is consistent with previous studies [26, 27], which showed that adsorption of protein on clay was greatest when the pH was approximately equal to the *pI*. It is mainly caused by the fact that protein at higher pH with more negative charges decreases the electrostatic attraction between protein and clay surface, thus reducing the amount of adsorption.

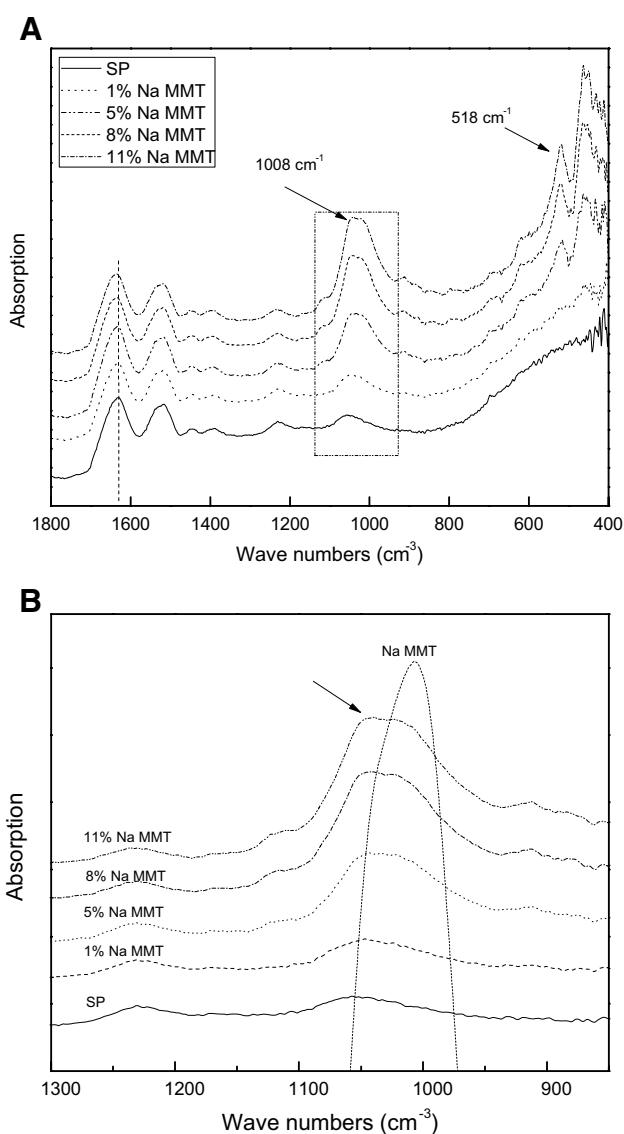
### Fourier Transform Infrared Spectroscopy

FTIR spectra of protein-clay adhesive are shown in Fig. 2a. Two typical absorption peaks (Amide I and II) that occurred at  $\sim 1630$  and  $1516\text{ cm}^{-1}$  were observed in soy protein, referred to as C=O stretching and N–H bending. Amide I peak in the protein-clay adhesive shifted to a higher value,  $1634.7\text{ cm}^{-1}$  at 8 % Na MMT (Fig. 2a), indicating the existence of hydrogen bonding between C=O groups in protein and polar groups (Si–O–Si and –OH) on MMT layered surfaces.

Pristine Na MMT demonstrated characteristic absorption bands at  $1008$  and  $518\text{ cm}^{-1}$  (Fig. 2a) that are related to in plane stretching vibrations and bending vibrations of Si–O groups, respectively [28]. Those peak intensities increased as Na MMT concentration increased in soy protein matrix (Fig. 2a). The peak at  $1008\text{ cm}^{-1}$  was non-split and uniform due to high symmetry of the dioctahedral MMT layer [12]. After incorporation with SP, this peak split and shifted to a higher value, thus illustrating the existence of hydrogen bonding (Fig. 2b). The band splitting suggests less order in the clay lamellas after penetration of soy protein polymer chains into the clay [12, 28].

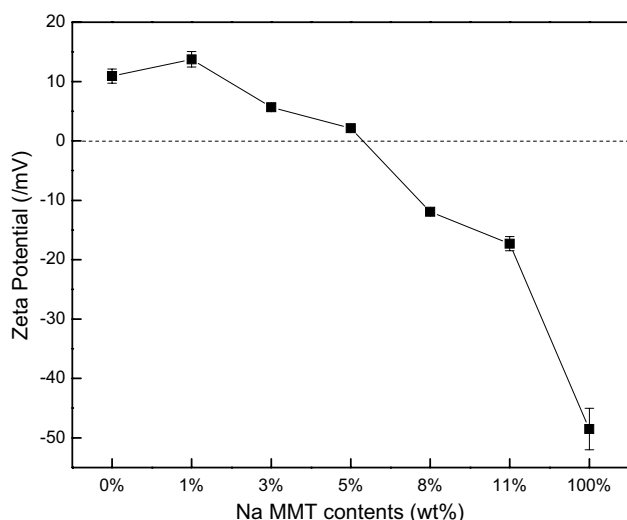
### Zeta Potential (ZP)

The pH of the control soy protein was 4.31. As the Na MMT increased from 0 to 11 %, the pH of SP/Na MMT complex slightly increased to 4.56, implying that the electrostatic surface potential of soy protein was barely altered [12]. Zeta potential of SP/Na MMT adhesives are shown in Fig. 3. Pristine Na MMT in water had negative charges, with ZP of  $-48\text{ mV}$ , which is in agreement with previous studies [12, 26]. Typically, zeta potential of control soy protein with pH 4.1 is  $11\text{ mV}$ , indicating a slightly positive

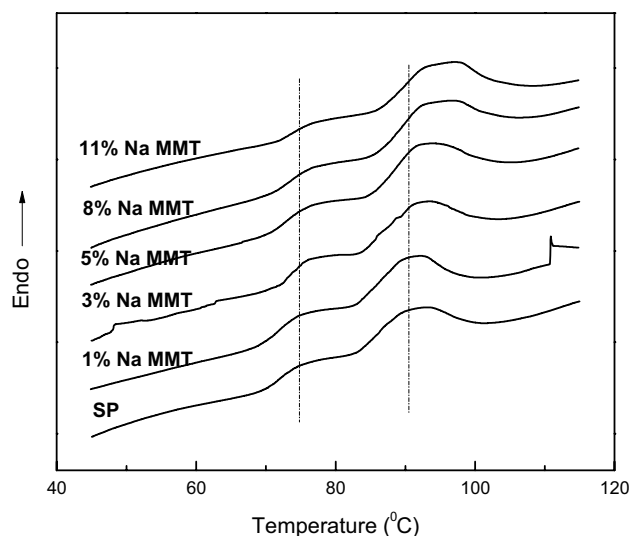


**Fig. 2** FTIR spectra of SP/Na MMT adhesives with various Na MMT contents

charged surface. With MMT content of 8–11 %, zeta potential of SP/Na MMT adhesives were far less negative than pure clay; it reached the point of zero charges at 5 % Na MMT and finally became positive at 1–3 % Na MMT. Compared to pure Na MMT, the significantly reduced zeta potential of SP/Na MMT complexes confirmed that the positively charged soy proteins were effectively adsorbed into the negatively charged MMT surface by the electrostatic interaction. All in all, the protein intercalation into the clay was attributed to electrostatic attraction and hydrogen bonding between the interfaces of clay and soy protein. The result is consistent with previous studies [26, 27].



**Fig. 3** Dependence of zeta potential of SP/Na MMT adhesives on MMT content



**Fig. 4** Differential scanning calorimetry thermogram of SP adhesives modified with various Na MMT contents

### Thermal Properties of SP/Na MMT

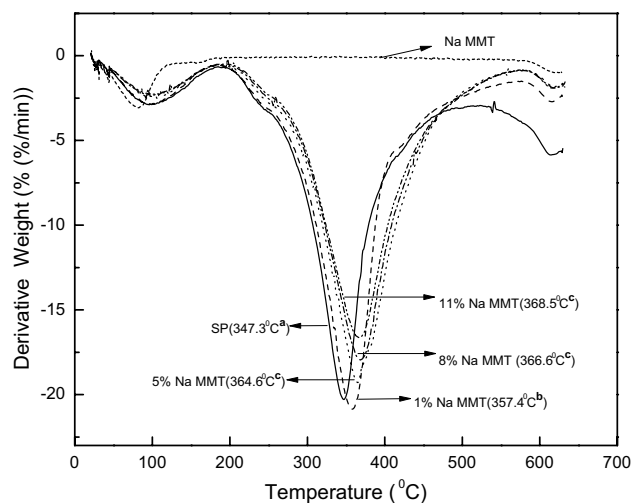
#### Thermal Denaturation Properties (DSC)

The typical DSC thermogram of SP (Fig. 4) showed two endothermic transitions ( $T_d$ ) at about 91 °C (11S) and 74 °C (7S) and had a total denaturation enthalpy ( $\Delta H_d$ ) of 8.12 J/g (Table 1). As the amount of Na MMT increased from 0 to 11 %,  $T_d$  for 11S gradually shifted to higher temperatures (95.7 °C at 11 % Na MMT) compared to

**Table 1** Denaturation temperature ( $T_d$ ) and enthalpy of denaturation ( $\Delta H$ ) of soy protein modified with Na MMT

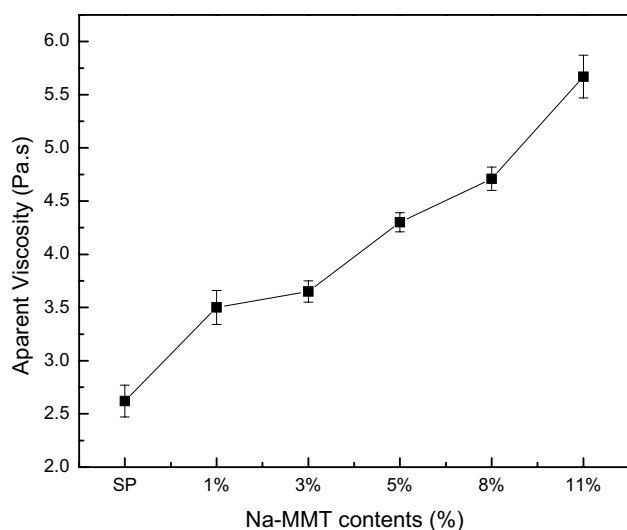
MMT (%)	$T_d$ (°C)		$\Delta H$ (J/g)
	7 s	11 s	
SP	74.77 ± 0.14 <sup>b</sup>	91.34 ± 0.42 <sup>d</sup>	1.34 ± 0.01 <sup>b</sup>
1 %	74.59 ± 0.42 <sup>b</sup>	90.69 ± 0.13 <sup>e</sup>	1.47 ± 0.01 <sup>b</sup>
3 %	76.09 ± 0 <sup>a</sup>	91.69 ± 0.16 <sup>d</sup>	1.59 ± 0.07 <sup>b</sup>
5 %	76.41 ± 0.01 <sup>a</sup>	92.14 ± 0.06 <sup>c</sup>	1.77 ± 0.01 <sup>a</sup>
8 %	76.55 ± 0.07 <sup>a</sup>	92.95 ± 0.06 <sup>b</sup>	1.80 ± 0.03 <sup>a</sup>
11 %	76.60 ± 0.01 <sup>a</sup>	95.67 ± 0.10 <sup>a</sup>	1.90 ± 0.03 <sup>a</sup>

Samples sharing the same superscripts were not significantly different ( $p > 0.05$ )



**Fig. 5** Derivative thermal gravimetric curves for SP/Na MMT adhesives with various Na MMT contents

91.3 °C of control SP. However, addition of Na MMT had a limited effect on the thermal properties of 7S;  $T_d$  slightly increased from 74.7 °C at 0 % Na MMT to 76.6 °C at 11 % Na MMT. The results suggested that Na MMT might interact with 11S and formed soy protein-clay complex, which need to be further investigated in the future. In addition, the increased total  $\Delta H_d$  of soy protein modified with Na MMT also confirmed the formation of SP/Na MMT complexes with higher thermal stability. Moreover, the increased total  $\Delta H_d$  of soy protein modified with Na MMT also confirmed formation of SP/Na MMT complexes with higher thermal stability. As indicated by FTIR and zeta potential data, it is believed that hydrogen bonding and electrostatic forces between protein macromolecules and the charged silicate are enough to restrict the segmental motion of soy protein chains, causing the increased thermal stability.



**Fig. 6** Apparent viscosity of SP/Na MMT adhesives with various Na MMT contents

#### Thermal Degradation Properties (TGA)

Thermal stability of SP/Na MMT adhesives was also investigated using TGA. Differential TGA (dTG) curves are shown in Fig. 5. Two typical weight loss stages were observed for SP in SP/Na MMT adhesives: water evaporation and protein decomposition. The first stage, around 100 °C, was attributed to evaporation of residual moisture in the powder sample. Soy protein decomposition involving broken intermolecular and intramolecular hydrogen bonds, electrostatic bonds, and cleavage of covalent bonding between peptide bonds of amino acid residues occurred primarily at the second stage, with temperatures between 200 and 400 °C. As shown in Fig. 5, the decomposition temperature of SP increased significantly from 347 to approximately 364 °C at 5 % Na MMT because of the strong interaction between SP and nano clay. Further increasing the clay concentration up to 11 % caused the decomposition temperature to increase only 4 °C further. This phenomenon indicated that the introduction of excessive Na MMT to soy protein may cause a steric hindrance effect on the

protein active groups, leading to limited thermal stability enhancement.

#### Apparent Viscosity

Figure 6 shows that the addition of Na MMT to soy protein substantially increased viscosity. It is known that the newly formed SP/nano adhesives had a more complex structure, thereby limiting the protein chain mobility in the solution and resulting in increased viscosity [29]. In addition, higher solid content in SP/Na MMT adhesives may also contribute to the higher viscosity. Despite the increased viscosity, SP/Na MMT adhesives retained good flowability by visual observation. Viscosity is an important physical property that governs the bonding behavior of wood adhesives. For industrial applications, adhesive viscosity is typically determined by various coating methods. Spray adhesives require low viscosity to allow proper atomization by the spray gun. Brush adhesives can have higher viscosity (7 Pa·s) to provide easy spreading and prevent soaking-in porous core materials. Our soy protein/Na MMT adhesives are brushable, with viscosities around 5 Pa·s at 8 % Na MMT.

#### Adhesion Strength

Table 2 represents adhesion performance of SP/Na MMT adhesives in terms of dry and wet adhesion strength using the press conditions of 170 °C for 10 min. As shown in Table 2, the dry strength of SP was 5.70 MPa with 100 % wood failure. The addition of 3 % of Na MMT extensively decreased the dry strength to 4.49 MPa with only 50 % wood failure at 3 % Na MM. However, dry strength began to increase when the Na MMT amount was increased 5–11 %; the highest dry strength occurred at 8 % of Na MMT. Wet strength of SP was also significantly improved at Na MMT concentrations of 5–11 %, and the greatest water resistance was observed at 8 % Na MMT.

Na MMT is known to have a hydrophilic nature with excellent swelling properties in water. The addition of Na MMT may improve the affinity of SP onto the hydrophilic surface of wood, thereby beneficially enhancing the

**Table 2** Dry and wet shear adhesion strength of soy protein modified with various Na-MMT contents, 0.28 % HEC and 1 % urea

Adhesive formulation	Dry strength (MPa)	Dry wood failure (%)	Wet strength (MPa)	Wet wood failure (%)
SP	5.70 ± 0.39 <sup>b</sup>	100	2.82 ± 0.23 <sup>c</sup>	0
1 %	5.39 ± 0.3 <sup>b</sup>	100	3.14 ± 0.39 <sup>bc</sup>	0
3 %	4.49 ± 0.22 <sup>c</sup>	50	3.05 ± 0.20 <sup>bc</sup>	0
5 %	5.95 ± 0.25 <sup>ab</sup>	100	3.50 ± 0.36 <sup>bc</sup>	Fiber pulling out
8 %	6.50 ± 0.20 <sup>a</sup>	75	4.34 ± 0.36 <sup>a</sup>	Fiber pulling out
11 %	6.38 ± 0.13 <sup>a</sup>	60	3.80 ± 0.18 <sup>ab</sup>	Fiber pulling out

Samples sharing the same superscripts were not significantly different ( $p > 0.05$ )

**Table 3** Effects of pH on dry and wet shear adhesion strength of soy protein modified with 8 % Na MMT, 0.28 % HEC and 1 % urea

pH of SP/Na MMT adhesives	Dry strength (MPa)	Dry wood failure (%)	Wet strength (MPa)	Wet wood failure (%)
4.4	7.26 ± 0.44 <sup>a</sup>	100	3.99 ± 0.38 <sup>a</sup>	Fiber pulling out
4.7	7.17 ± 0.69 <sup>a</sup>	100	4.12 ± 0.32 <sup>a</sup>	Fiber pulling out
5.0	6.02 ± 0.38 <sup>b</sup>	30	2.18 ± 0.15 <sup>b</sup>	0
5.4	4.58 ± 0.23 <sup>c</sup>	30–50	1.40 ± 0.20 <sup>c</sup>	0
6.0	1.91 ± 0.28 <sup>d</sup>	0	1.62 ± 0.12 <sup>bc</sup>	0

Samples sharing the same superscripts were not significantly different ( $p > 0.05$ )

interlocking between adhesives and veneer for increased adhesion strength. Moreover, with addition of 5–11 % of Na MMT to SP, the intercalation structure with protein through hydrogen bonding and electrostatic interaction was achieved as proved by XRD data. Those dense cross-linking protein structures could enhance the adhesion performance, especially for the water resistance, as a result of preventing the penetration of water into the interfacial layer between wood and adhesives. However, at 11 % Na MMT, the increased viscosity may decrease protein flowability and the ability of the adhesive to diffuse on a wood surface. Thus, the poor wetting ability of adhesives may limit further improvement of adhesion performance. For soy protein adhesive with 1–3 % Na MMT, the cross-linking density of adhesives may be not enough to overcome the hydrophilic nature of Na MMT (detrimental to wet strength of adhesives), thereby reducing water resistance and the adhesion of soy protein.

In our previous study, NaHSO<sub>3</sub>-modified soy protein adhesive with 33 % solid content and pH 4.3 was used as the starting material [20]. Soy protein adhesives prepared at or close to their *pI* (*pI* = 4.5) were proven to have the highest shear strength due to strong protein–protein interactions during the curing process [20, 30, 31]. However, soy protein was found to aggregate extensively at *pI* and became difficult to spread on a wood surface. Therefore, we used NaHSO<sub>3</sub> and urea to partly denature the highly aggregated soy protein to have a viscous, cohesive morphology and high protein content (35 %) [20] at *pI*, and HEC was then used to stabilize the protein and nano clay to become a dispersion adhesive system.

The effect of pH on the adhesion performance of SP/Na MMT adhesives was also investigated (Table 3). The highest adhesion strength of soy protein occurred at the *pI*. As pH increased above the *pI* value, electrostatic repulsions between protein molecules occur due to redundant surface charges [31]. We believe that the excessive surface charge enhanced protein–water interactions instead of protein–protein and protein–wood interactions, leading to reduced adhesion strength. In addition, the affinity between soy protein and clay was reduced as shown in Fig. 1b. As a result,

the weakened cross-linking structure of the soy protein/clay complex might also be detrimental to the adhesion performance of soy protein adhesive.

Overall, incorporation of 5–8 % Na MMT caused soy protein at *pI* to have a dense cross-linking network (Figs. 1, 2), resulting in significantly improved adhesion strength. Compared to traditional soy protein isolate (SPI) adhesives in previous studies [30, 31], SP/Na MMT adhesives have the following advantages: excellent water resistance (wet strength of 4.3 MPa compared to 2.0 MPa of SPI), solid content as high as 38 %, latex-like adhesives with good flowability, and storage at room temperature for over a month without mold due to the acidic environment and reducing effect of NaHSO<sub>3</sub>. Meanwhile, the bonding strength of soy protein adhesive with 8 % Na MMT also retained over a month (dry strength of 6.5 MPa and wet strength of 4.3 MPa).

## Conclusions

Novel SP/Na MMT adhesives with good adhesion performance, high protein content (about 30 %), and good flowability were investigated in this study. At Na MMT concentrations of 1–11 %, an intercalation structure between soy protein and nano clay was formed based on XRD patterns of the adhesives. Electrostatic interaction and hydrogen bonding between Si–O–Si (MMT) and –OH groups (soy protein) occurred as indicated by FTIR and ZP results. These two interactions were beneficial to the intercalation of MMT layers in the soy protein matrix. At 8 % Na MMT, an SP/Na MMT with pH close to *pI* demonstrated significantly improved adhesion strength, especially the water resistance with 4.3 MPa as compared to 2.9 MPa of control SP. Increasing pH beyond *pI* value resulted in decreased adhesion strength due to the redundant surface charges of soy protein and reduced interaction between SP and nano clay.

**Acknowledgments** The authors greatly appreciate the financial support from the USDA NIFA BRDI program (Grant No.: 2012-10006-20230).



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