

CO-EFFECTS OF CALCIUM CARBONATE AND SODIUM BISULFITE MODIFICATION
ON IMPROVING WATER RESISTANCE OF SOY PROTEIN ADHESIVES

by

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Abstract

Bio-based protein adhesives derived from renewable resources, especially soy protein are becoming more significant due to the concerns about environment and health related issues and the limit of petroleum resources. However, the relatively poor water resistance of soy-based protein adhesives limits its wide applications. The goal of this study was to improve the water resistance performance of soy-based protein adhesives by chemical modification. The specific objectives are 1) to modify soy protein with calcium carbonate (CaCO_3) and sodium bisulfite (NaHSO_3) as the denaturing agents; 2) to investigate the effects of calcium carbonate (CaCO_3) concentrations, curing time and curing temperatures on adhesion performance of the modified soy-based protein adhesives.

In this study, the co-effects of NaHSO_3 and CaCO_3 on adhesion properties of soy protein adhesives were investigated. NaHSO_3 was added to soy flour slurry at constant concentration 6g/L, while concentration of CaCO_3 was chosen in the range of 0 to 23g/L. Soy protein adhesives modified with 4g/L and 16g/L CaCO_3 were selected to characterize the adhesion performance on 3 ply yellow pine plywood using the Response Surface Method (RSM). The effects of curing temperature and curing time on the adhesion properties were also studied.

The major findings are 1) 4g/L CaCO_3 , 6g/L NaHSO_3 modified soy protein adhesives (MSPA) had better adhesion performance (both dry and wet) than 16g/L CaCO_3 , 6g/L NaHSO_3 MSPA; 2) Higher temperature (170°C) resulted in higher wet shear adhesion strengths; 3) Longer hot press time had positive impact on wet adhesion shear strength; and 4) 4g/L CaCO_3 , 6g/L NaHSO_3 MSPA showed better adhesion shear strength after 2 weeks storage than 16g/L CaCO_3 , 6g/L NaHSO_3 MSPA.

In general, 4g/L CaCO_3 , 6g/L NaHSO_3 MSPA, under longer hot press time and higher temperature would lead to a better adhesion performance.

Key words: Soy protein adhesive, Calcium Carbonate, Water resistance

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Dedication

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Preface

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1. INTRODUCTION

1.1. General background

Today's US wood adhesives markets are dominated by petroleum-based wood adhesives, mainly for plywood, particleboard, and the medium density fiberboard in construction industry (Sun, 2004). Phenol-formaldehyde, urea-formaldehyde and melamine-formaldehyde are the three major petroleum-based wood adhesives that are currently used in the market. The world crude oil price is excessively increased these years, and the oil demand was also increasing greatly from 1993 to 2009 (Figure 1.1). With the increasing of oil price, the price of petroleum-based products would also increase dramatically.

The lack of fossil resources for wood adhesives is not the only issue we are facing. Besides, the environmental and health issues related to production and the end-processing are drawing more attention. Petroleum-based adhesives are not biodegradable and pollute the soil and groundwater when they are disposed. This pollution can be a long-term and uneasy-to-handle liability for the society and human beings (CPC Aeroscience, 2005). The emission of formaldehyde from urea-formaldehyde adhesive is one of the major problems. The report from World Health Organization showed that formaldehyde is a kind of carcinogen that can cause nasopharyngeal cancer (International Agency for research on cancer, 2004).

Soybean is an abundant and renewable resource. The soybean production in 2010 is 3.329 billion bushels in US (USDA statics, 2011). Soybean protein is a byproduct of soy oil extraction, and it is a very good alternative product for the petroleum-based wood adhesives. Soy protein-based adhesive was first used in 1923 (Johnson, 1923). However, it had two disadvantages when compared to petroleum-based adhesives, which are the low adhesion and poor water resistance.

Efforts have been made to improve the adhesion properties of soy protein adhesive, including physical, chemical treatment, and enzymatic modification. Physical treatment method includes blending with commercial adhesives (Qi et al., 2010), pressure and heat. Enzymatic method includes protease and transglutaminase modification. Chemical modification is the most widely used and effective method for preparing soy protein adhesives. Kalapathy et al. (1995) used alkali-modifying method. Huang and Sun (2000 a, b) focused on using sodium dodecyl sulfate, sodium dodecylbenzene sulfonate, guanidine hydrochloride, and urea to modify soy protein.

Liu et al. (2008, 2010) used calcium carbonate as the modifying agent to improve the water resistance and adhesion strength. The core of modification is to change the conformation of the soybean protein. Exposing the buried hydrophobic groups of soy protein molecules to a certain extent is the most efficient way to improve the hydrophobicity of protein polymers, and thus improve the water resistance (Sun et al., 2008). Sun et al also indicated that ratio of hydrophobic group /hydrophilic group is an important factor influencing the wet adhesion properties of protein adhesives (Sun et al., 2008). Therefore, a good balance between adhesion and water resistance is often needed.

Although water resistance and adhesion have been greatly improved through those efforts, the wet adhesion strength of soy protein based adhesives is still not competitive with the petroleum-based adhesives. NaHSO₃ has been recently used to modify and partially unfold the soy protein molecules, which had significantly improved adhesion strength (Sun et al., 2008). Calcium carbonate (CaCO₃) has been used to coagulate soy protein to form tofo, and is expected to react with some functional groups of the soy proteins to cross-link with the protein molecules. On the other hand, CaCO₃ would penetrate through wood surface to interact with wood structure. Thus by adding CaCO₃, we would expect a better adhesion performance and improved water resistance. In this research, we introduced the CaCO₃ to this partially denatured soy protein induced by NaHSO₃, and study the co-effects of NaHSO₃ and CaCO₃ on the adhesion properties, particularly wet adhesion (water resistance) of soy protein adhesives.

1.2. Objectives

The overall objective of this research is to increase the adhesion strength and water resistance of soy protein adhesives. The specific objectives of this research are:

1. To investigate the co-effects of CaCO₃ and NaHSO₃ on the adhesion properties, particularly wet adhesion (water resistance) of modified soy protein adhesives.
2. To study the effects of curing temperature and time on the adhesion properties of the modified soy protein adhesives.

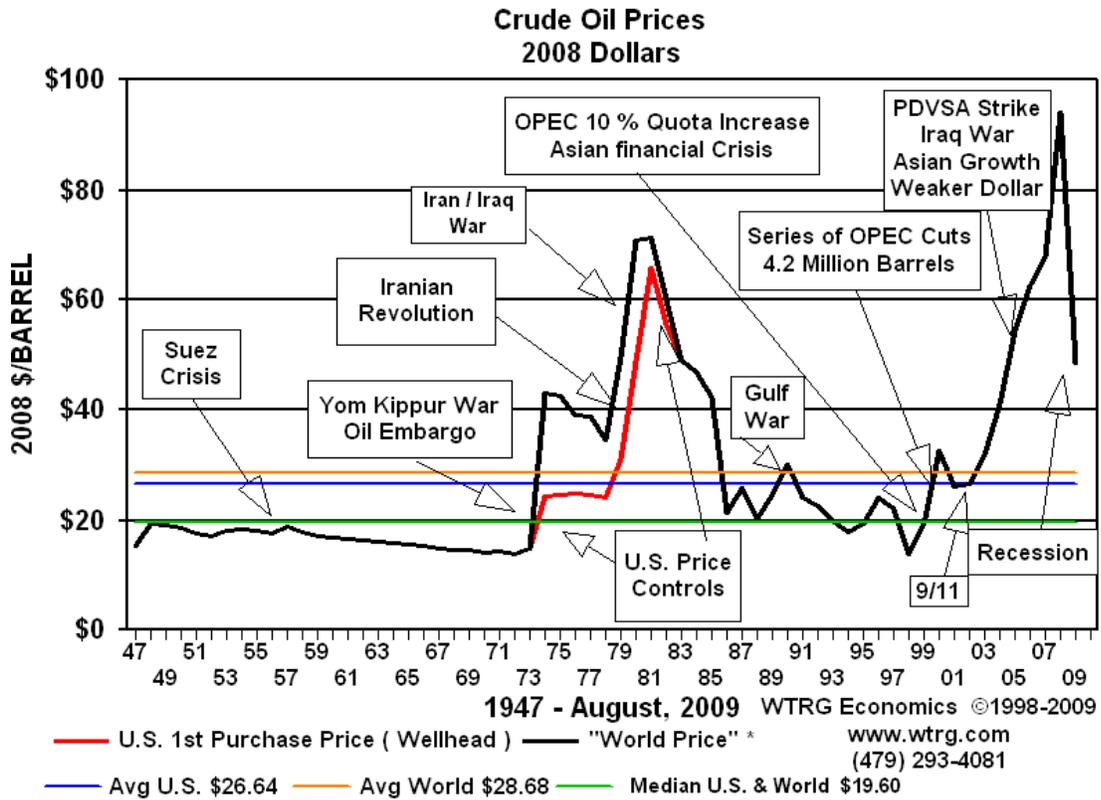


Figure 1.1 World Oil Price. (Resources: www.wtrg.com)

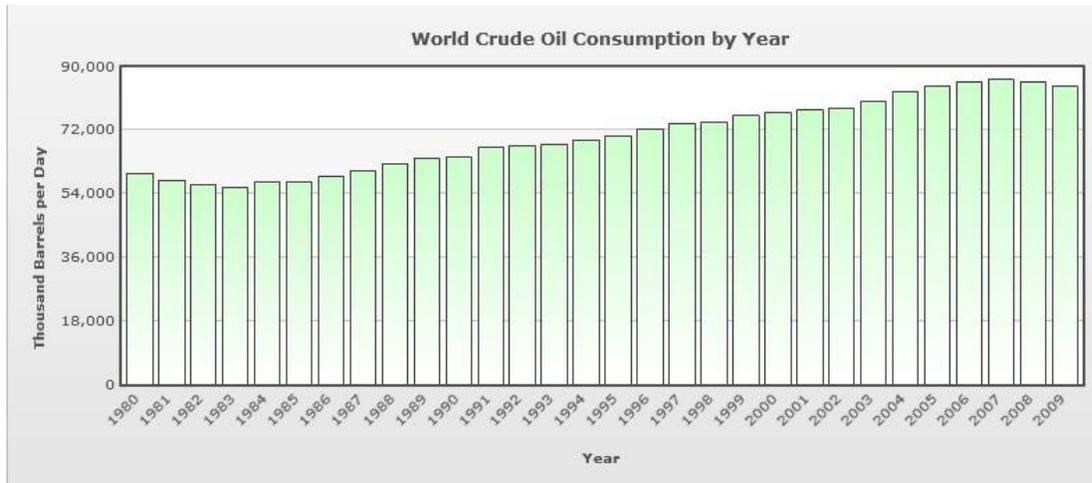


Figure 1.2 World Crude Oil Consumption by Year (Resource: <http://www.eia.gov>)

2. LITERATURE REVIEW

2.1 ADHESION MECHANISM

Adhesion is an attraction process between dissimilar molecular species that can potentially bring them in close contact. Adhesion is the tendency of dissimilar particles and/or surface to cling to one another. The forces that cause adhesion and cohesion can be divided into several types. The intermolecular forces responsible for the function of various kinds of stickers and sticky tape fall into the categories of chemical adhesion, dispersive adhesion, and diffusive adhesion. (Wikipedia)

There are five mechanisms of adhesion proposed by the scientists including mechanical adhesion, chemical adhesion, dispersive adhesion, electrostatic adhesion, and diffusive adhesion. Mechanical adhesion: The adhesives materials are filled to the surfaces by the mechanical interlocking. Mechanical interlocking is a very important driving force for the strength of the adhesion; Chemical adhesion: Chemical adhesion occurs when the surface atoms of two separate surfaces forms ionic, covalent, or hydrogen bonds. If the two surfaces could bond together, they formed new network of these bonds; Dispersive adhesion: The two adhesive materials are held by the van der Waals forces. The positive and negative charges are attracted by each other; Electrostatic adhesion: At the joining point of the two adhesive materials, a different electrical charge was formed; Diffusive adhesion: Two adhesive surfaces could be merged together by diffusion.

2.2 ADHESIVE PAST AND FUTURE

Adhesive had a long history of being utilized by human beings back to ancient China and Egyptians. The ancient adhesive materials including animal glues, vegetable protein glues, starch glues, and blood glues (McGuire, 1964). Soy-based protein adhesives had a long history since 1920s (Johnson, 1923). The application of these biobased adhesives is limited by their poor water resistance. The exterior application problem is still a challenge for today's biobased adhesives (Lambuth, 1989). These biobased adhesives were replaced by petroleum based adhesives in 1930s. After exploring the fossil resources, such as oil and nature gas, the petroleum-based adhesives became dominated in the US and world markets. The domination of the petroleum-based adhesives was not being changed until the energy crisis occurred. From

Figure 1.1, we can conclude that the world crude oil price is excessively increased these years. In addition, environmental pollution from these petroleum based adhesives also become acute. Thus, it is important to develop alternative adhesives to reduce reliance on fossil resources and to be environmental friendly, logistically feasible, and economically responsible.

2.3 SOYBEAN-RENEWABLE RESOURCE

Since soybean is an abundant and renewable resource in US. The soy protein based adhesives for wood products are considered as alternatives to reduce reliance on fossil chemicals and ease the formaldehyde emission problems. The major compositions of soybean include proteins (38%), oil (18%), soluble carbohydrates (15%), insoluble carbohydrates (15%), and other content (14%). In 2010, soybeans represented 58% of world oilseed production, and 35% of those soybeans were produced in the United States (Soystats, 2010).

Soybean protein contains about 20 amino acids, and they are attached to the side chain of the protein molecule through the functional groups. These functional groups include $-OH$, $-NH_2$, $-COOH$, and $-SH$. Among these functional groups, $-SH$ plays an important role affecting the interactions with the wood substrates resulting in adhesion strength. The major components of soybean protein are 7S globulin (β -conglycinin) and 11S globulin (glycinin). β -conglycinin is consists of three subunits: α , α' , and β . β -conglycinin have a molecular weight about 192,000; glycinin have a molecular weight about 350,000 . Table 2.1 shows the difference between 7S and 11S amino acid compositions. All two subunits have high content in aspartate/asparagin, glutamate/glutamine, leucine and arginine. Glycinin have six subunits. These subunits consist of acidic polypeptide and basic polypeptide. The two polypeptides are connected by disulfide bonds.

2.4 SOY PROTEIN BASED ADHESIVE MODIFICATION

Soybean protein can be modified by physical, chemical, enzymatic methods. Different modification methods have different effects on soybean protein viscosity, water resistance, and adhesion strength. Physical modification methods include heating, high pressure curing, blending with commercial wood adhesives; Chemical modification methods include alkali modification, acid modification, and inorganic salts modifications; enzymatic modification methods include

protease modification, papain & urease modification, and chymotrypsin modification (Kumar et al., 2004).

It is well known that the hydrophilic groups of soybean protein are in the outer layer of protein, while the hydrophobic groups are buried inside. The basic concept of modification is to use physical, chemical or enzymatic methods to break the covalent and non-covalent bonds to denature the soybean protein structures. The hydrophobic interactions are considered as an important factor affecting protein hydrophobicity. With the degree of soybean protein denaturation, more hydrophobic groups are exposed. The water resistance would be improved as the hydrophobic groups increased (Sun et al., 2008).

2.4.1 Physical Modification

Physically modification methods mainly include heat treatment, high pressure curing, and blending soybean protein based wood adhesives with commercial wood adhesives. The heat treatment is favored because of its simple adhesion mechanism. In general, denaturation is an endothermic process triggered and accelerated by providing heat. Heat treatment could provide the soybean protein extra thermal energy to break the disulfide bonds. High pressure (under 500 MPa) method could increase the soybean protein adhesive viscosity (Kajiyama et al., 1995). In their research, they found that high pressure would dissociate the soybean protein, and part of the dissociated proteins became coagulated. Blending is another efficient physical modification method. Soybean protein adhesives have been reported to blend with six commercial synthetic latex adhesives (Qi and Sun, 2010). The author compared different blending ratio of modified soy protein and press bond glue (MSP/PBG). The ratio of MSP/PBG at 40/60 had the highest wet adhesion strength than each adhesive alone. Blending modification methods have the advantages of reducing the formaldehyde emissions from commercial wood adhesives and at the same time, improving or maintaining wet adhesion properties of commercial adhesives.

2.4.2 Enzymatic Modification

Enzymatic modification method was developed after physical and chemical modification methods. Researchers have been compared the different effects of enzymes on the adhesion strength and viscosity performances of soybean protein (Kumar et al., 2004). Papain, urease, and chymotrypsin modified soybean protein adhesives were studied. . All three modified soy protein adhesives had lower viscosities than the unmodified soy protein adhesives. Papain and urease

modified soy protein adhesives had better adhesion strength than unmodified soy protein adhesives, while chymotrypsin-modified soy protein adhesives had no adhesion strength at all. However, the hydrophobicity did not have any correlations with the adhesion strength results. Chymotrypsin-modified soy protein adhesives had the maximum hydrophobicity among all four groups. Papain-modified soy protein adhesives had second high hydrophobicity. While the urease-modified soy protein adhesives only showed slight improvement when compared to the unmodified soy protein adhesives.

2.4.3 Chemical Modification

Chemical modification is the most efficient and widely used modification method. Chemical modification method include introducing a new functional group into the soybean protein structure, or removing a component from the protein, or breaking the disulfite bonds to acquire the denaturation. Acid, basic, inorganic salts and other chemicals agents have been used to improve soy protein adhesion strength by changing the structures and conformations of soy protein. Unfolding detergents are commonly used for protein modifications. Different concentrations of guanidine hydrochloride (GH), urea, and sodium dodecyl sulfate (SDS), and sodium dodecylbenzene sulfonate (SDBS) modified soy protein adhesives were studied by Huang and Sun (2000). The adhesion strength, water resistance and delamination results showed that 0.5 and 1M guanidine hydrochloride, 1 and 3M urea, 0.5% and 1% SDS, and 1% SDBS had the highest adhesion strength for the four chemicals. Alkali modified soy protein adhesives were reported by Kalapathy et al. (1995, 1996).

Disulfide bonds in native protein molecules have great effects on protein structures, stability and unfolding degrees (Koshiyama, 1972; Kella et al., 1986). Unlike with unfolding agents, reducing agents (i.e., sulfites, bisulfites and sulfates) could cleave the inter- and intra-disulfide bonds of protein. The results of disulfide bonds cleaved increase molecular flexibility, solubility, surface hydrophobicity, and decrease viscosity (Kella et al., 1986; Kalapathy et al., 1996). Sodium chloride, Sodium sulfulfite, sodium sulfate were added to the soy protein to obtain the disulfide bond-cleavage. On the other hand, alkali-modified soy protein and trypsin-modified soy protein were also investigated. The results proved that under different alkali modification, the

disulfide bond could be broken and resulted in improvements in water resistance and adhesion strength.

Cross-linking of protein is another commonly used method for protein modification. The compact protein structure after the cross-linking showed better adhesive performance than the unmodified protein (Rogers et al 2004, Zhong and Sun, 2007). 1,3-dichloro-2-propanol, Polyamide-epichlorohydrin (PAE) are some successful cross-link reagents (Wool and Sun, 2005). Liu and Li (2002, 2004) used organic synthesis method to acquire marine protein structures on soy protein adhesives, to improve water resistance and adhesion strength as well as reduce the price of this adhesive. Liu et al. (2008, 2010) found that calcium carbonate could crosslink with the protein structures to create novel soy protein-based nano-composites. Effects of two polymorph of calcium carbonate (aragonite and calcite) on structure, wet adhesion strength of the calcium carbonate modified soy protein hybrid adhesives were significantly improved. Chemical modification methods have a very bright future. With the advancement of chemistry, more chemical agents would be available to improve protein based adhesives.

Table 2.1 Amino acid compositions of 7S and 11S*.

Amino acid	7S	11S
Ala	7.49	5.17
Arg	7.29	6.32
Asn	5.18	7.35
Asp	0.14	4.31
Cys	8.86	1.48
Gln	13.15	9.89
Glu	4.84	8.74
Gly	2.32	7.27
His	5.72	1.97
Ile	8.72	4.56
Leu	6.13	6.90
Lys	0.34	4.31
Met	5.72	0.99
Phe	6.27	3.86
Pro	7.49	6.49
Ser	2.38	7.18
Thr	0.20	3.94
Trp	2.59	0.86
Tyr	5.18	2.55
Val	7.49	5.87

*Adapted from Utsumi et al., 1997. Expressed as percentages.

3. MATERIALS & METHOD

3.1 MATERIALS

Defatted soy flour (SF) from Cargill (Cedar Rapids, IA) was used as the starting material. The SF contained about 50% protein and 10% moisture with a dispersion index of 90. Sodium bisulfite (NaHSO_3) was obtained from Fisher Scientific (Fair Lawn, NJ). Calcium carbonate (CaCO_3) was purchased from Sigma-Aldrich (St. Louis, MO). Yellow pine veneers with dimensions of $50 \times 127 \times 5$ mm (width \times length \times thickness) were provided by Georgia Pacific Inc. (Decatur, GA).

3.2 ADHESIVES PREPARATION AND MODIFICATION

Soy Protein Adhesives (SPA) was prepared at pH 9.5 by mixing the defatted SF with distilled water at 6.25% solid content. The slurry was stirred for 1h at room temperature for the best mixing performance. NaHSO_3 was added to the slurry at 6 g/L on the basis of water volume. CaCO_3 was added into the slurry at different concentration, ranging from 0 g/L to 23 g/L. The slurry was stirred for 2hr at room temperature. The pH of the supernatant was adjusted to 5.4 by using 3 N HCl and stirred at room temperature for 20 min. Then it was centrifuged at 12,000 RCF. The supernatant was adjusted to 4.8 by using 3 N HCl and stirred for 20 min at room temperature. Then it was centrifuged again at 8,000 RCF. The precipitation was collected as SPA.

3.3 SPECIMEN PREPARATION

Cured soy protein film preparation: The modified soy protein adhesives were applied on the foil wrapped square iron plates. The modified SPA was left in air, at room temperature for 8 minutes. A square mold was put in between the two foil wrapped iron plates. The range of CaCO_3 concentrations was from 0g/L to 23g/L; curing temperatures was from 96°C to 164°C; curing time was from 1.3 minutes to 9.7 minutes.

Three ply plywood specimen preparation: Yellow pine wood veneers were pre-conditioned at room temperature and 30% relative humidity chamber for 7 days. The dimension of yellow pine wood veneer was $300 \times 300 \times 3.5$ mm. The adhesives were diluted by 1% urea on weight base. Then the adhesives were applied to two faces of the middle veneer. The amount of adhesives for

each surface of the yellow pine wood veneer was approximately 20 g/ ft². The three yellow pine wood veneers were following the order that, the middle veneer's grain was perpendicular to the upper and lower veneer. The assembled three ply veneers were conditioned at room temperature for 15 min and then cold press for 5 min and hot press for 4, 5, and 10 min. The conditions for hot press were 170°C and 150°C. 4g/L CaCO₃, 6g/L NaHSO₃ modified SPA and 16g/L CaCO₃, 6g/L NaHSO₃ modified SPA are being pressed at the same time under each condition. For each concentration of CaCO₃, 2 parallel three ply plywood specimens were prepared for better accuracy.

3.4 RESPONSE SURFACE METHOD

In this research, we used CaCO₃ concentrations, curing temperature, and curing time as the variables in the response surface method; 2 hr water absorption, 24 hr water absorption, and weight loss of the cured soy protein films were the three dependent variables. The specific experiment design using response surface method (Khuri and Conell, 1996) is listed in Figure 3.1

3.5 CHARACTERIZATION

Moisture Content: Moisture content of the modified SPA samples was determined by using AACC method 44-15A (AACC, 1999). Approximately 2 g to 3 g of each sample was placed in tared moisture dishes. Moisture content was determined as the weight loss of the sample when heated for 60 minutes at 130°C in an oven.

Water Absorption: Water absorption was determined following ASTM D570-81 (1993). The cured modified-SPI samples were preconditioned at 50°C for 24 hr, and then cooled in the desiccators before being weighed. The preconditioned SPA samples were submerged in distilled water at 25 °C for 2 hr and 24 hr. Then the extra water on the surface was removed with paper towels before weighing. Water absorption was calculated as the gram of absorbed water per gram of cured SPA samples. Soluble material loss of the sample was determined by weighing the specimens after drying them in an oven at 50 °C for 24 hr, which was subtracted in the total water absorption calculation for 24 hr water soaking.

Rheological Properties: Apparent viscosity measurements of CaCO₃ modified SPA samples were determined by the Bohlin CVOR 150 rheometer with a parallel plate (PP20, 20mm plate

diameter and 500 μm gap). The shear rate range of apparent viscosity was from 0.1 to 50 s^{-1} . The measurement was carried out at room temperature. A thin layer of silicone oil was applied to the circumference of the sample in order to prevent the dehydration of sample during the test. For each concentration of CaCO_3 modified SPA samples, 3 parallel groups were tested.

Differential Scanning Calorimetry (DSC): Thermal denaturation properties of CaCO_3 modified SPA samples were evaluated by DSC (Q200, TA instrument, Schaumburg, IL) calibrated with indium and zinc. CaCO_3 modified SPA samples were hermetically sealed in T-zero aluminum hermetic pans. Each samples was held at 20 $^\circ\text{C}$ for 1 min and then scanned from 20 $^\circ\text{C}$ to 130 $^\circ\text{C}$ at a heating rate of 10 $^\circ\text{C}/\text{min}$. Peak temperatures (T_d) and denaturation enthalpies (ΔH) were calculated from thermograms.

Adhesion Shear Strength: The bonded wood samples were conditioned at 23 $^\circ\text{C}$ and 50% relative humidity for 48h before cutting. From each panel, 10 specimens (4 for dry strength and 6 for wet strength) with dimensions of 82.6 \times 25.4 mm were obtained according to ASTM Standard Method D 906-98 (2004) as shown in Figure 3.1. The cut specimens were conditioned in 23 $^\circ\text{C}$ and 50% chamber for 48h. Dry shear adhesion strength at maximum load was recorded.

Water resistance of the three ply plywood samples was evaluated in terms of wet shear strength and three-cycle soak test. Six preconditioned specimens were soaked in water at 23 $^\circ\text{C}$ for 24 hr, and wet strength was tested immediately after soaking. Another 4 specimens with 50 \times 127 mm cut from each panel were for three-cycle soak test in according with the American National Standard for Hardwood and Decorative Plywood; Hardwood Plywood & Veneer Association; 2004 (ANSI/HPVA HP-1). Four specimens were soaked in water at room temperature for 4 hr, and then dried in chamber at 50 $^\circ\text{C}$ with good air circulation for 19 hr. The soaking/drying cycle was repeated until three cycles were completed. All specimens were inspected to see whether they were delaminated after each cycle. The water soaking and dry (WSAD) cycle are evaluated on a 0-10 scale. 0 represents no visible delamination; 5 is the maximum allowable delamination to pass per the ANSI/HPVA procedure; 10 means a completely separation of the veneer. We recorded the pass rate to evaluate the performance of wood veneer.

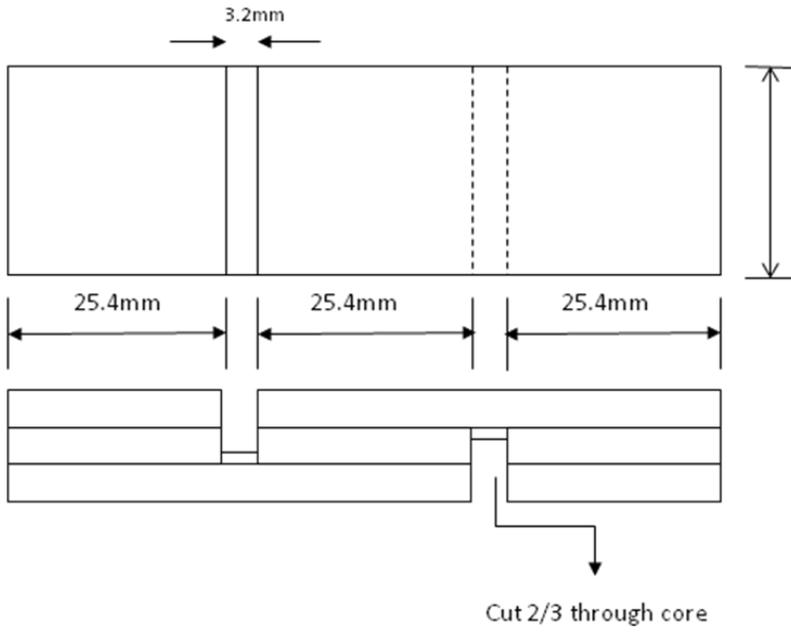


Figure 3.1 Schematic diagram of three ply plywood specimen for shear adhesion strength test

Table 3.1 Response Surface Method Experiment Design.

Samples	CaCO ₃ (g/L)	Curing Temp (°C)	Curing Time (min)
1	16	110	8
2	16	150	8
3	4	110	3
4	4	150	3
5	4	150	8
6	20	130	5.5
7	0	130	5.5
8	16	150	3
9	10	130	5.5
10	4	110	8
11	10	130	130
12	10	130	130
13	10	130	130
14	10	130	130
15	10	96	96
16	10	130	130
17	10	164	164
18	10	130	130
19	16	110	110
20	10	130	130

4. RESULTS AND DISCUSSION

4.1. RESOPONSE SURFACE METHOD

Response surface method was used to study the effects and interactions of CaCO₃ concentration, curing temperature, and curing time on the water resistance of the modified SPA film. The optimized curing conditions were applied to 3 ply plywood for the wood adhesive application.

4.1.1. Water resistance of SPA film at 2-hour water absorption

The regression equation of 2hr water absorption was expressed in equation 1:

$$Y_1 = -36.24 + 7.88 \times X_1 + 1.17 \times X_2 - 0.02 \times X_3 - 0.34 \times X_1^2 - 3.66 \times 10^{-3} \times X_2^2 \quad (1)$$

Y_1 : 2hr water absorption; X_1 : CaCO₃ concentration; X_2 : Curing Temperature; X_3 : Curing Time

The results indicated that all of the CaCO₃ concentration, curing temperature, and curing time had a significant effect on the water resistance of the modified SPA film with R-squared value of 0.700 and $P < 0.050$. As shown in Figure 4.1, the lowest water absorption was obtained with 4 g/L CaCO₃ modified SPA, and it increased as the CaCO₃ amount increased until around 13 g/L, beyond which it started to decrease. Therefore, 4 g/L and 16 g/L had the better water resistance than others. As the curing temperature increased, the water absorption of modified SPA increased, indicating the curing temperature had negative effect on the water resistance.

4.1.2. Water resistance of SPA film at 24-hour water absorption

The equation 2 displayed the regression equation of 24 hr water absorption.

$$Y_2 = -147.26 + 0.06 \times X_1 + 3.17 \times X_2 + 5.62 \times X_3 + 0.02 \times X_1 \times X_2 - 0.10014 \times X_1^2 - 0.012 \times X_2^2 - 0.44 \times X_3^2 \quad (2)$$

Y_2 : 24hr water absorption; X_1 : CaCO₃ concentration; X_2 : Curing Temperature; X_3 : Curing Time

The results showed that all of the CaCO₃ concentration, curing temperature, and curing time had a significant effect on the water resistance of the modified SPA film during 24 hours soaking with R-squared value 0.713 and $P < 0.050$. Similar to the 2 hr water absorption, 4 g/L and 16 g/L

CaCO₃ modified SPA had the best water resistance, and the curing temperature had a negative relationship with the water absorption (Figure 4.2).

4.1.3. Weight loss of cured SPA film

The regression equation of weight loss was expressed in equation 3:

$$Y_3 = +127.84 + 0.62 \times X_1 - 1.80 \times X_2 + 0.80 \times X_3 - 0.03 \times X_1^2 + 6.40 \times 10^{-3} \times X_2^2 - 0.09 \times X_3^2 \quad (3)$$

Y_3 : Weight loss; X_1 : CaCO₃ concentration; X_2 : Curing Temperature; X_3 : Curing Time

Results showed that all of the CaCO₃ concentration, curing temperature, and curing time had a significant effect on the SPA film weight loss with R-squared value of 0.669 and $P < 0.050$. Increasing temperature from 110 °C to 150 °C resulted in the obviously decreased weight loss of modified SPA; while CaCO₃ concentration played an insignificant effects on the weight loss.

The worse performance of high temperature curing was because the protein structures were buried. As we all know that heat spreaded very fast on iron. However, the plywood was much thicker than the iron plate. The thermal conductivities of plywood and iron were also different. Thus, if we carried the same temperature we got based on the response surface method, the actual temperatures of the modified SPA in the middle of plywoods would be lower than the metal temperature. So if we continued to carry the same curing temperatures on the 3 ply plywood test, we would not have the results we expected since the central temperatures might be too low to trigger the reaction that happened during the curing.

4.2. RHEOLOGICAL PROPERTIES

As shown in Figures 4.4 and 4.5, the apparent viscosity of all samples decreased as the shear rate increased in the range of 0.1 s⁻¹ to 100 s⁻¹, indicating their shear thinning behavior. Soy protein adhesive modified with pure 6 g/L NaHSO₃ had the lowest viscosity; while with the co-modification of CaCO₃, the protein viscosity increased obviously. CaCO₃ is expected to induce the cross-link of the soy protein molecules (Figure 4. 6), leading to the strong protein-protein interaction and then having the increased viscosity.

The increased viscosity of SPA had the poor flow-ability and hand-ability, having the adverse effects on the adhesion properties. As a protein denaturant, urea is known to interact with hydroxyl groups of soy protein and break down the hydrogen bonding, resulting in the partly

unfolded protein structure. From Figures 4.4 and 4.5, we can see that the viscosity of SPA decreased significantly by addition of 1% urea. The urea modified SPA had the enhanced hand-ability and wetting ability on the wood surface, favoring the adhesion strength improvement.

4.3. THERMAL PROPERTIES

The typical DSC thermograms of SPA are shown in Figure 4.7. Two endothermic transitions with denaturation temperature (T_d) about 80 °C and 99 °C were designated as β -conglycinin (7S) and glycinin (11S) globulin, respectively. The addition of 4 g/L and 16 g/L of CaCO_3 did not have a significant effect on the T_d of these two components in SPA. However, the total denaturation enthalpy (ΔH) of SPA was increased from 10 J/g to 14 J/g with addition of CaCO_3 (Table 4.1), suggesting that a slightly more complicated protein structure was formed through the cross-linking affected by CaCO_3 . In addition, the change of protein composition in SPA can also be reflected from the thermogram. The ratio of 7S and 11S was reduced significantly as the addition of CaCO_3 , indicating the alteration of protein solubility during the protein extract process. Lower amount of 11S was precipitated out along with fiber and carbohydrates at pH 5.4 in the CaCO_3 -modified soy flour slurry, therefore, the ratio of 11S and 7S in the final soy protein adhesive at pH 4.8 increased.

4.4. SHEAR ADHESION STRENGTH

The choice of CaCO_3 concentrations were based on results obtained from response surface methods test. As we discussed in part 4.1, for 3 ply plywood test, the low curing temperature 110 °C may not reach the soy protein denaturation temperature. Therefore, we used the curing temperatures of 150 °C and 170 °C instead of 110 °C. This change would assure us the reaction of denaturation protein structures occurred as we expected in the 3 ply plywood test.

4.4.1. Effects of CaCO_3 concentration, curing temperature, and curing time on adhesion properties of modified SPA.

The shear strength test was followed the schematic diagram shown in Figure 4.8. Dry and wet shear adhesion strength of CaCO_3 (4 g/L, 16 g/L) and NaHSO_3 (6 g/L) modified SPA samples were summarized in Table 4.2. As we can see, 4 g/L CaCO_3 modified SPA sample with curing temperature 170 °C and 10 min curing time had the best adhesion performance in terms of both

dry and wet adhesion strength (1.405 MPa and 0.709 MPa, respectively). Under higher curing temperature 170 °C, longer curing time would lead to better dry and wet adhesion strength of 4 g/L CaCO₃ modified SPA, and better wet adhesion strength of 16 g/L CaCO₃ modified SPA. However, at 150 °C, curing time had negative impact on dry adhesion strength for both 4 g/L and 16g/L modified SPA. In the case of wet strength, curing time had a positive impact on 16 g/L modified SPA. Furthermore, increasing temperature from 150 °C to 170 °C affected the wet adhesion strength for both 4 g/L and 16 g/L modified SPA positively, because the higher temperature favors the protein molecules entanglement and cross-linking during curing, leading to the higher wet strength.

The 3 cycle water soaking and dry test of SPA are summarized in Table 4.3. Only 4 g/L CaCO₃ modified SPA had the 100% passing rate for all the three cycles under 3 curing conditions: 170 °C, 10 min; 150 °C, 4 min; and 150 °C 10 min. And consistent with the wet shear strength results, higher curing temperature and curing time led to a better water resistance.

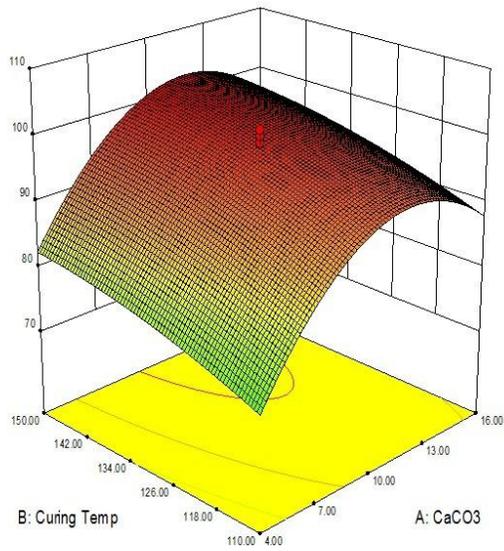
4.4.2. Co-effects of CaCO₃ and NaHSO₃ on the adhesion performance of modified SPA.

The comparison between the co-effects of CaCO₃ (4 g/L), NaHSO₃ (6 g/L) and their sole effects on the adhesion properties of SPA were also studied (Table 4.4). The curing condition was selected as 170 °C press temperature and 10 min pressing time. Results showed that the dry adhesion strength was enhanced to 1.4 MPa when both chemicals exerted on the soy protein, in comparison of 1.1 MPa and 1.2 MPa for NaHSO₃ and CaCO₃, respectively. The wet strength was also increased by around 0.13 MPa. NaHSO₃ is known to partly unfold the protein structure, and expose more functional groups on the surface of protein surface (i.e., -COOH and -OH), resulting in more reaction with CaCO₃ and inducing more cross-linking reactions among soy protein, which will favor the adhesion strength improvement. Furthermore, calcium cation would penetrate through wood surface to interact with wood structure, thus having the improved dry adhesion strength and water resistance as well.

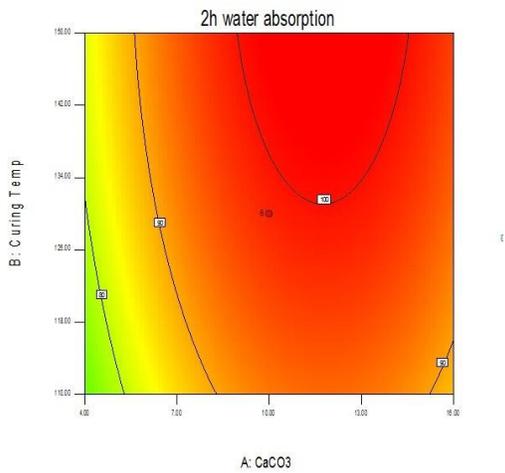
4.4.3. Modified SPA aging study

The aging effects on the adhesion performance of 4g/L and 16g/L CaCO₃ modified SPA were also studied (Table 4.5). Both samples had the improved dry adhesion strength after two weeks storage; and the wet strength of 4 g/L CaCO₃ modified SPA also increased slightly from 0.31 MPa to 0.48 MPa. However, two weeks aging of 16 g/L CaCO₃ modified SPA caused the largely

decreased wet strength (too low to be accurately determined by Instron). Therefore, results indicated that high CaCO_3 concentration (16 g/L) modified SPA was not as stable as the low CaCO_3 concentration (4 g/L) modified SPA.

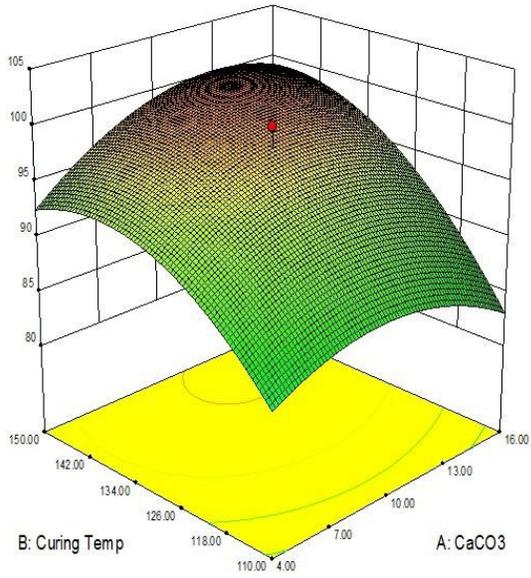


Curing time was set at 5.5 min.

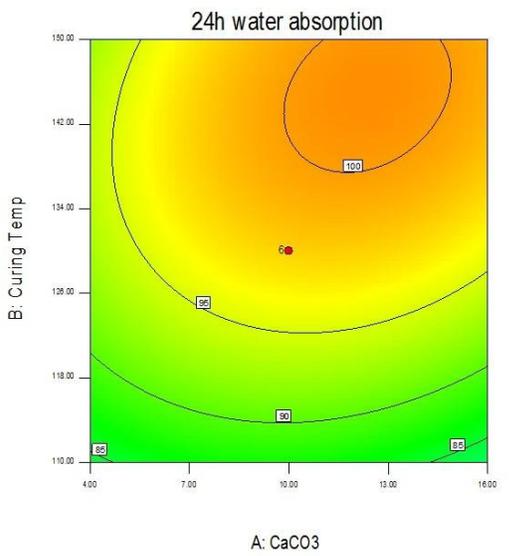


Curing time was set at 5.5 min.

Figure 4.1 2hr water absorption of cured SPA film as affected by curing temperature and CaCO_3 concentrations.

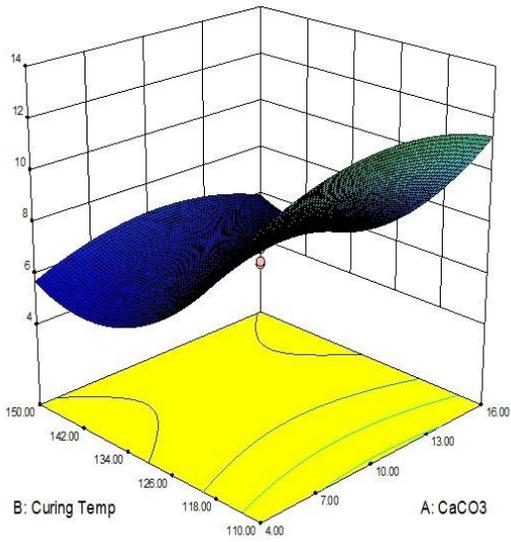


Curing time was set at 5.5 min.

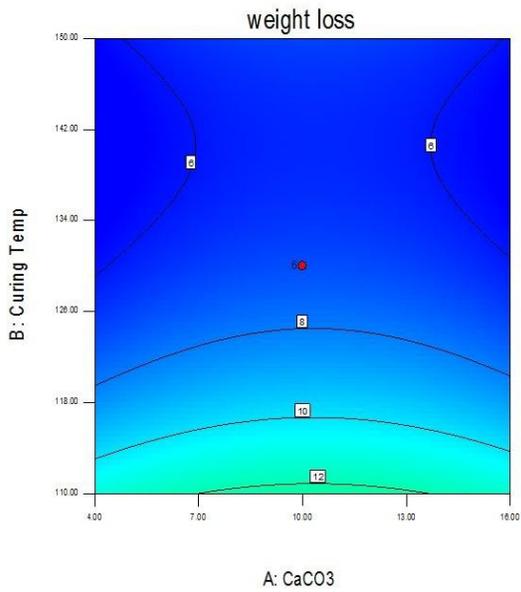


Curing time was set at 5.5 min.

Figure 4.2 24 hr water absorption of cured SPA film as affected by curing temperature and CaCO_3 concentrations.



Curing time was set at 5.5 min.



Curing time was set at 5.5 min.

Figure 4.3. Weight loss of cured SPA film as affected by curing temperatures and CaCO₃ concentrations.

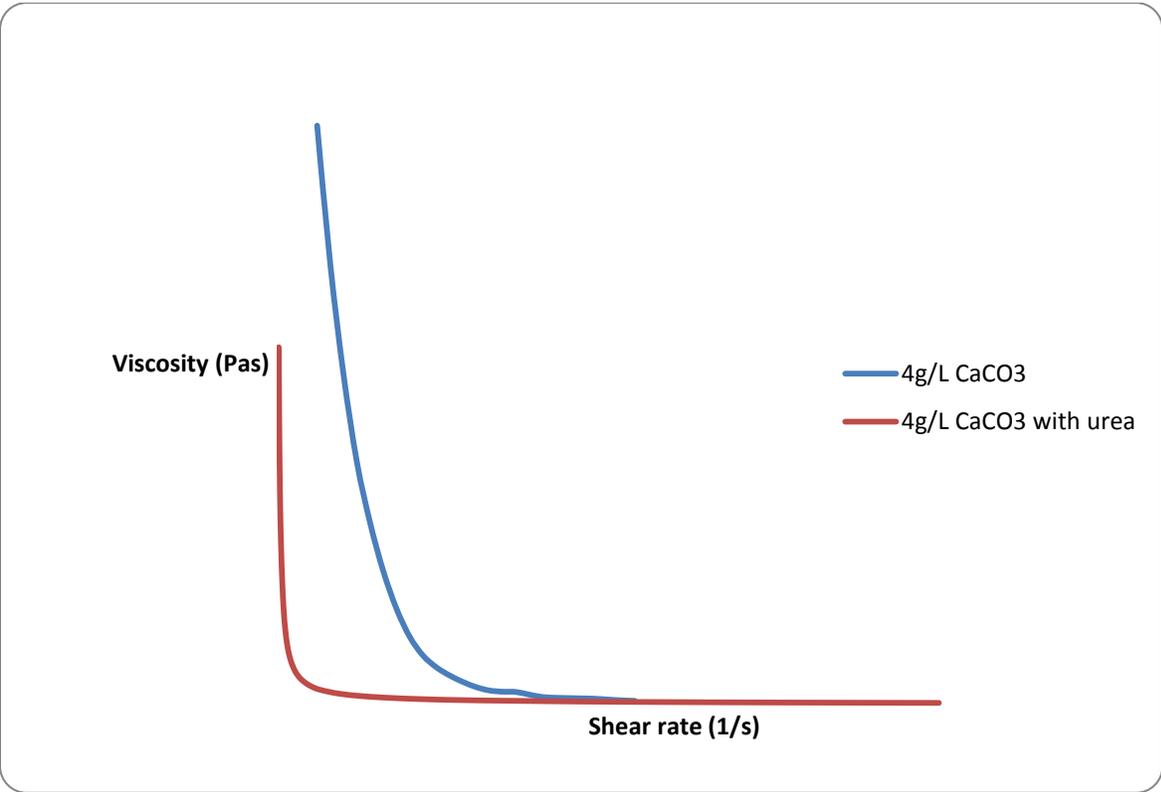


Figure 4.4. Apparent viscosity data of 4g/L CaCO₃, 6g/L NaHSO₃ modified SPA.

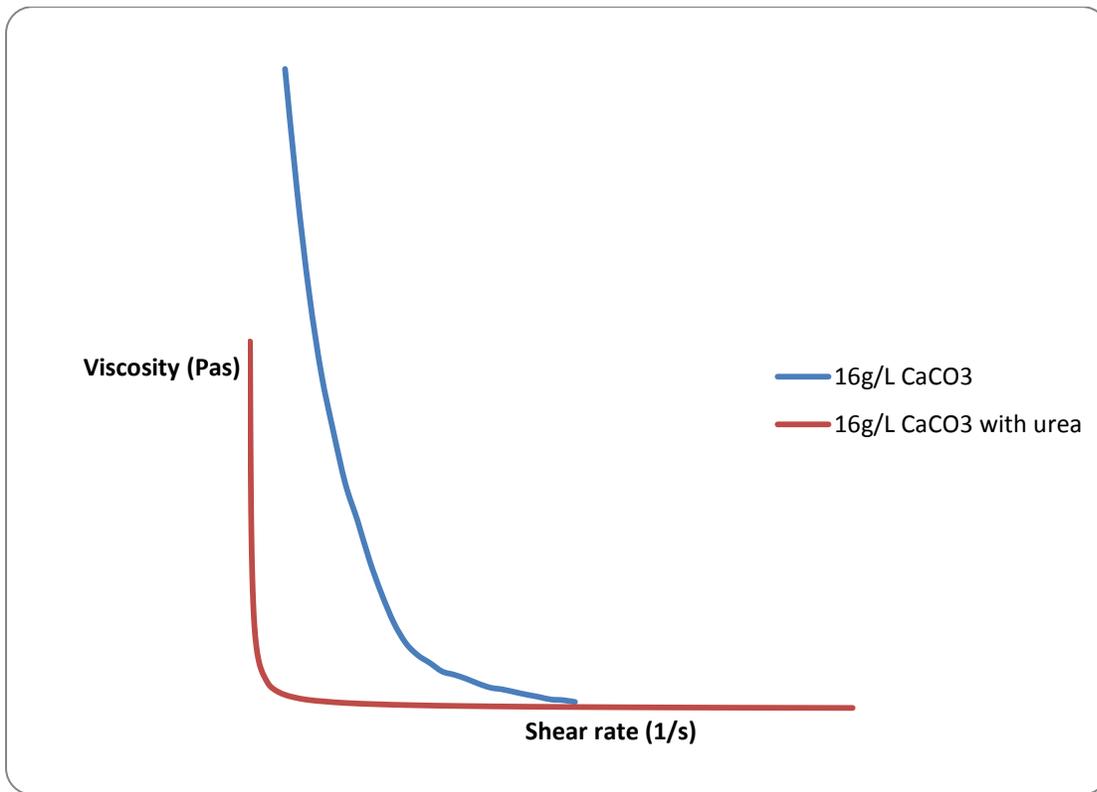


Figure 4.5 Apparent viscosity Data of 16g/L CaCO₃, 6g/L NaHSO₃ modified SPA.

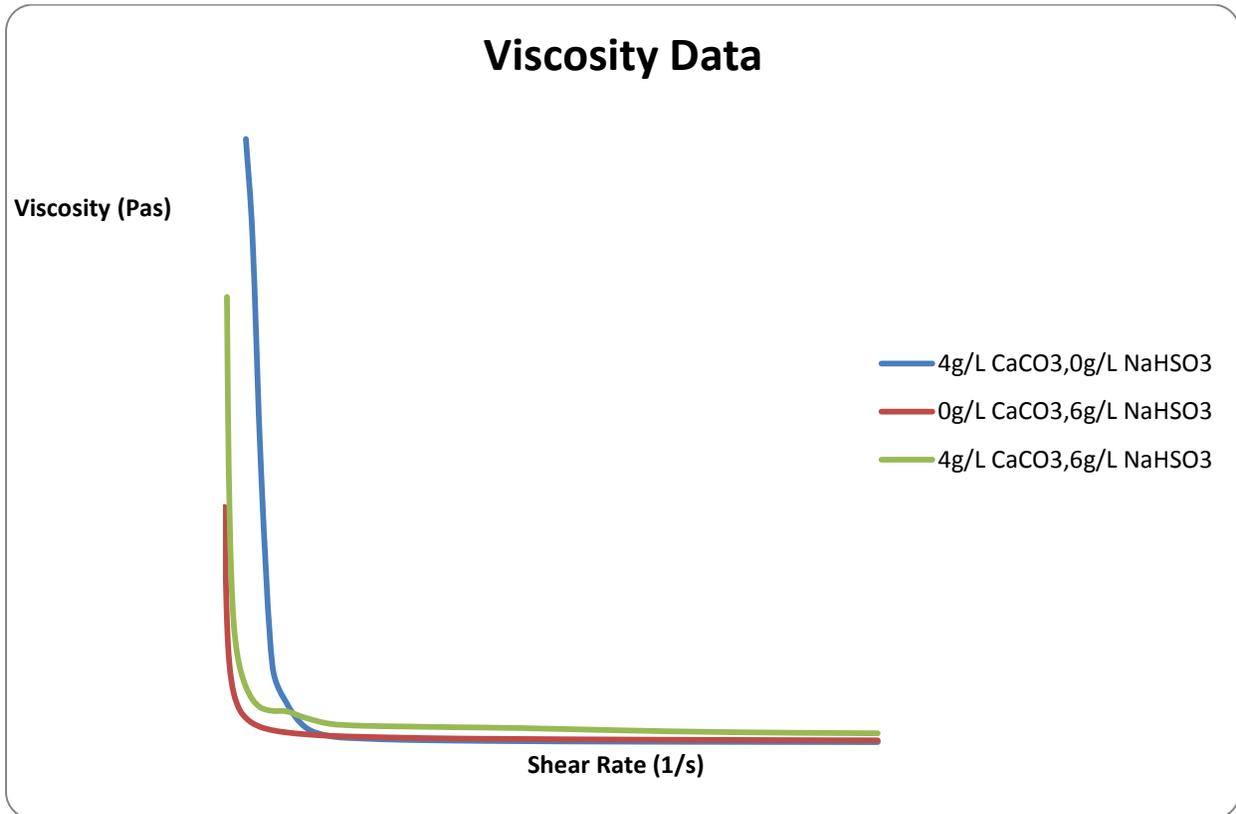


Figure 4.6 Apparent viscosity of two single salts and co-effect of two salts modification

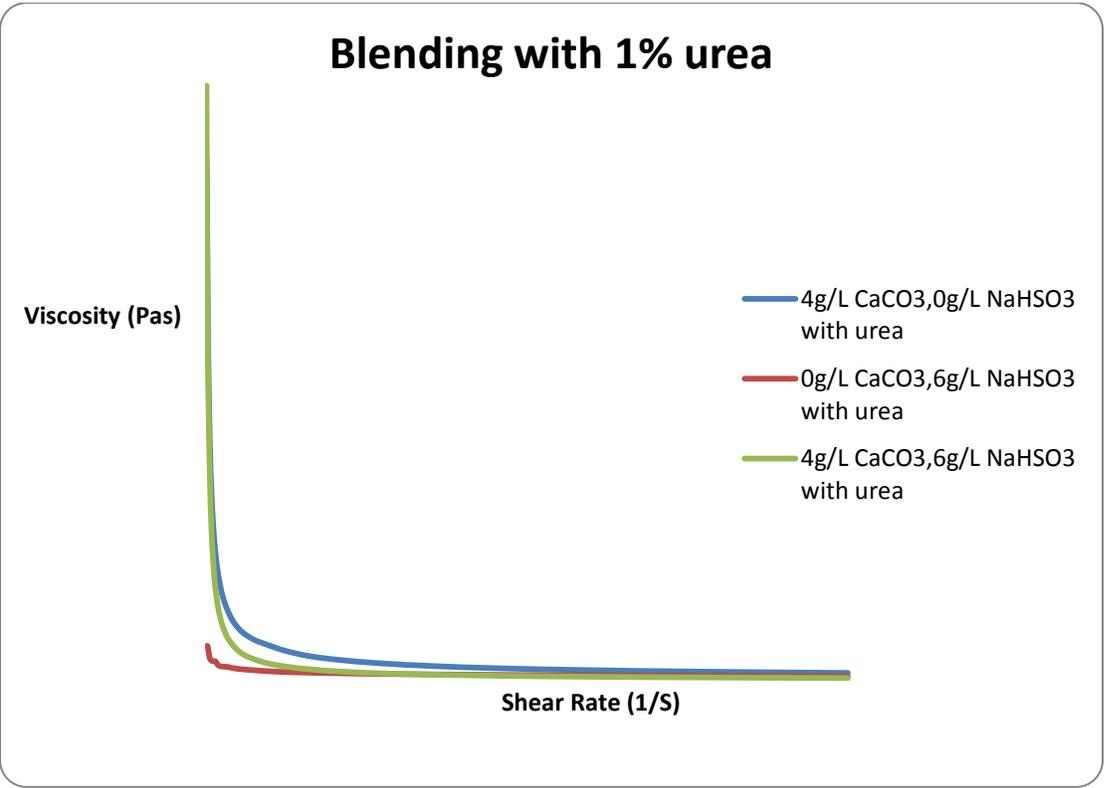


Figure 4.7. Apparent viscosity of two single salts and co-effect of two salts modification after blending with 1% urea.

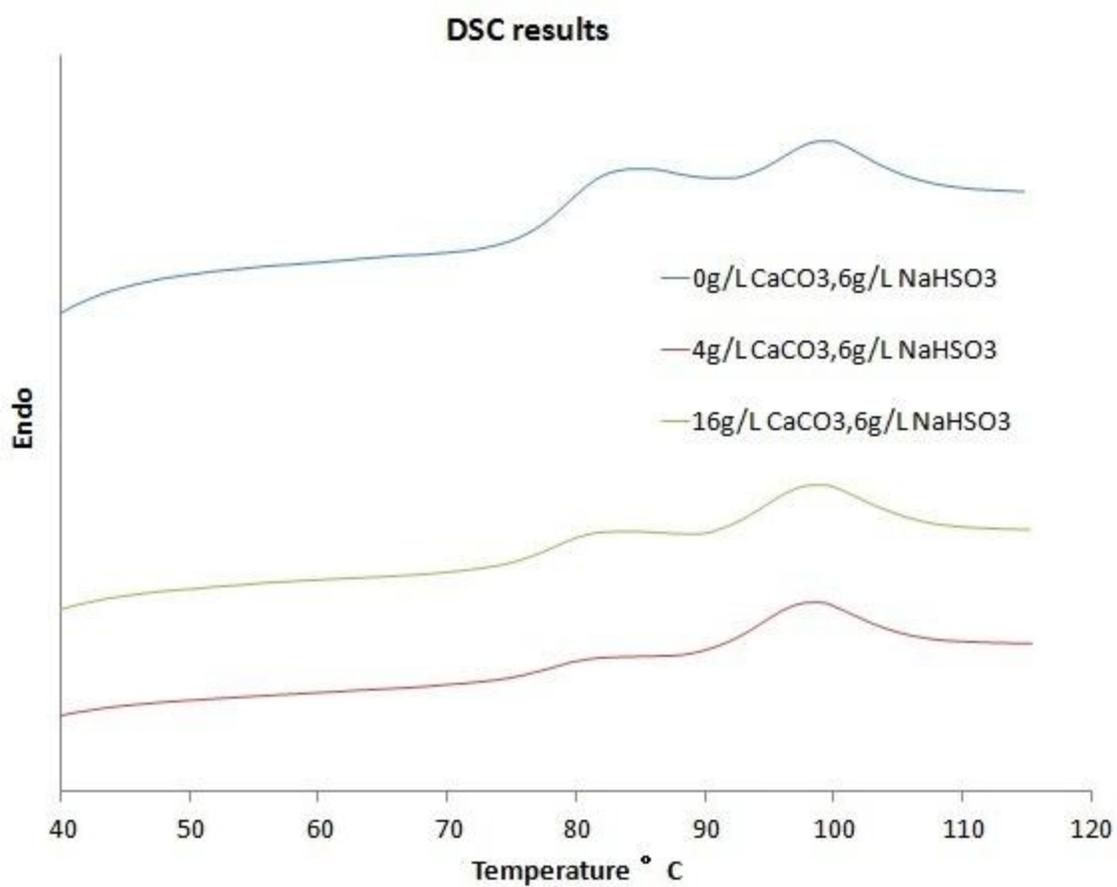


Figure 4.8. DSC thermogram of SPA modified with CaCO₃ at different concentrations.



Figure 4.9. Speculated reaction mechanisms occurred between soy protein and CaCO₃.

Table 4.1 Enthalpy of denaturation (ΔH) of SPA modified with CaCO_3 at different concentrations.

Samples	CaCO_3 (g/L)	NaHSO_3 (g/L)	7S ΔH (J/g)	11S ΔH (J/g)
1	0	6	4.79	5.39
2	4	6	0.85	13.45
3	16	6	0.66	10.85

Table 4.2 Effects of CaCO₃ concentration and curing condition on the adhesion strength of modified SPA.

Curing temperature (°C)	Curing Time (min)	CaCO ₃ (g/L)	Dry strength (MPa)	Wet strength (MPa)
170	4	4	0.991±0.168	0.282±0.049
170	4	16	1.096±0.220	0
170	5	4	0.815±0.153	0.311±0.063
170	5	16	1.024±0.149	0.294±0.086
170	10	4	1.405±0.345	0.709±0.219
170	10	16	0.755±0.161	0.324±0.125
150	4	4	1.60±0.251	0.293±0.166
150	4	16	1.269±0.326	0
150	5	4	0.871±0.287	0
150	5	16	0.757±0.238	0
150	10	4	0.983±0.265	0.30±0.147
150	10	16	1.07±0.192	0.386±0.125

Table 4.3 Water Soaking and Dry Cycle Evaluation of modified SPA as affected by CaCO₃ concentration and curing condition.

Curing temperature (°C)	Curing Time (min)	CaCO ₃ (g/L)	Cycle I Passing Rate	Cycle II Passing Rate	Cycle III Passing Rate
170	4	4	50%	25%	0%
170	4	16	75%	12.5%	0%
170	5	4	12.5%	0%	0%
170	5	16	100%	62.5%	37.5%
170	10	4	100%	100%	100%
170	10	16	87.5%	62.5%	62.5%
150	4	4	100%	100%	100%
150	4	16	25%	12.5%	12.5%
150	5	4	0%	0%	0%
150	5	16	0%	0%	0%
150	10	4	100%	100%	100%
150	10	16	87.5%	87.5%	75%

Table 4.4 Comparison between the co-effects of CaCO₃, NaHSO₃ and their sole effect on the adhesion performance of modified SPA.

CaCO ₃ (g/L)	NaHSO ₃ (g/L)	Dry Strength (MPa)	Wet strength (MPa)	Cycle I Passing Rate	Cycle II Passing Rate	Cycle III Passing Rate
0	6	1.090± 0.177	0.575±0.227	100%	100%	100%
4	0	1.234±0.122	0.586±0.146	100%	100%	100%
4	6	1.405±0.345	0.709±0.219	100%	100%	100%

Table 4.5 Two weeks aging effect on the adhesion performance of 4 g/L CaCO₃ and 6 g/L NaHSO₃ modified SPA.

Samples	CaCO ₃ (g/L)	Dry Strength (MPa)	Wet strength (MPa)	Cycle I Passing Rate	Cycle II Passing Rate	Cycle III Passing Rate
1	4	0.815±0.153	0.311±0.063	12.5%	0%	0%
2	4	1.13±0.637	0.481±0.303	75%	37.5%	25%
3	16	1.024±0.149	0.294±0.086	100%	62.5%	37.5%
4	16	1.311±0.374	0	100%	87.5%	87.5%

1. represented the original 4 g/L CaCO₃ and 6 g/L NaHSO₃ modified SPA;
2. No.1 sample after two weeks aging.
3. represented the original 16 g/L and 6 g/L NaHSO₃ modified SPA;
4. No.3 sample after two weeks aging.

5. CONCLUSION

5.1 CONCLUSION

Co-effects of CaCO_3 and NaHSO_3 on improving the water resistance of SPA was reported in this thesis. Both curing temperature and curing time were compared at different level to get to a better water resistance performance.

Modified SPA was prepared at different CaCO_3 and NaHSO_3 concentrations from 0 g/L to 23 g/L. The change of protein composition in SPA was confirmed by DSC. Via response surface methods, we narrowed the concentrations of CaCO_3 into two levels: 4 g/L and 16 g/L.

The higher viscosity of modified SPA had poor flow-ability and hand-ability. Thus, in the shear adhesion test, we blended modified SPA with 1% urea in order to solve this problem. The rheology test proved our point. In rheology test, we figured out that the apparent viscosity of all samples decreased as the shear rate increased in the range of 0.1 s^{-1} to 100 s^{-1} . In order to acquire better conditions of increasing water resistance of modified SPA. We carried out three sets of test.

- 1) Shear adhesion strength test results: 4 g/L CaCO_3 modified SPA sample with curing temperature $170 \text{ }^\circ\text{C}$ and curing time 10 min had the best adhesion performance in terms of both dry and wet adhesion strength.
- 2) The 3 cycle water soaking and dry test results: 4 g/L CaCO_3 modified SPA was the only concentration that have 100% passing rate for all three cycles under three different conditions of $170 \text{ }^\circ\text{C}$, 10min; $150 \text{ }^\circ\text{C}$, 4min; and $150 \text{ }^\circ\text{C}$ 10min.
- 3) Modified SPA aging study results: after two weeks aging, the wet strength of 4 g/L CaCO_3 modified SPA increased slightly from 0.31 MPa to 0.48 MPa; however, the wet strength of 16 g/L CaCO_3 after two week aging was too low for Instron to determine. This indicated that 4 g/L CaCO_3 modified SPA was more stable than that of 16 g/L CaCO_3 .

Based on the comprehensive results of all three sets of tests, we could conclude that 4 g/L CaCO_3 combined with 6 g/L NaHSO_3 had the relatively best adhesion performance in terms of dry and wet adhesion strength. Longer curing time and higher curing temperature improved the water resistance performance of modified SPA evaluated on the 3 ply plywood.

The Co-effects of CaCO_3 and NaHSO_3 on the shear adhesion strength performance of modified SPA were also studied. By comparing the co-effects of CaCO_3 and NaHSO_3 and the

solo effect of each chemical, we found the fact that both dry and wet shear adhesion strength was higher than that of any single chemical modified SPA. During the reaction, NaHSO_3 played the part of unfolding protein structure. By this way, more functional groups were exposed on the surface of protein. These exposed functional groups inducing more cross-linking with CaCO_3 . As we know, the hydrophilic groups were buried inside of protein structure. This was the reason why that without NaHSO_3 unfolding the protein structure, solo CaCO_3 modified SPA had poor shear adhesion performance. The co-effects of CaCO_3 and NaHSO_3 modification on SPA had the improved adhesion performance in terms of dry and wet adhesion strength

Overall, this thesis showed the methods to enhance the water resistance performance of SPA. In a certain range, higher curing temperature, and longer curing time had positive effects on shear adhesion performance on 3 ply plywood test. In the future, the commercialization of these bio-based protein adhesives would be a must-happen process due to the energy crisis. With the increasing water resistance, protein adhesives would replace the current petroleum-based commercial adhesives step by step.

5.2 RECOMMENDATIONS ON FUTURE STUDIES

Based on the experience and results of this study, future research on the following topics should be considered:

- 1) To investigate the different reaction time during the modified SPA preparation. In this dissertation, the CaCO_3 and soy protein reaction time was set at 2 hr. This choice was according to former literatures. Comparison between reaction time might be another factor affects water resistance performance of modified SPA.
- 2) To further understand the mechanism of CaCO_3 and NaHSO_3 combined reaction with soy protein. Although we proved that the co-effects of these two chemicals were better than any single modification. However, we are still far from understating the comprehensive reaction mechanism of CaCO_3 and NaHSO_3 modification.

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