Development of a Chitosan-Based Adhesive. Application to Wood Bonding

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ABSTRACT: The potential of chitosan (CS) as wood adhesive was investigated in this study. The measurement of the bond strength of different CS-based formulations was carried out using double-lap shear tests. The best formulation tested was an adhesive composed of 6% of CS, 1% of glycerol, and 5 mmol/L of trisodium citrate dehydrate. Best bond strength from this formulation was found to be equal to 6.0 MPa in dried conditions and 1.6 MPa in wet conditions (specimens immersed for 3 h at 30°C in water). The failure in the double-lap joint tests mainly occurred in wood. Finally, the penetration of the rhodamine-labeled CS at 4% in the pinewood matrix was also studied using microtome and microscopy techniques to show interactions between CS-based adhesive and wood. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

INTRODUCTION

Synthetic wood adhesives available for the consumers are mostly derived from depleting petrochemical resources. They present many advantages, such as high bond strength and high water resistance. However, most of them are also made of volatile organic compounds or other toxic compounds viz. epichlorohydrin, methylene diphenyl diisocyanate, formaldehyde, toluene diisocyanate etc and cause environmental problems. The growing health concern and the increasing concern over environmental pollution also encourage wood industry to develop environmentally friendly wood adhesives. Thus, the development of natural and renewable source adhesives exhibiting good bonding properties is actually an industrial challenge and an important edge of the current research on adhesives.[1,2] However, the use of natural polymer-based adhesives is currently limited because of low water resistance. Studies on new natural and renewable source adhesives are still necessary for the development of new wood adhesives.

Chitosan (CS) is a copolymer of β-(1,4)-linked 2-acetamido-2-deoxy-D-glucopyranoses and 2-amino-2-deoxy-D-glucopyranoses. This polysaccharide, obtained by alkaline deacetylation

(DD) of chitin,[3] is the sole cationic polysaccharide due to its positive charges (NH_3^+) at acidic pH (pH < 5). The pKa of primary amine group of D-glucosamine residues of CS is 6.5 according to Claesson and Ninham.[4] It has received wide attention for several commercial applications as it is one of the highest abundant biopolymer on earth after cellulose.[5,6] The main developments of bioadhesives were carried out in the biomedical field[7–9] and more recently in wood industry.[10–14] Parameters influencing the attractive characteristics of CS in the field of adhesives are its molecular weight (M_w) as well as its degree of DD. Indeed, several studies clearly showed that adhesive properties were altered when DD and M_w decreased.[15–17] This phenomenon was partially explained by the entanglement of macromolecules and the hydrogen bonding between hydroxyl groups and amino groups.[18] Commercial CS extracted from crab and shrimp shells exhibits a wide molecular weight range (g/mol) and its degree of DD is generally greater than 75%. The adhesive properties of CS in a swollen state were shown to persist better during repeated contacts of CS and adherend. Furthermore, its cationic charges increase retention at the site of application.[19] Wood is characterized as a suitable adherend for polysaccharides and so for CS. The reason is that, cellulose has a
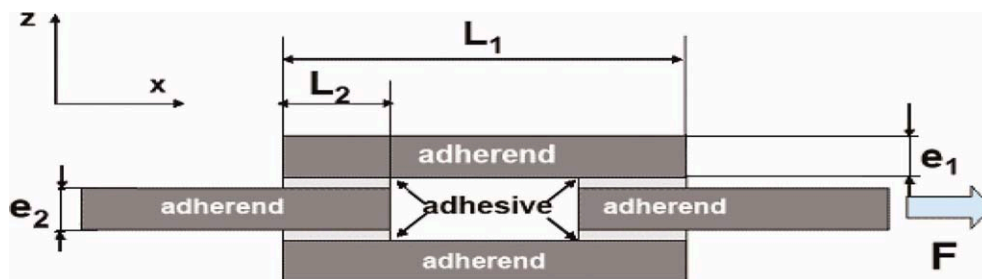
Figure 1. Schematic view of the double-lap bonded specimen (L_1: length of adherend; L_2: lap area of adherend; e_1: outer adherend thickness; e_2: inner adherend thickness). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

strong tendency to form intramolecular and intermolecular hydrogen bonds through the hydroxyl groups on linear polysaccharide chains.[20,21] Hence, the H-bonding facilitates electrostatic interactions and affinity between wood and CS.

Once wood is exposed to moisture, it can be attacked by several wood-decaying microorganisms. So, it needs proper protection to find long-lasting life. The conventional safeguard methods are principally based on the use of hazardous chemicals (viz. vinyl acetate-ethylene, styrene-acrylic etc.), which are undesirable for consumers. In this context, CS-based adhesives seem to be an interesting alternative in a next future for wood preservation.[22] Singh et al.[23] reported that the additional advantage of CS by impregnation into *Pinus radiata* tissues ensured its protection against wood-deteriorating microorganisms. It is important to mention that the bioactive agents did not only fill cell lumens and cavities but also impregnated cell walls. Umemura et al.[2] reported that the dry bond strength of CS with various molecular weights (M_w 35,000–350,000) ranged between 1 and 1.6 MPa. This result was obtained with three-ply plywood of *Shorea spp*. These strengths were enhanced up to 1.89 MPa by adding 70% (W) glucose to a low-molecular CS featuring a low molecular weight. The wet bond strength of the modified low-molecular weight CS exhibited a maximum value of 1.1 MPa.

Glycerol has been used as a renewable resource for wood adhesives.[24] For this purpose, they have developed an original approach allowing conversion of glycerol to polyglycerols. Obtained hyperbranched polyglycerols could substitute petroleum-based polyols for wood adhesive.[24] They have then cross-linked these polyglycerol-based adhesive with hexamethoxymethyl melamines to enhance water resistance.[25]

The bonding properties, mechanical strength, and penetration of a high-molecular weight CS on wood have not been well described till now to the best knowledge of the authors. Hence, this study mainly focuses on the measurement of the bond strength, water resistance, molecular weight determination, labeling, and penetration in pinewood matrix of such a type of high-molecular weight CS-based adhesive associated with glycerol and a polyanionic compound.

EXPERIMENTAL

Adhesive Preparation

Commercial CS, 75% deacetylated and derived from chitin of shrimp shells (Sigma Aldrich), was used for adhesive formula-

tion. Three different formulations were tested: (1) 4% (w/v) CS, (2) 6% (w/v) CS, and (3) CS 6% (w/v) + glycerol 1% (v/v) + trisodium citrate dehydrate (Sigma Aldrich) 5 mmol/L (CS 6% + gly 1% + cit 5 mmol/L). First, two solutions were made with 1 and 2% (v/v) acetic acid (Ac), respectively. For the last formulation, CS (6%) was dissolved in 5 mmol/L citrate (cit) containing 2% Ac (v/v) solution. Moreover, 1% (v/v) glycerol (Sigma Aldrich) was added to it (glycerol acts as a plasticizer). Each of these three individual adhesive formulations was thoroughly mixed for 10 min before further solubilizations. These solutions were then degassed by centrifugation during 20 min, at 10,000 rpm and room temperature. The pH of CS formulations 4, 6, and 6% + 1% gly + 5 m Mcit were, respectively, 5.2, 4.8, and 4.79 and viscosity 35.31, 91.15, and 91.05 Pa s at 10 s^{-1} shear rate. They were then applied on the adherends. Each CS formulation was individually used in the experiments. The rationale was to investigate their ability to obtain a better resulting polymer in terms of adhesion performance, either alone or with accommodated plasticizers, or chemical modifiers.

Adherend Preparation

The adherends used to prepare the specimens were from softwood species of European pine (*Pinus pinaster*) also known as maritime pine purchased in the local market (Clermont-Ferrand, France). This wood species has already often been used for adhesive characterization purposes.[26–29] It is also widespread in many countries for wood applications. For all these reasons, it was decided to use this species for this first attempt of CS bonding characterization. Each specimen was manufactured with four pieces of wood before testing (Figure 1). The dimensions were 124 × 18 × 4 mm^3. The fibres of the selected wood were aligned with the longest dimension of each piece. The estimated average moisture content of the wood was about 8%. This percentage was determined after drying wood pieces of 2 cm^3 in a conditioning chamber (110°C) until stabilization of the mass. The percentage moisture content was calculated by the following equation:

$$MC = (M_0 - M_f)/M_f \times 100 \qquad (1)$$

where MC: moisture content; M_0: mass of specimen before drying; and M_f: mass of specimen after 48 h of drying.

Double-Lap Specimen Preparation

Double-lap specimens were used here to characterize the bonding properties of CS. This type of geometry leads to a low

peeling effects. For each bonded specimen, the overall length L_1 was equal to 124 mm and L_2 was equal to 20 mm. The thicknesses of the adherends e_1 and e_2 were equal to 4 mm. These dimensions were chosen to be close to those published in literature: $150 \times 20 \times 10$ mm^3 [32]; $50 \times 20 \times 10$ mm^3 [30]; $115 \times 25 \times 3$ mm^3 [26,27]; and $170 \times 25 \times 1$ mm^3.[31] The specimens were prepared as depicted in Figure 1 and then dried for 24 h at 40°C with no pressure applied to accelerate the drying process of the adhesive. It has been checked through some preliminary tests that this conditioning did not change the bonding properties of the joints compared with the properties of joints obtained with a drying process performed at 20°C (data not shown). The dimensions of the lap area were $20 \times 18 = 360$ mm^2 according to literature: 10×10 mm^2 [32]; 20×20 mm^2 [30]; 25×25 mm^2 [26,27]; and 25×25 mm^2.[31] Four other lap areas ($25 \times 18 = 450$ mm^2, $30 \times 18 = 540$ mm^2, $35 \times 18 = 630$ mm^2, and $40 \times 18 = 720$ mm^2) were also tested to observe the different failure modes depending on the lap lengths (see section below). Three adhesive formulations (CS 4%, CS 6% and CS 6% + gly 1% + cit 5 mmol/L) were used in turn for gluing the four wood adherends before preparing the specimens.

Assessment of the Bond Strength Test

The tests were carried out using a universal Zwick Roell testing machine. The specimens were fixed in the grips of the machine. The crosshead speed was 0.05 mm/s up to failure. The Testxpert V11.02 software was used to drive displacement of moving grip and to record the force versus time. The value of the force applied at failure was measured and used to deduce the bond strength of the adhesive (σ_a). Each experiment was repeated 10 times and the corresponding standard deviation was calculated in each case. Bond strength was deduced by calculating shear stress σ_a that was estimated using the following equation:

$$\sigma_a = F/(2A')$$ (2)

where F is the applied force (N) and A' the lap area (m^2).

Measurement of Water Resistance of CS

The response of the (1) CS 4%, (2) CS 6%, and (3) CS 6% + gly 1% + cit 5 mmol/L adhesive formulations was evaluated by water immersion test prior to determine their bond strength. Moreover, in formulation 3, higher glycerol concentrations were added (3, 5, and 10%) to obtain formulations (4) CS 6% + gly 3% + cit 5 mmol/L, (5) CS 6% + gly 5% + cit 5 mmol/L, and (6) CS 6% + gly 10% + cit 5 mmol/L. The objective here was to measure the effect of higher glycerol concentration in terms of protection of the adhesive against water. Likewise, in another approach, the lap surface of the specimens of formulation 3 was coated with soybean oil prior to observe the retaining strength by protecting its interaction with water (this is the 7th formulation tested in this study). First, double-lap specimens of the above formulations were prepared and dried for 24 h at 40°C. The water immersion test of these specimens was conducted for 3 h at 30°C and subsequently cooled in water according to Umemura et al.[11] This procedure is similar to that used in other studies dealing with wood adhesive characterization.[31,33–36] The bond strength was then determined as described above. Five specimens were tested for each formulation. The average value

and standard deviation of bond strengths (dry and wet) and the average wood failure were calculated for each formulation. This wet bond strength value is close to the value reported previously on 80–90% deacetylated low-molecular weight CS (35 kDa) by Umemura et al.[11]

CS's Molecular Weight Determination

The average molecular weight of CS was determined by high-pressure size exclusion chromatography with online multiangle laser light scattering (SECMALLS) filled with a K5 cell (50 µL) and two detectors: a He–Ne laser ($\lambda = 690$ nm) and a differential refractive index. The columns [OHPAK SB-G guard column, OHPAK SB806, 804, and 803 HQ columns (Shodex)] were eluted with 65 µM ammonium acetate (pH 4.5) at 0.7 mL/min. The solvent was filtered through 0.1 µm filter, degassed, and finally filtered through a 0.45 µm filter upstream column. The specimen was injected through a 100 µL full loop. The collected data were analysed using the Astra 4.50 software package.

Synthesis of Rhodamine-Labeled CS

One gram of dried CS powder was dissolved in 100 mL of acetic acid at 100 mmol/L. Subsequently, 100 mL of dehydrated methanol was slowly added in this solution of CS with continuous stirring. Rhodamine isothiocynate (RITC, Sigma Aldrich) dissolved in methanol at 1.0 mg/mL was then slowly added to the CS solution. The final concentration of RITC in the reaction medium was controlled to give a label to D-glucosamine residue ratio equal to 1 : 50. The reaction between the isothiocyanate group of RITC and the primary amine group of the D-glucosamine residue was allowed to proceed for 1 h in dark at room temperature. RITC-labeled CS was then precipitated in a solution of sodium hydroxide at 100 mmol/L. The precipitate was washed extensively with deionized distilled water until complete absence of free RITC signal was observed in the washing medium. The labeled CS was then freeze dried. The yield of labeled CS was 85% as described.[37]

Analysis of rhodamine-Labeled CS penetration in wood

CS-impregnated pinewood was stuck and microtomed perpendicularly into 20 µm wide cuts with a microtome machine (Leica Microsystems, Bannockburn, Ill., model RM 2165) using R35 blades. The obtained cuts were stained with 0.05% aqueous toluidine blue for 5 min, rinsed in successive ethanol baths until the excessive stains washed off. Cuts were then mounted by Eukitt mounting medium (contains 5% Acrylic Resin and 55% Xylenes). Fluorescent microscope (Leitz: loborlux K, United States) was used to take some pictures of the bonding area to investigate the adhesive penetration beneath the adherend surface.

RESULTS AND DISCUSSION

Assessment of Shear Test

In this study, four different types of failure were observed: adhesive failure, cohesive failure of the bonded joint itself, failure of one arms of the specimen, and failure parallel to the bonded joint in the bulk of one of the wooden piece (Figure 2). This last type of failure is referred to as a structural failure in the following. When the shear strength of the adhesive was greater than the shear strength of the wooden adherends, the
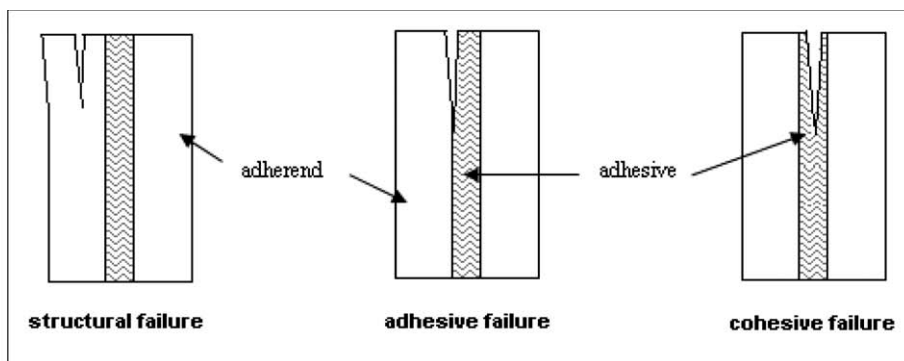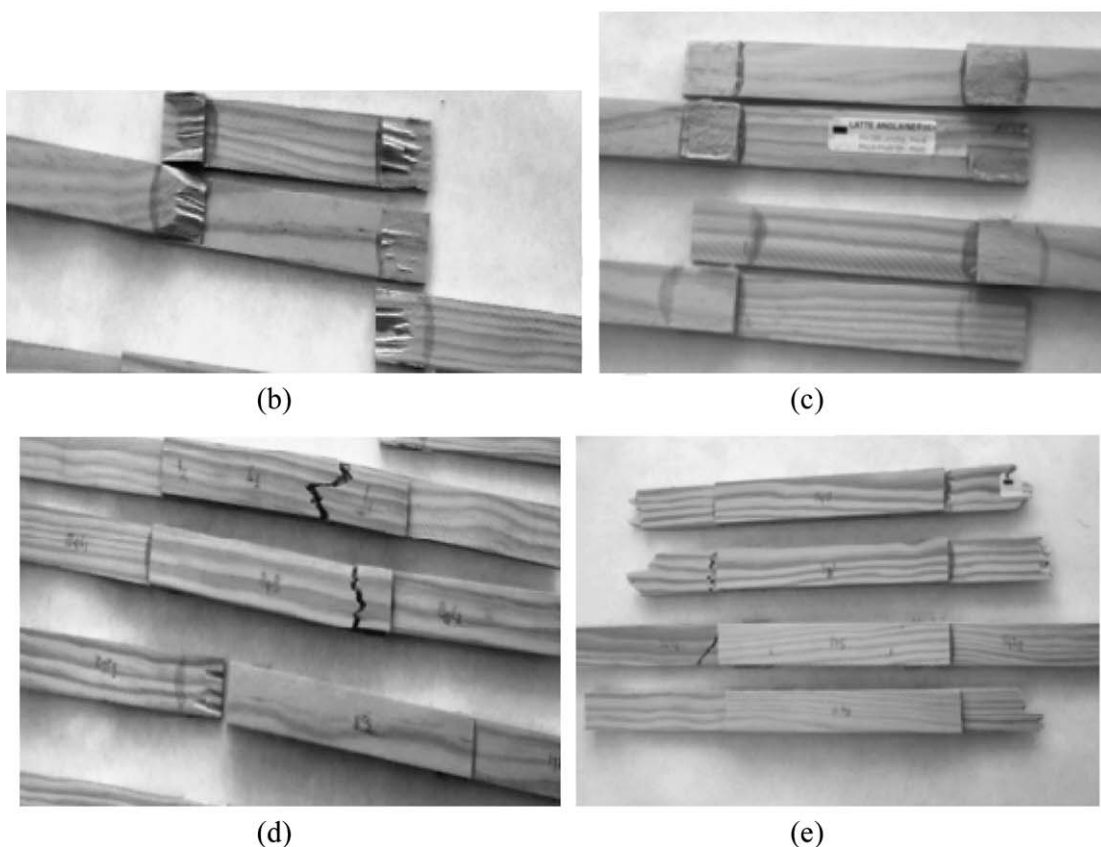
Figure 2. Failure types; (a) schematic figure of the mode of failure (b) structural failure in the lap region (c) cohesive failure (d) structural failure in the lap region and adherend arm existing between two lap ends (e) structural wood failure only in the specimen arms and not in the lap area.

measurement reflected the integrity of the wooden matrix rather than the strength of the adhesive itself. In this study, structural failure was generally observed with the different types of tested adhesive formulations described above. This kind of failure is generally the most appreciated mode because the bonded joint is not the weakest link. However, the shear strength deduced in this case is in fact lower than that the adhesive itself.

The goal of the addition of citrate and glycerol in formulation 3 was to enhance the adhesive performance of the resultant polymer. Literature supports that citric acid reacts with CS amine groups to form an acyclic amide structure.[38] The hydrophilic and hydrophobic interaction are addressed between $-CH_2OH$ and $>CHOH$ functional groups of glycerol and $-CH_2OH$, $-NH_2$, $-NH_3^+$, $-OOCCH_3$, $-NHCOCH_3$ functional groups of CS. Glycerol establishes three H-bonds with a glucosamine unit of CS and takes part in curing process.[39] It was found to be the best plasticizer that affects the mobility of acetamide groups, thus playing a major role in the formation of H-bonds between adjacent chains. It acts then as an internal plasticizer.[39] Plasticizers or dispersants are additives that increase both the plasticity and the fluidity of the material to which they are added.

As explained above, particular attention was paid to the lap length, because it was observed that it influenced the failure
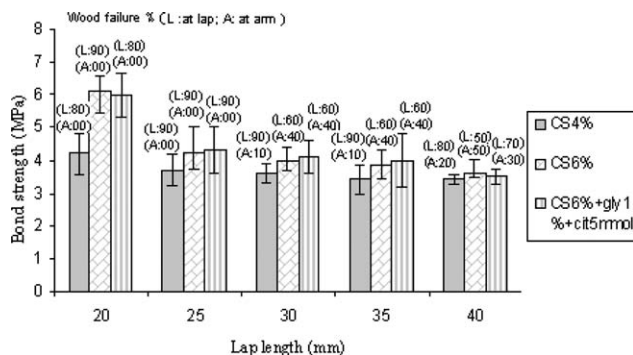
Figure 3. Effect of lap length on shear strength.

mode. For correct assessment of an adhesive strength, the aim was focused to achieve an optimum lap length where the failure should fall within the lap area with more regularity in cohesive mode [Figure 2(a)]. As explained in "Experimental Section" above, softwood was chosen here as adherend, because it has been routinely used for adhesive characterization.[26–29] It was, therefore, necessary to achieve a structural failure mode because this type of adherend exhibited a lower strength than the adhesive. The following comments can be drawn from the obtained results:

- with a lap length equal to 20 and 25 mm: formulation 1 exhibited a bond strength of 4.2 and 3.7 MPa, formulation 2, 6.1 and 4.2 MPa and formulation 3, 6.0 and 4.3 MPa, respectively. Eighty to ninety percent of the specimens exhibited a structural failure and 10–20% a cohesive/adhesive failure in the lap area [Figure 2(b, c)]. These two lap lengths were better for bond strength assessments because 100% of the failure occurred in the lap region. Moreover, the results show that the 20 mm lap length is more appropriate than the 25 mm lap length (Figure 3) because higher values of the bond strength were obtained;

- with a lap lengths equal to 30 and 35 mm: the bond strength of formulation 1 was equal to 3.6 and 3.4 MPa, respectively, whereas formulation 2 leads to bond strength of 4.0 and 3.8 MPa, respectively. Ninety percent of the specimens with formulation 1 exhibited a structural wood failure within the lap area and 10% in the arms. Sixty percent of the specimens prepared with formulation 2 had a structural wood failure within the lap area and 40% a structural wood failure in the arms. The highest bond strength of formulation 3 was 4.1 and 4.0 MPa for these two lap lengths, respectively, with 60% of the specimens exhibiting a structural wood failure in the arms and 40% in the lap area (including 10% in the adhesive itself). With formulations 2 and 3, some of the lap areas failed near the middle of the wooden arms, as depicted in Figure 2(d);

- with a lap length equal to 40 mm, the specimens prepared with formulation 1 exhibited a bond strength equal to 3.4 MPa. Eighty percent of them had a wood failure within the lap area and 20% in the arms. Likewise, the mean bond strength of formulation 2 was equal to 3.6 MPa with 50% of the specimens exhibiting a wood failure in the arms and 50% in the lap region. Formulation 3 had a bond strength

of 3.5 MPa with 70% of the specimens exhibiting a wood failure in the arm [Figure 2(e)] and 30% in the lap region.

The value of the bond strength of CS (M_w up to 1200,000 g/mol) collected in the literature has a maximum value of 1.9 MPa. Moreover, similar results are obtained with a CS solution supplemented with glucose and konjac glucomannan. This bond strength value was obtained from specimens cut in three-sheet plywood preparations made up of *Shorea spp.* and pressed at hot temperature.[2,11] This study shows comparatively higher bond strengths with the tested CS.

For comparison purposes with synthetic commercial adhesives, it must be noted that the bond strength values of polyvinyl acetate (PVA) and polyurethane adhesives measured by compression loading mode are reported to be equal to 11.8 and 6.01 MPa, respectively.[40] Titebond[TM] is a well-known brand of PVA adhesive. Its mean bond strength obtained on Douglas fir softwood spp was equal to 13.4 MPa.[41] By adopting the same testing standard, Lee et al.[42] reported that the dry bond strength of urea–melamine–formaldehyde (MF), MF, phenol–formaldehyde, and resorcinol–formaldehyde resin in softwood species of Korean pine (*Pinus koraiensis*) was equal to 1.80, 1.22, 0.75, and 5.69 MPa, respectively. With reference to the values from these synthetic adhesives in softwood species, the bond strengths obtained in this work appear to be significant. These values associated to the failure mode generally observed in wood mean that the apparent bond strengths deduced from the shear tests are lower than the actual bond strength of the adhesive itself.

Measurement of Water Resistance of CS

The bond strength of the three formulations was evaluated after water immersion to observe the influence of moisture. The response varies significantly with the type of formulation. Results show that formulation 3 exhibits the best wet bond strength (0.55 MPa) compared to other adhesive preparations. Moreover, it was declined by adding glycerol with increasing concentrations, although this did not show significant difference compared to the wet bond strength reported above. Only a cohesive mode failure is observed in these tests. According to Cui et al.,[38] higher glycerol concentrations in "chitosan + citric acid + glycerol" films led to a water absorption of 44% in weight against 96% for pure CS. After soaking, the modified film swelled by only 11%. This is lower than the 42% for pure CS. Hence, additional glycerol concentrations of 3, 5, and 10% were tested in formulations 4, 5, and 6, respectively, as described in "Experimental Section." These formulations featured lower mechanical properties compared with formulations 1, 2, and 3. This phenomenon can certainly be attributed to an excess of glycerol (Figure 4). It clearly shows that we have an optimum for the ratio glycerol-CS. Specimens prepared with formulation 3 protected with oil led to the best wet bond strength (1.6 MPa). They retrieved 27% of their dry bond strength. Moreover, 50% of these specimens exhibited a structural wood failure. This result suggests that coating could be an effective means to explore water-sensitive adhesive systems. This bond strength in wet conditions is close to the value (1.74 MPa) previously
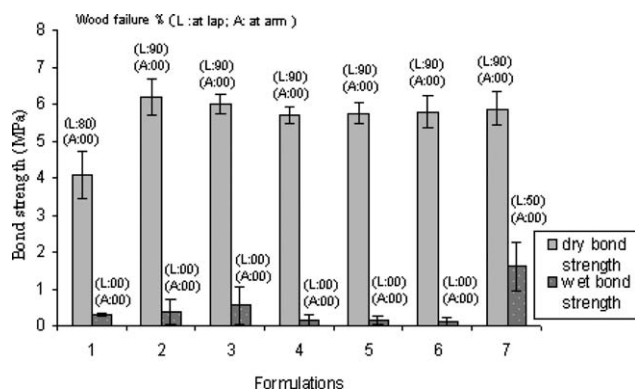
Figure 4. Dry and wet bond strengths of CS-based adhesive formulations: 1: CS 4%; 2: CS 6%; 3: CS 6%+gly 1%+cit 5 mmol/L; 4: CS 6%+gly 3%+cit 5 mmol/L; 5: CS 6%+gly 5%+cit 5 mmol/L; 6: CS 6%+gly 10%+cit 5 mmol/L; 7 CS 6%+gly 1%+cit 5 mmol/L+ oil coated.

reported by Umemura et al.,[11] on 80–90% deacetylated low-molecular weight CS (35 kDa).

### Determination of the Molecular Weight of CS

The average molecular weight was determined using a SEC-MALLS analysis. The CS fraction consisted of a high-molecular weight polysaccharide of weight-average molecular weight (M_w) and number-average molecular weight (M_n) calculated at 3.038×10^5 g/mol and of 2.251×10^5 g/mol, respectively. The polysaccharide fraction was heterogeneous. The measured polydispersity index (I_p) was equal to 1.35.

Rhodamine-Labeled CS Penetration in Wood

The solution of unlabeled parent CS appears in light golden colored polymer when it adheres to a surface. It is, however, not visible in the wood matrix. Hence, for the sake of visibility, RITC-labeled CS was synthesized using the reactivity of isothiocynate functional groups of RITC moiety with the primary amine group of D-glucosamine residues of CS (Figure 5). The
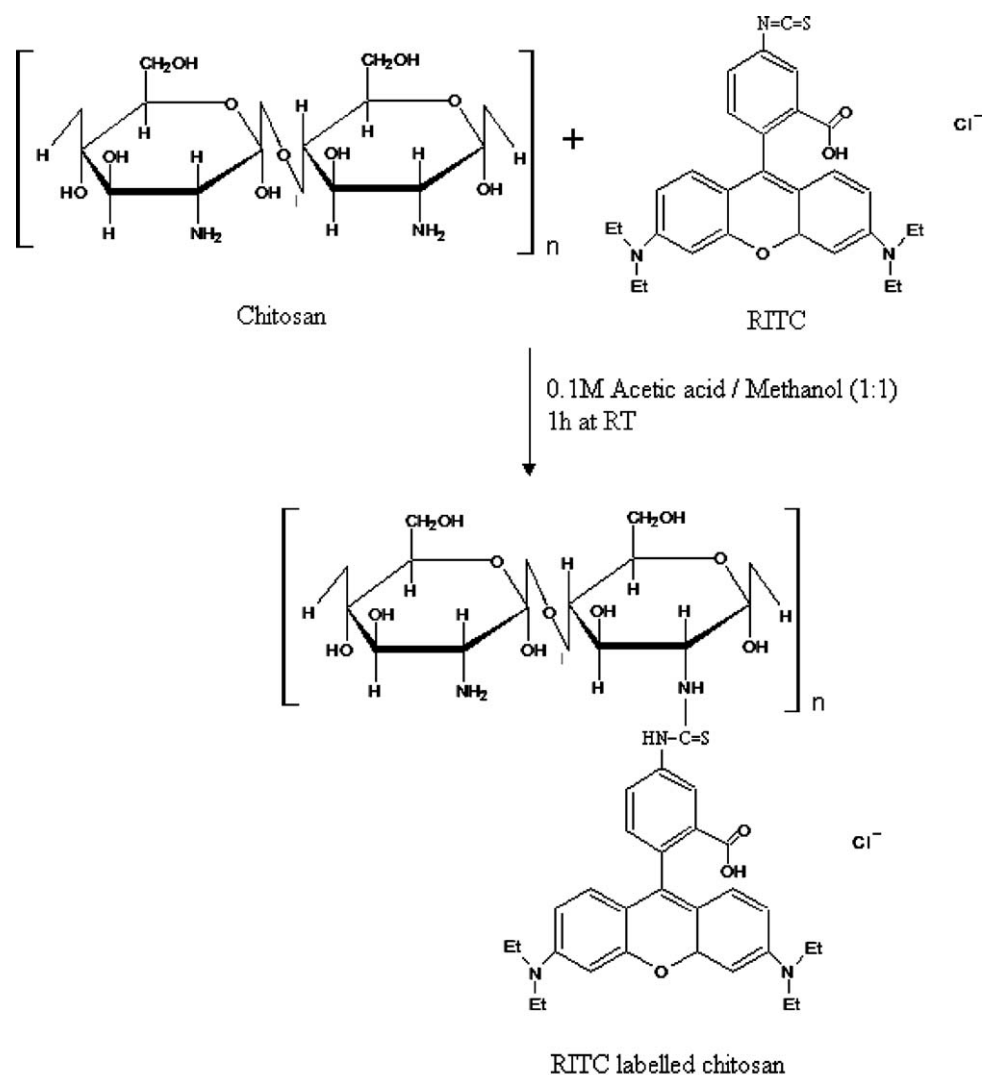
Figure 5. Schematic view of the chemical synthesis of RITC-labeled CS. (Et: CH_3CH_2).
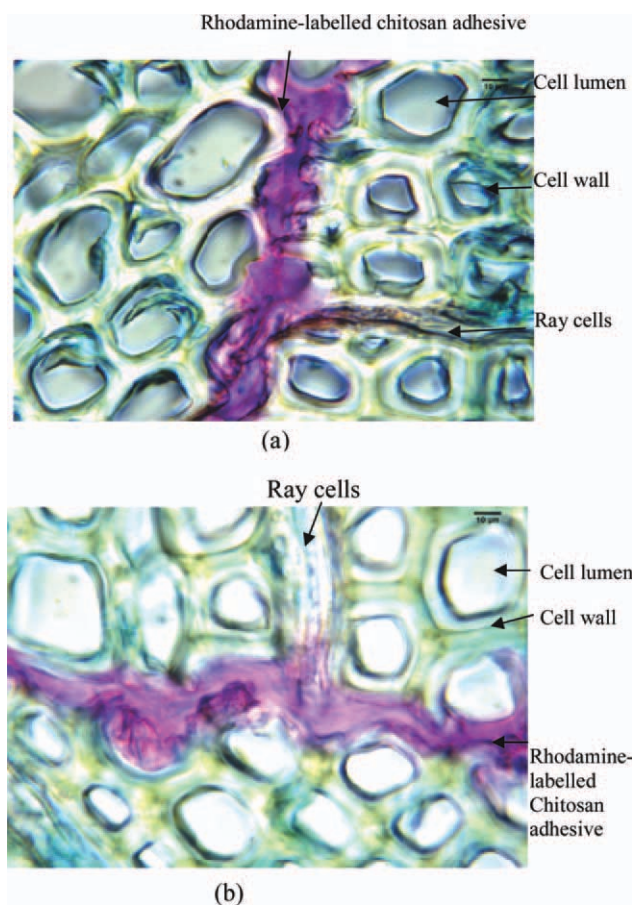
Figure 6. Penetration of labeled-CS into wood cells; (a) horizontal pink-colored rhodamine-labeled CS layer adhering the outer layer of lower and upper adherends and also filling to sectioned lumens and ray cells (X: 10 μm); (b) vertical view of pink-colored rhodamine-labeled CS layer adhering vertically both the left and right adherends (X: 10 μm). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

obtained bright pink-colored polymer was explored to investigate its penetration level into wood matrix.

Although the CS used in this study was a high-molecular weight polymer, it was not expected to go through the cell wall. Effectively, high-molecular CS polymer is known to be quite flexible in an aqueous solution. Moreover, Larnøy et al.[14] showed that media with high-molecular weight CSs can elasticate into cell wall, enter into intercellular capillary spaces, and expend, thus being trapped. A microimaging technique was used to visualize the fine wood structure as well as the penetration level of a solution of rhodamine-labeled CS at 4% (w/v). Figure 6(a, b) clearly shows that the labeled CS appeared along a single line at the outside of the cell wall of each adherend. This result provides deeper insight and understanding of linear distribution of applied CS in wood tissues. It was possible to detect the bright pink-colored rhodamine-labeled CS along a single line between the light blue-stained sections of both the upper and lower wood adherends. The staining of the section with toluidine blue stain is in common practice in laboratories to visualize lignified

plant tissues.[43] This facilitates, for instance, the discrimination of pink-colored CS and bluish cell walls. The image provides evidence that rhodamine-labeled CS did not penetrate the cell wall and, therefore, did not reach the cell lumens. It only interacted with the outer surface of the plant cell. It could penetrate the ray cells of the wood matrix up to some extent. Rhodamine-labeled CS filled the sectioned lumens of the cells found in the outer surface, but it neither penetrated subsequent cell walls nor entered in the cell lumens found inside the surface. It is, therefore, obvious that the CS used in this study had a high molecular size compared to the pores present in the cell wall. This finding is contrary to that reported by Singh et al.,[23] where toluidine blue or OsO_4 labeled-CS (50–190 kDa) impregnation was described in the cell lumens of *Pinus radiata*. Larnøy et al.[14] also investigated the chlorine-labeled CS penetration into Scot pine sapwood using energy-dispersive X-ray spectroscopy technique. They reported increasing interactions (covalent bonding) of CS molecules into cell wall with their increasing molecular weights from 3–111 kDa. In these studies, CS was not detected in the cell lumens. It was only present up to cell walls. However, in this study, CS had a rather high molecular size (3.038 $\times 10^5$ g/mol) and could therefore only scarcely penetrate the wood matrix. Mehrtens[44] also described the penetration of the CS in Scot pine up to the outermost tracheids. This phenomenon was probably due to the interaction of very high-molecular weight CS, for which it is difficult to penetrate into the wood matrix. Mehrtens[44] used Lugol's solution (Iodine, potassium iodide and water) to stain CS prior to visualize the lateral penetration of the reactant, but the method was not quantitative.

CONCLUSIONS

The bonding properties of high-molecular weight CS-based formulations were investigated in this study. Lap lengths ranging from 20 to 40 mm were tested to obtain the best lap length value for correct bond strength determination purposes. A 20 mm lap length has been found to be the best value compared to the others which were tested. Formulations CS 4%, CS 6%, and CS 6% + gly 5% + cit 5 mmol/L showed a dry bond strength equal to 4.2, 6.1, and 6.0 MPa, respectively, with 100% structural wood failure using a 20 mm lap length. Moreover, a larger variation was observed in the type of failures. Water resistance property of all the formulations was found to be lower, although formulation 3 gave maximum bond strength up to 0.55 MPa. The average molecular weight (M_w) of CS was equal to 3.038 $\times 10^5$ g/mol. The rhodamine labelling with CS was remarkably useful to understand the interaction of adhesive with the adherend surface. The in-depth penetration of rhodamine-labeled CS 4% CS into the cell lumen was not observed. It only adhered on the outer cell walls of each adherend. The bond strength of the high molecular weight dry CS-based formulations exhibited an acceptable level of strength compared to the synthetic adhesives available in the market. Hence, they are showing a promising potential for wood industries in the near future.

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