MECHANISMS FOR CELLULOSE-REACTIVE POLYVINYLAMINE-GRAFT-TEMPO ADHESIVE

MECHANISMS FOR CELLULOSE-REACTIVE POLYVINYLAMINE-GRAFT-TEMPO ADHESIVE

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Abstract

Weak wet strength of paper is one of the major challenges limiting the increase use of paper products. It is difficult to form strong adhesive joints between hydrophilic wet cellulose surfaces. Previous research disclosed an approach using polyvinylamine (PVAm) with grafted TEMPO for oxidation of cellulose to improve wet cellulose adhesion. The object of this research is to further develop new and more eco-friendly approaches to induce adhesion between wet cellulose surfaces.

PVAm-graft-TEMPOs (PVAm-TEMPO) with various TEMPO grafting extents were prepared and characterized by electron paramagnetic resonance (EPR) and conductometric titration. The stability studies of fully hydrolysed PVAm in sodium hypochlorite (NaClO) environment were conducted. PVAm can be oxidized and degraded by NaClO in alkaline solution.

Furthermore, PVAm-TEMPO was applied into the TEMPO/laccase/O_2 oxidation of cellulose. Increased wet adhesion between cellulose surfaces were achieved with this enzyme catalyzed approach and the mechanism of this approach was investigated. PVAm-TEMPO and laccase works together as mediators catalyzing the oxidation of primary alcohol groups on cellulose into aldehyde groups that react to form covalent bonds with primary amines on PVAm. However, cationic PVAm-TEMPO and anionic laccase can form complexes during the oxidation process.

Grafted TEMPO in enzyme catalyzed approach offers three significant advantages over small molecule TEMPO (free TEMPO). First, as PVAm has high molecular weight, the oxidation of porous fibers is restricted to the exterior surfaces only, which avoids the excessive oxidation of interior surfaces and prevents from weakening the mechanical property of fibers. Second, TEMPO is concentrated on cellulose surfaces by tethered it to PVAm, compared with water-soluble free TEMPO. Thus the total dose of TEMPO required to oxidize fibers by PVAm-TEMPO is much less than that required by free TEMPO. Third, the enzyme catalyzed approach not only avoids the harmful halide reagents, but also improves the chemical stability of PVAm in oxidation solution because of the high substrate selectivity of the enzyme.

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1 Introduction

Modern paper products such as liquid packaging paper, tissue and towels are required to retain some level of mechanical strength when subjected to high humidity or when soaked in water.[1] Without special treatment, paper loses 90% of its original strength when it is wet and this is mostly due to the high hydrophilicity of cellulose. The fiber-fiber joint including hydrogen bonding,[2] van der Waals force and inter-diffusion of surface polymers swells in water.[3] Thus, inducing strong adhesion between wet cellulose surfaces is a very important challenge. Rising to the challenge, chemical wet-strength additives have been developed.

Wet strength of paper can be increased by either modifying fiber surfaces with small molecules or with macromolecules. Water-soluble small molecules that are smaller than 1000 Da can access most of the interior surfaces of a fiber. However, polymers with high molecular weight are excluded from pores of fibers. In industry, wet-strength additives are applied to improve the wet strength of paper. Most of them are linear cationic polymers which can adsorb onto wood fibers to enhance the fiber-fiber joints.[3] Polyvinylamine (PVAm), a linear polymer containing primary amine groups was reported to increase the wet strength of paper 50 years ago.[4] DiFlavio et al.'s investigation indicated the mechanism of PVAm wet-strengthening. Amine groups on PVAm can react with aldehyde/hemiacetal groups on cellulose to form covalent bonds.

In Ren's previous study, a new paper additive, PVAm-*graft*-TEMPO (PVAm-TEMPO) was developed and applied into TEMPO/sodium hypochlorite/sodium bromide oxidation of cellulose. In his research, PVAm-TEMPO was relatively stable under oxidation conditions. The wet-strength between cellulose films was improved from almost 0 N/m to around 40 N/m. In addition, treatment conditions such as sodium bromide concentration and pressing temperature were optimized. Furthermore, the catalytic activity of the immobilized TEMPO was demonstrated by the conversion of methylglyoxal to pyruvic acid.[5]

1.1 Literature review

1.1.1 Structure of fibers

Paper is a thin material produced by pressing together moist fibers, typically cellulose pulp derived from wood, rags or grasses, and drying them into flexible sheets.[6] As illustrated in Figure 1-1, a wood fiber is composed of several structured layers. The middle lamella, very high in lignin content, is a hollow tube, surrounded by a primary wall P and three secondary wall layers S1, S2 and S3. All three secondary wall layers consist of cellulosic micro fibrils embedded in an amorphous matrix of hemicelluloses and lignin.

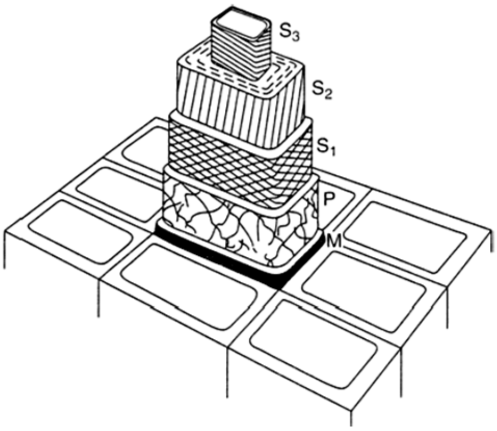


Figure 1-1 Diagram of cell wall organization of a wood fiber.[7] P, primary wall; S1, S2, S3, layers of secondary wall.

It is the cellulose that determines the character of the fiber and permits its use in papermaking.[8] Cellulose is a polysaccharide consisting of many repeating β-D-glucopyranose sugar units. The structure of cellulose molecule is illustrated in Figure 1-2. Each glucose unit contains three hydroxyl groups, composed of one primary alcohol group at the C6 position and two secondary alcohol groups, which are arranged in positions 2 and 3. The hydroxyl groups in the cellulose molecule undergo typical alcohol reactions, i.e., oxidation, ester formation and alcoholate formation etc.[9]

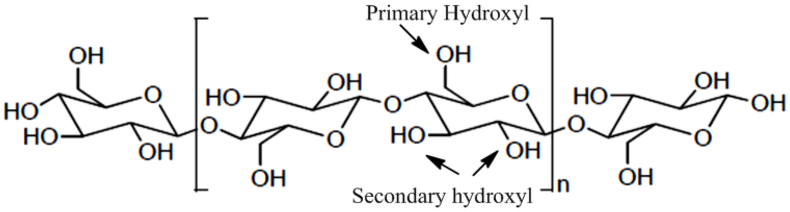


Figure 1-2 the molecular structure of cellulose

Hemicelluloses are composed of glucose, mannose, galactose, xylose and arabinose. The polymeric structures of these sugars along with uronic acids are various depending on the plant species. Dramatic changes of the structure, the quantity and the location of hemicellulose take place during the production of pulp with chemical treatment. Thus, the hemicelluloses are easy to be degraded and removed.[8]

The chemistry of lignin is extremely complex. Linked in together in three dimensional, the structure of lignin is composed primarily of phenyl propane units. Lignin is insoluble in most solvents due to its high cross-linking degree. The aromatic components of lignin protect wood from adsorbing water. However, lignin is a highly reactive substance containing hydroxyl, methoxyl groups etc.[9] Therefore, its structure can be degraded by chemical treatment called delignification.[10]

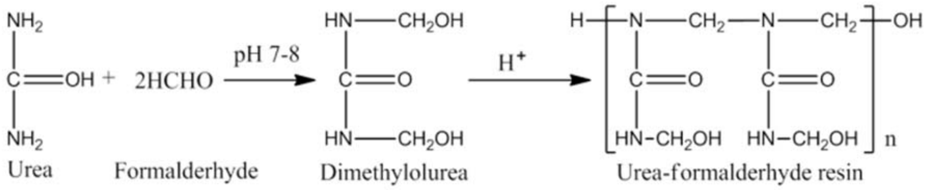
1.1.2 Wet-strength additives

Paper is a network of cellulose fibers bonded together by inter-molecular forces, such as van der Waals, hydrogen bonding. These inter-molecular interactions are commonly very sensitive to water. Only 3-10% of the original dry strength remains when the water wets the fiber, because fibers swell when it is wet and the inter-molecular bonds are broken. Some of papers' original dry strength can be maintained when wet by strengthening the existing bonds, protecting the existing bonds, forming new bonds that are insensitive to water or producing a network of material that physically entangles with the fibers.[11] Therefore, generally speaking, paper wet-strength additives are water-soluble chemically reactive polymers which can either react with themselves to form homo-cross-linking or react with cellulose interfaces to form co-cross-linking. There are some commercial chemicals which are used as wet-strength additives as follows.

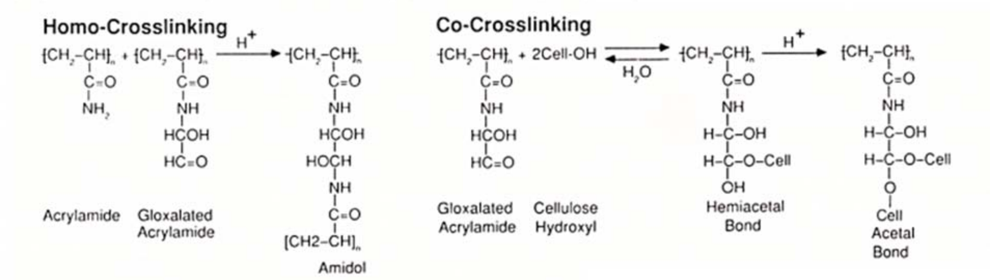
Urea-formaldehyde resins As the earliest process to produce paper wet strength, paper fibers are heated to high temperatures or immersed in dilute acidic solution with urea-formaldehyde to achieve networks by homo-cross-linking of the wet-strength additive itself, as shown in Scheme 1-1 (a).[12] However, there are several drawbacks such as brittleness of paper after low pH treatment and offensive odours.

Glyoxalated polyacrylamide resins Glyoxalated polyacrylamide resin is a widely accepted wet-strength additive in paper and board industry.[13] Synthesized by cross-linking a glyoxal with a low molecular weight polyacrylamide, glyoxalated polyacrylamide is a copolymer of acrylamide. It can form both homo-cross-linking with itself and co-cross-linking with cellulose hydroxyls as illustrated in Scheme 1-1 (b). There is clear evidence that glyoxalated polyacrylamide reinforces the wet strength of paper mostly through covalent bonds formed with paper fibers. However, this additive contains cationic polymers, which means that a retention aid has to be used in its application. Furthermore, the additive is not stable in storage and continues to crosslink with itself with time.

(a) Reactions of Urea-formaldehyde resins



(b) Crosslinking reactions of glyoxalated polyacrylamide resins



Scheme 1-1 (a) the preparation and structure of urea-formaldehyde resins; (b) crosslinking reactions of glyoxalated polyacrylamide

Epoxidised polyamide resins Poly (aminoamide)-epichlorohydrin (PAE) resins is the most widely used commercial paper wet-strength additive. According to the conclusions of previous studies, PAE can generate cross-linking networks with itself and reacts with carboxylic acid groups on cellulose fibers[14]. It is generally considered that the formation of covalent bonds between PAE and cellulose fibers are the mechanism how PAE improves the wet strength. However, the use of PAE is now limited in paper making industry because of the organic chloride environmental hazard..[15]

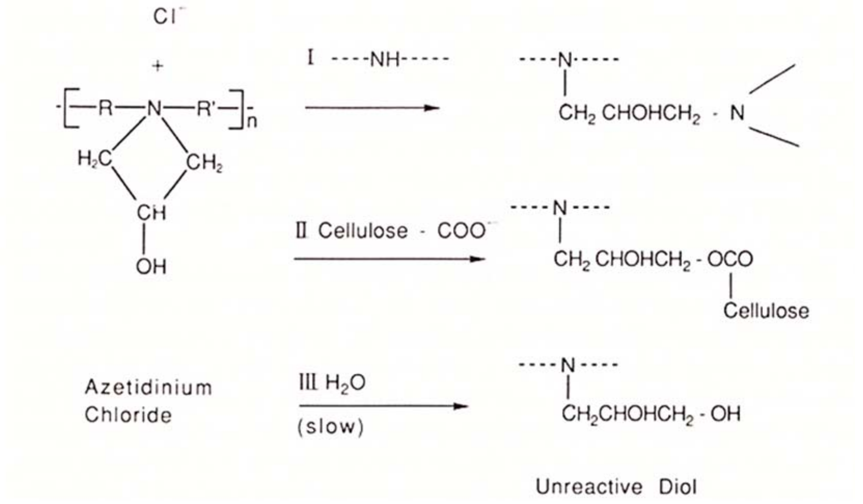


Figure 1-3 The reaction of PAE, I: reaction through the amine groups of other resin molecules (homo-crosslinking); II: reaction with carboxyl group in cellulose (co-crosslinking); III: slow reaction with water.[11]

1.1.3 Measuring of paper strength

There are various methods to measure the strength of paper, such as tensile tests, tearing tests, peeling tests, etc. The strength of paper is classified as in-plane strength and out-of-plane strength depending on how the measurements are conducted.

In-plane tensile strength shows the capacity of papers to hold together under the stress. The tensile properties of paper are significant for packaging papers, boards, printing papers and other paper product uses in web form. Tensile strength as it presents itself, measures the maximum load that a paper specimen can hold, as illustrated in Figure 1-4 (a). However, the results have no direct significance, since paper and board are seldom loaded to tensile failure. It is usually triggered by flaws in papers at stress levels as a fraction of tensile strength. Thus, paper tensile strength is dominated by both the fiber rupture and the inter-fiber bond failure. As papermaking operators affect fiber and bond properties and even sheet structure, it is difficult to control separately of the two failure modes.[6] The external load can transmit across the fiber to fiber, through shear forces at the inter-fiber bonds, as shown in Figure 1-4 (b). Bond failure is carried out when the shear force on the inter-fiber bond exceeds its strength with the increase of external load.

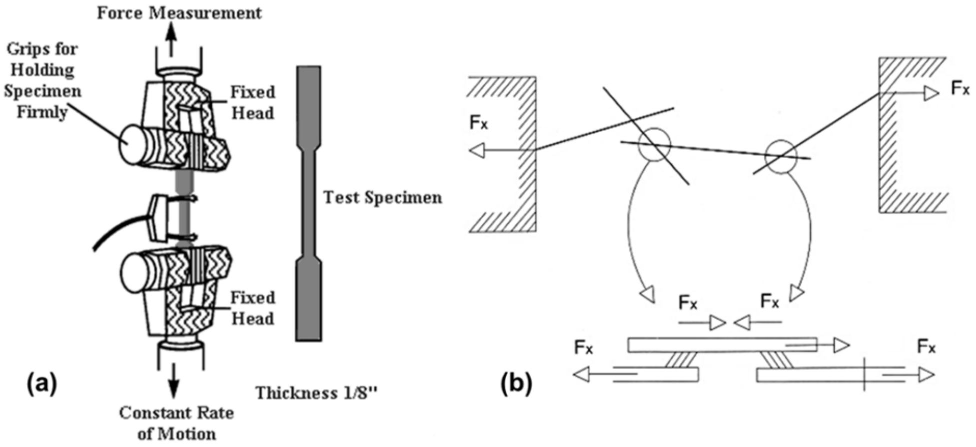


Figure 1-4 (a) measurement of paper tensile strength; (b) stress transfer across paper along a chain of fibers. The external load transmits through shear forces at inter-fiber bonds.[6]

Out-of-plane strength presents the resistance of paper in the thickness direction to load. A tensile force must be applied on the two surfaces of the specimen in the measurement of out-of-plane strength by attaching suitable metal blocks with an adhesive or double-sided tape. Figure 1-5 shows 4 different methods of strength measurement. Figure 1-5 (a) shows a typical tensile test measuring the stress of out-of-strength failure. However, as the displacements of two solid bodies are too small, this method can neither provide enough information for the load-elongation plot nor determine the delamination energy. Figure 1-5 (b) presents the mechanism of a Scott bond tester. The delamination of sample takes place very rapidly when a pendulum swings down and hits a fixed-angle aluminum block adhesive to the paper sample. The delamination energy can be achieved through this method. The peel-wheel test and the cantilever beam test are illustrated in Figure 1-5 (c) and (d), respectively. Both of these two tests measure the energy consumption during a controlled and stable delamination process. During the peel-wheel test, the recorded delamination force is constant.

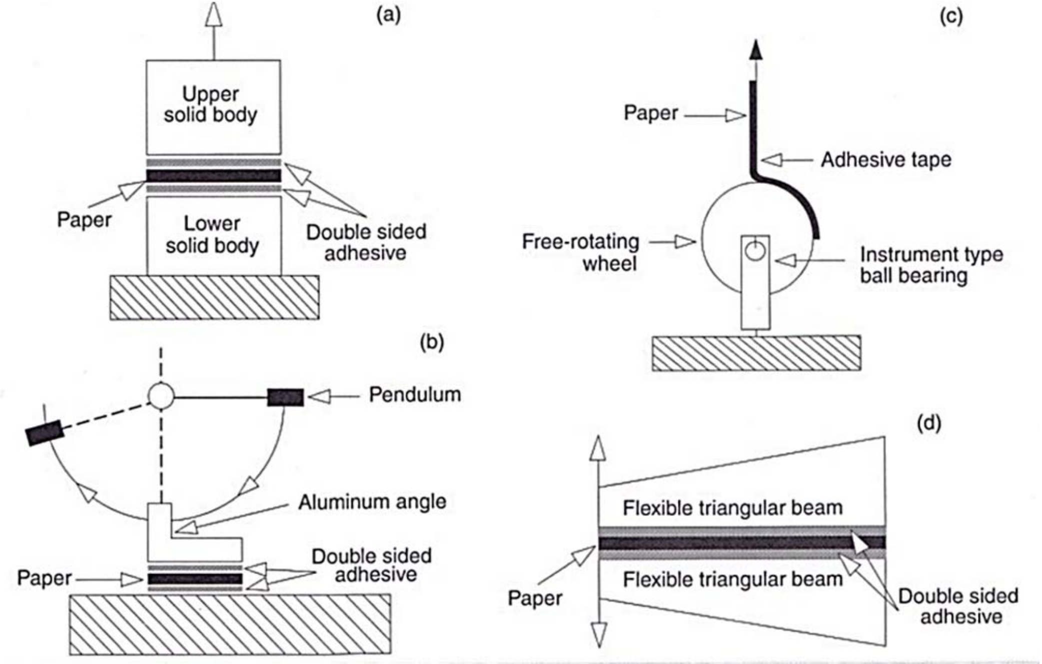


Figure 1-5 (a) measurement of out-of-plane strength (b) Scott bond delamination energy measurement. (c) peeling wheel. (d) cantilever beam technique.[6]

1.1.4 Paper wet strength measurement

As illustrated in previous sections, there are various paper wet strength measurements, measuring either the in-plane strength or the out-of-plane strength. For in-plane paper strength, paper tensile test in wet state is a typical method. However, either fiber failure or fiber-fiber bonds failure may contribute to the paper sample failure in the tensile test. It comes to be difficult to analyse or identify the failure location. When considering the effect of using polymer additives, it is even more difficult using this method. Identifying key factors which can change paper wet strength requires not only more information of polymer content and its location and fiber chemistry, but also homogeneous and stochastic paper samples. To better investigate the polymer adhesion to cellulose, a 90^{o} peeling test has been developed as shown in Figure 1-6. This peeling test has been accepted as a model measurement of bonding interactions between cellulose fibers.[16]

In this 90^{o} peeling test, two pieces of cellulose membranes are laminated with a thin adhesive film between them. The dimensions of membranes and the laminate preparation procedure are illustrated in Figure 1-7. The laminates are rewetted after being dried out under a desired condition. The 90^{o} delamination force of the wet laminate is recorded by an Instron system on the aluminum wheel at a desired constant peeling rate.

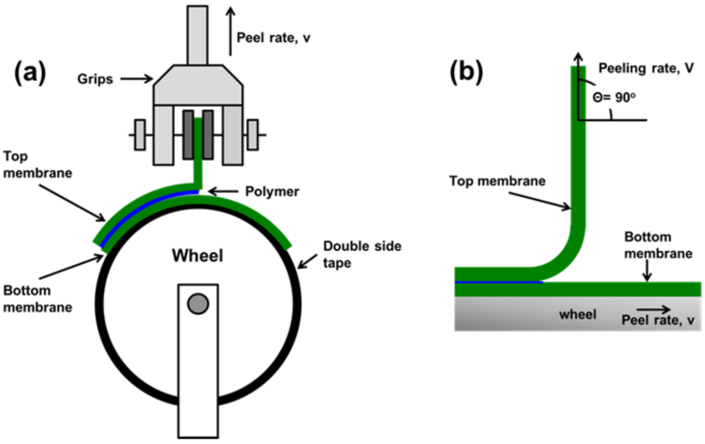


Figure 1-6 Scheme of cellulose peeling test

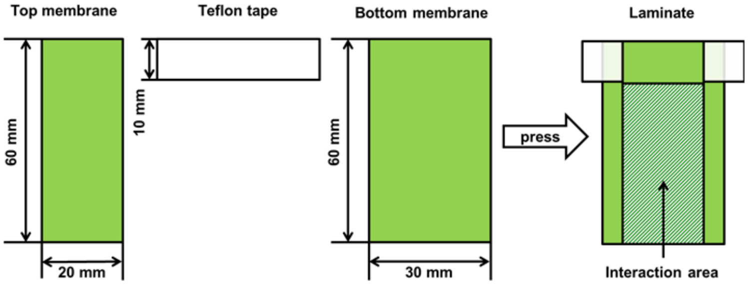


Figure 1-7 Dimensions of the cellulose materials for the laminate preparation

A typical peeling test data plot is shown in Figure 1-8. A relatively stable peeling force is achieved. The delamination force was an average value of steady-state peeling load divided by the width of the interaction area (20 mm)

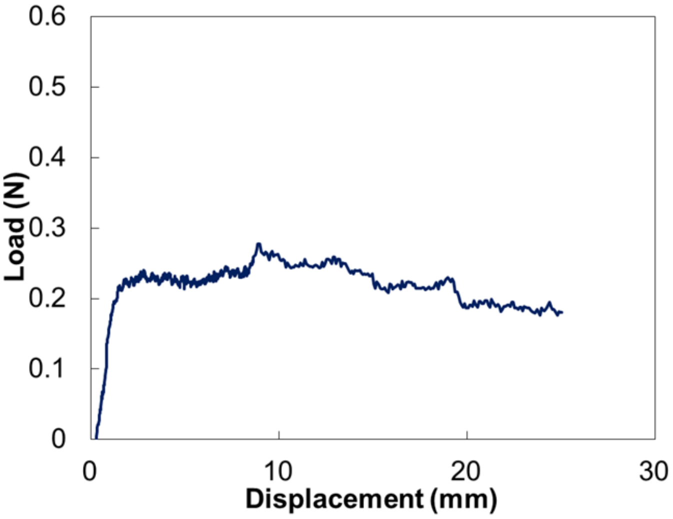
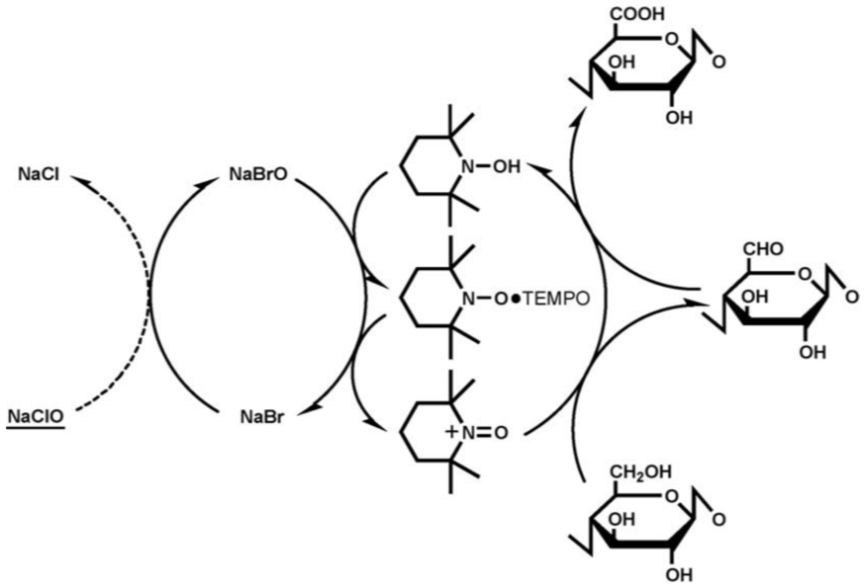


Figure 1-8 Force versus the displacement data of a typical peeling test

1.1.5 TEMPO oxidation of alcohol

2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and its analogues belong to a class of compounds generally referred to as nitroxide or nitroxyl radicals. There is an unpaired electron delocalized between the nitrogen and the oxygen atom.[17] Thus, TEMPO can be detected by Electron Paramagnetic Resonance (EPR). However, TEMPO is much more soluble in organic solutions rather than aqueous solutions.[18] The water solubility of TEMPO can be improved by the modification with hydrophilic groups, such as 4-hydroxyl-TEMPO and 4-amino-TEMPO.[19]

TEMPO catalyzed oxidation is one of the most promising procedures to selectively oxidize primary alcohol groups into aldehydes and/or carboxyl groups. Contrary to other oxidation, TEMPO-oxidation is a catalytic process, which has high selectivity, high reaction rate and high yield.[17] The mechanism of TEMPO mediated oxidation of alcohol groups on polysaccharides is complicated under different conditions. Generally speaking, TEMPO and its analogues are utilized as oxidation catalyst. In this process, the nitrosonium ion (oxoammonium, $TEMPO^+$) is continuously regenerated in situ by a primary oxidant, e.g. sodium hypochlorite. The amount of primary oxidant determines the yield of oxidation reaction.[20] Even though, the oxidation potential of the TEMPO radical is not negligible; the nitroxyl radical has to be oxidized to the more powerful oxoammonium to function as efficient oxidant of substrate. Oxoammonium ion is mostly considered as the direct oxidant for alcohol. The product of TEMPO-mediated oxidation of alcohol varies with reaction conditions, e.g. primary oxidant, reaction medium and pH. In aqueous solution, the reaction product contains either carbonyl or carboxyl groups. However, as the conversion of aldehyde groups to carboxyl groups mainly take place in aqueous solution, the aldehyde or keto groups are formed in homogeneous organic solution without subsequent oxidation to the carboxyl groups.[21] As to the primary oxidant, there are various oxidants that have been used in this process for the oxidation of TEMPO and the regeneration of TEMPO, e.g. $NaClO_2/NaClO$,[22] NaClO/NaBr and oxidative enzymes.



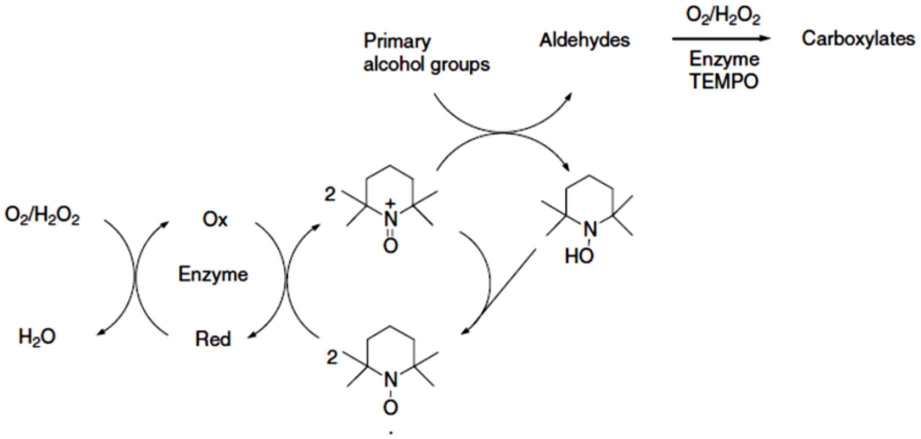
Scheme 1-2 TEMPO-mediated oxidation of cellulose to form C6-carboxylate groups via C6-aldehyde groups[23]

NaClO/NaBr/TEMPO oxidation of primary alcohol on cellulose (Anelli's oxidation)

The TEMPO oxidation of cellulose was first reported by Chang and Robyt.[24] Isogai's group has published a series of papers disclosing many details on TEMPO oxidation of cellulose and its use in paper making industry.[25] According to their research result, the C6 primary hydroxyl groups on cellulose are first oxidized to aldehyde group in this oxidation system as illustrated in Scheme 1-2.[23] As a mediator, TEMPO must be in molecular contact with the target alcohol groups. The selectivity for the primary alcohols depends on the steric demand of the secondary alcohol.[26] Furthermore, the ratio of aldehyde and carboxyl groups generated by TEMPO oxidation depends upon conditions such as pH, amount of sodium hypochlorite etc. However, side reactions including the depolymerisation of cellulose was observed in Saito's research, which broke down cellulose fibers into shorter fragments.[25d] It was also found that the dry strength of paper was improved by TEMPO oxidation, while with the addition of wet-strength resins, the wet strength of paper can be dramatically increased as well.[27]

O_2/laccase/TEMPO oxidation of primary alcohol on cellulose

Oxidative enzymes (e.g., oxidase and peroxidises) together with oxygen or hydrogen peroxide respectively were utilized as primary oxidants in TEMPO oxidation due to the environmental awareness of harmful halide reagent system. The oxidation mechanism of enzyme catalyzed TEMPO oxidation is similar to the hypochlorite process as shown in Scheme 1-3. Laccases are a group of oxidative enzymes belonging to the multinuclear copper-containing oxidases. The reactions catalyzed by laccases proceed by the monoelectronic oxidation of a suitable substrate molecule (phenols, aromatic amines) to the corresponding reactive radical.[28] They were first introduced to the pulp and paper industry for pulp delignification. Using TEMPO as a mediator, this technology was applied in the oxidation of polysaccharides.[29] Aracri et al. first reported the wet-strength development in bleached cellulose fibers by effect of a laccase-TEMPO treatment.[30] In their research, the reaction rate was slow (18 hours) and the oxidation yield was low.



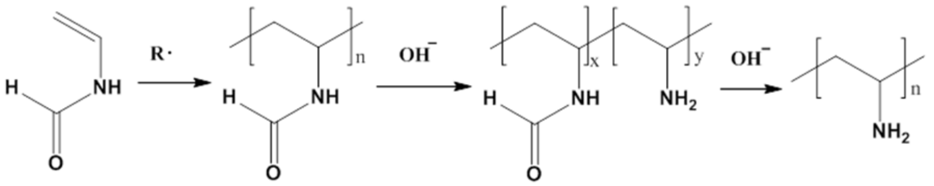
Scheme 1-3 TEMPO catalyzed oxidation of primary alcohol groups in carbohydrates to C6-aldehydes and carboxylates using oxidative enzymes[17]

1.1.6 Oxidation using immobilized TEMPO derivatives

TEMPO and its derivatives are usually water soluble and are used as homogenous catalysts. However, immobilized TMEPO moieties have also been recognized as heterogeneous catalysts for the oxidation of primary alcohols. In comparison with homogeneous catalysts, heterogeneous catalysts offer various advantages, such as the possibility of catalyst reusing, easier product isolation and purification and less toxicity of reagent.[31] It has been reported that TEMPO moieties are covalently bounded to polymers or silica surfaces.[17] Silica serves as the carrier of TEMPO moieties in most of the reported works. The application of silica-supported TEMPO as a recyclable catalyst in the Anelli oxidation of alcohols was reported by Fey et al.[31] However, the immobilized TEMPO catalysts studies are mainly limited in the oxidation of small molecule substrates. Polymeric substrates are generally precluded due to the diffusional limitations.[17] In addition, the reaction rate of small alcohols is lower compared with the homogeneous system.[17]

1.1.7 Polyvinylamine

Polyvinylamine (PVAm) is a commercialized primary amine containing polymer, which is produced from *N*-vinylformamide. In the producing process, *N*-vinylformamide is readily polymerized to polyvinylformamide (PVFA) via free radical polymerization. PVFA is then hydrolyzed to polyvinylamine (PVAm) in warm base solution. The reactions are shown in Scheme 1-4.[32] Fully hydrolyzed PVAm is known to carry a high density of amine groups, which is approximately 50% ionized at neutral pH. Thus, PVAm can rapidly adsorb onto anionic charge cellulose fibers driven by electrostatic interactions.



Scheme 1-4 the preparation of PVAm from the base hydrolysis of polyvinylformamide

PVAm was first reported to increase the wet strength of paper by Weisgerber in 1955.[4] An earlier study by DiFlavio systematically showed the mechanism by which PVAm increases paper wet strength. It was proposed that the covalent bonds formed between amine groups on PVAm and hemiacetal/aldehyde groups on oxidized cellulose contribute the most to the increasing of cellulose wet adhesion. However, electrostatic interaction between amine and carboxyl groups was believed to improve cellulose wet adhesion as well.[3, 33]

1.2 Thesis outline

This thesis continues Ren's previous study on PVAm-TEMPO and its application in improving the wet adhesion between cellulose. In Ren's research, he prepared PVAm-TEMPO which was confirmed by FTIR spectrum and applied PVAm-TEMPO in NaClO/NaBr/TEMPO oxidation system for cellulose wet strength improvement. In this thesis, the properties of PVAm-TEMPO were further characterized including the grafting extents and stability of PVAm in NaClO environment. The oxidation conditions of PVAm-TEMPO/NaClO/NaBr approach were optimized. Furthermore, a new eco-friendly enzyme catalyzed oxidation approach with PVAm-TEMPO was designed and give high wet adhesion between cellulose as well.

Chapter 1: Introduction. This chapter presents the background of this project and relevant literature. The outline of this thesis is also given here.

Chapter 2: Experiments This chapter provides all the detailed experimental information in this research including PVAm-TEMPO characterization and its application in PVAm-TEMPO/NaClO/NaBr and PVAm-TEMPO/laccase/O_2 system.

Chapter 3: Results. Experimental results achieved in this study are described in this chapter including the grafting extents of PVAm-TEMPO, PVAm stability, oxidation mechanism and factors affecting the wet adhesion of cellulose.

Chapter 4: Discussion. Results achieved in this study are discussed in terms of NaClO oxidation of PVAm, cellulose surface oxidation, TEMPO concentration on cellulose surface, oxidation mechanism of PVAm-TEMPO/laccase/O_2 system and the possible reason of one-step method failure.

Chapter 5: Conclusion. Conclusions of this research are made on the basis of experimental data and available literature in this chapter.

2 Experiments

2.1 Synthesis and characterization of PVAm-TEMPO

2.1.1 Degree of hydrolysis of PVAm

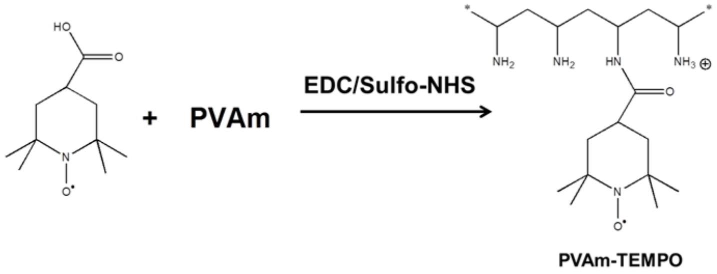
PVAm was obtained from BASF, Ludwigshafen (Lupamin® 5095, 45 kDa). Further purification by dialysis against water after adjusting the pH of polymer solution to 12 with sodium hydroxide and lyophilisation were conducted before PVAm was used in the experiment. However, Lupamin® 5095 is partially hydrolyzed PVFA, which was used in most of the experiments without further hydrolysis. In some experiments, PVAm was fully hydrolyzed in 5% NaOH solution at 75°C for 5 days. ^{1}H NMR characterization was conducted to determine the degree of hydrolysis according to Equation 2-1. [34]

$$DH = 1 - \frac{2 \times amide\ group\ peak\ area}{\mathrm{CH_2} - peak\ area}$$

Equation 2-1 [34]

2.1.2 Synthesis of PVAm-TEMPO

Polyvinylamine graft TEMPO derivative (PVAm-TEMPO) was synthesised by EDC/sulfo-NHS-mediated conjugation of 4-carboxy-TEMPO to PVAm (see Scheme 2-1).



Scheme 2-1 Scheme of the systhesis of PVAm-TEMPO

In a typical experiment, 200 mg PVAm (75% DH) and a desired amount of 4-carboxy-TEMPO (as shown in Table 2-1) were first dissolved in 200 mL of DI water. The conjugation reaction was initiated by quickly adding 1 g of *N*-(3-Dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC) powder and 217 mg *N*-Hydroxysulfosuccinimide sodium salt (sulfo-NHS) powder to the reaction solution. The reaction was maintained at pH 6 by addition of 1 N HCl solution for 80 minutes. After the conjugation reaction, the mixture solution was dialyzed against DI water for 14 days, then freeze-dried and stored in a desiccator.

Table 2-1 TEMPO grafting reaction recipes

Batch No.	Solution volume (mL)	PVAm (mg)	4-carboxy-TEMPO (mg)	EDC (mM)	Sulfo-NHS (mM)
4	200	200	120.8	32.21	5.00
6	200	200	241.8	32.21	5.00
9	100	100	17.8	16.10	1.87
10	100	100	8.9	8.05	0.94
11	100	100	3.6	64.42	0.27
14	200	200	149.0	32.21	5.00
16	200	200	150.0	32.21	5.00

2.1.3 Grafting extent of TEMPO moieties

The amine contents of PVAm-TEMPO freeze dried samples were determined by conductometric titration. Taking PVAm-TEMPO 10 as an example, 6.7 mg PVAm-TEMPO 10 was dissolved in 50 mL of 0.1 mM KCl solution. PVAm-TEMPO 10 solution was then titrated by 0.1 M NaOH and the amount of amine groups in this solution was determined by a conductivity plot.

To determine the TEMPO content of PVAm-TEMPO samples, Electron Paramagnetic Resonance (EPR) spectroscopy was performed on a Bruker ELEXSYS E580 spectrometer at 140K in an EPR tube. In a typical experiment, a desired concentration of PVAm-TEMPO solution (see Table 2-2) was prepared for the EPR spectroscopy in water-glycerol mixed solvent (50 vol %glycerol). Then, 120 μL of PVAm-TEMPO solution was frozen in an EPR tube by liquid nitrogen and then thawed in a vacuum for degasification. Signals were collected and double integrated by program. Using a 8.7 mM (1.4 g/L) TEMPO solution as a standard, TEMPO concentrations in PVAm-TEMPO solutions were determined by double integration of the EPR signals.

Table 2-2 Solution concentrations of PVAm-TEMPO samples for EPR measurements

Sample Name	Concentration (g/L)
PVAm-TEMPO 4	5.0
PVAm-TEMPO 6	5.1
PVAm-TEMPO 9	8.3
PVAm-TEMPO 10	10.8
PVAm-TEMPO 11	9.3
PVAm-TEMPO 14	6.0
PVAm-TEMPO 16	2.6
TEMPO (Reference)	1.4

2.1.4 Stability of PVAm in NaClO environment

FT-IR spectroscopy, intrinsic viscosity measurements and conductometric titrations were utilized to study the functional group reaction and degradation of PVAm in NaClO environment. PVAm (75% DH) from BASF was first fully hydrolyzed by hot

NaOH (75°C) as described previously to achieve a fully hydrolyzed PVAm, PVAm (100% DH). In a typical stability experiment, 1 mL of 1.7 mM NaClO was added to 25 mL of 10 g/L PVAm (100% DH) solution to start the reaction. The reaction was carried out at room temperature and maintained at pH 10.5 for 3 hours. The mixture solution is then placed in dialysis tubes and dialysed against DI water for 2 weeks. Dry solid sample for FT-IR and intrinsic viscosity analysis was achieved by freeze drying.

2.1.4.1 The stability of functional groups on PVAm with NaClO

As qualitative characterizations, FT-IR spectra of PVAm (100% DH) before and after NaClO treatment were recorded and compared. Samples for FT-IR spectroscope were conducted with KBr pellet method.

Besides of FT-IR spectroscopy, the amine content of PVAm (100% DH) before and after NaClO treatment were determined by conductometric titration using a PC titrate (ManTech Associates Inc) as quantitative characterizations. 25mg of PVAm (100% DH) before NaClO treatment or 2 mg of sample after NaClO treatment were dissolved in 50 mL of 0.1 mM KCl solution respectively and the pH of both solutions were adjusted to 3 with 0.1N HCl before the measurement. 0.1N NaOH solution was used as the titrant and the conductivity data were collected using a base-into-acid titration method with interval injection of 2 min until pH 11.5. The amount of amine groups in the solution was determined by the conductivity plot.

2.1.4.2 Degradation of PVAm by NaClO

In a typical viscosity measurement, PVAm (100% DH) was dissolved in 10ml solvent consisting of 0.1 M NaCl and 0.01 M NaOH to reach a concentration of 10 g/L. The flow time (t) was recorded by Ubbelohde viscometer (Size: 75, Cannon Instrument Company) in 25°C water bath. Then PVAm solution was diluted to a designed concentration by adding a known volume of solvent. With the same procedure, flow times of polymer solutions with known concentrations were measured. Using blank solution as a reference, the relative viscosity (η_r) and specific viscosity (η_{sp}) of polymer solutions were calculated according to Equation 2-2 and Equation 2-3.

$$\eta_{rel} = t_{sol'n} / t_{solvent} \qquad \textbf{Equation 2-2}$$

$$\eta_{sp} = \frac{\eta_{sol'n} - \eta_{solvent}}{\eta_{solvent}} = \frac{t_{sol'n} - t_{solvent}}{t_{solvent}} \qquad \textbf{Equation 2-3}$$

The intrinsic viscosity [η] of PVAm (100% DH) was determined by the plotting of relative and specific viscosity at different concentrations. The number average molecular weight of PVAm (100% DH) was estimated with Mark-Houwink equation.[35] The molecular weights of degraded PVAm with known NaClO concentrations were determined in the same method by replacing PVAm (100% DH) to degraded PVAm samples with corresponding NaClO treatment.

2.2 Wet adhesion of PVAm-TEMPO oxidized cellulose

2.2.1 PVAm-TEMPO application in NaClO/NaBr/TEMPO oxidation of cellulose

Regenerated cellulose membrane (Spectra/Por® 2, 12-14 kDa MWCO) used in this study was purchased from Spectrum Laboratories (Rancho Dominguez, CA) and was cut into strips (20 mm × 60 mm rectangles for top, 30 mm × 60 mm rectangles for bottom) along the lengthwise direction. Prior to use, membranes were boiled and washed with DI water to remove the impurities. The inside tubing surfaces were used to form the joints. A pair of membranes was composed of a top and a bottom membrane. The carboxyl content of membranes was 1-3 meq/100g cellulose, determined by Kurosu.[16]

In a typical experiment, four pairs of washed regenerated cellulose membrane strips were soaked in 200 mL aqueous solution containing 50 mg/L of NaBr and 20 mg/L of PVAm-TEMPO. The solution was stirred for 30 min to give a monolayer of PVAm-TEMPO adsorbed on cellulose surfaces. Oxidation of cellulose was initiated by adding 1.370 mmol NaClO. All reactions took place at room temperature and the pH of the oxidation solution was maintained at 10.5 with 0.1N sodium hydroxide (NaOH). After 30min of oxidation time, the reaction was quenched by adding 20 mL ethanol and the cellulose membranes were rinsed with water for three times.

Using *Direct Application* method, 15 μL of PVAm solution (1 g/L PVAm, 3 mmol/L NaCl, pH=7.5), which corresponded to 15 mg/m^2, was directly applied and spread between the top and bottom membranes. During this method, the bottom cellulose membrane was placed on a stainless steel plate and excess surface water was adsorbed by KimWipes (Kimberly-Clark Inc., Ontario) from the edge of the membrane. A piece of Teflon^{TM} tape (Width: approx. 10 mm) was then placed at the edge of the bottom membrane and a 15 μL of adhesive solution was applied by drawing an approximately 30 mm line in the middle of bottom membrane from the other end of the membrane with a micropipette (Eppendorf Research, 20) as shown in Figure 2-1. The top membrane was progressively placed over the bottom membrane starting at the end without the Teflon tape. Adhesive solution can uniformly spread between two membranes with negligible loss of polymer solution or air bubbles. The laminate was then placed between two TAPPI standard blotters and pressed (20000 pounds) for 30 minutes in a Carver press. The pressed membranes were dried at 23^{o}C and 50% humidity for 24 hours.

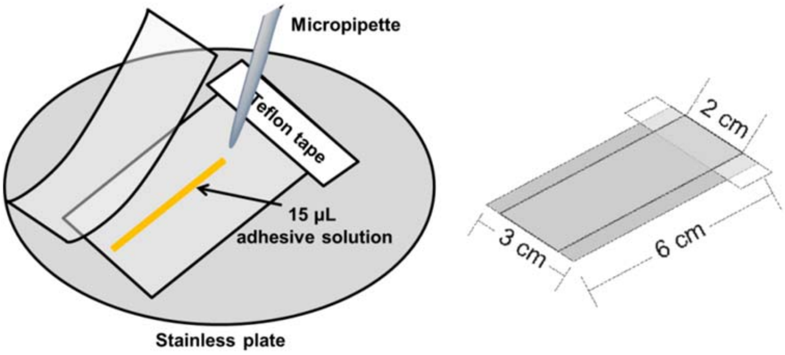


Figure 2-1 Illustration showing the direct application of PVAm (left) and the final dimensions of the laminates used in this study (right). (adapted from DiFlavio[3])

Before the wet adhesion test, the laminates were soaked in pH 7.5 Tris-HCl buffer (3 mmol/L) for 30 min. The excess water on cellulose laminates was removed by pressing with a 2.4 kg stainless steel roller between blotting paper. The laminates were attached onto a freely rotating aluminum wheel using a double-side tape (3M

Polyethylene Medical Double Coated Tape, Model No: 1522). The peeling loads between wet laminates were measured immediately with a 90 degree peeling apparatus using an extensional rate of 20 mm/min by Instron 4411 Universal Testing System. The delamination force was an average value of steady-state peeling load divided by the width of the interaction area (20 mm). At least triplicates were conducted for each sample. The whole typical procedures for wet delamination test were illustrated in Figure 2-2.

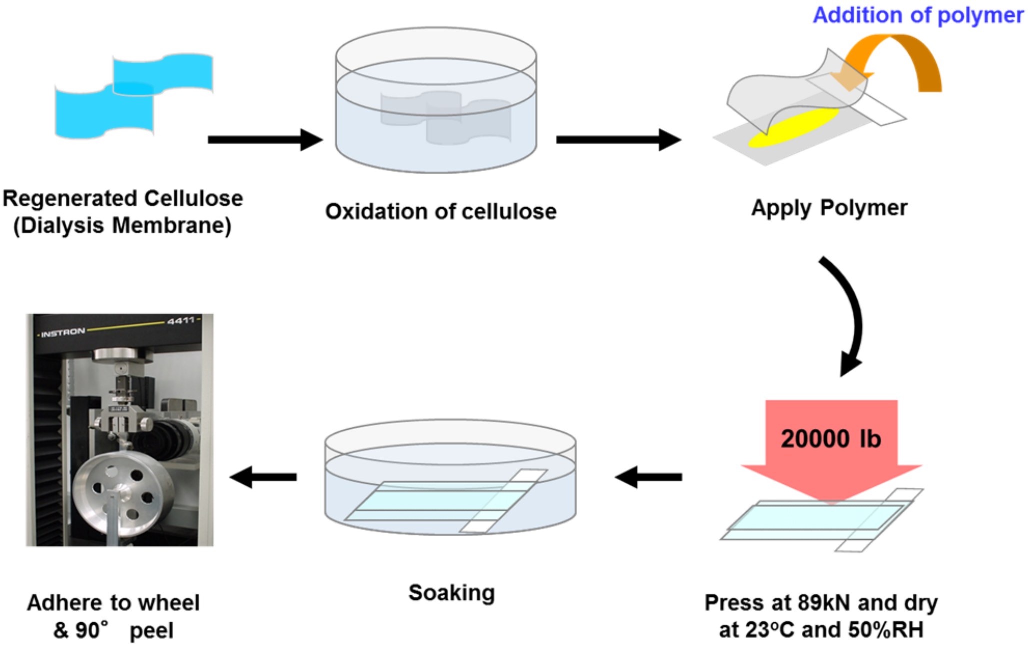


Figure 2-2 the procedures for a typical wet delamination test. (adapted from Chen[36])

2.2.1.1 Effect of oxidation time on wet adhesion

In this series of experiments, cellulose membranes were oxidized in 200 mL solution consisting of 50 mg/L of NaBr and 20 mg/L of PVAm-TEMPO 6 solution with various reaction times. A typical direct PVAm application method was used to prepare laminated samples. For sodium hypochlorite (NaClO) blank experiments, membranes were soaked in the PVAm-TEMPO solution for 30 minutes without NaClO addition and applied extra PVAm solution before being laminated. The delamination forces of wet laminates were recorded by an Instron system.

In addition, scanning electron microscopy (SEM, JEOL 7000F) was performed after the peeling test to observe the features of cellulose membrane surfaces treated by PVAm-TEMPO or free TEMPO with various oxidation times. Membranes for SEM observation were conditioned in at 23^{o}C and 50% humidity for more than 24h and glued to SEM metal stub by silver paint. 10 nm Pt was coated on the surface ahead of observation.

2.2.1.2 Effect of NaClO concentration on wet adhesion

In this series of experiments, oxidation of cellulose was started by adding desired amounts of NaClO. Other oxidation conditions were controlled as a typical experiment.

A typical direct PVAm application method was used to prepare laminated samples. The delamination force measurements were conducted as a typical experiment as well.

2.2.1.3 Effect of reaction pH on cellulose wet adhesion

In this series of experiments, the oxidation pH was maintained at desired values (7.5, 8.5, 9.5, 10.5 and 11.5) with addition of 0.1N sodium hydroxide (NaOH) during the oxidation. Other oxidation conditions were controlled in a typical experiment. A typical direct PVAm application method was used to prepare laminated samples after 30 minutes of oxidation. The delamination forces of re-wet laminates were measured to investigate the effect of reaction pH on cellulose wet adhesion.

2.2.1.4 Effect of TEMPO grafting extent on cellulose wet adhesion

PVAm-TEMPO with different TEMPO grafting extents varying from 0.7% to 16.4% (per mole) were used in this series of experiments with a same final concentration of 20 mg/L. After a typical oxidation procedure, 15 µL of PVAm solution (1 g/L) was directly applied between two cellulose stripes before pressing. The delamination forces of re-wet laminates were measured to investigate the effect of TEMPO grafting extent on cellulose wet adhesion.

2.2.1.5 Effect of oxidation yield and amine content of PVAm-TEMPO on cellulose wet adhesion by the adsorption method

In order to investigate the influence of oxidation yield of cellulose and TEMPO grafting extent of PVAm-TEMPO on cellulose wet adhesion, a set of experiment was designed and conducted as shown in Table 2-3. In the first series of experiment, cellulose membranes were oxidized in 200 mL solution consisting of 0.24 mM TEMPO, 6.8 mM NaCl and 0.49 mM (50 mg/L) NaBr for 30 min. The pH of solution was maintained at 10.5 with 0.1N NaOH. After the oxidation, membranes were rinsed 3 times and immersed in corresponding adsorption solution (pH 7.2, 10 mM NaCl) composed of 0.1 g/L of corresponding polymer for 30 min. The membranes were then rinsed 3 times to remove the excess polymer before lamination. Membranes were laminated and pressed without extra application of PVAm solution. The delamination tests were conducted as typical experiments described in previous sections. In the second series, the primary oxidant, NaClO, and catalyst, TEMPO, dosages were reduced to 0.042 mM of TEMPO and 3.4 mM of NaClO. Other experimental parts were conducted as same as the first series.

Table 2-3 Experimental design of oxidation recipe and adhesive adsorption method

Oxidation		Adhesive application method	
TEMPO (mM)	NaClO (mM)	Method	Solution (0.1g/L)
0.24	6.8	Adsorption	PVAm (75% DH)
0.24	6.8	Adsorption	PVAm-TEMPO 10
0.24	6.8	Adsorption	PVAm-TEMPO 6
0.042	3.4	Adsorption	PVAm (75% DH)
0.042	3.4	Adsorption	PVAm-TEMPO 10
0.042	3.4	Adsorption	PVAm-TEMPO 4
0.042	3.4	Adsorption	PVAm-TEMPO 6

2.2.2 PVAm-TEMPO application in Laccase/O_2/TEMPO oxidation of cellulose

2.2.2.1 Laccase activity assay

Laccase used in this study was from *Trametes versicolor* (Sigma-Aldrich). In most cases, laccase used in this thesis was dissolved in DI water and filtered with 0.45 µm syringe filters without other further purification. The laccase activity was determined by monitoring the oxidation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) at 420 nm (ε_{420}=36 000 M^{-1} cm^{-1})[37]. The ABTS test was conducted in 0.05 M sodium acetate pH 5 buffer consisting of 0.5 mM ABTS at 25°C. One unit of laccase activity is defined as the amount of enzyme required to oxidize 1 µmol of ABTS/min at 25°C.[37-38]

$$A = \varepsilon c l \qquad \textbf{Equation 2-4}$$

The UV/Vis absorbance of a sample is directly proportional to its concentration (Beer's law) and to sample thickness (Lambert's Law). The relationship between the absorbance and sample concentration was shown in Beer-Lambert equation (Equation 2-4). Where ε = extinction coefficient; c = concentration of substrate in mol/L; l = thickness of the sample in cm (1 cm in this study). The substrate conversion rate can be calculated according to Beer-Lambert equation with absorbance change data at 420 nm.

2.2.2.2 Wet adhesion between cellulose oxidized by PVAm-TEMPO/laccase system and its mechanism

In a typical PVAm-TEMPO/laccase oxidation experiment, 20 mg PVAm-TEMPO was dissolved in 130 mL of sodium acetate buffer (50mM, pH 5) and four pairs of cellulose membranes were immersed in the solution for 30 min. Then, 20 mL of 1 mg/mL laccase solution (190 Units) filtered by syringe filter (0.45 µm) was dropwise added to initiate the oxidation at room-temperature. The final oxidation mixture was 150 mL solution consisting of 133 mg/L PVAm-TEMPO and 133 mg/L laccase. The oxidation solution was stirred under oxygen purging (1 bubble/second) for 24 h. After the oxidation, membranes were immersed in sodium acetate buffer (50 mM, pH 5) for 5 min and rinsed to the buffer three times to remove excess unabsorbed polymer. 15 µL PVAm solution (1 g/L) was directly applied between two cellulose stripes before strips were pressed and dried out. Typical delamination tests as illustrated in previous section were conducted in this series of experiments.

In order to investigate the mechanism of PVAm-TEMPO/laccase oxidation of cellulose, a group of controlled experiments was designed as shown in Table 2-4. Batch 1 was a typical procedure for cellulose oxidation by PVAm-TEMPO/laccase system, while batch 2 was a blank experiment without any treatment. In batch 3 and 4, the reaction solution contained either no laccase or no PVAm-TEMPO, respectively. In batch 5 reaction, the laccase solution was denatured by boiling in 100°C water bath for 30 min before adding to the system. In batch 5 reaction, laccase and PVAm-TEMPO were separated by putting the laccase solution in a dialysis tube (MWCO 12-14 kDa).

Table 2-4 Controlled experiments of cellulose oxidation by PVAm-TEMPO/laccase

Batch No.	Laccase (mg/L)	Laccase activity (U/mL)	PVAm-TEMPO (mg/L)	Coated PVAm (mg/m^2)
1	133	1.27	133	15
2	0	0	0	15
3	0	0	133	15
4	133	1.27	0	15
5†	133	0	133	15
6‡	133	1.27	133	15

† laccase was denatured at 100°C

‡ laccase and PVAm-TEMPO were separated by dialysis tube

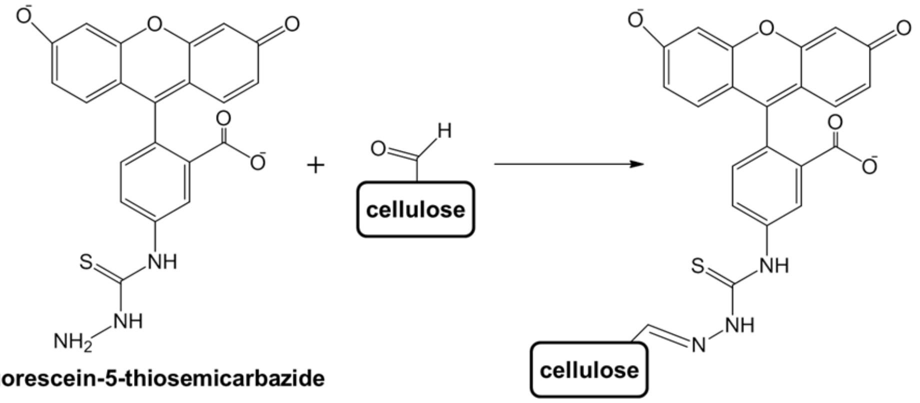
2.2.2.3 The comparison of cellulose wet adhesion between cellulose membranes oxidized by PVAm-TEMPO and free TEMPO

In this section, a typical PVAm-TEMPO oxidation experiment was conducted as illustrated previously. In free TEMPO oxidation experiment, PVAm-TEMPO was replaced by TEMPO and the final TEMPO concentration was 21 mg/L. While in free TEMPO/PVAm oxidation experiment, PVAm-TEMPO was replaced by TEMPO and PVAm. The final TEMPO and PVAm concentration was 21 mg/L and 112 mg/L respectively. The oxidation recipe is shown in Table 2-5. Reactions were carried out at room temperature for 24 h with oxygen purging. 15 μL PVAm solution (1 g/L) was directly applied between two cellulose strips before strips were pressed and dried.

Table 2-5 the oxidation recipe of PVAm-TEMPO and free TEMPO comparison

Experiments	PVAm-TEMPO 6 (15.77% w/w) (mg/L)	TEMPO (mg/L)	PVAm (mg/L)	Laccase (U/mL)
Blank	0	0	0	1.27
TEMPO	0	20	0	1.27
TEMPO/PVAm	0	20	113	1.27
PVAm-TEMPO	133	0	0	1.27

Cellulose membrane samples with different oxidation treatment for Confocal Laser Scanning Microscope (CLSM, Zeiss LSM 510) observation were prepared by immersing sample membranes in fluorescein probe solution consisting of 0.5 g/L of fluorescein-5-thiosemicarbazide overnight at pH 8. The conjugate reaction between fluorescein probe and aldehyde group was illustrated in Scheme 2-2.[39] The excess fluorescein probe was removed by immersing in and rinsing membranes against pH 8 Na phosphate buffer 10 times. The cross-section of cellulose membranes was observed under a laser beam at the wavelength of 488 nm. The images of PVAm-TEMPO treated, free TEMPO treated and un-oxidized cellulose membranes were taken under the same parameter settings of CLSM.



Scheme 2-2 Reaction of fluorescein probe labelling aldehyde groups.[39]

2.2.2.4 Effect of reaction time on cellulose wet adhesion

In this section, PVAm-TEMPO/laccase oxidation experiment was conducted with various reaction times from 1 min to 48 hours. In a typical experiment, four pairs of cellulose membranes were immersed in 130 mL sodium acetate buffer (50mM, pH 5) consisting of 10 mg PVAm-TEMPO 14 for 30 min. 20 mL of 1 mg/mL Laccase solution (9.5 U/mL) was added to start the oxidation at room-temperature. The oxidation solution was stirred under oxygen purging (1 bubble/second) for a desired time. 15 μL PVAm solution (1 g/L) was directly applied between two cellulose strips before strips were pressed and dried out. A typical delamination test was conducted to measure the wet adhesion between cellulose membranes. A controlled test absence of laccase was performed in parallel. In addition, the turbidity variation of laccase and PVAm-TEMPO 14 mixture was characterized by UV/Vis spectroscopy at wavelength of 500 nm with time.

2.2.2.5 Effect of grafting extent on wet adhesion

The influence of TEMPO grafting extent on cellulose wet adhesion was investigated in this series of experiments. Four pairs of cellulose membranes were immersed in 130 mL sodium acetate buffer (50mM, pH 5) consisting of 20 mg PVAm-TEMPO (batch No. 4, 6, 9, 10 or 16) for 30 min. 20 mL of 1 g/L laccase solution (9.5 U/mL) was added to start the oxidation at room temperature. The oxidation solution was stirred under oxygen purging (1 bubble/second) for 24 hours. 15 μL PVAm solution (1 g/L) was directly applied between two cellulose stripes before stripes were pressed and dried out. A typical delamination test was conducted to measure the wet adhesion between cellulose membranes. A controlled test, in which the PVAm-TEMPO was replaced by PVAm, was conducted in parallel.

2.2.2.6 Effect of PVAm concentration on wet adhesion

The property of PVAm-TEMPO/laccase complex varies with the ratio of PVAm-TEMPO and laccase. Therefore, the PVAm-TEMPO concentration variation with a fixed laccase concentration may affect the property of complex and further affect the oxidation of cellulose. In this series of experiments, cellulose membranes were oxidized in 150 mL, pH 5, 50 mM sodium acetate buffer consisting of 5 mg, 7.5 mg, 10 mg or 20 mg of PVAm-TEMPO 6 and 20 mg of laccase (approx. 190 U) with oxygen purging for 24 hours. Direct application of 15 µL 1 g/L PVAm solution was conducted after oxidation. Standard lamination and delamination experiments were then performed. A controlled test absence of PVAm-TEMPO 6 was performed in parallel. In addition, the electrophoretic mobility values of PVAm-TEMPO/laccase complex from reaction solution in each experiment were directly measured with a ZetaPlus Analyzer (Brookhaven Instruments Corp.) operating with the phase analysis light scattering (PALS) model. The charge density of PVAm-TEMPO and laccase in a pH 5 50 mM sodium acetate buffer were measured by polyelectrolyte titration (Mütek PCD titrator) using a 1 mM poly(diallyldimethyl ammonium chloride) (PDADMAC) as the titrant for the laccase solution (1 g/L) and a 1 mM potassium poly(vinyl sulphate) (PVSK) as the titrant for PVAm-TEMPO 6 (0.06 g/L) solution.

2.2.2.7 Effect of PVAm concentration on laccase catalyzed oxidation

2.2.2.7.1 Inhibition of laccase activity by PVAm

In order to study the inhibition of laccase activity by PVAm, the laccase-PVAm mixture solution consisting of 2.54 U/mL (0.3 g/L) laccase and desired concentrations of PVAm from 0 g/L to 3 g/L were prepared in pH 5 50 mM sodium acetate buffer. Using ABTS as the substrate, the reaction was run at 25°C and started by adding 40 µL laccase-PVAm mixture solution to 3 mL sodium acetate buffer (50 mM, pH 5) consisting of 0.5 mM ABTS. The oxidation rate was determined spectrophotometrically (420nm) by monitoring the coloured oxidation products for 2 min. A controlled PVAm blank test was conducted in parallel.

$$I = (\mathrm{d}A_{CT} - dA_{IT}) / dA_{CT} \times 100\% \qquad \textbf{Equation 2-5}$$

The inhibition percentage was calculated by means of the Equation 2-5. Where I: inhibition (in %), dA_{IT}: average absorbance change (dAbs/min) in inhibition test during 2 min, and dA_{CT}: average absorbance change (dAbs/min) in controlled test. At least triplicates were conducted for each sample.

2.2.2.7.2 Kinetic study of PVAm influence on laccase oxidation of TEMPO

The oxoammonium TEMPO cation was obtained by adding bromine to the TEMPO n-hexane solution.[40] (See Scheme 2-1) In this reaction, TEMPO was oxidized to oxoammonium bromide TEMPO, which formed precipitates in n-hexane. The new formed oxoammonium bromide precipitates were filtered and washed with n-hexane and subsequently dried with nitrogen. In order to remove bromide ions, oxoammonium bromide was dissolved in water and subjected to an ion-exchange column (DOWEX 1-8 exchange resin) saturated with 1 M NaH_2PO_4–Na_2HPO_4 at pH 4.5. Oxoammonium phosphate eluate was collected and drying under reduced pressure followed by freeze drying yielded the oxoammonium phosphate salt that was used in the experiments.

Scheme 2-3 Preparation of oxoammonium TEMPO

To study the kinetic influence of PVAm concentration on laccase oxidation of TEMPO, UV/Vis spectra of mixtures in the range of 380–600 nm was recorded for concentration analysis.[41] The reaction mixture consisting of 10mM of TEMPO as the substrate and 0.2 g/L of PVAm were buffered at pH 5 with 50mM sodium acetate buffer. The reaction was carried out by the addition of 50 μL laccase solution (1.6 g/mL) to 2 mL reaction mixture at room temperature with constant air purging. The concentration of TEMPO and oxoammonium were determined using a classical least squares (CLS) method in the 380-600 nm range. The spectra of 10 mM TEMPO and 10 mM oxoammonium TEMPO were used as standard curves. A controlled PVAm blank test was conducted in parallel.

2.2.2.8 Wet adhesion between cellulose membranes fabricated by layer-by-layer self-assembly of PVAm-TEMPO and laccase

In order to investigate the reaction mechanism of PVAm-TEMPO/laccase approach, layer-by-layer self-assembly of PVAm-TEMPO and laccase for cellulose oxidation was conducted. In this experiment, wet cellulose membranes were dipped in PVAm-TEMPO for 30 min and then rinsed with pH 5, 50 mM sodium acetate solution for 15 min. After removing surface water, the membranes were dipped in laccase solution for 30 min, followed by rinsing with pH 5, 50 mM sodium acetate solution for 15 min. The same procedures were repeated for multilayer fabrication.

Four pairs of layer-by-layer fabricated membrane were soaked in 130 mL pH 5, 50 mM sodium acetate buffer for 24 hours with oxygen purging, follow by direct applying extra PVAm solution to 15 mg/m^2. The lamination and delamination experiments were conducted as typical experiments.

2.2.2.9 The purification of laccase and the comparison with unpurified laccase on cellulose adhesion

Laccase was further isolated with liquid chromatography on an ÄKTA prime system (GE Healthcare) in this section. Filtered laccase used in previous sections was first dissolved in sodium phosphate buffer (20 mM, pH 7) with a concentration of 3g/L. The laccase solution was then applied to a 5 mL Bio-Scale Mini Macro-prep DEAE column (Bio-Rad, Laboratories, Inc) equilibrated in sodium phosphate buffer (20 mM, pH 7) as buffer A at 2.5 mL/min mobile phase flow rate. The eluate absorbance of UV light at 280 nm wavelength was monitored. After unbounded proteins were washed off, the column was eluted with a linear buffer gradient up to 200 mM sodium phosphate buffer (pH 7) to a volume of 100 mL. The eluate was collected into fractions of 5 mL. The laccase activity assay of each fraction was conducted immediately after the purification. The specific activity of each fraction was plotted together with eluate UV absorbance. Fractions in activity peak and absorbance peak at the same time were ultra-filtrated and desalted by centrifuging with centrifugal filter units (cut-off molecular weight: 3 kDa) from Millipore Ireland Ltd. The desalted purified laccase was frozen for storage.

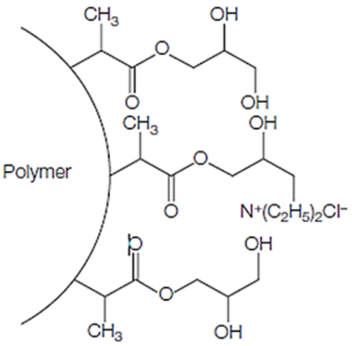


Figure 2-3 the macro-prep epoxide bead is derivatized with diethylamine to form the Macro-prep DEAE weak anion exchange support. (Bio-rad)

To compare the difference between purified and un-purified laccase on oxidation of cellulose, oxidation of cellulose was initiated by the addition of 20 mL, 190 Units of purified laccase to the oxidation solution (130 mL) consisting of 10 mg PVAm-TEMPO 14. The reaction solution was stirred under oxygen purging for 30 min. All other reaction conditions, laminate preparation and delamination procedures were controlled as a typical experiment.

Table 2-6 Comparison of purified and unpurified laccase on cellulose oxidation

Samples	Laccase (U/mL)	PVAm-TEMPO (mg/L)	Coated PVAm (mg/m^2)
Purified Laccase	1.27	66.67	15
Unpurified Laccase	1.27	66.67	15

2.2.2.10 Effect of adhesive application method on wet adhesion

Three methods were used to apply adhesive between oxidized cellulose and their influence on wet adhesion was studies. The detailed experimental processes were listed in Table 2-7. The oxidation of cellulose membranes were conducted as a typical experiment in 150 mL of 50 mM, pH 5 sodium acetate buffer consisting of 20 mg of PVAm-TEMPO 6 and 20 mg of laccase for 24 hours at room temperature with oxygen purging. In direct application method, 15 µL of 1 g/L PVAm solution was directly applied between oxidized cellulose membranes before lamination. In the adsorption method, oxidized cellulose membranes were immersed in 50 mM, pH 5 sodium acetate buffer consisting of 0.5 g/L PVAm for 30 min followed by rinsing three times. The membranes were then laminated by pressure. In a “one step” method, oxidized cellulose membranes were directly laminated after oxidation without extra polymer solution applying or soaking. All laminates were dried in condition room at 23°C and 50% humidity for 24 hours followed by standard delamination tests.

Table 2-7 Experimental processes of different adhesive application method

Oxidation		Adhesive application method	
PVAm-TEMPO (mg/L)	Laccase (U/mL)	Method	Solution
133	1.27	Direct Application	PVAm (15 mg/m^2)
133	1.27	Adsorption	PVAm (0.5 g/L, pH 5)
133	1.27	"one step"	N/A

3 Results

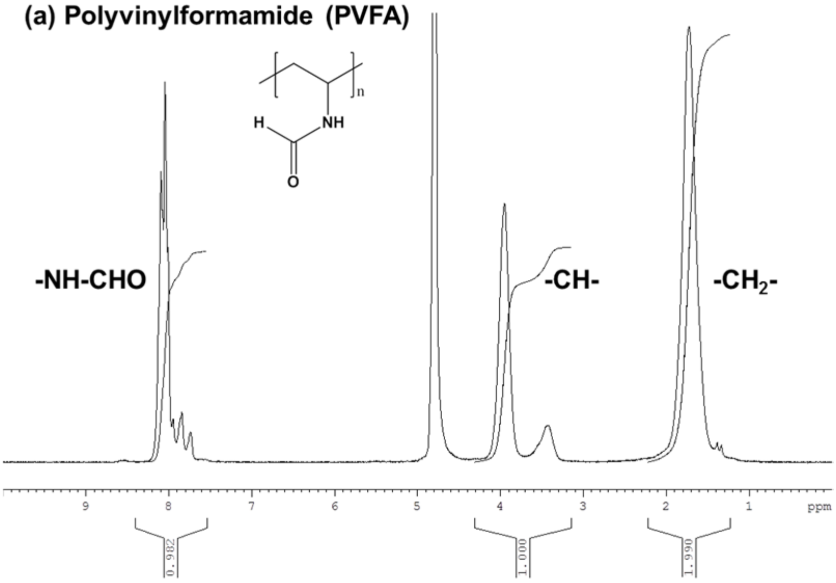
3.1 Synthesis and characterization of PVAm-TEMPO

3.1.1 Degree of hydrolysis of PVAm

The degree of hydrolysis (DH) of PVAm was determined by proton nuclear magnetic resonance (^{1}H NMR). ^{1}H NMR spectra of polyvinylformamide(PVFA), fully hydrolyzed PVAm and Lupamin® 5095 (partially hydrolyzed PVAm) are shown in Figure 3-1.

The chemical shift of polymer backbone -CH_2- groups is around 1.2-2.4, while the chemical shift of the amide groups is at 7.4-8.2. Determined by Equation 3-1, the degree of hydrolysis (DH) of Lupamin® 5095 (partially hydrolyzed PVAm) from BASF was 75.46% (average over duplicate measurements). Figure 3-1 (b) shows no signal around chemical shift 7.4-8.2 indicating that Lupamin® was 100% hydrolyzed in 75°C 5% NaOH solution.

$$DH = 1 - \frac{2 \times amide\ group\ peak\ area}{\mathrm{CH_2} - peak\ area} = 75.46\% \qquad \textbf{Equation 3-1}$$



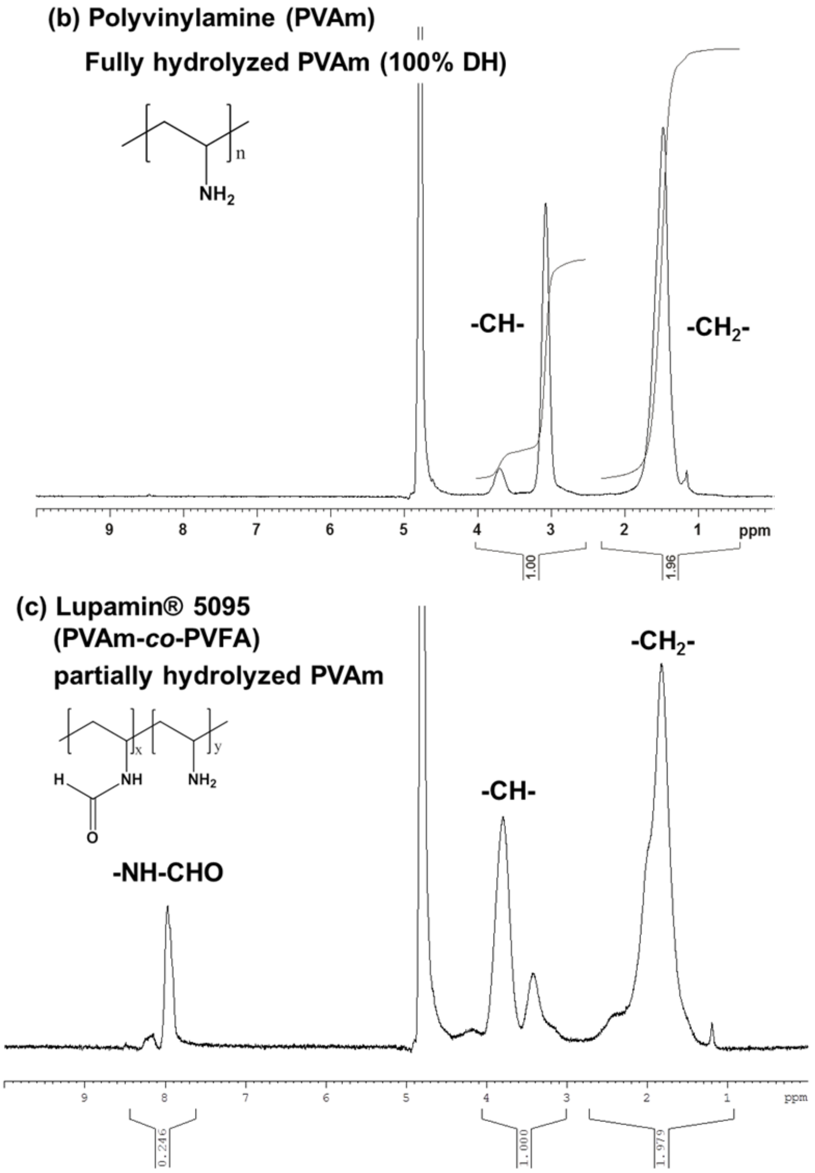


Figure 3-1 ^{1}H NMR of (a) PVFA, (b) PVAm (100% DH) and (c) partially hydrolyzed PVAm (PVAm 75% DH).

3.1.2 Amine contents of PVAm-TEMPO samples

3.1.3 Grafting extent of TEMPO moieties

To determine the equivalent amine content of freeze dried PVAm-TEMPO samples, PVAm-TEMPO samples was dissolved in 0.1 mM KCl solution and titrated by 0.1 M NaOH. The figure below gave an example of conductometric titration curve of PVAm-TEMPO 10. The volume of NaOH consumed by 6.7 mg PVAm-TEMPO 10 was 0.52 mL. Therefore, the amine content of PVAm-TEMPO 10 is 7.8 mmol NH_2/g sample. Results are shown in Table 3-1.

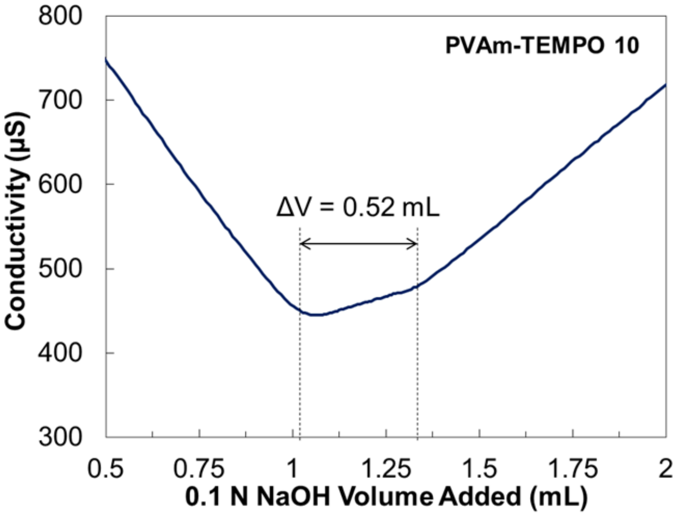


Figure 3-2 Base-into-acid (charging) conductometric titration of PVAm-TEMPO 10

Table 3-1 Amine contents of PVAm-TEMPO samples

Sample Name	Amine content (mmol NH_2/g sample)
PVAm-TEMPO 4	4.6
PVAm-TEMPO 6	3.6
PVAm-TEMPO 9	6.4
PVAm-TEMPO 10	7.7
PVAm-TEMPO 11	7.3
PVAm-TEMPO 14	4.4
PVAm-TEMPO 16	5.7

EPR spectroscopy was performed to determine the TEMPO grafting extent of PVAm-TEMPO. Figure 3-3 gives an example of EPR spectra of TEMPO (1.4 g/L) and PVAm-TEMPO 4 (5.0 g/L). TEMPO (radical) concentration is proportional to the double integral ($\iint$) of EPR intensity.[42] Using a 8.7 mM (1.4 g/L) TEMPO solution as a reference, TEMPO moieties concentrations in PVAm-TEMPO solutions can be calculated according to Equation 3-2.

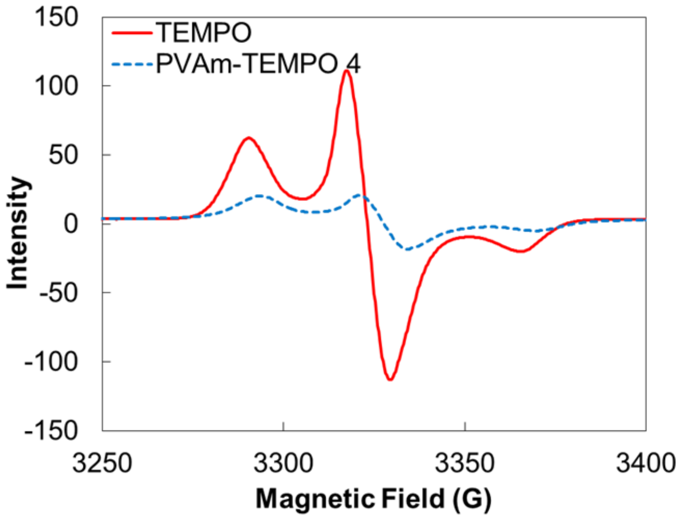


Figure 3-3 EPR spectra of TEMPO and PVAm-TEMPO 4

$$TEMPO\ moieties\ concentration\ (mM)=\frac{\iint_{PVAm\text{-}TEMPO}}{\iint_{TEMPO}}\cdot\frac{C_{TEMPO}}{Mw_{TEMPO}}$$ **Equation 3-2**

Table 3-2 The TEMPO moieties concentrations in PVAm-TEMPO sample solutions

PVAm_TEMPO Batch No.	Double integration ($\iint\times10^{-4}$)	$C_{\text{TEMPO moieties}}$ (mmol/L)
4	2.6	3.4
6	3.9	5.1
9	2.4	3.2
10	0.6	0.8
11	0.6	0.8
14	3.4	4.4
16	1.0	1.3
TEMPO (Reference)	6.7	8.7

$$GraftingExtent(\text{mole percent})=\frac{C_{TEMPOmoieties}}{(\alpha_{PVAm\text{-}TEMPO}\cdot C_{PVAm\text{-}TEMPO}+C_{TEMPOmoieties})/DH}$$ **Equation 3-3**

$C_{PVAm\text{-}TEMPO}$: Concentration of PVAm-TEMPO in solution (g/L), refer to Table 2-2
$C_{TEMPO\ moieties}$: TEMPO moieties concentration in solution (mmol/L), refer to Table 3-2
$\alpha_{PVAm\text{-}TEMPO}$: Amine content of PVAm-TEMPO sample (mmol NH_2/g sample), Table 3-1
DH: PVAm hydrolysis degree

Using Equation 3-3 to calculate the grafting extent of TEMPO substitution in mole percent, the results are shown in Table 3-3. The grafting extent of PVAm-TEMPO samples ranged from 0.007 to 0.164 TEMPO moieties per mole amine group, alternatively from 1.2% to 15.8% w/w. The grafting reaction yield ranged from 15.0% to 44.9% depending on the recipe ratio. With similar recipes, the conjugation yields of Batch 4, 6, and 14 were around 25%. As the concentration of EDC increased to 64.4 mM, the yield of Batch 11 turned out to be 44.9%, which was much higher than that of Batch 10. Meanwhile, the conjugation yield may be affected by other reaction conditions such as pH and reaction time etc. However, the yield of batch 16 was only 15% as a repeat batch. It indicates that the reaction conditions used in this study required better design and controlling.

Table 3-3 The TEMPO grafting extents of PVAm-TEMPO samples

PVAm-TEMPO Batch No.	Grafting Extent % (w/w)	Grafting Extent % (mole percent)	Grafting Yield %
4	10.5	9.6	29
6	15.8	16.4	25
9	5.9	4.2	43
10	1.2	0.7	15
11	1.4	0.9	45
14	11.6	10.8	26
16	7.9	6.1	15

3.1.3.1 The stability of amine groups on PVAm with NaClO

As sodium hypochlorite (NaClO) is a strong oxidant, there has been a concern that the amine oxidation reaction may undergo with the presence of NaClO. In this stability study, PVAm (100% DH) solution showed a slightly yellow color from colorless with the addition of NaClO. FT-IR spectroscopy was used to study the chemical reaction between PVAm (100% DH) and NaClO.

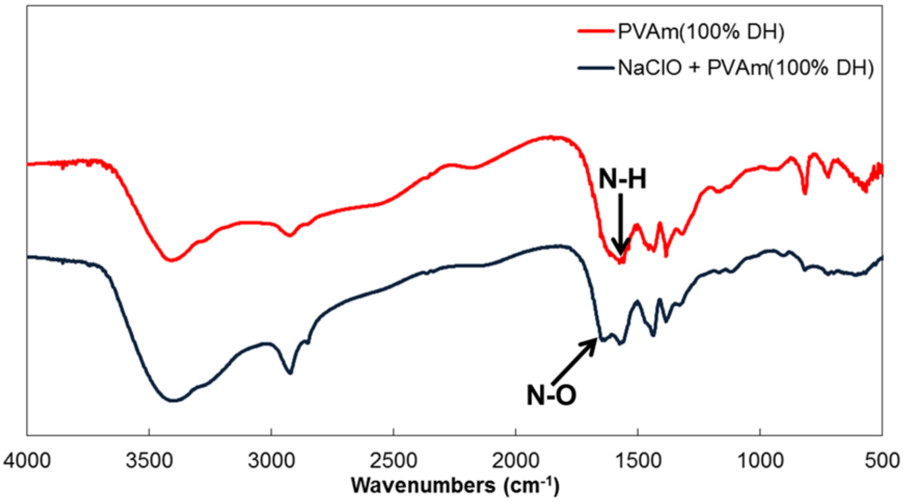
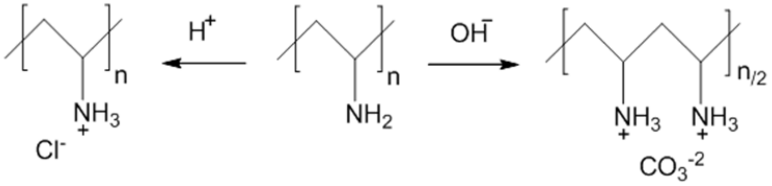


Figure 3-4 FTIR spectra of PVAm(100% DH) before and after NaClO oxidation

Figure 3-4 shows the FT-IR spectra of PVAm (100% DH) before and after NaClO oxidation. Compared with the spectrum of PVAm before oxidation, oxidized PVAm showed a new absorbance band at 1600 cm^{-1}. The absorbance for the N-O stretch is around 1500-1600 cm^{-1}, compared to the C=O stretch at 1650-1800 cm^{-1}.[43]

The amine contents of PVAm (100% DH) before and after NaClO treatment were determined by conductometric titration as shown in Figure 3-5. The amine content of PVAm (100% DH) before oxidation was 16.73 mmol NH_2/g, while the amine content after NaClO oxidation was 7.58 mmol NH_2/g, which was only 45.3% of the original amine content. The result indicates that a large number of amine groups were oxidized by NaClO during the process. The titrated amine content of PVAm (100% DH) is lower than the theoretic value, 23 mmol NH_2/g. It may be due to the polymer solution adsorbed carbon dioxide from air during the dialysis and formed carbonate groups. Those carbonate groups interacted with amine groups (see Scheme 3-1) and cannot be removed during freeze drying. Another possible reason was that there was still some moisture remaining in freeze dried sample as PVAm is very hydrophilic. Similar phenomena were observed in PVAm-TEMPO amine content measurements. It may be due to the pH of conjugation solution were maintained at 6 by adding HCl solution. Chlorite ions interacting with amine groups and cannot be removed by dialysis and freeze drying.



Scheme 3-1 Possible existed ions in freeze dried PVAm sample

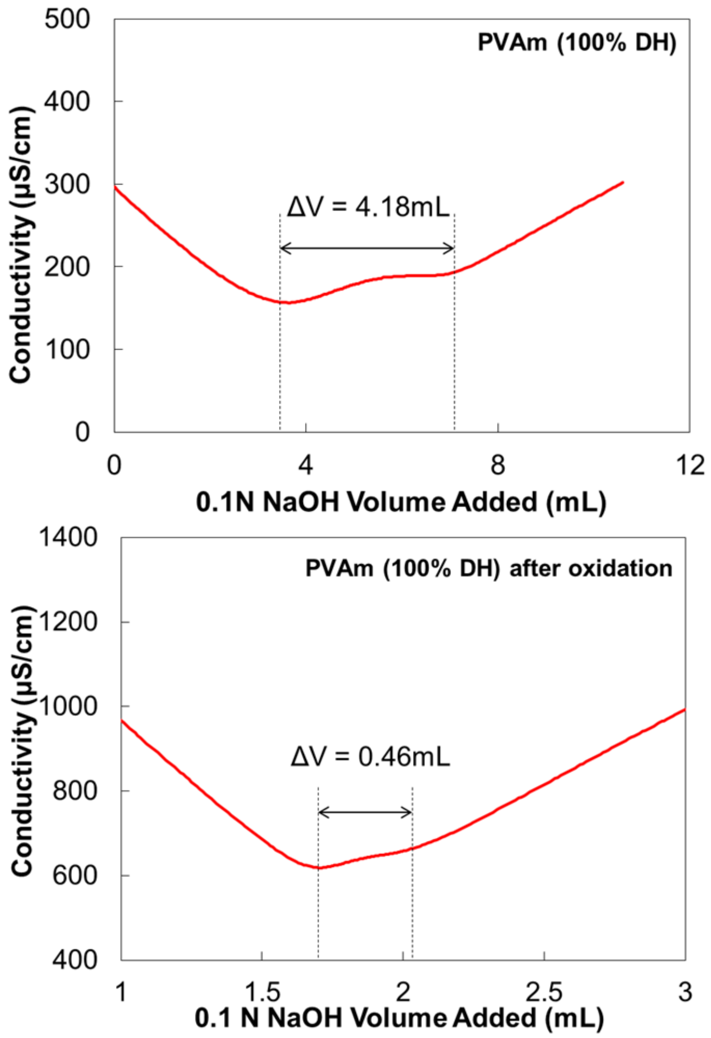


Figure 3-5 Base-into-acid (charging) conductometric titration of PVAm (100% DH) before and after NaClO treatment

3.1.3.2 Degradation of PVAm by NaClO

As reported by some papers, NaClO may degrade the backbone structure of PVAm.[44] In to order investigate the degradation of PVAm by NaClO, molecular weights of PVAm (100% DH) with various NaClO concentration treatment were determined by intrinsic viscosity measurement. An Ubbelohde capillary viscometer was used to measure the viscosity of polymer solutions.

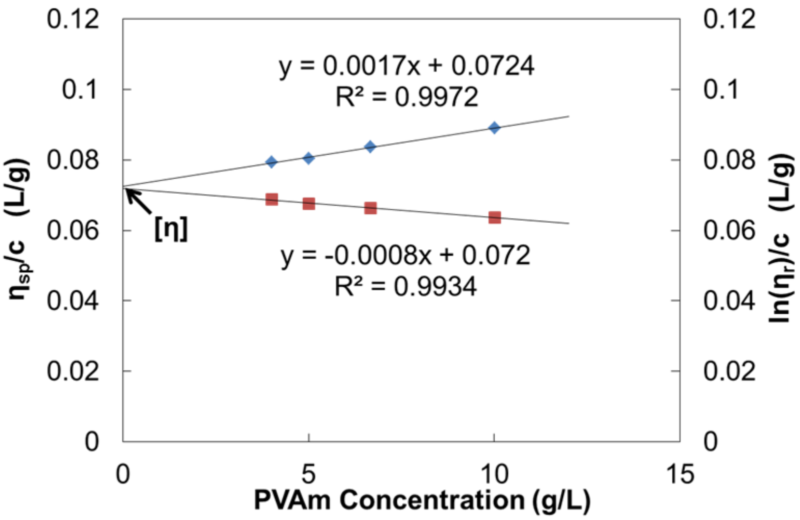


Figure 3-6 Intrinsic viscosity of PVAm (100% DH)

The intrinsic viscosity [η] of PVAm (100% DH) in 0.1M NaCl and 0.01M NaOH solution was determined to be 72 mL/g by the plotting shown in Figure 3-6. According to the modified Mark-Houwink Equation (Equation 3-4, which is valid for PVAm with 1.5) reported by Bloys van Treslong and Morra[35] , the number average molecular weight of PVAm (100% DH) was 42 kDa.

$$[\eta] = 6.2 \times 10^{-3} \cdot \overline{M}_n^{\ 0.88}$$ **Equation 3-4**

The molecular weights of degraded PVAm with known NaClO concentrations were measured in the same method converting PVAm (100% DH) to degraded PVAm. The results were plotted in Figure 3-7. Figure 3-7 shows a decreasing of polymer molecular weight from around 42 kDa to 7 kDa with the increasing of NaClO concentration from 0 mM to 68 mM. Polymer solution turned to be yellow from transparent with NaClO treatment as shown in Figure 3-7.

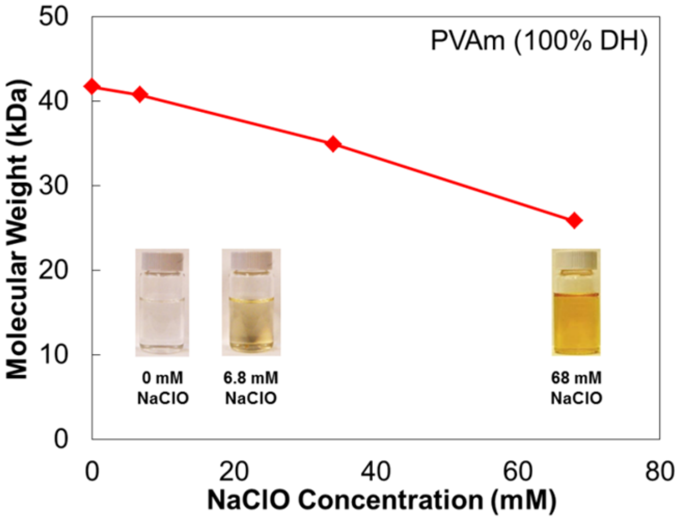


Figure 3-7 Effect of NaClO concentration on degradation of PVAm

3.2 Wet adhesion of PVAm-TEMPO oxidized cellulose

In this study, cellulose wet adhesion was improved by applying PVAm as an adhesive on oxidized cellulose. There are several factors that will affect the wet adhesion of cellulose, such as oxidation conditions, adhesive application method etc. Two oxidation approaches were developed in this study, PVAm-TEMPO/NaClO/NaBr approach and PVAm-TEMPO/laccase/O_2 approach. Factors affecting wet adhesion of cellulose in these two approaches are shown as follows.

3.2.1 Application in NaClO/NaBr/TEMPO oxidation of cellulose

The application of PVAm-TEMPO in NaClO/NaBr/TEMPO oxidation of cellulose was first developed by Ren in his earlier study.[5] Following his research, further study of this oxidation approach was conducted. Results are shown in this section.

3.2.1.1 Effect of oxidation time on wet adhesion

Figure 3-8 shows the influence of oxidation time on cellulose wet adhesion. In this figure, PVAm-TEMPO catalyzed reaction results are represented by the solid diamonds, while free TEMPO catalyzed reaction results adapted from DiFlavio et al.[3] are represented by the solid spheres. In both cases, the oxidation of cellulose took place in a short time and increased wet adhesion was achieved after about 10 min oxidation. Free TEMPO catalyzed oxidation improved wet adhesion faster than PVAm-TEMPO did. However, instead of delamination, substrate failure occurred during the peeling test on the samples oxidized by free TEMPO with a long time. However, no substrate failure has been observed for the sample oxidized by PVAm-TEMPO.

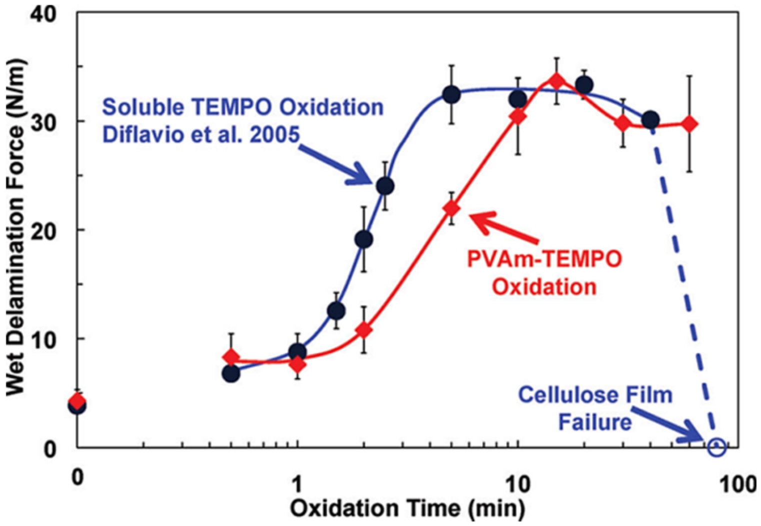


Figure 3-8 Influence of oxidation time on cellulose-PVAm-cellulose wet adhesion

SEM images in Figure 3-9 show some topological features of cellulose membrane surfaces after delamination test with various treatment conditions. Without TEMPO oxidation, cellulose membrane surface was relatively smooth. The membrane surface appeared to remain intact with small fragments after 5 min oxidation by free TEMPO or after 120 min oxidation by PVAm-TEMPO. Whereas, serious membrane cohesive failures were observed after 120 min oxidation by free TEMPO. Cellulose membranes were separated from the bulk during peeling test. It indicates that the mechanical strength of cellulose membrane itself was weakened by long time oxidation with free TEMPO.

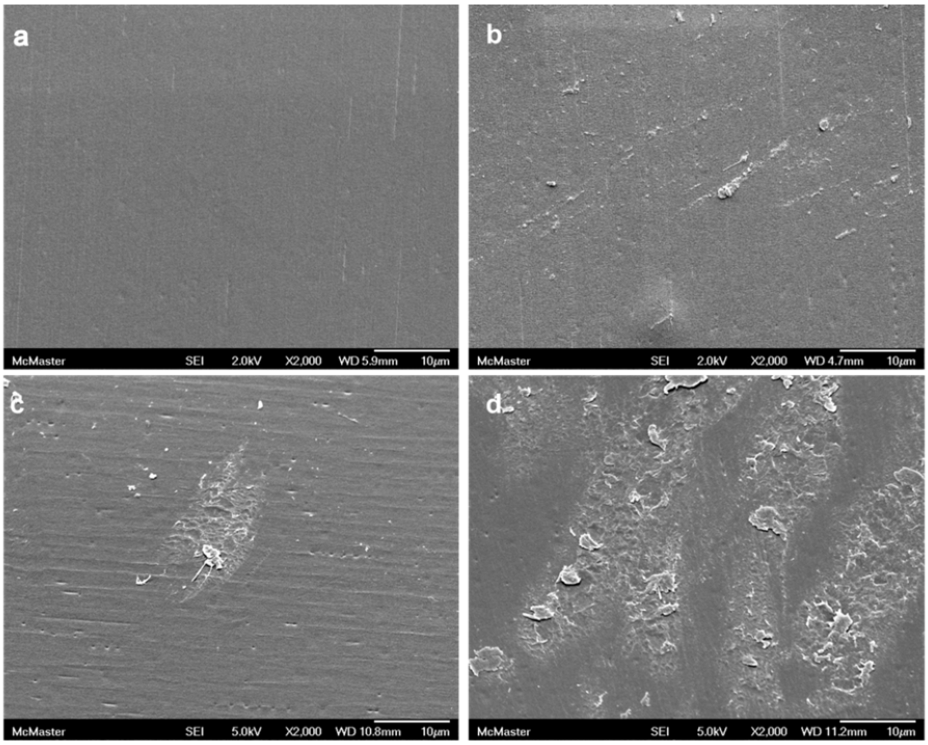


Figure 3-9 SEM images of cellulose membrane surfaces after delamination. The oxidation times were (a) 0 min (b) 10 min by free TEMPO, (c) 120 min by PVAm-TEMPO, and (d) 120 min by free TEMPO

3.2.1.2 Effect of NaClO concentration on wet adhesion

Figure 3-10 shows the effect of NaClO concentration on cellulose wet adhesion. Performed as a primary oxidant, NaClO concentration determined the yield of the oxidation reaction. As shown in Figure 3-10, the wet delamination force between cellulose membranes increased with the increasing of NaClO added to the oxidation medium and reached a plateau level of about 36 N/m. However, no wet adhesion was obtained without the addition of NaClO.

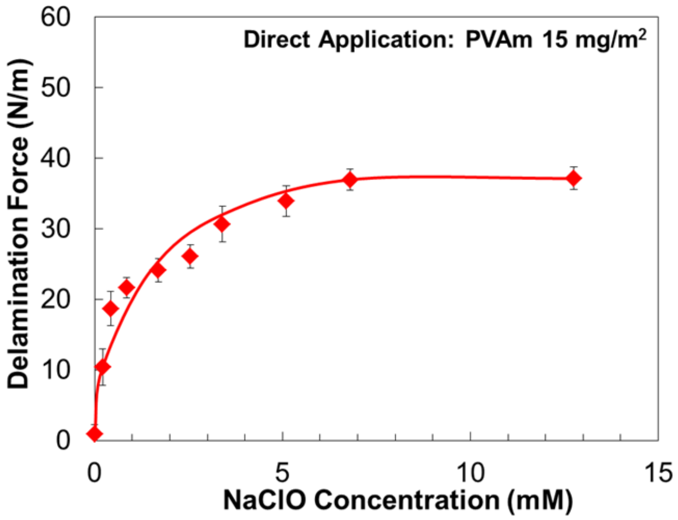


Figure 3-10 Effect of NaClO concentration on cellulose wet adhesion. Each experiment was conducted in 200 mL solution consisting of 50 mg/L NaBr, and 20 mg/L PVAm-TEMPO. A desired amount of NaClO was added to each system to initiate the reaction. . The oxidations were maintained at pH 10.5 by adding 0.1N NaOH for 30 min. A typical direct PVAm application method was used to prepare laminated samples.

3.2.1.3 Effect of reaction pH on cellulose wet adhesion

Figure 3-11 shows the effect of reaction pH on cellulose wet adhesion. The oxidation reaction carried out at alkaline pH gave slightly higher wet adhesion. Particularly, when the PVAm-TEMPO mediated oxidation reaction was carried out at pH 9.5, wet delamination force showed a maximum value of 32 N/m. TEMPO mediated oxidation of primary alcohol was reported to be carried out mostly in alkaline environment from pH 8.5 to pH 11.5.[26] The optimized reaction pH is 9.5. It may be because the reaction rate at pH 8 and 11.5 was much lower than that at pH 9.5. The reaction rate of hypochlorite oxidation of TEMPO is limited at pH 11.5.[45] While at pH 8.5, the abstraction of proton in the complex formed between alcohol and TEMPO$^+$ is retarded.[26]

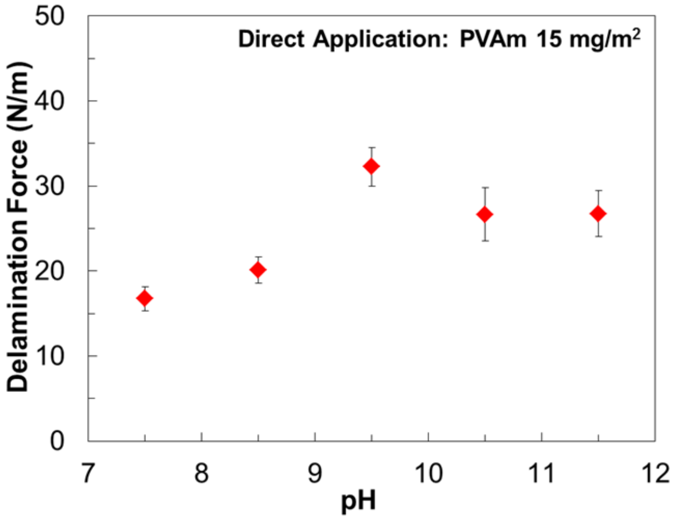


Figure 3-11 Effect of reaction pH on cellulose wet adhesion. Each experiment was conducted in 200 mL solution consisting of 50 mg/L NaBr, and 20 mg/L PVAm-TEMPO, and 0.68 mmol NaClO was added to initiate the reaction. Each oxidation system was maintained at a desired pH by adding 0.1N NaOH for 30 min. A typical direct PVAm application method was used to prepare laminated samples.

3.2.1.4 Effect of TEMPO grafting extent on wet adhesion

Figure 3-12 shows the effect of TEMPO grafting extent on cellulose wet adhesion. Without TEMPO moieties, little wet adhesion was achieved. Increased wet adhesion of 24 N/m was achieved with as low as 0.9% TEMPO moieties (per mole) grafting extent. Furthermore, PVAm-TEMPO with higher TEMPO grafting extents did not further increase wet adhesion significantly. This is because TEMPO performs as a mediator in the oxidation system. Without TEMPO moieties, the primary oxidant NaClO, cannot efficiently convert alcohol groups to aldehyde groups. However, as NaClO is small molecule, it has sufficient mobility to move around and convert immobilized TEMPO moieties to TEMPO$^+$. Thus, as few as catalytic amounts of TEMPO grafting extent are required to obtain increased wet adhesion.

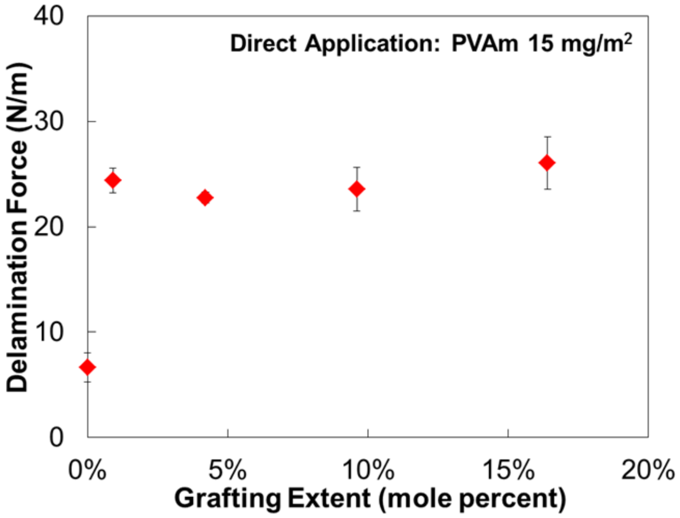


Figure 3-12 Effect of TEMPO grafting extent on cellulose wet adhesion. Each experiment was conducted in 200 mL solution consisting of 50 mg/L NaBr, and 20 mg/L PVAm-TEMPOs with different TEMPO grafting extents varying from 0.7% to 16.4% (mole percent), and 0.68 mmol NaClO was added to initiate the reaction. Each oxidation system was maintained at pH 10.5 by adding 0.1N NaOH for 30 min. A typical direct PVAm application method was used to prepare laminated samples.

3.2.1.5 Effect of oxidation yield and grafting extent of PVAm-TEMPO on cellulose wet adhesion by the adsorption method

Two series of experiments were designed to study the influence of oxidation yield of cellulose and grafting extent of PVAm-TEMPO on cellulose wet adhesion. The results are shown in Figure 3-13. It can be observed that with high dose of TEMPO and NaClO oxidation, adsorbed PVAm-TEMPO with different grafting extents gave similar wet adhesion of around 30 N/m to that of PVAm. However, with fewer doses of TEMPO and NaClO oxidation, adsorbed PVAm-TEMPO 6 with 16.4% TEMPO moieties (per mole) resulted in 18.42 N/m of delamination force, which is smaller than that of PVAm. Comparing the conditions of two results with PVAm-TEMPO 6 adsorbed in each series, the different oxidation recipe made the wet adhesion different. It is probably because cellulose membranes were oxidized to be more attractive to low amine content adhesive with a higher oxidant and catalyst dose. This result indicated that in the studied range, the wet adhesion of cellulose with high oxidation yield of cellulose is not sensitive to the amine content of adsorbed adhesive, while with lower oxidation yield of cellulose, the low amine content of adsorbed adhesive may cause a drop of wet adhesion of cellulose.

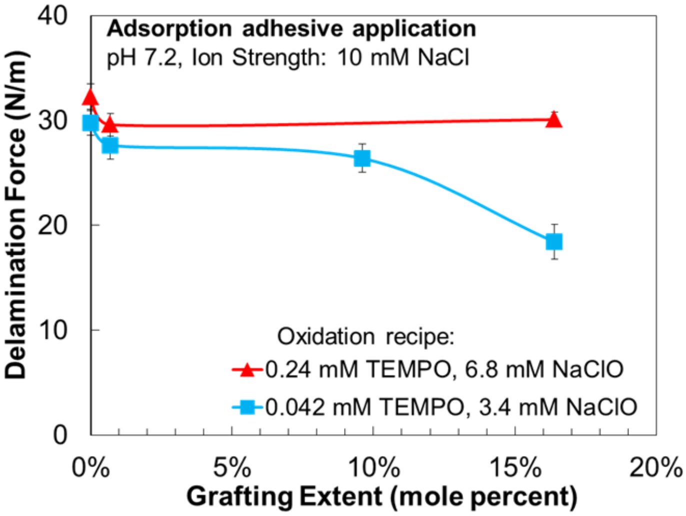


Figure 3-13 Effect of oxidation recipe and amine content of adhesive on wet adhesion

3.2.2 Application in Laccase/O_2/TEMPO oxidation of cellulose

3.2.2.1 Laccase activity assay

A spectroscopic method for measuring laccase activity using ABTS has been utilized.[37-38] The oxidation of ABTS by laccase to a green color product corresponds with the increase in absorbance at 420 nm. Figure 3-14 shows a typical set of triplicate laccase activity assay results. The absorbance at 420 nm showed a linear increase with time during the initial 2 min. The average initial rate of absorbance variation was 0.9 ΔA/min. According to the Beer-Lambert equation, the average initial reaction rate was approximately 77 µmol/min (also known as 77 Unit).

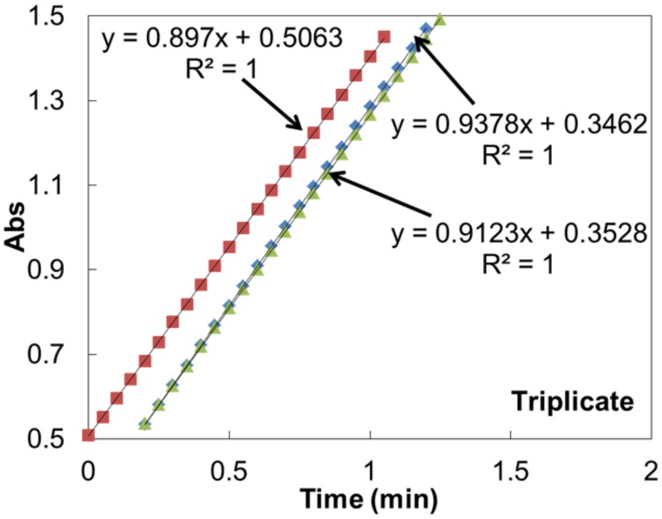


Figure 3-14 A typical laccase activity assay plot, the absorbance values variation at 410 nm using ABTS as the substrate (triplicate). Refer to the experimental part for detailed conditions.

3.2.2.2 Wet adhesion between cellulose oxidized by PVAm-TEMPO/laccase system and its mechanism

Table 3-4 presents the wet adhesion between cellulose membranes oxidized by PVAm/laccase/O_2 and some control experiments. The experimental conditions are shown in Table 2-4. The peeling results for batch 1 was 35.5 N/m, while the result of un-oxidized cellulose membranes in batch 2 was only 1.9 N/m. In order to investigate the mechanism of this approach, more controlled experiments were conducted. The average delamination force of batch 3 and batch 4 were as low as 3.4 N/m and 1.2 N/m in which laccase or PVAm-TEMPO was absent, respectively. In batch 5, laccase was denatured by boiling in a 100°C water bath. The delamination result shows no wet adhesion. In batch 6, laccase and PVAm-TEMPO was separated by a dialysis tube (MWCO 10-12 kDa) and ended up achieving no wet adhesion, neither. These results indicate that the wet adhesion is increased through the PVAm-TEMPO/Laccase/O_2 approach due to the oxidation of cellulose. Without oxidation reaction, the PVAm-TEMPO/laccase complexes cannot physically increase the wet adhesion. Furthermore, to carry out the reaction, active laccase must be in molecular contact with the TEMPO moieties on PVAm-TEMPO to mediate the oxidation reaction. This may be because enzymes are usually very specific as to which reactions they catalyze and the substrates that are involved in these reactions. Both enzyme and substrate possess specific complementary geometric shapes that fit exactly in to one another. This is often referred to as "the lock and key" model.[46]

Table 3-4 Controlled experiments of cellulose oxidaiton by PVAm-TEMPO/laccase

Batch No.	Laccase (mg/L)	Laccase activity (U/mL)	PVAm-TEMPO (mg/L)	Coated PVAm (mg/m^2)	Average delamination force (N/m)
1	133	1.3	133	15	35.5±2.1
2	0	0	0	15	1.9±0.5
3	0	0	133	15	3.4±1.4
4	133	1.3	0	15	1.2±1.6
5†	133	0	133	15	4.4±1.4
6‡	133	1.3	133	15	1.0±0.4

† laccase was denatured at 100°C

‡ laccase and PVAm-TEMPO were separated by dialysis tube (MWCO 12-14 kDa)

3.2.2.3 The Comparison of cellulose wet adhesion between cellulose membranes oxidized by PVAm-TEMPO and free TEMPO

The PVAm-TEMPO and free TEMPO catalyzed oxidation were compared as descried in Table 2-5. The results are shown in Figure 3-15. With the equivalent TEMPO moieties concentration (0.128 mM), PVAm-TEMPO catalyzed oxidation resulted in much higher wet adhesion of 35.5 N/m, while free TEMPO catalyzed oxidation ended up with 15.0 N/m, which is very similar to the result of membranes with equivalent TEMPO (0.128 mM) and PVAm (113 mg/L) existing in the oxidation system.

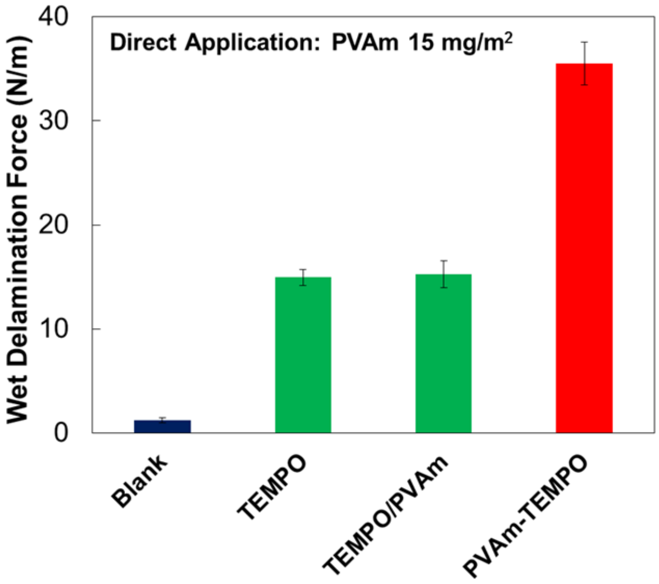


Figure 3-15 PVAm-TEMPO versus free TEMPO on oxidation of cellulose. Refer to **Table 2-5** for detailed conditions

The distribution of aldehyde groups through the thickness of the cellulose membranes was characterized by confocal microscopy. Membranes after free TEMPO and PVAm-TEMPO oxidation were immersed in fluorescein probe solution overnight to label the newly generated aldehyde groups by chemical reaction. The images are shown in Figure 3-16. A plot of grey values versus position through the membrane is shown as well. It can be observed that there was almost no aldehyde group existing on cellulose membrane before oxidation. The oxidation of cellulose by free TEMPO generated a large amount of aldehyde groups and those aldehyde groups distributed evenly throughout the interior and exterior part of cellulose membrane. However, oxidized by PVAm-TEMPO, aldehyde groups were only introduced on the exterior surface of cellulose membrane.

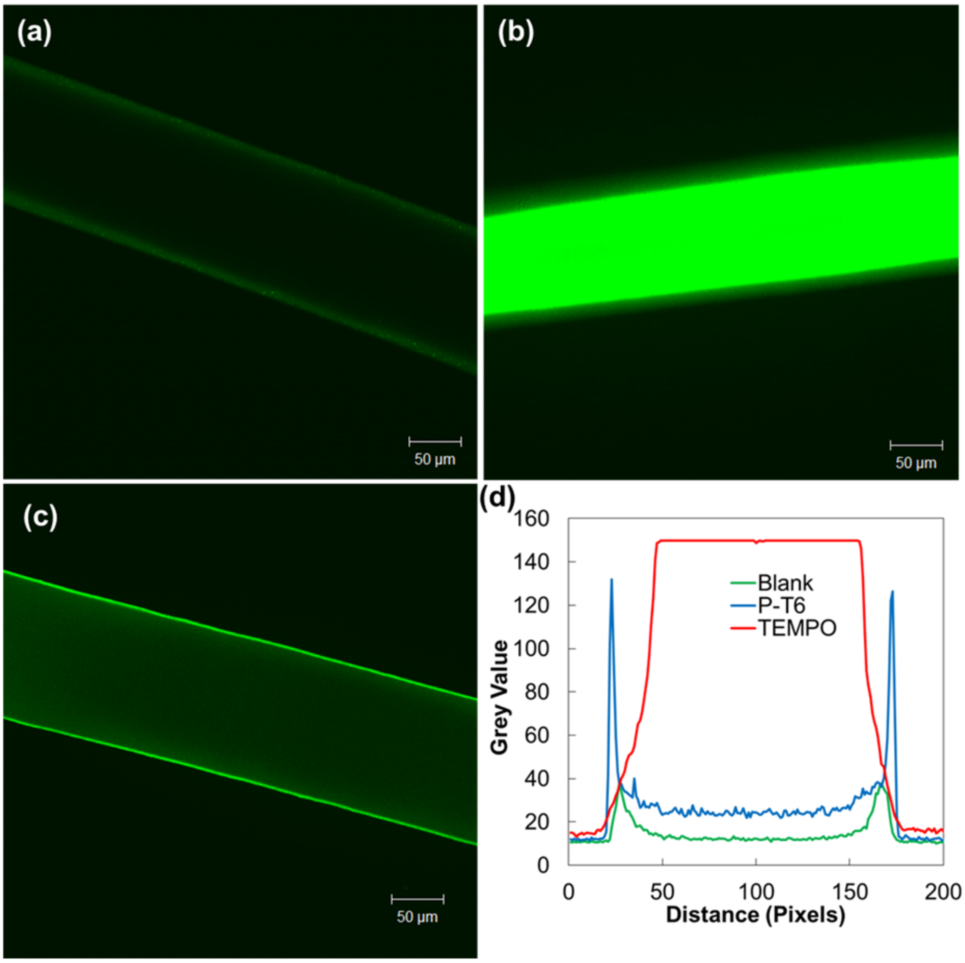


Figure 3-16 CLSM images of (a) unoxidized membrane cross-section, (b) free TEMPO oxidized membrane cross-section, (c) PVAm-TEMPO oxidized membrane cross-section and (d) the fluorescence intensities as a function of distance through the membranes. Aldehydes were labeled with green fluorescein-5-thiosemicarbazide (excited at 488 nm)

3.2.2.4 Effect of reaction time on cellulose wet adhesion

Figure 3-17 shows the delamination force as a function of oxidation time. Each point represents the average of at least 3 delamination experiments with the error bars representing the standard error. The wet adhesion between cellulose showed an increase from 8.9 N/m to a maximum of 29.1 N/m with oxidation time from 5 min to 30 min. The wet adhesion slightly dropped to a plateau level of about 26 N/m with oxidation times longer than 30 min. It indicates 30 min of oxidation is sufficient to obtain a relative high level of wet adhesion.

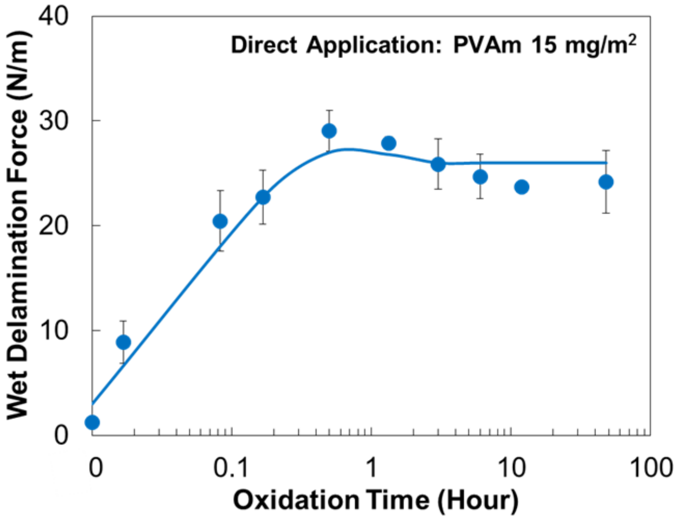


Figure 3-17 Effect of oxidation time on cellulose wet adhesion. All experiments were conducted in pH 5 50 mM sodium acetate buffers consisting of 66.7 gm/L PVAm-TEMPO 14 and 133.3 mg/L laccase. The oxidation time varied from 0 min to 48 hours.

Laccase has been reported to have an isoelectric point (pI) near 4.3-4.5.[47] It is net negatively charged at pH 5.[48] And PVAm is cationic at pH 5. Thus, laccase and PVAm-TEMPO can complex together forming colloid. As laccase/PVAm-TEMPO complex aggregation was observed, turbidity characterization of samples during the oxidation was conducted at wavelength of 500 nm by UV/Vis spectroscopy. As shown in Figure 3-18, the turbidity of laccase/PVAm-TEMPO complex increased with the time from the value of 0.06 to a maximum value of 0.27 at 3 hours oxidation. After that, the turbidity dropped as the complex continued to aggregate and formed precipitates.

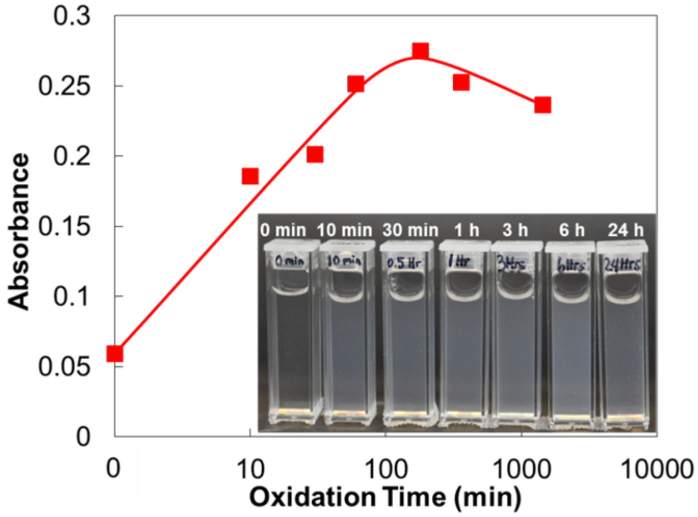


Figure 3-18 Turbidity (500 nm) variation with oxidation times. The PVAm-TEMPO : laccase mass ratio was 1:2

3.2.2.5 Effect of grafting extent on wet adhesion

Using PVAm-TEMPO with different grafting extents in oxidation system, the effect of grafting extent on cellulose wet adhesion was investigated and the results are described in Figure 3-19. As shown in Figure 3-19, PVAm-TEMPO with grafting extent lower than 4.2% (mole %), almost did not increase the wet adhesion. Whereas the wet adhesion was improved to around 30 N/m as the grafting extent of PVAm-TEMPO increased to 9.6% or higher. It indicates that the grafting extent of PVAm-TEMPO is an important factor in the enhancement of wet adhesion. This may be because PVAm-TEMPO can complex with laccase. Laccase must in molecule contact with TEMPO moieties on PVAm-TEMPO to catalyze the oxidation reaction. Thereby, the catalytic efficiency of laccase/TEMPO system may be limited due to the less ability of laccase and TEMPO moieties to move around. In other words, there must be sufficient amount of TEMPO moieties around laccase to achieve an ideal yield of oxidation.

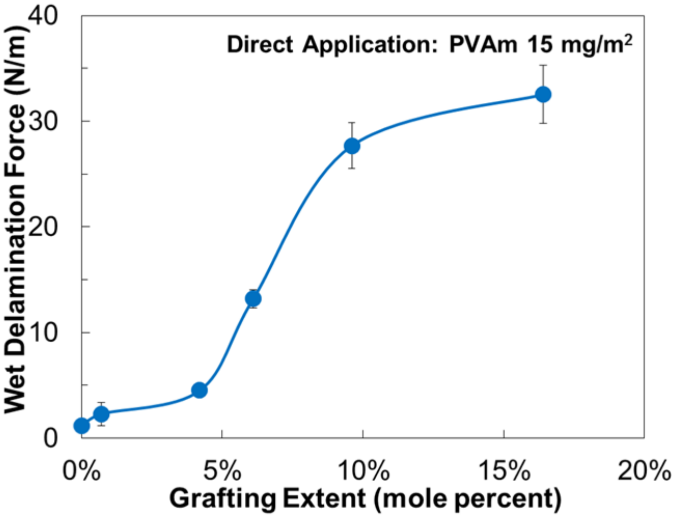


Figure 3-19 Effect of TEMPO grafting extent on cellulose wet adhesion. Experiments were conducted in pH 5 50 mM sodium acetate buffers consisting of 133 mg/L PVAm-TEMPOs with different TEMPO grafting extents varying from 0.7% to 16.4% (mole percent) and a fixed laccase concentration of 133 mg/L. A typical direct PVAm application method was used to prepare laminated samples.

3.2.2.6 Effect of PVAm concentration on wet adhesion

The effect of PVAm-TEMPO concentration on laccase catalyzed oxidation was studied as shown in Figure 3-20. No wet adhesion was achieved between un-oxidized cellulose. The wet delamination force showed a sharp increase from the initial value to a maximum of 35 N/m with the increase of PVAm-TEMPO concentration.

Measured by polyelectrolyte titration, the charge density of PVAm-TEMPO 6 at pH 5 is +2.7 meq/g sample, while the charge density of filtered laccase is -0.9 meq/g sample. The electrophoretic mobility result indicates that with the fixed laccase concentration of 133 mg/L, the concentration of PVAm-TEMPO determined the mobility of laccase/PVAm-TEMPO complex. When the PVAm-TEMPO concentration was 33 mg/L, the theoretical charge ratio was 0.74:1 (cations: anions) and the complex surface carried negative charge. When the PVAm-TEMPO concentration was 50 mg/L, the complex was almost uncharged. Compared with polyelectrolyte titration result, the charge ratio at this point was 1.12:1 (cations: anions), which was very close to 1:1. With the increasing of PVAm-TEMPO concentration, the mobility of complex increased to more positive.

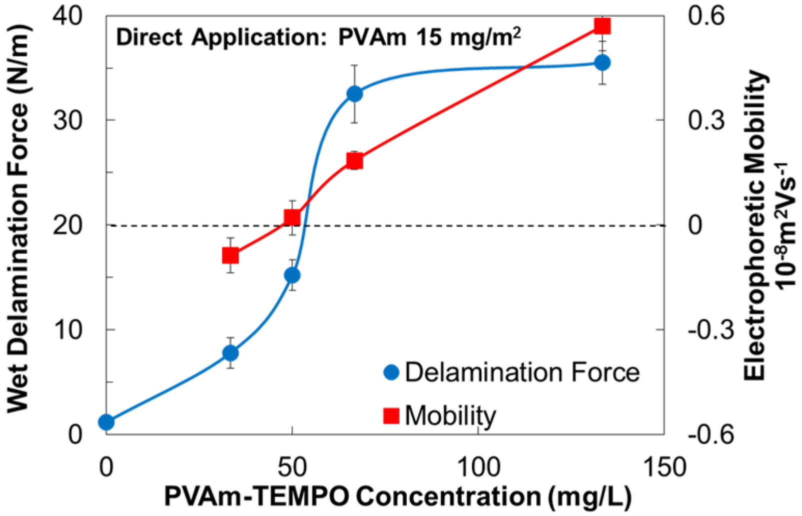


Figure 3-20 Effect of PVAm concentration on wet adhesion. All experiments were conducted in pH 5 50 mM sodium acetate buffers consisting of a fixed laccase concentration of 133 mg/L. The PVAm-TEMPO 6 concentration varied from 0 to 133 mg/L.

3.2.2.7 Effect of PVAm concentration on laccase catalyzed oxidation

3.2.2.7.1 Inhibition of laccase activity by PVAm

In previous section, it was found that negatively charged laccase complexes with cationic PVAm laccase. The complex formation may affect the configuration of enzyme and thereby affect the affinity between enzyme and substrate. Thus, the effect of PVAm concentration on laccase activity was studied in this section and the results obtained with ABTS as substrate are shown in Figure 3-21. As can be observed, PVAm has an inhibitory effect on laccase activity. The inhibition increased with increasing PVAm concentration. This may be because the bonding formed between laccase and PVAm changes the configuration of laccase and limits the catalyze rate of laccase.

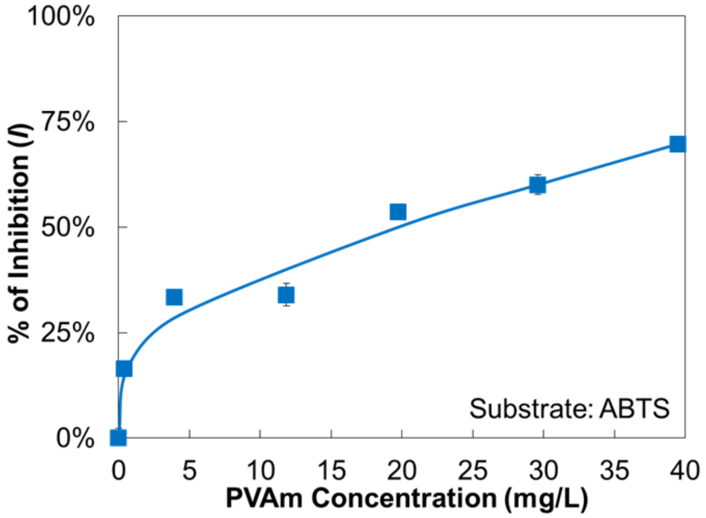


Figure 3-21 Inhibition of laccase activity by PVAm with fixed laccase concentration: 3.9 mg/L and various PVAm concentrations

Further kinetics study was conducted to investigate the influence of the present of PVAm on laccase activity using TEMPO as the substrate. No colloidal phenomenon was observed during the experiment. The concentration of TEMPO and oxoammonium TEMPO in solution were measured by UV/Vis spectroscopy. The results are shown in Figure 3-22. In TEMPO/Laccase/O_2 oxidation system, oxygen performs as the primary oxidant. As a mediator, laccase transfers oxidant potential from O_2 to TEMPO. TEMPO is activated by laccase oxidation to oxoammonium TEMPO. Compared with the original initial reaction rate of laccase mediated oxidation of TEMPO, the initial reaction rate was inhibited by the addition of PVAm from 0.18 mM/min to 0.07 mM/min. Furthermore, the final oxoammonium TEMPO conversion was lowered to 53%, which indicated all laccase were deactivated by PVAm.

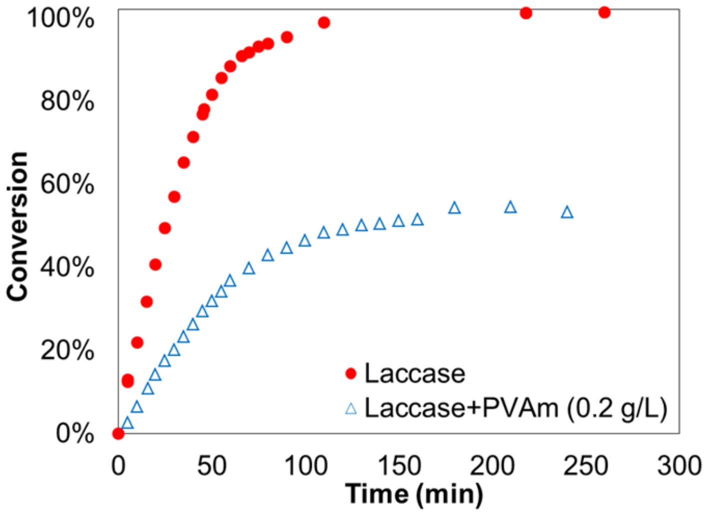


Figure 3-22 Effect of PVAm on laccase oxidation of TEMPO (Experiments started with10 mM TEMPO, 0.04 g/L laccase or 10 mM TEMPO, 0.04g/L laccase, 0.2 g/L PVAm)

3.2.2.8 Wet adhesion between cellulose membranes fabricated by layer-by-layer self-assembly of PVAm-TEMPO and laccase for cellulose oxidation

In this study, layer-by-layer (LbL) self-assembly method was used to fabricate PVAm-TEMPO and laccase LbL structure on cellulose membrane surface. The wet adhesion between cellulose was slightly improved from 1.0 N/m without LbL structure to 5.9 N/m with three PVAm/Laccase layers. Compared with previous results, it shows that PVAm-TEMPO/laccase layer-by-layer structure cannot oxidize cellulose membrane to achieve increased wet adhesion. The monolayer structure adsorbed on cellulose surface is not the mechanism of oxidation in previous PVAm-TEMPO/laccase/O_2 approach.

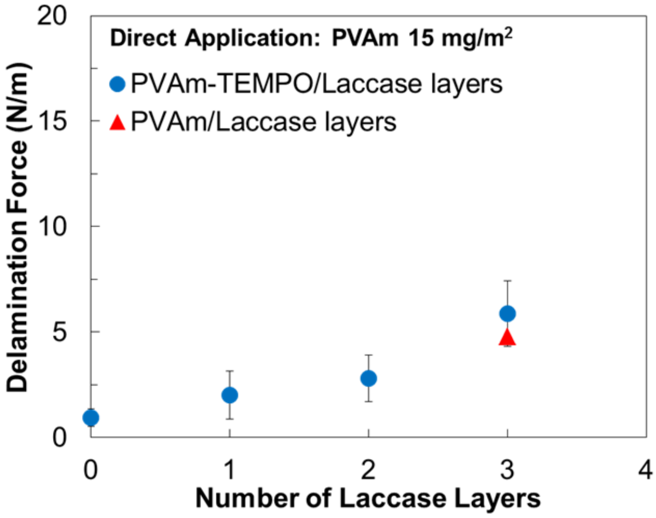


Figure 3-23 Wet adhesion between cellulose membranes fabricated by layer-by-layer self-assembly for oxidation

3.2.2.9 The purification of laccase and the comparison with unpurified laccase on cellulose oxidation

Laccase from *Trametes versicolor* was purified by liquid chromatography on a DEAE ion exchange column. Both the UV absorbance at 280 nm and specific activity of eluate were monitored. The results were plotted and shown in Figure 3-24. The fractions from 125 mL to 160 mL were desalted and concentrated by ultrafiltration.

The effect of purification of laccase on cellulose wet adhesion was studies and the result was shown in Table 3-5. With the same units of laccase addition, the wet adhesion showed no significant difference, which indicate purification of laccase was unnecessary in PVAm-TEMPO/Laccase/O_2 approach.

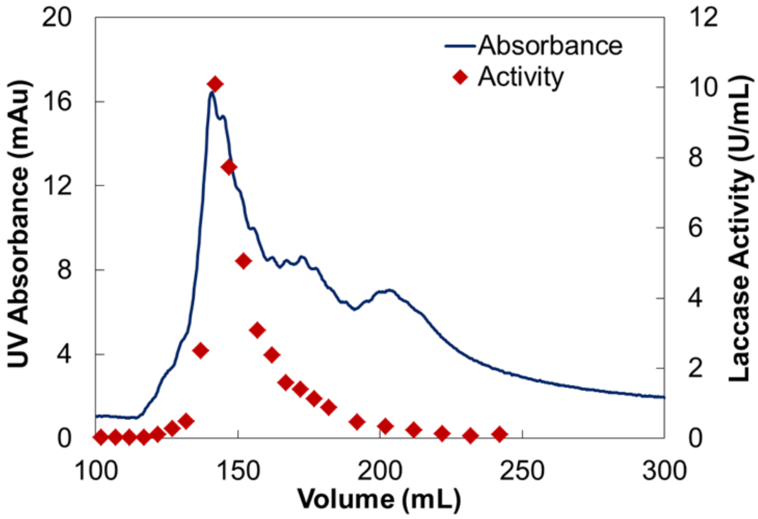


Figure 3-24 Purification of laccase: chromatography on DEAE column. Profiles corresponding to optical density at 280 nm (solid line) and laccase specific activity (solid diamond)

Table 3-5 Comparison between purified and unpurified laccase on cellulose oxidation

Samples	Laccase (U/mL)	PVAm-TEMPO (mg/L)	Coated PVAm (mg/m^2)	Average Delamination force (N/m)
Purified Laccase	1.27	66.67	15	28.2±1.6
Unpurified Laccase	1.27	66.67	15	29.0±2.0

3.2.2.10 Effect of adhesive application method on wet adhesion

Three methods (direct coating, adsorption, one step) were used to apply adhesive between cellulose after the same oxidation process. The influence of different methods on cellulose wet adhesion were summarised in Table 3-6. The direct coating method resulted in a huge increase of wet adhesion, 35.5 N/m, which meant there were enough aldehyde groups and amine groups in this system to form covalent bonds linking cellulose and PVAm together. The adsorption method resulted in a lower level of wet adhesion of 12.6 N/m with the same yield of oxidation, which meant the adsorbed PVAm amount was not as much as directly applied PVAm. However, with the one step method, laminates showed a very small wet adhesion of 5.1 N/m, which meant the PVAm-TEMPO 6 absorbed on cellulose in oxidation system cannot offer enough amine groups to improve the wet adhesion.

Table 3-6 Effect of adhesive application method on wet adhesion

Adhesive application method		Average delamination force (N/m)
Method	Solution	
Direct Application	PVAm (15 mg/m^2)	35.5±2.1
Adsorption	PVAm (0.5 g/L, pH 5)	12.6±2.0
"one step"	N/A	5.1±1.3

4 Discussion

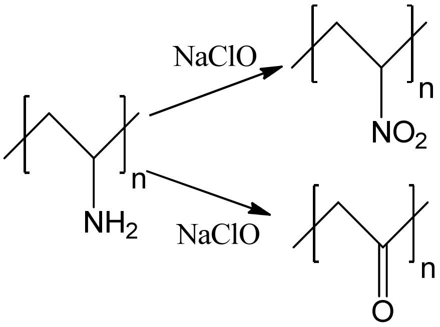
4.1 The grafting extent of PVAm-TEMPO

The previous study has shown that 4-carboxyl-TEMPO can be successfully grafted onto PVAm chain by conjugation. The grafting extents of PVAm-TEMPO were determined to be around 30%, by conductometric titration based on the assumption that all loss in titratable amine groups was because of TEMPO grafting. It should be noted that amine groups can form complex and chelates with some metal ions, hydrochloride, etc.[49] The existing of these complex and chelates affect the titration result.

In this study, the grafting extents of PVAm-TEMPO samples were determined by EPR spectroscopy and conductometric titration. According to the signal of TEMPO derivatives in PVAm-TEMPO solution, the concentration of TEMPO in solution can be calculated. The TEMPO grafting extents by weight percent were determined. Combining EPR results with titration results, the TEMPO grafting extents by mole percent were estimated. Compared with titration method in Ren's study, EPR method provided more straightforward information about TEMPO content for grafting extent calculation.

4.2 The stability of PVAm in sodium hypochlorite (NaClO)

The stability study result indicates amine groups are not stable in NaClO environment at pH 10.5. The oxidation of primary amine by NaClO has been reported by several papers.[50] Most of them concluded that the oxidation reaction is complicated with a lot of side reactions existing, as NaClO is a strong oxidant. The final reaction products may vary with different situations. In this study, the color change of PVAm solution and the new band showed at 1600 cm^{-1} in FT-IR spectrum after oxidation both suggest that some of amine groups might be oxidized to nitro or ketone groups.[50a, b] The possible reactions are showed in Scheme 4-1.



Scheme 4-1 scheme of possible reactions of PVAm during sodium hypochlorite oxidation

4.3 Surface oxidation of cellulose membranes through PVAm-TEMPO oxidation

In paper making industry, paper strength is a function of individual fiber strength and fiber-fiber bond strength. The mechanical strength of cellulose fibers is one of the most important features. The longer and stronger fibers used in paper making process will result in a higher strength paper product. However, the shorter fibers will generally end up with a significant loss in tensile strength and fracture toughness of paper.[6] In order to increase the wet-strength of paper, fiber-fiber bonds should be introduced among cellulose fibers. Whereas, the fiber-fiber bond strength only depends on surface properties of fibers.[51] Thus, cellulose modification is required only on the fiber exterior surface to build up interfibers bonds. However, cellulose fibers are porous. The average pore volume on cellulose fibers is in the range of approximately 2-20 nm.[52] It is estimated that molecules bigger than 20 kDa can hardly penetrate into the interior surface of fibers.[53]

In the free TEMPO oxidation, TEMPO and its derivatives with low-molecular-weight can easily permeate into the inner structure of cellulose fibers and oxidize both exterior and interior parts. In this study, regenerate cellulose dialysis membranes (MWCO 10 - 12 kDa) were used as a model of cellulose fibers. From the CLSM images, aldehyde groups generated by free TEMPO oxidation distributed evenly all-over the membrane. However, membrane failures were observed during the peeling tensile. This may be because the oxidation and depolymerisation in interior area weakened the bulk characteristics of cellulose membranes. Furthermore, Isogai et al. have reported the extended oxidation of free TEMPO mediated oxidation resulting in the depolymerisation of cellulose fibers.[25b]

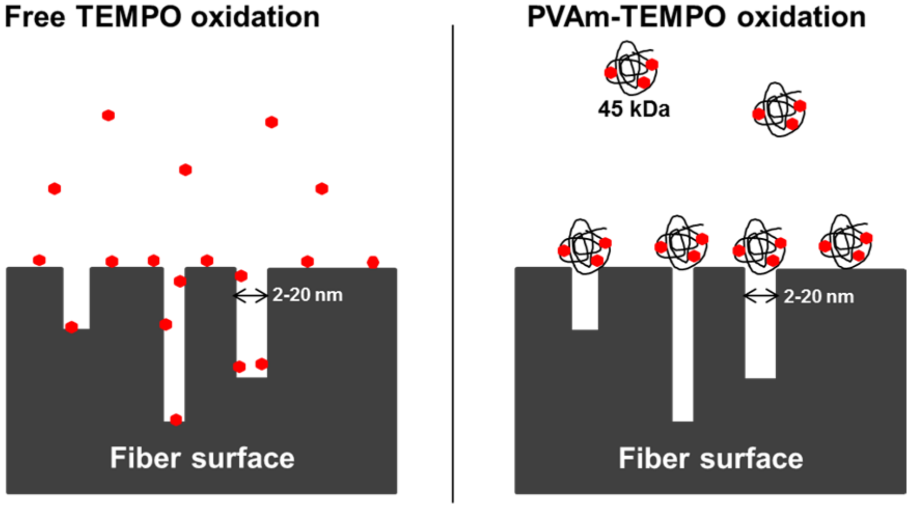


Figure 4-1 distribution of free TEMPO and PVAm-TEMPO on nanopores of a cellulosic fiber surface

Comparing with free TEMPO oxidation, PVAM-TEMPO mediated oxidation is restricted to the exterior surfaces of porous fibers, as confirmed by CLSM images shown in previous section. In addition, membrane failure has never been observed with PVAm-TEMPO approaches. This is because the molecular weight of PVAm-TEMPO is around

45 kDa. The radius of gyration of 22.7 kDa PVAm in 0.2 M HCl and 0.1 M NaCl solution is 14.6 nm.[54] Thus radius of gyration of 45 kDa PVAm in reaction solution should be much larger than the pour size. PVAm-TEMPO is hard to penetrate into the inner structure of cellulose fibers as illustrated in Figure 4-1. Thus, the fiber mechanical property weakening by oxidation of fiber interior surfaces can be prevented.

4.4 TEMPO concentration on cellulose surface during free TEMPO and PVAm-TEMPO oxidation

TEMPO is a hydrophobic and non-charged small molecule. It has no special affinity to cellulose substrate.[55] However during TEMPO mediated oxidation, TEMPO is oxidized to TEMPO$^+$ (oxoammonium TEMPO) by the primary oxidant. The cationic TEMPO$^+$ might be electrostatically attracted into the slightly anionic cellulose substrate.[56] The adsorption of 4-amino-TEMPO on cellulose fibers was published by Hu et. al[57], which is supposed to attach more amount on cellulose than the adsorption of free TEMPO due to the ionic bonding between its amino ($-NH_2$) groups and the pulp carboxyl (-COOH) groups. No TEMPO adsorption on cellulose data has been found. Assuming the attached amount of TEMPO on cellulose membrane (carboxyl contents: 20meq/kg cellulose)[58] equals to that of 4-amino-TEMPO on the same cellulose, the free TEMPO concentration on cellulose surface is estimated to be 3 mM. However, in the case of PVAm-TEMPO mediated oxidation, TEMPO is much more concentrated at the cellulose surface by tethering TEMPO to PVAm. Assuming that the saturated adsorbed density of PVAm on cellulose is 1 mg/m^2 and the thickness of the adsorbed monolayer is 5 nm,[59] the concentration of TEMPO moieties in the absorbed PVAm-TEMPO 6 monolayer is 173 mM. Both cases were illustrated in Figure 4-2 and detailed calculation was shown in Appendix section.

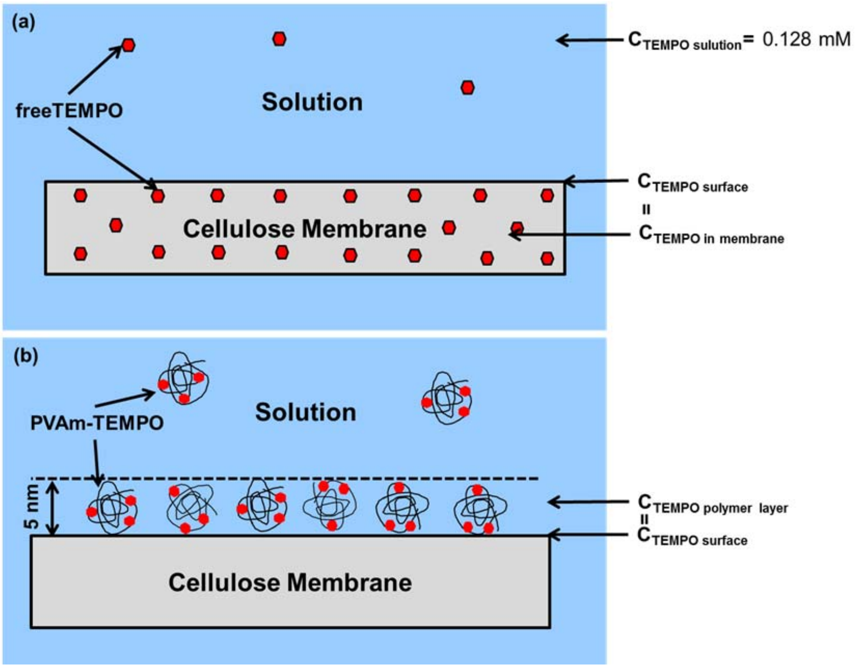


Figure 4-2 TEMPO concentration on cellulose surface during free TEMPO (a) and PVAm-TEMPO (b) oxidation

4.5 Mechanism of PVAm-TEMPO/laccase/O_2 oxidation of cellulose

The mechanism of oxidation of cellulose through PVAm-TEMPO/laccase/O_2 approach was explained in Figure 4-3. In this approach, PVAm-TEMPO first adsorbs onto cellulose surface electrostatically. PVAm is a weak polybase, whose overall cationic charge density is varying through the whole pH range. It was confirmed that PVAm is cationic at pH 5. While, cellulose surface normally carries negative charge at pH 5.[59] The detailed adsorption kinetics, adsorption amount and effects of conditions of PVAm adsorption onto cellulose were published in several papers.[59-60] Therefore, there is electrostatic attraction between PVAm-TEMPO and cellulose. In the second step, the TEMPO moieties mediated oxidation is initiated by the addition of laccase. The isoelectric point of laccase was reported to be around 4.3-4.5.[47] It indicates that laccase is net negatively charged at reaction condition, pH 5.[61] Thereby, anionic laccase can form complexes with cationic PVAm-TEMPO. In this approach, a saturated PVAm-TEMPO adsorbed layer is formed at first with PVAm-TEMPO greatly in excess of the saturation level. The excess of cationic PVAm-TEMPO in solution forms complex with the subsequently is then added to the anionic laccase in the bulk phase, and that the complex subsequently deposited onto fiber surfaces.[62] The PVAm-TEMPO/laccase catalyzed oxidation of cellulose takes place at the same time and converts the primary alcohol groups on cellulose to aldehyde or carboxyl groups. In the end, amine containing polymer is provided to form covalent bonds with aldehyde groups to increase the wet adhesion. According to the experimental results, the oxidation is necessary to achieve increased wet adhesion. The property of PVAm-TEMPO/laccase complex varies with the charge ratio of acidic and basic groups and affects the wet adhesion result. Further study about PVAm-TEMPO/laccase complexes is required in the future.

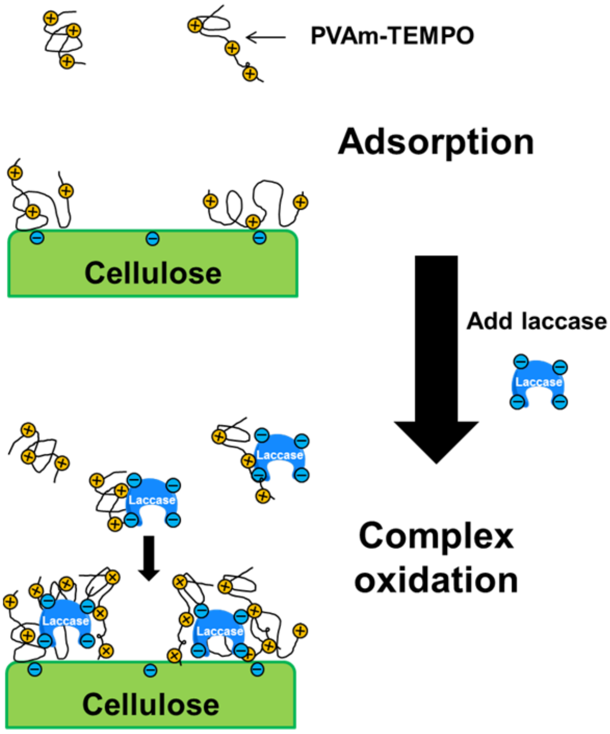


Figure 4-3 Proposed mechanism of PVAm-TEMPO/laccase/O_2 oxidation of cellulose

4.6 Comparison of two oxidation systems

As shown in the previous sections, either NaClO/NaBr/TEMPO oxidation system or O_2/laccase/TEMPO oxidation system has its pros and cons. For the NaClO/NaBr/TEMPO system, short time of oxidation and low TEMPO grafting extent are required to give relatively high wet adhesion between cellulose. However, the large amount of NaClO used in this system affect the stability of PVAm-TEMPO during the oxidation process. In addition, halide reagents and high pH solution used in this system are environmental harmful. While, the O_2/laccase/TEMPO oxidation system not only avoids the harmful halide reagents, but also improves the chemical stability of PVAm in oxidation solution because of the high substrate selectivity of the enzyme. However, in laccase mediated system, it required a relatively high TEMPO substitute degree.

In order to better compare the TEMPO grafting extent effect between NaClO/NaBr/TEMPO and O_2/laccase/TEMPO oxidation systems, two sets of data described in previous sections were plotted in Figure 4-4. As shown in Figure 4-4, the cellulose wet adhesion is not sensitive to the TEMPO grafting extent in NaClO/NaBr/TEMPO oxidation system. A very little TEMPO substitute degree is enough to oxidize well and give strong wet adhesion between cellulose. However, the TEMPO grafting extent is important for O_2/laccase/TEMPO oxidation system. About 10% TEMPO substitute is required. This is probably because in NaClO/NaBr/TEMPO oxidation system, TEMPO performs as a catalyst mediating the reaction. With NaClO, a small molecule, playing as a role of primary oxidant, TEMPO moieties can efficiently get in touch with NaClO and catalyze the reaction. However, as laccase is net negatively charged at reaction condition, it can complex with cationic PVAm-TEMPO. In laccase catalyzed oxidation system, laccase must in molecule contact with TEMPO moieties to catalyze the oxidation reaction. Thereby, the catalytic efficiency of laccase/TEMPO system may be limited due to the less ability of laccase and TEMPO moieties to move around. In other words, there must be sufficient amount of TEMPO moieties around laccase to achieve an ideal yield of oxidation. This might be the reason why a relatively higher TEMPO substitute degree is required in laccase mediated oxidation system.

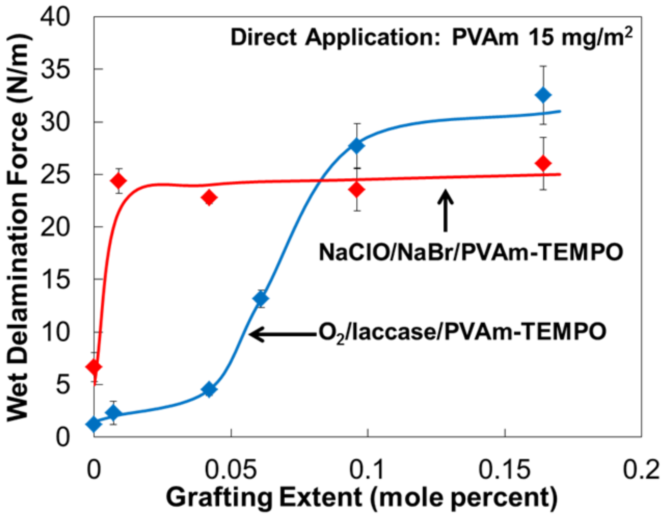


Figure 4-4 Comparison of the influence of TEMPO grafting extent on cellulose wet adhesion in NaClO/NaBr/TEMPO and O_2/laccase/TEMPO oxidation system.

4.7 Possible reasons of one-step failure

The simplified one-step approach combining PVAm-TEMPO adsorption, catalyzing oxidation and covalent bonding without extra application of PVAm has never succeeded in this study. Summarizing the experimental results achieved in this study and previous research conclusion, here are two possible reasons listed as follows.

Low amine content of polymer The amine groups on PVAm play a very important role in this study including polymer adsorption and covalent bonding with aldehyde groups. As the adsorption of cationic PVAm onto anionic cellulose surface is driven by electrostatics, the amine content on polymer directly affects the adsorption amount and configuration. In addition, wet adhesion achieved in this study is mostly due to the covalent bonds between aldehyde groups on cellulose and amine groups on PVAm. Therefore, the amine content of polymer may affect the bond amount as well. However, during the whole approach, there are several possibilities that the amine content of PVAm is lowered. For instance, amine is a reducer which may react with strong oxidant in the oxidation reaction of cellulose. The grafting of TEMPO derivative on PVAm also lowers the amine content of polymer. With the lower amine content, there might be less amount of PVAm-TEMPO adsorbed on cellulose and less amine provided to form covalent bonds with modified cellulose. The effect of PVAm hydrolysis degree on wet adhesion by DiFlavio is shown in Figure 4-5. The lower PVAm hydrolysis degree ended up lowering wet adhesion. This phenomenon can be explained by the drop of amine content with the lower hydrolysis degree. This theory can also explain the delamination force difference achieved between one-step method and PVAm adsorption method in this study.

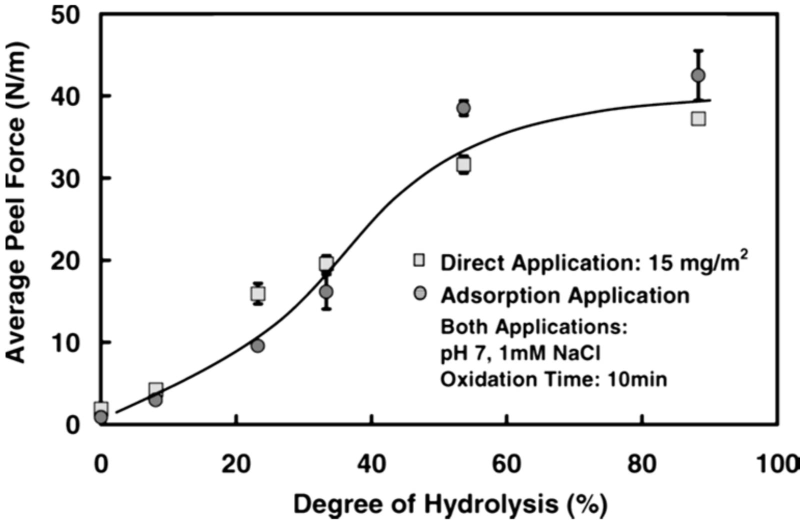


Figure 4-5 The effect of PVAm hydrolysis degree on the delamination force [3]

Low attraction of cellulose for PVAm-TEMPO One of the very critical steps in one-step approach is adsorption of PVAm-TEMPO onto cellulose. Besides of the amine content of PVAm-TEMPO, the attraction feature of cellulose membranes may also be a factor affecting the final result. It has already been reported by several papers that the wet adhesion of cellulose has very little relationship with carboxyl group content of cellulose.[23] Only surface aldehyde groups contribute to the paper wet-strength development.[23, 63] However, the carboxyl group content affects the adsorption behaviour of cationic polymer onto cellulose. Marton and Marton have reported that increasing the negative charged character of fibers can be used as a way to enhance the ability to adsorb cationic starch.[64] Furthermore, Retulainen et al. showed that higher-yield fibers, having a higher density of negative charge, were able to adsorb cationic starch more efficiently compared to low-yield, bleached kraft fibers.[65] However, Tanaka et al. has shown the influence of charge density on the adsorption of cationic polymer into anionic polystyrene latex, which the higher charge density resulted in a lower adsorption due to the high fraction of flat configuration of adsorbed polymer. Therefore, the carboxyl content of cellulose may affect the adsorption of PVAm onto cellulose membranes. According to the result of Geffroy et al.'s research, the maximum saturated adsorption of PVAm onto unoxidized cellulose was around 1 mg/m^2.[59] Whereas, around 10 mg/m^2 of PVAm coverage was required to observe increased wet adhesion. The lower PVAm coverage resulted in lower wet adhesion with the same yield of oxidation.[3] (Figure 4-6) Thus, the wet adhesion difference between direct application method and PVAm adsorption method may be a result of the different PVAm coverage.

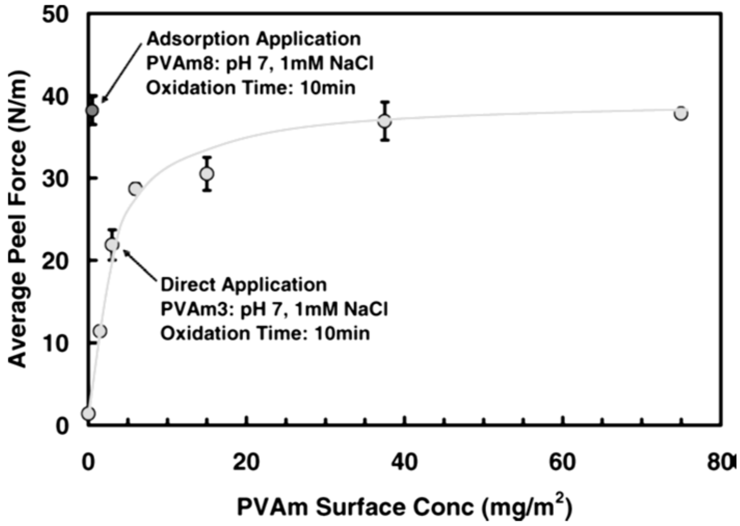


Figure 4-6 the influence of PVAm coverage on peeling force[3]

5 Conclusions

In order to improve paper wet strength through a more eco-friendly and efficient approach, this study further investigated the influences of oxidation conditions on cellulose wet adhesion and developed a new approach combining an enzyme mediated oxidation of cellulose with supported TEMPO and a new commercial wet-strength additive, polyvinylamine. Following previous research, the properties of PVAm supported TEMPO were further characterized as well. The main conclusions are summarized as follows.

5.1 Synthesis and characterization of PVAm-TEMPO and stability of PVAm in NaClO

1) PVAm was not stable in sodium hypochlorite environment at pH 10.5. Amine groups can be oxidized to nitro groups and PVAm chains can be degraded by NaClO into a shorter chain with lower molecular weight.

5.2 PVAm-TEMPO application in NaClO/NaBr/TEMPO oxidation of cellulose

Following previous research, the influences of PVAm-TEMPO oxidation conditions on cellulose wet adhesion with extra PVAm applied as an adhesive were investigated in this study.

1) The oxidation of cellulose by PVAm-TEMPO/NaClO/NaBr system took place in a short time. Oxidation of about 10 minutes was enough to achieve a high wet adhesion between cellulose. At longer oxidation times, PVAm-TEMPO treatment reduced cohesive film failure to cellulose compared with free TEMPO treatment.

2) In PVAm-TEMPO/NaClO/NaBr oxidation system, NaClO performed as a primary oxidant. There was almost no wet adhesion between cellulose without addition of NaClO. Wet adhesion between cellulose increased with the raising of NaClO concentration.

3) The oxidation of cellulose by PVAm-TEMPO/NaClO/ NaBr system took place in basic environment. The reaction pH was optimized at pH 9.5 with the highest wet adhesion between cellulose membranes.

4) Performed as mediators, TEMPO moieties were necessary for the oxidation of cellulose. However, the wet adhesion between cellulose membranes was not sensitive to

the TEMPO grafting extent. As low as 0.9% TEMPO moieties per amine group were enough to oxidize cellulose membranes and achieve a high wet adhesion.

5.3 PVAm-TEMPO application in O_2/laccase/TEMPO oxidation of cellulose

In this study, PVAm-TEMPO was applied into the O_2/laccase/TEMPO oxidation system, which can avoid harmful halogen salts and high pH solution resulting in a more eco-friendly approach.

1) Wet adhesion between cellulose membranes can be raised from no adhesion to 35 N/m with a proper treatment by PVAm-TEMPO and laccase mixture. In this approach, laccase and PVAm-TEMPO both played very important roles as mediators transporting oxidant potential energy from oxygen to cellulose. Furthermore, activated laccase must touch PVAm-TEMPO to catalyze the oxidation.

2) With the same TEMPO moiety concentration, wet adhesion between cellulose membranes treated by PVAm-TEMPO was 100% higher than those treated by free TEMPO. PVAm-TEMPO and laccase mixture treatment to cellulose can generate aldehyde groups on cellulose. Furthermore, the aldehyde groups formed by PVAm-TEMPO approach distributed only on the exterior surfaces of cellulose membranes, while aldehyde groups formed by free TEMPO approach distributed evenly all over the exterior and interior parts of cellulose membranes.

3) The oxidation of cellulose by PVAm-TEMPO/laccase/O_2 approach took place immediately after the mixing of PVAm-TEMPO with laccase. 30 min of oxidation was needed to achieve the highest wet adhesion result.

4) The TEMPO grafting extent of PVAm-TEMPO played a significant role in PVAm-TEMPO/laccase approach. High wet adhesion between cellulose were obtained when the grafting extents were above about 4% per amine group, while with grafting extents lower than 2%, very small wet adhesion was achieved.

5) Cationic PVAm can bond with anionic laccase at pH 5 and generated complexes, which aggregated and formed precipitates. PVAm deactivated laccase as a noncompetitive inhibitor. The initial reaction rate was slowed down with appearance of PVAm. After a period of time, when the entire enzyme was deactivated, laccase catalyzed oxidation reaction stopped.

6) Very small wet adhesion was gained between cellulose membranes fabricated by layer-by-layer self-assembly of PVAm-TEMPO and laccase approach. This result indicates that PVAm-TEMPO and laccase complex formed in previous approach contributes the most mediators in oxidation reaction of cellulose. The ratio of PVAm-TEMPO and laccase affected the oxidation result of cellulose by affecting the property of their complexes.

5) The comparison of different adhesive application methods showed that direct coating 15 μL of 1 g/L PVAm onto oxidized cellulose gave the highest wet adhesion. While no wet adhesion was gained between cellulose membranes treated by "one step" method in which laminates were prepared directly after oxidation without extra PVAm application. With the same oxidation yield of cellulose, adhesive with higher amine content, PVAm, gave a higher wet adhesion than that with lower amine content, PVAm-TEMPO, did.

On the basis of experimental data and the data available in literature, suggestions can be made for the possible mechanism of PVAm-TEMPO/laccase catalyzed oxidation of cellulose. In the first step, cationic PVAm-TEMPO adsorbs on cellulose surface. In the second step, anionic laccase is added to the system. Laccase and PVAm-TEMPO form complexes and further deposit on cellulose surface. At the same time, laccase in molecular contacts with TEMPO moieties on PVAm-TEMPO and converts TEMPO moieties to activated $TEMPO^+$. Performing as a mediator, activated $TEMPO^+$ contacts with and oxidized the primary alcohol groups on cellulose surface to aldehyde and/or carboxyl groups. After the oxidation, extra PVAm solution is applied between cellulose as adhesive. Because of the covalent bond formation between aldehydes on oxidized cellulose and primary amine groups, the increased wet adhesion between cellulose is achieved.

In summary, grafted TEMPO in enzyme catalyzed approach offers four significant advantages over small molecule TEMPO (free TEMPO). First, as PVAm has high molecular weight, the oxidation of porous fibers is restricted to the exterior surfaces only, which avoids the excessive oxidation of interior surfaces and prevents from weakening the mechanical property of fibers. Second, TEMPO is concentrated on cellulose surfaces by tethered it to PVAm, compared with water-soluble free TEMPO. Thus the total dose of TEMPO required to oxidize fibers is much less than that required by free TEMPO. Third, the enzyme catalyzed approach not only avoids the harmful halide reagents, but also improves the chemical stability of PVAm in oxidation solution because of the high substrate selectivity of the enzyme. Fourth, TEMPO stays with solid phase by bounding it with polymer. It is less toxic than free TEMPO.

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Appendix

TEMPO concentration on cellulose surface

Cellulose membrane dimensions:

membrane volume:

$$v := 20\ mm \cdot 60\ mm \cdot 0.13\ mm + 30\ mm \cdot 60\ mm \cdot 0.13\ mm = \left(3.9 \cdot 10^{-4}\right)\ L$$

membrane weight: $m := 0.32\ gm$

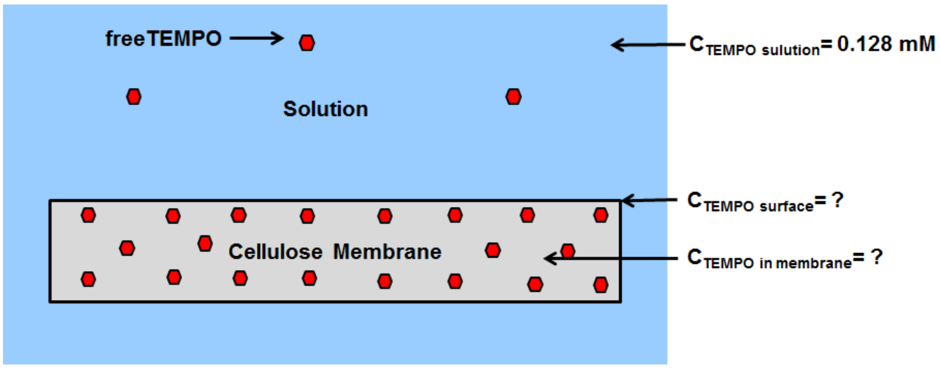
1. Free TEMPO:

4-Amino-TEMPO attached on bleached kraft pulp (acid group: 35 meq/kg pulp) (from reference)

$$a_{amino\_TEMPO} := \frac{3.5}{1000}\ \frac{mol}{kg} = \left(3.5 \cdot 10^{-6}\right)\ \frac{mol}{gm} \qquad \text{pulp}$$

Assumption:
TEMPO attached on cellulose: $a_{TEMPO} := a_{amino\_TEMPO} = \left(3.5 \cdot 10^{-6}\right)\ \frac{mol}{gm}\ pulp$

$C_{\text{TEMPO on surface}} = C_{\text{TEMPO in membranes}}$



Adsorbed freeTEMPO concentration:

$$C_{TEMPO\_in\_membrane} := a_{TEMPO} \cdot \frac{m}{v} = 0.003\ \frac{mol}{L}$$

2. PVAm-TEMPO:

(TEMPO grafting extent: 15.77% w/w)

Assumption:

PVAm-TEMPO adsorbed density: $a := \frac{1}{1000} \frac{gm}{m^2}$

Polymer monolayer thickness: $l := 5\ nm = \left(5 \cdot 10^{-9}\right)\ m$

Molecular weight of TEMPO moiety: $m_w := 182.25\ \frac{gm}{mol}$

Adsorbed PVAm-TEMPO, TEMPO concentration on surface:

$$C_{TEMPO\_polymer\_layer} := \frac{a \cdot 15.77\%}{m_w} \frac{1}{l} = 0.173\ \frac{mol}{L}$$