

# REACTIVE POLYVINYLAMINE- GRAFT-TEMPO/LACCASE COMPLEX GIVING WET CELLULOSE ADHESION

*Jieyi Liu and Robert Pelton*

McMaster University, Hamilton, Ontario, Canada

## ABSTRACT

Cellulose surfaces are activated for wet adhesion, bioconjugation and other applications by the introduction of a “primer” layer consisting of a covalently bonded polyelectrolyte complex based on laccase and polyvinylamine with pendent TEMPO groups, PVAm-T. The laccase, in conjunction with dissolved oxygen, activates the TEMPO moieties on PVAm-T, facilitating the oxidation of primary hydroxyl groups on the cellulose surface. The resulting cellulosic aldehydes are free to couple covalently with amine groups on the PVAm-T. The generally accepted mechanism of TEMPO oxidation is that the primary oxidant converts TEMPO into a reactive oxoammonium ion that shuttles an electron to primary alcohols. Since the translational mobility of TEMPO will be limited when grafted to a polymer and present as a polyelectrolyte complex with laccase, it is proposed that the activated oxoammonium ions jump along the PVAm-T chain, from TEMPO to neighbouring TEMPO. Wet adhesion of laminated regenerated cellulose sheets was used as the primary assay indicating the presence of covalent bonding.

## INTRODUCTION

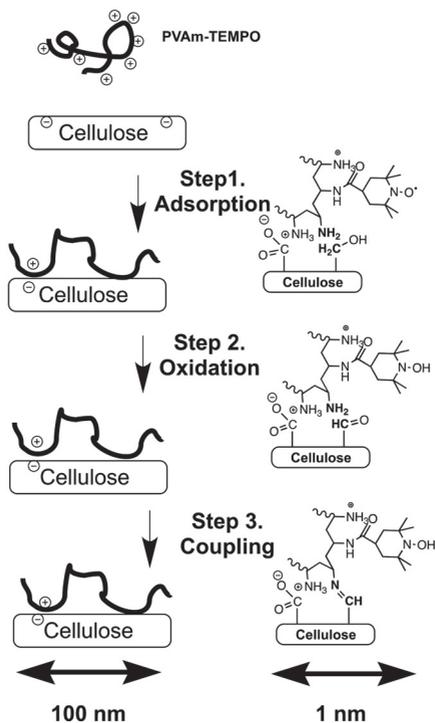
Many of the most interesting potential new applications of cellulose fibers, whiskers, and nanocrystals require chemical modification of the cellulose surfaces [1]. One of the most promising approaches, championed by Isogai and his

coworkers, has been TEMPO mediated oxidation [2]. Under relatively mild conditions, the primary (C6) hydroxyls are sequentially converted first to aldehydes, and then to carboxyl groups, while inducing little main chain damage. Taken to an extreme, TEMPO mediated oxidations convert wood pulp fibers into nano-fibrillated cellulose [3], and ultimately to water-soluble polyglucuronic acid [4]. Our interest in TEMPO mediated oxidation arose from observations in our laboratory [5], and by Isogai [6], that amine containing polymers formed strong, covalent attachments to lightly TEMPO oxidized cellulose. Strong polymer binding to cellulose is important for wet adhesion (i.e. wet strength), and for functional paper surfaces such as bioactive paper. Although most cationic (positively charged) polymers will adsorb onto pulp fibers, the physical attachment is relatively weak. For example, polyDADMAC, a common cationic water soluble polymer used in papermaking to improve retention and for fixing pitch, contributes nothing to wet [7] or dry [8] paper strength.

The early TEMPO oxidation recipes have problems which limited TEMPO application in conventional papermaking. TEMPO is an oxidation mediator that must be activated by a primary oxidant. The early recipes used bleach at alkaline pH as the primary oxidant; these conditions are more extreme than the wet-end conditions in most mills. In addition, bleach is a potent oxidizer. Herein we will show that polyvinylamine, PVAm, a popular papermaking polymer, is slowly decomposed in the presence of bleach.

In addition to the bleach, TEMPO itself causes difficulties when applied to conventional papermaking. Active TEMPO concentrations are usually in the range of 1 to 8% on pulp, which, considering the water volumes on in the white-water system, is a significant expense. In addition, there are some environmental impacts associated with TEMPO [9]. Finally, TEMPO is a low molecular weight water-soluble chemical. Herein we will show that TEMPO can penetrate into porous cellulose, causing significant damage to fiber interiors.

In previous work we proposed that some of the drawbacks of TEMPO mediated, cellulose fiber oxidation could be alleviated by covalently attaching the TEMPO to PVAm, giving a graft copolymer, PVAm-T[10]. Our vision, shown in Scheme 1, was that the cationic PVAm adsorbs onto cellulose, and in the presence of bleach, the grafted TEMPO moieties catalyze the oxidation of the cellulose surface, generating aldehyde groups that immediately react with the abundant primary amine groups remaining on the PVAm-T. Our particular interest in this work was to increase wet adhesion between cellulose surfaces. The initial results showed that PVAm-T was indeed effective at promoting cellulose oxidation in the presence of dilute bleach, as evidenced by strong wet adhesion with PVAm. Furthermore, this work suggested that much less PVAm grafted TEMPO was required compared to free TEMPO oxidations, because the polymer confined the TEMPO moieties near the cellulose surface. Nevertheless, the use of bleach as a primary oxidant was not attractive.



**Scheme 1.** Interaction of activated PVAm-T with cellulose.

In an effort to replace bleach as a primary oxidant, we evaluated laccase in the presence of oxygen as the primary oxidant [11]. Laccase catalyzed oxidations have been extensively studied in the forest products sector for bleaching, lignin reactions, pitch removal and paper strength enhancement [12] Furthermore, it has been long known that the wet adhesion between cellulose surfaces can be enhanced by mild oxidation, presumably because of covalent crosslinking from aldehyde groups, both without [13] and with strength enhancing polymers [2]. Therefore it is not surprising that laccase plus mediators increase the paper wet strength [14–16] In all of the prior work the mediator, usually TEMPO, was present as a small molecule that could easily shuttle between the enzyme and the ultimate targets of the oxidation. At the outset of this work we expected that PVAm-T would not function as a mediator for laccase catalyzed oxidation because the highly cationic PVAm-T forms polyelectrolyte complexes with laccase, an anionic protein. We expected that the PVAm-T would block the active sites on the laccase and, more importantly, that the immobilized TEMPO moieties would

not have enough translational freedom to interact with both laccase and with the cellulose surfaces. Nevertheless laccase + oxygen+ PVAm-T does indeed oxidize cellulose, and the results herein suggest laccase offers many advantages over bleach as the primary oxidant.

## EXPERIMENTAL

**Materials** Polyvinylamine (PVAm) with a number-average molecular weight of 45 kDa, and a degree of hydrolysis of 75% was obtained from BASF, Ludwigshafen (Lupamin® 5095). Fully hydrolyzed PVAm (100% DH) was achieved by being treated in 5% NaOH solution at 75°C for 5 day. PVAm samples were exhaustively dialyzed and freeze-dried for storage. Conductometric titrations were used to measure the concentrations of PVAm stock solutions.

Regenerated cellulose dialysis tubing (Spectra/Por® 2, 12–1400 Da MWCO) was purchased from Spectrum Laboratories. The tubing was cut into two sizes of rectangles: top membranes (2 cm×6 cm) and bottom membranes (3 cm×6 cm), and then were boiled in deionized water to remove plasticizer.

Laccase from *Trametes versicolor* (EC 1.10.3.2), TEMPO, 4-carboxy-TEMPO, N-Hydroxysulfosuccinimide sodium salt (sulfo-NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma-Aldrich. Other salts for buffer preparation were purchased from Caledon Laboratories Ltd. Water was purified with a nanopure purification system to a specific resistance of at least 18MΩ cm<sup>-1</sup>.

**PVAm-T Preparation and Characterization** As described in our previous publication,[17] PVAm-T was prepared by EDC/sulfo-NHS-mediated conjugation of 4-carboxy-TEMPO to PVAm. The recipes are shown in **Table 1**. PVAm-T product was dialysed for two weeks, freeze-dried and stored in a desiccator.

Conductometric titration and Electron Paramagnetic Resonance (EPR) spectroscopy were performed to determine the TEMPO content of product. Using PC titrate (Man-Tech Associates), conductometric titration was conducted to determine the primary amine content in lyophilized PVAm-T [17]. EPR spectroscopy was performed on a Bruker ELEXSYS E580 spectrometer at 140K in an EPR tube. Normally 10 g/L PVAm-T solutions were prepared for EPR spectroscopy. 120 μL solutions in EPR tubes were frozen by liquid nitrogen then thawed in a vacuum for degasification. TEMPO concentrations in PVAm-T solutions were determined by double integration of the EPR signal using a 8.7 mM TEMPO solution as a standard.

**Laccase Activity Assay** Laccase activity was determined by monitoring the oxidation of ABTS at 420 nm ( $\epsilon_{420}=36\ 000\ \text{M}^{-1}\ \text{cm}^{-1}$ ). 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  $\mu$ mol of ABTS/min at 25°C.

**Cellulose Oxidations.** Two primary oxidants were used to oxidize cellulose membranes, bleach (NaClO + NaBr) or laccase + O<sub>2</sub>. In addition, two forms of TEMPO were employed, free TEMPO and TEMPO grafted onto polyvinylamine (PVAm-T).

**Bleach Based Oxidations.** 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-T. The solution was stirred for 30 min to give a monolayer of PVAm-T adsorbed on the cellulose surfaces. Oxidation of cellulose was initiated by adding 0.68 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 deionized water three times.

**Laccase Based Oxidations.** In a typical 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 30min. Then 20 mL of 1 mg/mL laccase solution (190 Units) filtered by syringe filter (0.45  $\mu$ m) was added dropwise 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 with buffer three times to remove excess unabsorbed polymer.

**Laminate Preparation and Delamination.** Wet cellulose adhesion test laminates consisting of two cellulose membranes were prepared using direct application methods. In this method, the bottom cellulose membrane was placed on a polished TAPPI standard stainless-steel drying plate. The excess surface water was removed by gently dabbing with Kimwipes. To give two cellulose membrane tails for attachment to Instron clamps and a uniform crack in the laminate, a piece of Teflon tape (40 $\times$ 12.7 mm, G.F. Thompson, TWB480P) was placed across top edge of the bottom membrane. Then a 15  $\mu$ L drop of PVAm solution (1g/L) was applied using a 20  $\mu$ L micropipette (ependorf) onto the bottom membrane. The top membrane was progressively placed over the bottom membrane carefully and the polymer solution between membranes spread uniformly with negligible loss of polymer solution. Then the laminate was pressed (89 kN) between two TAPPI standard blotters for 30 min in a Carver press and dried at 23°C and 50% humidity

for 24 h. The delamination tests and data analysis were conducted as illustrated in a previous publication [17].

**Confocal Laser Scanning Microscope (CLSM) Images of Cellulose Membranes** Cellulose membrane samples with different oxidation treatments for 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 excess fluorescein probe was removed by immersing and rinsing membranes against pH 8 Na phosphate buffer 10 times. A cross-section of the cellulose membranes was observed under a laser beam at a wavelength of 488 nm. The images of PVAm-TEMPO treated, soluble TEMPO treated and un-oxidized cellulose membranes were taken with the same CLSM settings.

**PVAm Degradation in Bleach** FT-IR spectroscopy and intrinsic viscosity measurements were used to study the functional group reaction and degradation of PVAm in NaClO. PVAm (75% DH) from BASF was first fully hydrolyzed with 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 M 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 was then placed in dialysis tubes and dialysed against DI water for 2 weeks. A dry solid sample for FT-IR and intrinsic viscosity analysis was achieved by freeze-drying.

Intrinsic viscosity measurements were used to indicate PVAm chain scission. In a typical viscosity measurement, 10 g/L PVAm (100% DH) was prepared in 10ml of 0.1 M NaCl and 0.01 M NaOH and the specific viscosity was measured as a function of dilution with an Ubbelohde viscometer (Size: 75, Cannon Instrument Company) in 25°C water bath. The corresponding intrinsic viscosity values and molecular weights were determined.

## RESULTS

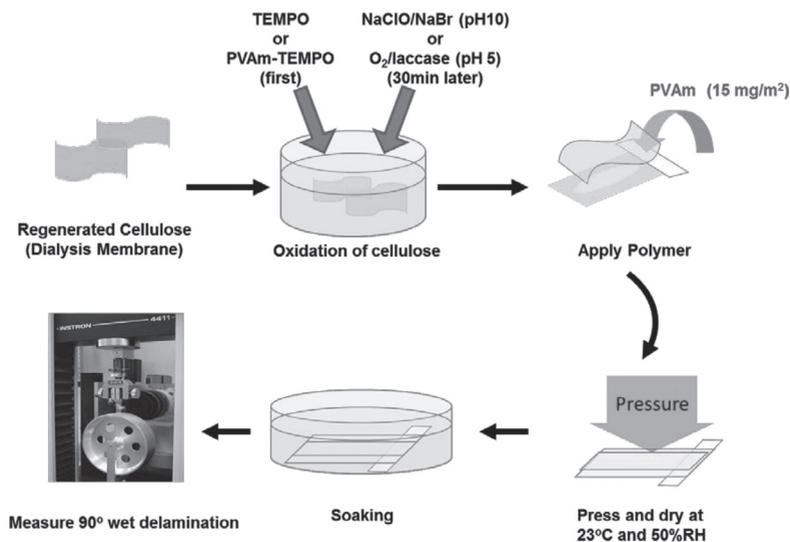
4-carboxy-TEMPO was grafted onto 45 kDa PVAm using EDC/S-NHS catalyzed coupling. Details of the grafting chemistry, polymer purification and characterization were published previously [10] [18]. Table 1 summarizes the grafting conditions and the TEMPO contents of polymers. The grafting extents ranged from 0.007 to 0.164 TEMPO moieties per mole amine group, corresponding to TEMPO mass fractions of 1.2% to 15.8%.

Although this work is directed towards modifying wood fiber surfaces to improve wet adhesion, all the adhesion experiments here involve delamination experiments in which oxidized, polymer coated, cellulose surfaces are laminated,

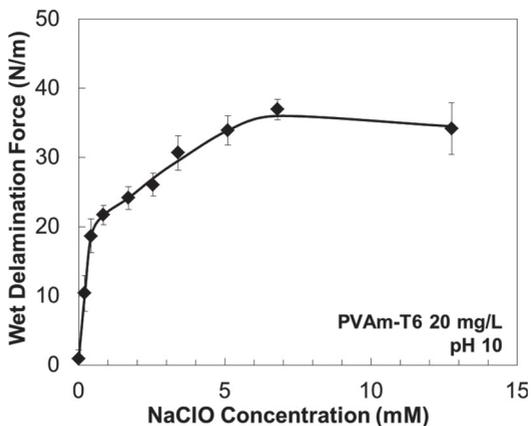
**Table 1.** Grafting conditions and TEMPO contents of PVAm-TX oxidation mediators. The TEMPO moiety contents are expressed as the mole fraction of amine groups bearing a TEMPO

PVAm-TX	PVAm (mg)	4-carboxy-TEMPO (mg)	EDC (mM)	Sulfo-NHS (mM)	TEMPO Content (mole %)
T4	200	120.8	32.21	5.00	9.6
T6	200	241.8	32.21	5.00	16.4
T9	100	17.8	16.10	1.87	4.2
T10	100	8.9	8.05	0.94	0.7
T11	100	3.6	64.42	0.27	0.9
T14	200	149.0	32.21	5.00	10.8
T16	200	150.0	32.21	5.00	6.1

dried at room temperature, and re-wetted before testing. This method was first described at an FRC meeting many years ago [5] and follows from very old work [19]. The sample preparation steps are illustrated in Figure 1. The cellulose laminates are considered as physical models for fiber-fiber bonds in papermaking. The advantage of this approach is the composition of the model joint can be



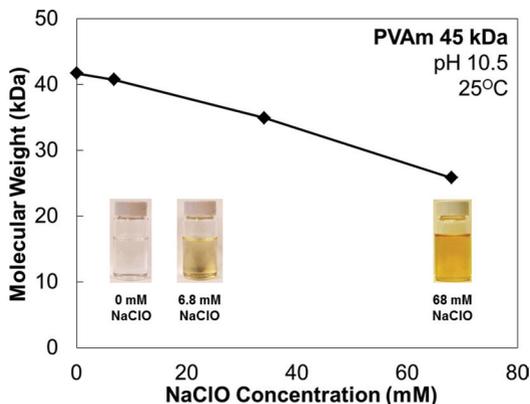
**Figure 1.** Oxidation, laminate preparation and adhesion testing.



**Figure 2.** 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-T. 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.

controlled and measured. In addition, there are no complications, such as poor formation, which complicates handsheet testing in the presence of polymers.

Figure 2 shows examples of delamination force results for a series of experiments designed to show the influence of bleach concentration on adhesion. Without bleach the delamination forces were very low. However, under these conditions, very high wet adhesion values were obtained with as little as 5 mM NaClO. Oxidation kinetics are a function of both bleach concentration and exposure time; the effects of oxidation time will be shown later. Although both the results in Figure 2 and our previous work [10] showed significant increases in wet adhesion when using bleach as the primary oxidant with PVAm-T as the mediator, we were concerned that the NaClO would decompose the PVAm and other functional materials in a papermaking furnish. A series of PVAm 45kDa solutions was exposed to various concentrations of bleach at room temperature for 3 hours. PVAm stability was monitored by measuring the intrinsic viscosity, a measure of polymer molecular weight. Figure 3 shows the resulting viscosity average molecular weights as a function of bleach concentration. PVAm decomposition was apparent from the >25% decrease in MW and the appearance of colour in the solution. The FT-IR spectra (available in a thesis [18]) of the PVAm (100% DH) 45 kDa sample before and after exposure to bleach indicate the formation of N-O bonds, a further indication of the instability of PVAm in bleach.



**Figure 3.** Effect of NaClO concentration on degradation of PVAm. 25 mL of 10 g/L PVAm (100% DH) solution was exposed to various concentrations of NaClO at pH 10.5, 25°C for 3 hours.

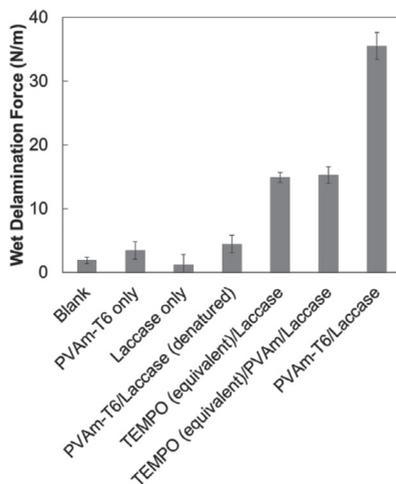
### Laccase/Oxygen as Primary Oxidants

A series of cellulose oxidation experiments was performed to illustrate the critical features, and the wet delamination results are summarized in Figure 4. PVAm-T6 oxidation with laccase gave the highest wet adhesion, even higher than TEMPO. Denaturing the enzyme or eliminating either PVAm-T or laccase gave very low adhesion. Finally, conducting a conventional laccase + TEMPO oxidation was not influenced by the presence of PVAm.

In all the adhesion experiments in Figure 4 and in the previous figures, extra PVAm 45 kDa was placed between the oxidized films before lamination. Our original goal was to have PVAm-T perform as both an oxidation mediator and as a strength-enhancing adhesive. Table 2 compares results from three adhesion experimental conditions. In our standard method, labeled “direct application”, 15 mg/m<sup>2</sup> PVAm was placed between the oxidized cellulose films before lamination. This approach gave the highest delamination forces. By contrast, if the

**Table 2.** The influence of “extra PVAm” on wet adhesion

<i>Adhesive application method</i>		<i>Average delamination force (N/m)</i>
<i>Method</i>	<i>Solution</i>	
Direct Application	PVAm (15 mg/m <sup>2</sup> )	35.5±2.1
Adsorption	PVAm (0.5 g/L, pH 5)	12.6±2.0
None	N/A	5.1±1.3



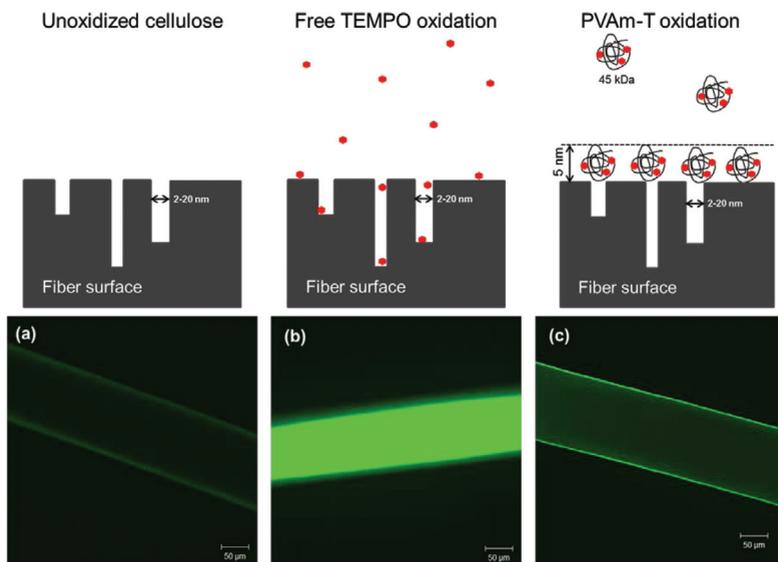
**Figure 4.** Comparing wet adhesion with PVAm-T6 with control experiments. Laccase was denatured at 100°C for 30 min.

oxidized membranes that were coated with PVAm-T/laccase complex receive no further treatment before lamination (i.e. “none”), the resulting wet adhesion was very low. Adsorbing additional PVAm from solution gave intermediate results. Therefore the PVAm-T/laccase treatment of cellulose is functionally equivalent to adding an adhesion primer to cellulose that improves the performance of strength enhancing polymers.

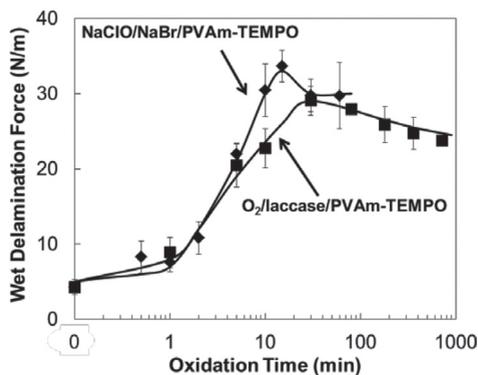
One of the advantages of PVAm-T over TEMPO is that the large polymer cannot migrate into the interior of fibers or porous films. This was demonstrated by oxidizing cellulose films with either TEMPO or PVAm-T mediators. The location of the resulting aldehyde groups was mapped by exposure to a reactive fluorescent dye. Laser scanning confocal images of the film crosssections are shown in Figure 5. The TEMPO mediated oxidation produced aldehydes throughout the thickness of the cellulose membrane, whereas the PVAm-T/laccase complex was restricted to the exterior surface. This result is important because it suggests that excessive oxidation would deteriorate wood fiber strength because the complexes are too big to enter small pores in the fiber walls.

### Comparing Bleach and Laccase Primary Oxidants

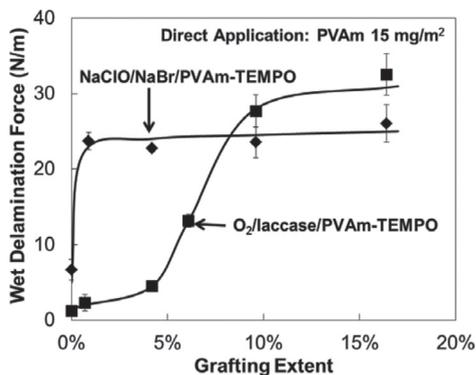
Figure 6 compares bleach and laccase as primary oxidants as functions of the oxidation time. Both oxidants generated strong adhesion after about 10 minutes. Bleach gave a slightly higher maximum adhesion. Both systems showed only



**Figure 5.** Distribution of fluorescein labeled aldehyde groups in cellulose membrane cross sections. CLSM images of (a) un-oxidized membrane cross-section, (b) free TEMPO oxidized membrane cross-section, (c) PVAm-T oxidized membrane cross-section. Aldehydes were labeled with green fluorescein-5-thiosemicarbazide and excited at 488 nm.



**Figure 6.** Influence of oxidation time on adhesion for bleach and laccase activation of PVAm-T. NaClO mediated oxidation experiments were performed in solutions containing 50 mg/L of NaBr and 20 mg/L of PVAm-T and 6.8 mmol/L NaClO at pH 10.5.[17] Laccase mediated oxidation experiments were conducted in pH 5 50 mM sodium acetate buffers consisting of 66.7 gm/L PVAm-T 14 and 133.3 mg/L laccase. The oxidation time varied from 0 min to 12 hours.



**Figure 7.** Influence of TEMPO grafting extent on cellulose wet adhesion comparing NaClO/NaBr/TEMPO(■) and O<sub>2</sub>/laccase/TEMPO (◆) oxidation system. NaClO mediated oxidation experiments were performed in solutions containing 50 mg/L of NaBr and 20 mg/L of PVAm-T and 6.8 mmol/L NaClO at pH 10.5. Laccase mediated oxidation experiments were conducted in pH 5 50 mM sodium acetate buffers consisting of 66.7 gm/L PVAm-T 14 and 133.3 mg/L laccase. The oxidation time varied from 0 min to 12 hours.

minor degradation at very high oxidation times because the immobilized TEMPO moieties on PVAm-T cannot migrate into the interior of the films.

The TEMPO contents in the PVAm-T graft copolymers were expressed as the extent of TEMPO grafting, defined as the fraction of amine groups bearing a TEMPO moiety. Figure 7 shows the influence of the extent of TEMPO grafting on resulting wet adhesion for both bleach and laccase based oxidations. Bleach, as a primary oxidant, required only 1/10th the extent of TEMPO grafting, as did the laccase. In this respect, bleach has a clear advantage as a primary oxidant.

## DISCUSSION

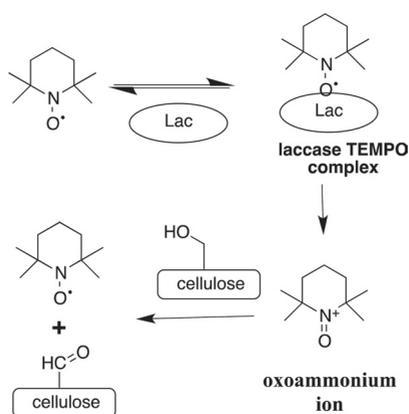
The laccase/PVAm-T cellulose oxidation system is unusual because the specific enzyme/substrate interactions between laccase and the TEMPO moieties are superimposed on the non-specific polyelectrolyte complex formation between laccase, a negatively charged polymer, and the very cationic PVAm-T. Polyelectrolyte complex formation between oppositely charged polymers is a general feature of water-soluble polymers. Complexation is universal, irreversible, and is driven by the large entropy gain from the release of small, mobile counterions that occurs when negative polymer groups form ion pairs with positive polymer groups. The properties of the resulting complexes are sensitive to the charge ratio

of the polymers, the stoichiometric ratio, and the intrinsic water solubility of the polyelectrolyte backbones. If the polymer concentrations are below the overlap concentrations, and if the mixture is not in stoichiometric balance, the complexes are often present as colloiddally stable complexes that are hydrogels. High polymer concentrations, or a stoichiometric balance, often lead to macroscopic precipitate or macrogel formation.

Proteins, including enzymes, are polyelectrolytes and readily form irreversible complexes with oppositely charged polymers. Kulys and coworkers showed that cationic polymers inhibit laccase oxidation of phenolics because of complex formation [20]. Their paper did not speculate about the deactivation mechanism; two obvious possible mechanisms are that with complexation, the enzyme active site is blocked and the substrate access to enzyme buried in colloidal complexes or precipitates is inhibited. We will now consider how complexation might influence oxidation when TEMPO is covalently attached to the complexing polymer.

The conventional mechanism for TEMPO/laccase mediated oxidation involves two consecutive reactions – see Scheme 2. In the first, TEMPO forms an enzyme substrate complex with laccase in the presence of oxygen and the TEMPO is released as the corresponding oxoammonium ion. In the second reaction, the oxoammonium ion forms a transition state adduct with the target alcohol that decomposes to give the corresponding aldehyde and regenerating the TEMPO radical. Thus, some publications describe TEMPO as a shuttle that goes between laccase and the oxidation target [21].

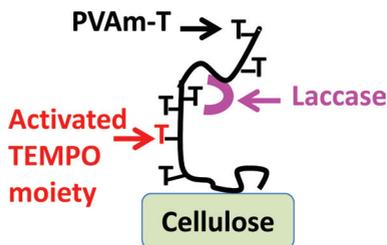
We anticipated that grafting TEMPO to PVAm would impact both reactions in Scheme 2 because the translational mobility of the TEMPO moieties is greatly



**Scheme 2**  $O_2$ /laccase/PVAm-T oxidation of cellulose.

reduced. It is conceivable that during polyelectrolyte complex formation between PVAm-T and laccase, the TEMPO moieties have an opportunity to contact the active site on laccase and then form an oxoammonium ion. It seems less possible that activated oxoammonium ions can then come in molecular contact with the cellulose. Nevertheless, our results show that oxidation is indeed occurring. In view of these restraints it is not surprising that the TEMPO grafting density on PVAm-T must be ten times higher with laccase compared to small, mobile, non-complexing NaClO – see Figure 7. A speculative explanation of our results is that TEMPO moieties oxidized to the oxoammonium form, can pass the electron to a neighbouring TEMPO moiety if the neighbour is sufficiently close – this mechanism illustrated in Figure 8. If this explanation is correct, the results in Figure 7 suggest that one TEMPO on every tenth amine group is sufficient to facilitate the transfer process. By this mechanism we can envisage that when a laccase/TEMPO enzyme substrate complex dissociates, the activated oxoammonium ion only has to have enough mobility to contact a neighbouring TEMPO, it does not have to find the cellulose surface. By randomly moving along the PVAm-TEMPO chain, the oxoammonium ion has an enhanced chance of contacting cellulose.

The covalent attachment of PVAm-TEMPO to cellulose surfaces greatly enhances the utility of cellulose fibers. Herein we have focused on wet adhesion, however, the primary amine groups offer many other opportunities including: surface primary amine groups are excellent sites for bioconjugation of bioactive materials; [22] amines have a high affinity for metal ions facilitating removal from water; amine groups have antibacterial activity; amine groups render pulp fibers cationic, facilitating the removal of anionic trash in papermaking. Because of these potential applications, we view the PVAm-TEMPO/laccase treatment as analogous to “priming a surface” for other uses.



**Figure 8.** Proposed mechanism – activated TEMPO (oxoammonium ion) travels along PVAm-T chain by electron transfer between neighbouring TEMPO moieties, explaining how immobilized TEMPO interacting with immobilized laccase can “shuttle” to a remote cellulose surface.

The principal advantage of using PVAm-TEMPO compared with PVAm + TEMPO is that the total required dose of TEMPO is less, and the cost and problems associated with the loss of TEMPO in the aqueous phase are minimized. Both advantages arise because, by virtue of being grafted, the TEMPO moieties are fixed near the cellulose surface. PVAm-TEMPO can be activated by either bleach or laccase/O<sub>2</sub>; the enzyme functions at much less extreme pH values and does not appear to degrade the PVAm.

## **CONCLUSIONS**

The following conclusions arise from the analysis of our experimental results:

1. Cationic PVAm-T binds to anionic laccase at pH 5 producing polyelectrolyte complexes. Adsorption of the complex onto cellulose competes with the slow phase separation of complex in solution. The oxidation and grafting reaction occurs near neutral pH and ambient temperature in less than an hour.
2. The oxidation and grafting reaction is restricted to the exterior fiber surfaces because of the size of the PVAm-T/laccase complex.
3. Compared to processes involving soluble TEMPO, the total dose of TEMPO required to oxidize fibers by PVAm-T is 74% less than that required by free TEMPO because the polymer/laccase complex is adsorbed onto the cellulose.
4. O<sub>2</sub>-laccase offers many advantages over bleach (NaClO) as a primary oxidant, including neutral pH vs high pH for bleach, and no evidence of PVAm-T degradation, whereas bleach degrades PVAm.
5. The cellulose grafted PVAm-T/laccase complexes can perform as a surface primer layer, activating cellulose surfaces for wet adhesion, and possibly for other applications such as bioconjugation (bioactive paper), metal ion removal, and any other applications requiring a reactive surface polymer.

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## Transcription of Discussion

# REACTIVE POLYVINYLAMINE- GRAFT-TEMPO/LACCASE COMPLEX GIVING WET CELLULOSE ADHESION

*Jieyi Liu and Robert Pelton*

McMaster University, Hamilton, Ontario, Canada

*Gil Garnier*      Monash University

Excellent presentation, Bob, thanks a lot. Firstly, can you still repulp?

*Bob Pelton*

Yes.

*Gil Garnier*

No need to change your process?

*Bob Pelton*

I do not know. For important conclusions from our cellulose film delamination work, we made a few handsheets to show that we get the same result with cellulose fibres. We have not actually done any repulping. So if we're getting good covalent bonding then repulping should be an issue, but these amine and amino groups should be relatively easy to hydrolyze. In other words, I do not know

*Gil Garnier*

Second question, about the morphology, I do not recall the molecular weight, but from what I recall polyvinylamine is very short, probably less than a million, and you work with proteins with very big molecular weight, perhaps 50,000. My

### *Discussion*

question: what would be the effect of the component, or is there any component, of the enzyme as macroparticulate system?

*Bob Pelton*

I think for most of this work, the molecular weight of the PVAm was around 45,000. Now, the repeat unit is quite small, so that is quite a long chain, and I think the enzymes are not that high of a molecular weight, I vaguely recall 15 kiloDaltons. This acts like any other polyelectrolyte complex system. I am not sure that I understood your question.

*Gil Garnier*

What I was wondering is, if you form a bigger complex and change the morphology of the polymer using the protein as a structure, which of the components plays an important role in the mechanism?

*Bob Pelton*

No, I do not think so. We did control experiments. One of the early questions we asked was: is this just a two-component strength resin, base on negatively charged protein and positively charged PVAm? However, we killed the enzyme by heating and did the same experiments. We get no adhesion, so the oxidation is an important part of the story.

*Janet Preston*      Imerys Minerals (from the chair)

You said that you are possibly going to commercialise this work for different applications, could you speculate on a possible application?

*Bob Pelton*

I did not say we are going to commercialize. My experiences have been pretty bad commercializing anything. But I suspect conventional paper will not be an application but there is a lot of interest now in making all sorts of materials based on cellulose and nano-cellulose and this is a particularly nice way of grafting polymer onto cellulose, particularly if you want a reactive enzyme to stay on the cellulose surface. So, I challenge the audience; I am sure there have to be some good applications of this technology.