

PREPARATION AND CHARACTERISTICS OF ANIONIC POLYACRYLAMIDES CONTAINING DIRECT DYE WITH A HIGH AFFINITY FOR CELLULOSE

Shingo Yokota, Takefumi Ohta, Takuya Kitaoka,* Toshihiro Ona, and Hiroyuki Wariishi

Direct dye with a high affinity for cellulose substrate was utilized as a cellulose anchor to promote retention of paper strengthening additives under various conditions associated with the wet end of a paper machine. Direct Red 28 (DR) was covalently linked to anionic polyacrylamide (A-PAM) via a condensation reaction using water-soluble carbodiimide. The DR-conjugated A-PAM (DR-A-PAM) demonstrated good retention efficiency, resulting in strength enhancement of handsheets. Anionic trash showed no interference with the performance of DR-A-PAM in the wet end, while the additive performance was sensitive to calcium ions. Surface plasmon resonance analysis gave useful information on the cellulose-anchoring ability of DR-A-PAM. Dye molecules were irreversibly adsorbed onto the cellulose substrate under aqueous conditions, while A-PAM possessed no significant affinity for cellulose. These results suggest that anionic DR moieties in DR-A-PAM molecules served as a cellulose-anchor, possibly due to multiple CH- π interaction between hydrophobic face of cellulose substrate and π -conjugated system of dye molecules. Such a unique interaction of direct dye and cellulose provides a new insight into the wet end system, and does not depend on conventional electrostatic attraction.

Keywords: Direct dye; Cellulose; Papermaking additive; Retention; Interfering substances; Surface plasmon resonance

Contact information: Department of Forest and Forest Products Sciences, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan *Corresponding author: tkitaoka@agr.kyushu-u.ac.jp

INTRODUCTION

Cellulose is the most abundant renewable resource, and hence has long been recognized as a sustainable energy source and an environmentally friendly material (Klemm et al. 2005). Fibrous cellulose is a raw material for paper products whose functional design has been achieved using a variety of papermaking additives in the wet end process. The wet end of the paper machine system is currently attracting increasing attention as an effective place to implement “green” processes for the modification of cellulose fiber-based materials. However, the wet end has been found to be significantly contaminated by charged colloidal substances derived from recycled fibers and water in practical papermaking (Pelton et al. 1981). Such charged contaminants have greatly interfered with the adsorption of papermaking additives on pulp fibers, because conventional wet end additives are retained on pulp fiber surfaces via electrostatic

interaction in an aqueous system (Zhang 1999). Consequently, a novel concept for additive retention systems that does not depend on electrostatic interaction is required.

Non-electrostatic attraction to cellulose is commonly found in nature, and many researchers have reported a diverse array of substances having such interaction with cellulose. Carbohydrate binding modules (CBMs) of glycohydrolases are the most common macromolecules that have a high and specific affinity for cellulose (Tomme et al. 1988; Boraston et al. 2004). Schwarz et al. (2003) reported that a certain kind of antibody (immunoglobulin G) was able to adsorb strongly to β -1,4 glucan. Yang et al. (1998) discovered cellulose-binding activity of some nucleic acid aptamers. Such biomolecules have high affinity and specificity for cellulose, owing to their rigorously controlled supramolecular architectures. In our previous work CBMs originating from cellulases and xylanase were utilized as effective promoters of pulp-specific retention of papermaking additives, even in the contaminated wet end (Kitaoka and Tanaka 2001; Yokota et al. 2008, 2009). Although those trials were successful, such enzymatic functions are difficult to directly apply in practice because of their disadvantages of cost and productivity. In addition, there are difficulties in regulating the interaction of high molecular weight biomolecules with cellulosic substrates. For those reasons we are investigating other approaches as potential solutions.

Direct dyes generally are able to strongly adsorb to cellulose surfaces without dye mordants (Vickerstaff 1954), and have been widely applied in the textile and clothing industries for coloring. Particular types of direct dyes have been used for histochemical observations of plant cell walls (Earp et al. 1983; Wood et al. 1983), as molecular probes to evaluate the pore sizes of cellulose powders (Yu and Atalla 1998), and for regulation of the crystal structure of microbial cellulose (Mondal and Kai 2001). Recently, structure-activity relationship studies of dye-cellulose interactions have been carried out to predict dye adsorption properties (Funar-Timofei and Schuurmann 2002). Direct Red 28 (DR) is a typical direct dye that contains anionic sulfonate groups as illustrated in Fig. 1. However, it is well known that such type of direct dyes with a negative charge firmly stain negatively-charged pulp fibers in an aqueous system despite electrostatic repulsion, enabling their industrial applications to produce papers having a desired color or shade (Roberts 1996; Scott 1996). The specific attraction of direct dyes to cellulose substrates is attracting much attention from the viewpoints of cellulose and paper science.

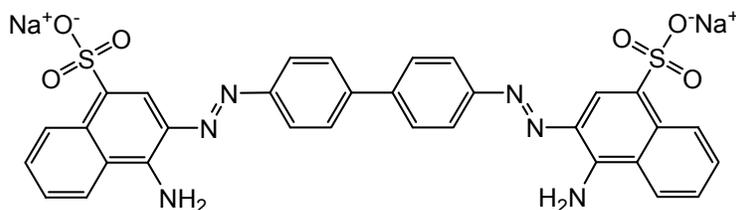


Fig. 1. Chemical structure of Direct Red 28 (DR).

In the present study, DR was conjugated with anionic polyacrylamide (A-PAM) by a condensation reaction. Handsheets were prepared from a pulp suspension by adding the obtained polymer (DR-A-PAM) as a paper strength agent, and its additive retention and paper strengthening performance were investigated. Retention of DR-A-PAM was

estimated under wet end conditions contaminated with anionic trash or inorganic ions. The affinity of DR-A-PAM for cellulose substrate is discussed on the basis of the results of surface plasmon resonance (SPR) analysis.

EXPERIMENTAL

Materials

Commercial DR was purchased from Sigma-Aldrich Co. Ltd. Figure 1 shows the structure of DR. Bleached hardwood kraft pulp with 450 mL of Canadian Standard Freeness was used for making handsheets. A-PAM (HH-351, molecular weight ca. 4×10^6 g mol⁻¹; charge density 0.83 meq. g⁻¹) was kindly provided by Kurita Co. Ltd. Lignosulfonic acid sodium salt (Sigma-Aldrich) and calcium chloride (CaCl₂, Wako Co. Ltd.) were used as model contaminants of anionic trash and multivalent cation, respectively. Cellulose thiosemicarbazone (Cel-TSC, degree of polymerization ca. 190) was synthesized from mercerized cellulose as previously reported (Yokota et al. 2007). *N*-Methylmorpholine-*N*-oxide (NMMO) was obtained from Sigma-Aldrich. Other chemicals were reagent grade and were used without further purification.

Methods

Synthesis of DR-conjugated A-PAM (DR-A-PAM)

As-purchased DR (content ca. 50% w/w) was purified by three times salting-out using a 40% w/v sodium acetate aqueous solution (Robinson and Mills 1931). The final purity of DR was at least 95% w/w, as evaluated by elemental analysis. Purified DR (3.0 μmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, 9.4 mmol) were gradually added to a 0.1% w/w A-PAM aqueous solution (150 mL) with gentle stirring at room temperature for 3 h, keeping the pH of the reaction medium constant at 4.75 by addition of dilute hydrochloric acid. Figure 2 depicts schematically the synthesis of DR-A-PAM: amine groups of DR were reacted with carboxyl groups of A-PAM by EDC-activated condensation. The resulting DR-A-PAM was purified by three centrifugations using isopropanol, prior to handsheet-making.

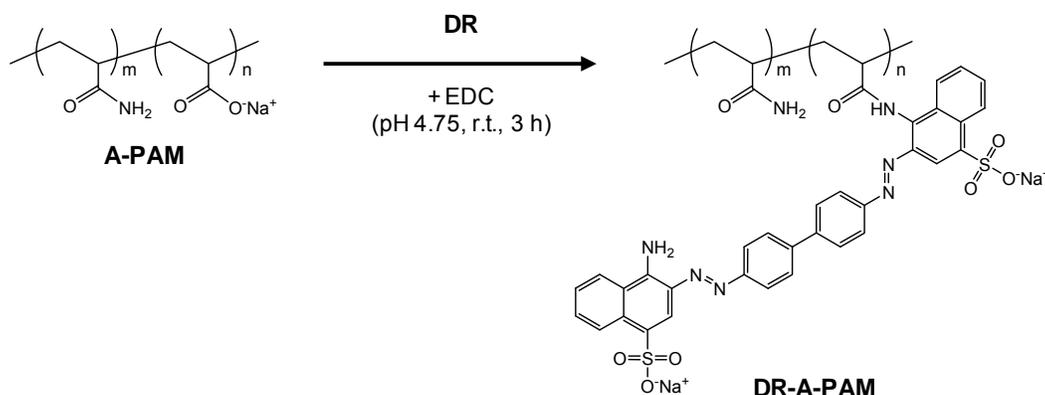


Fig. 2. Schematic representation of the preparation of DR-A-PAM from DR and A-PAM via EDC-mediated condensation reaction.

Preparation and characterization of handsheets

Designated amounts of DR-A-PAM (0-0.8% based on dry weight of pulp) were added to 0.15% w/v pulp suspension. Handsheets with a basis weight of 60 g m⁻² were prepared according to TAPPI test method T205 sp-95. The cationic demand of the pulp suspension was adjusted to 20-100 µeq. L⁻¹ by adding lignosulfonic acid sodium salt as a model of anionic contaminants (anionic trash), before addition of DR-A-PAM. The conductivity of the suspension was adjusted to 0.4-4.0 mS cm⁻¹ using CaCl₂. The handsheets obtained were subjected to the dry tensile strength test according to TAPPI test method T494 om-88 (n=4). Total additive retention was determined by combustion nitrogen analysis (Yokota et al. 2008).

Surface plasmon resonance (SPR) analysis

The affinity of DR molecules for cellulose was evaluated by SPR analysis using a model cellulose substrate. A flat cellulose nanolayer was prepared on a gold-coated sensor chip for SPR (Moritex, Co. Ltd.) by self-assembling chemisorption of Cel-TSC as reported previously (Yokota et al. 2007). The SPR chip was immersed for 12 h at room temperature in 80% w/w NMMO/H₂O solution containing 0.1% w/w Cel-TSC diluted with dimethylsulfoxide. The cellulose-modified SPR chip was thoroughly washed with 80% NMMO solution at 105°C for 30 min, then with water. Adsorption phenomena at the cellulose/water interface were detected as a variation of resonance angles via the SPR instrument (SPR 670, Nippon Laser & Electronics Lab.). All measurements were carried out using deionized water or 20 mM aqueous CaCl₂ at 25°C (constant flow rate 50 µL min⁻¹).

RESULTS AND DISCUSSION

Retention and Paper-strength Characteristics of DR-A-PAM

Purified DR molecules were successfully introduced into A-PAM via EDC-mediated condensation. The degree of DR-substitution was calculated from the nitrogen content of DR-A-PAM determined by elementary analysis as approximately three of every 100 A-PAM units. The DR-A-PAM was introduced to the fibrous suspension for the preparation of handsheets. Figure 3 shows the additive retention and the dry tensile strength of the handsheets prepared with internal addition of DR-A-PAM under clean wet end conditions. Very little DR-free A-PAM was retained in the paper sheet because of its electrostatic repulsion with negatively-charged pulp fibers, resulting in negligible effect of A-PAM on paper strengthening. This observation is in accordance with a previously reported result (Yokota et al. 2008). The addition of DR molecules alone likewise did not give physical enhancement of handsheets, due to the inherent ineffectiveness of DR as a paper strengthening agent. Although little retention of polymer-free DR was detected due to the very low concentration of DR added to paper furnish, the resultant paper sheets were red-stained by addition of DR, implying slight but significant retention of DR molecules alone on pulp fiber. In the case of DR-A-PAM, increasing the addition level of DR-A-PAM to paper furnish proportionally increased its retention ranging from 0.2 to 0.8% on dry pulp, resulting in good physical enhancement of paper sheets. These

results suggested that the DR moieties in the DR-A-PAM had an attractive interaction with cellulosic pulp fibers, even though DR-A-PAM is an anionic polymer.

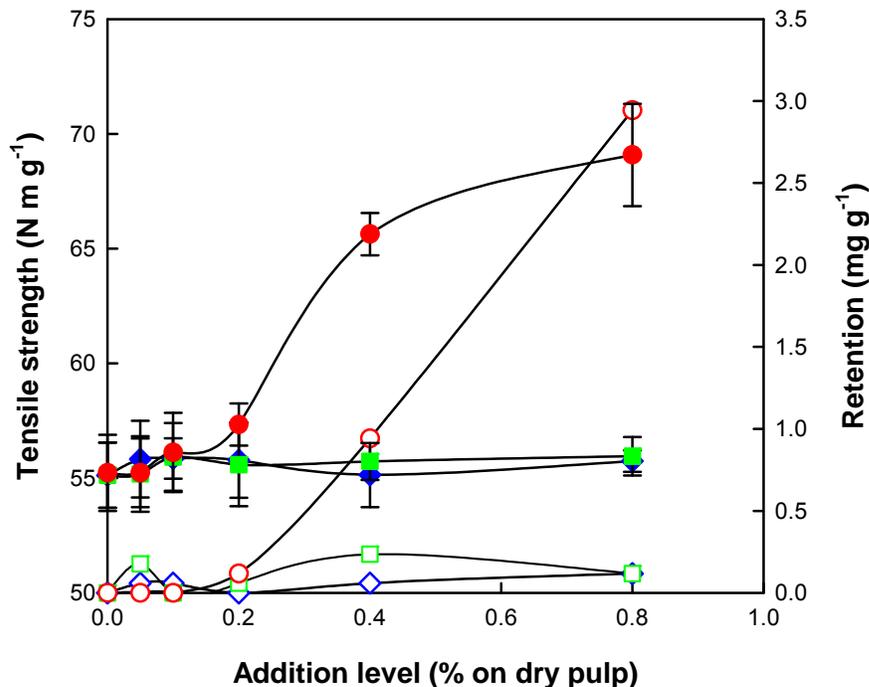


Fig. 3. Effects of additives on paper strength (closed) and retention (open): DR-A-PAM (red circles); A-PAM (blue diamonds); DR (green squares).

While DR molecules alone exhibited slight adsorption to pulp fiber, DR-A-PAM demonstrated strong retention, and thus the synergistic effect of multiple DR units in each A-PAM chain apparently promoted retention of DR-A-PAM. Subsequently, the acrylamide moieties in DR-A-PAM presumably form hydrogen bonds to cellulose, leading to the improvement of paper tensile strength. Many researches of the interaction between ionic dyes and water-soluble polymers in aqueous media have been carried out extensively, e.g. for spectroscopic applications (Scott 1996; Chmilenko et al. 2004) and decoloration of wastewaters (Yu et al. 2001; Shen et al. 2006); major dye-polymer interactions are derived from electrostatic attractions. However, in this case there may not be significant attraction between DR and A-PAM, since both have a negative charge. Free DR molecules were obviously adsorbed to pulp fibers; however they would make little contribution to the retention of DR-free A-PAM to pulp fibers. Therefore, the retention of DR-A-PAM (Fig. 3) was possibly attributed to the specific interactions of DR moieties in DR-A-PAM with cellulosic pulp fibers. The amount of DR-A-PAM retained in handsheets was considerably lower than that of polyamideamine-epichlorohydrin (PAE), a commercial cationic additive (Yokota et al. 2008). However, DR-A-PAM had a greater effect than PAE on paper strength, suggesting favorable performance of DR-A-PAM as a paper strengthening additive.

Additive Performance of DR-A-PAM in Contaminated Wet End

Most commercial papermaking additives are retained on pulp fibers via electrostatic attraction. Thus both accumulation of anionic trash and high electrical conductivity in the wet end system can inhibit the functions of papermaking additives. In this work, an aqueous papermaking system containing either organic or inorganic compounds as model contaminants was employed to elucidate the influence of such interfering substances on the performance of DR-A-PAM. Cationic demand and conductivity of the wet end system were adjusted using lignosulfonic acid sodium salt and CaCl_2 , respectively.

Figure 4 shows the influence of anionic trash on the additive retention and tensile strength of handsheets prepared with DR-A-PAM. The magnitudes of both properties were virtually independent of cationic demand in the range 20 to 100 $\mu\text{eq. L}^{-1}$.

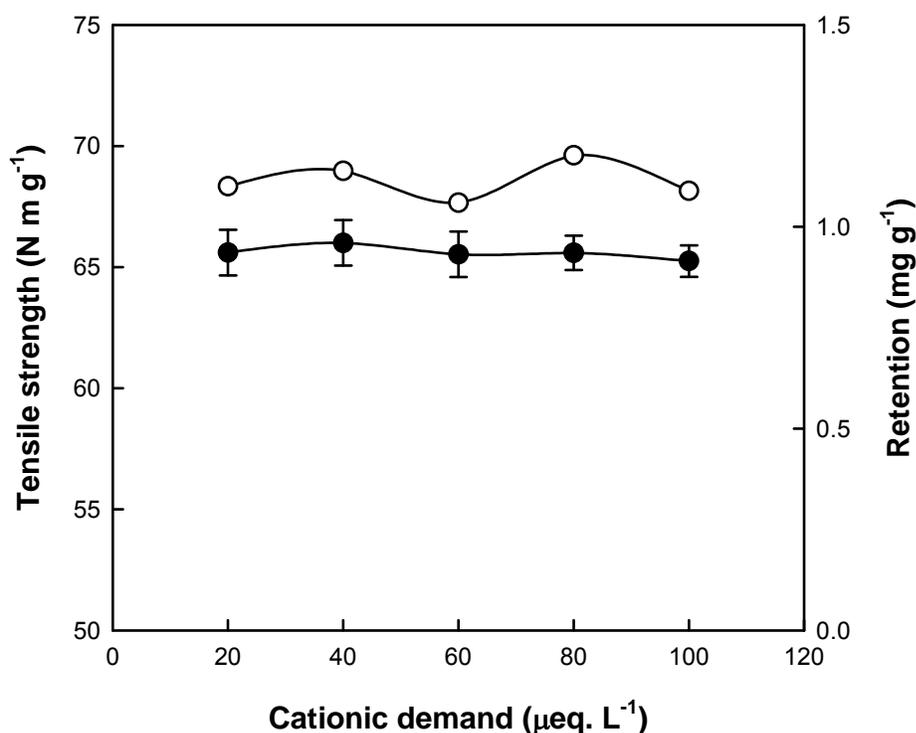


Fig. 4. Influence of anionic trash on the tensile strength (closed) and retention (open) of paper sheets prepared with DR-A-PAM (0.4% on dry pulp).

It was reported that retention of additives and development of paper strength of paper sheets treated with PAE significantly decreased with increasing cationic demand (Yokota et al. 2008). In general, PAE is fixed on negatively-charged pulp fibers by electrostatic attraction; hence PAE easily interacts with not only pulp fiber but also anionic trash, resulting in a very marked reduction of additive retention. By contrast, the anionic trash gave almost no interference with DR-A-PAM performance in the wet end due to electrostatic repulsion. Anionic dyes generally precipitate on pulp fibers in the

presence of cationic mordants and various auxiliary agents (Roberts 1996); however the adsorption of DR-A-PAM to pulp was insensitive to lignosulfonic acid sodium salt as a model of anionic trash. Thus DR-A-PAM was strongly and stably adsorbed to cellulosic substrate via non-electrostatic forces that are intrinsic to DR even under the severe conditions at a high cationic demand.

The influence of multivalent ions such as Ca^{2+} on the additive performance of DR-A-PAM was investigated. Both tensile strength and additive retention were significantly reduced by addition of CaCl_2 (Fig. 5). It is well known that such inorganic cations neutralize or cationize the surfaces of anionic pulp fibers, causing poor retention of cationic polymer additives in the paper furnish. On the other hand, the adsorption of anionic dyes to pulp fibers can be improved by adding multivalent cations such as Ca^{2+} ions due to their mordanting (cross-linking) effect (Roberts 1996). Although DR molecules have anionic sulfonate groups that can interact with a positively-charged surface, the addition of DR-A-PAM showed insufficient effectiveness, as shown in Fig. 5. It is presumed that the direct dye might form undesirable insoluble salts at a high salt concentration, resulting in partial deposition. Consequently, intra- and/or intermolecular aggregation of DR-A-PAM may occur in the wet end with high conductivity, eventually leading to reduction of additive performance. Hence, DR-A-PAM was unaffected by accumulation of anionic trash, but sensitive to cationic interferences.

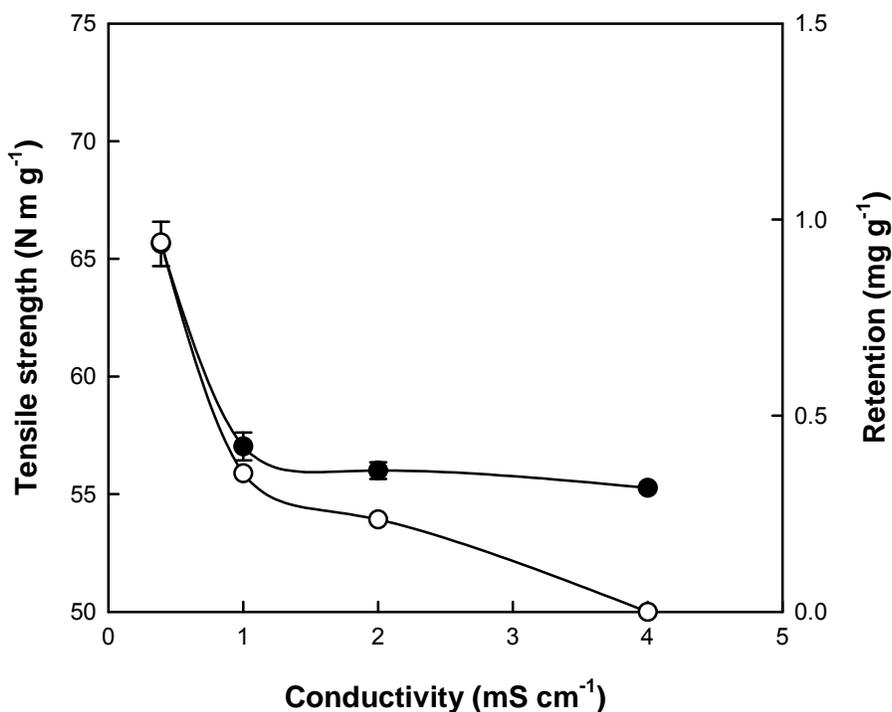


Fig. 5. Influence of paper stock conductivity on the tensile strength (closed) and retention (open) of paper sheets prepared with DR-A-PAM (0.4% on dry pulp).

Affinity of DR for Cellulose Substrate

DR-A-PAM served as a good paper strengthening agent under the various wet end conditions, including uncontaminated, contaminated with anionic trash, or contaminated with Ca^{2+} (Figs. 3, 4 or 5, respectively), which indicates that the retention mechanism of DR-A-PAM to pulp fibers differs from conventional additive retention via electrostatic attraction. SPR analysis is a powerful tool for elucidating molecular level interaction at a liquid/solid interface, and thus was utilized to elucidate the interaction between DR and cellulose. A cellulose I model substrate was prepared on a gold-coated SPR sensor chip (Yokota et al. 2007). X-Ray photoelectron spectroscopy (XPS) confirmed that the cellulose nanolayer was composed of pure cellulose molecules (Fig. 6), implying that there were few carboxyl groups (charged sites) on the model cellulose surface (Yokota et al. 2009), unlike commercial pulp fibers containing carboxyl groups. Porous morphology and electric charges of cellulose fibers in the wet state are of great importance for dye-adsorption behavior (Roberts 1996; Ougiya et al. 1998; Inglesby and Zeronian 2002). However, in this work, pure and smooth cellulose substrate was subjected to the SPR analysis in order to determine the direct interaction between DR and cellulose surface without charged groups, regardless of electrostatic attraction.

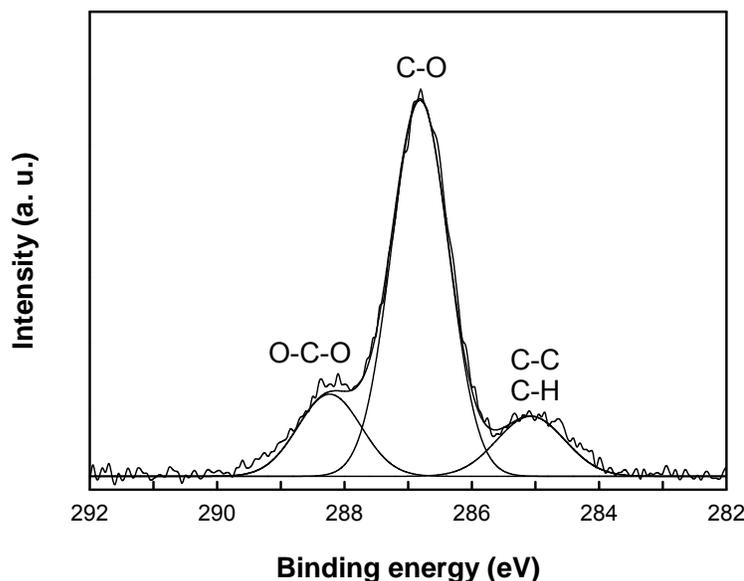


Fig. 6. XPS C1s narrow region spectrum of the model cellulose nanolayer formed on a gold-coated SPR sensor chip. The binding energy was determined with respect to the reference signal (unoxidized C-C and/or C-H band) at 285.0 eV.

Figure 7 shows the SPR sensorgrams obtained in deionized water. A-PAM was initially attached to, and then immediately detached from the substrate, presumably due to the weak attractive force of A-PAM to cellulose. By contrast, the resonance angle was drastically changed by injection of the DR aqueous solution; the SPR angle shift eventually reached a plateau. The results indicate that DR molecules were adsorbed onto

the uncharged cellulose surface in the aqueous system, possibly owing to molecular recognition of cellulose by DR that is independent of electrostatic interaction. Levitt and Perutz (1988) have proposed that π electrons may interact with axial CH groups at the hydrophobic face of cellulose chains (termed CH- π interaction). The DR molecule has a highly π -conjugated system (Fig. 1), so that CH- π interaction should afford high affinity of DR for cellulose. Besides, amino groups of a DR molecule can interact with cellulose via hydrogen bondings (Roberts 1996; Allan et al. 2001), and thus such strong adsorption of DR to cellulose without carboxyl groups is possibly attributed to synergistic effect of non-electrostatic interactions, resulting in the high retention efficiency of DR-A-PAM at the wet end (Fig. 3). Unfortunately, it proved not to be possible to obtain a reliable sensorgram of DR-A-PAM. A very large amount of DR-A-PAM was assumed to adsorb strongly onto the model cellulose substrate, resulting in excessive thickening of polymer layer on the sensor chip and thus inhibiting the SPR analysis. DR-A-PAM was prepared via a condensation reaction between amino groups of DR and carboxyl groups of A-PAM (Fig. 1), and thus a CH- π interaction may be more significant in the case of DR-A-PAM. Further investigations are required for the elucidation of the adsorption mechanisms of DR-A-PAM.

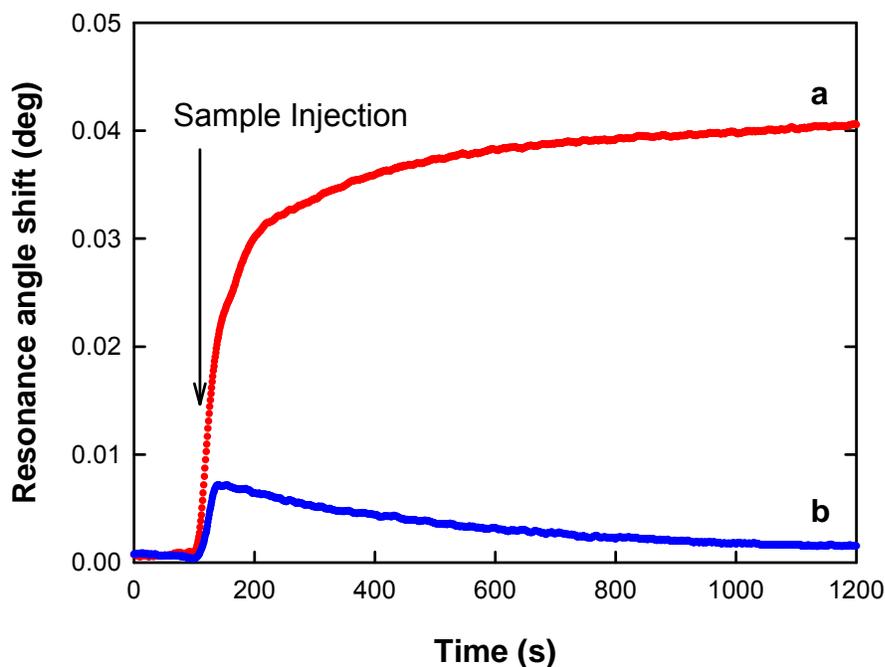


Fig. 7. SPR sensorgrams of DR (a) and A-PAM (b).

The association constant of DR to the model cellulose surface was determined as $7.7 \times 10^5 \text{ M}^{-1}$ from the results of SPR analysis (Table 1). This value indicates that the affinity of DR for cellulose is comparable to that of biological recognition systems such as antigen-antibody (10^5 - 10^{10} M^{-1}), enzyme-substrate (10^2 - 10^6 M^{-1}), and lectin-saccharide (10^3 - 10^5 M^{-1}). Moreover, the low dissociation rate constant ($9.0 \times 10^{-4} \text{ s}^{-1}$, Table 1) indicates that negligible desorption of DR molecules adsorbed to the model cellulose

occurred in an aqueous medium, in accordance with the general understanding that it is extremely difficult to decolorize cellulose textile fibers once stained with direct dyes.

Table 1. Association Constants (K_{ass}) of DR for Cellulose Substrate

Flow solvent	K_{ass} (M^{-1}) *	k_{ass} ($\text{M}^{-1} \text{s}^{-1}$)	k_{diss} (s^{-1})
Deionized water	7.7×10^5	6.9×10^2	9.0×10^{-4}
20 mM CaCl_2 aq.	2.7×10^4	1.8×10^2	6.7×10^{-3}

* K_{ass} was calculated by dividing association rate constant (k_{ass}) by dissociation rate constant (k_{diss}) under the pure and contaminated conditions.

SPR analysis was performed in a 20 mM CaCl_2 aqueous solution (conductivity 4 mS cm^{-1}). The association content in CaCl_2 solution was one order of magnitude lower than the value in deionized water (Table 1). Ca^{2+} ions are able to interact electrostatically with sulfonate groups in DR molecules. That might give rise to association of DR molecules due to dissipation of electrostatic repulsion, resulting in inhibition of DR-cellulose interaction. The adsorption of anionic dyes to pulp fibers with negatively-charged groups in the paper machine wet end is frequently improved in the presence of multivalent cations such as Ca^{2+} ions as a mordant due to their cross-linking effect (Roberts 1996); however, in the case of DR-A-PAM the negative impact of cations on the affinity for cellulose was observed, possibly due to intra- and/or intermolecular aggregation of polymer itself via blocking of charged groups of DR moieties in the polymer, eventually reducing the accessibility of DR to cellulose substrates (Fig. 5). Insufficient effectiveness of DR-conjugated polymers under high conductivity conditions would be overcome by the molecular design of polymer to prevent its self-aggregation. Direct dyes having a high affinity for cellulose are expected to provide a new concept for a novel wet end system that does not depend wholly on electrostatic interaction.

CONCLUSIONS

1. Direct Red 28 (DR) was successfully conjugated with A-PAM via an EDC-mediated reaction. DR-A-PAM thus obtained demonstrates good retention to cellulosic pulp fibers, resulting in enhancement of paper strength.
2. The intrinsic affinity of DR for cellulose allows the dye to bind to cellulose fiber in the presence of large amounts of anionic trash. The binding weakens at high salt concentration, possibly due to polymer aggregation.
3. The DR-cellulose interaction was evaluated by SPR analysis using a model cellulose substrate. The attraction of DR to cellulose is presumed to involve CH- π interaction between the π -conjugated system of DR and axial CH groups at the hydrophobic face of cellulose chains. Such a wet end system has a novel aspect, being independent of conventional electrostatic interaction.

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REFERENCES CITED

- Allan, G. G., Stoyanov, A. P., Ueda, M., and Yahiaoui, A. (2001). "Sugar-cellulose composites V. The mechanism of fiber strengthening by cell wall incorporation of sugars," *Cellulose* 8(2), 127-138.
- Boraston, A. B., Bolam, D. N., Gilbert, H. J., and Davies, G. J. (2004). "Carbohydrate-binding modules: fine-tuning polysaccharide recognition," *Biochemical Journal* 382(3), 769-781.
- Chmilenko, F. A., Korobova, I. V., and Nazarenko, S. V. (2004). "Spectrophotometric determination of polyacrylamide in aqueous solutions using cationic dyes," *Journal of Analytical Chemistry* 59(2), 124-128.
- Earp, C. F., Doherty, C. A., Fulcher, R. G., and Rooney L. W. (1983). "β-Glucans in the caryopsis of *Sorghum bicolor* (L.) moench," *Food Microstructure* 2(2), 183-188.
- Funar-Timofei, S., and Schuurmann, G. (2002). "Comparative molecular field analysis (CoMFA) of anionic azo dye-fiber affinities I: Gas-phase molecular orbital descriptors," *Journal of Chemical Information and Computer Sciences* 42(4), 788-795.
- Inglesby, M. K., and Zeronian, S. H. (2002). "Direct dyes as molecular sensors to characterize cellulose substrates," *Cellulose* 9(1), 19-29.
- Kitaoka, T., and Tanaka, H. (2001). "Novel paper strength additive containing cellulose-binding domain of cellulase," *Journal of Wood Science* 47(4), 322-324.
- Klemm, D., Heublein, B., Fink, H.-P., and Bohn, A. (2005). "Cellulose: Fascinating biopolymer and sustainable raw material," *Angewandte Chemie International Edition* 44(22), 3358-3393.
- Levitt, M., and Perutz, M. F. (1988). "Aromatic rings act as hydrogen bond acceptors," *Journal of Molecular Biology* 201(4), 751-754.
- Mondal, Md. I. H., and Kai, A. (2001). "Control of the crystal structure of microbial cellulose during nascent stage," *Journal of Applied Polymer Science* 79(9), 1726-1734.
- Ougiya, H., Hioki, N., Watanabe, K., Morinaga, Y., Yoshinaga, F., and Samejima, M. (1998). "Relationship between the physical properties and surface area of cellulose derived from adsorbates of various molecular sizes," *Bioscience, Biotechnology, and Biochemistry* 62(10), 1880-1884.
- Pelton, R. H., Allen, L. H., and Nugent, H. M. (1981). "Novel dual-polymer retention aids for newsprint and groundwood specialties," *TAPPI Journal* 64(11), 89-92.

- Robert, J. C. (1996). *Paper Chemistry Second edition*, Blackie Academic & Professional, London.
- Robinson, C., and Mills, H. A. T. (1931). "The colloid chemistry of dyes: The aqueous solutions of benzo-purpurine 4B and its isomer prepared from *m*-tolidine Part I," *Proceedings of the Royal Society of London Series A, Containing Papers of a Mathematical and Physical Character* 131(818), 576-595.
- Schwarz, M., Spector, L., Gargir, A., Shtevi, A., Gortler, M., Altstock, R. T., Dukler, A. A., and Dotan, N. (2003). "A new kind of carbohydrate array, its use for profiling antiglycan antibodies, and the discovery of a novel human cellulose-binding antibody," *Glycobiology* 13(11), 749-754.
- Scott, W. E. (1996). *Principles of Wet End Chemistry*, TAPPI Press, Atlanta.
- Shen, J.-J., Ren, L.-L., and Zhuang, Y.-Y. (2006). "Interaction between anionic dyes and cationic flocculant P(AM-DMC) in synthetic solutions," *Journal of Hazardous Materials* 136(2), 809-815.
- Tomme, P., Van Tilbeurgh, H., Pettersson, G., Van Damme, J., Vandekerckhove, J., Knowles, J., Teeri, T., and Claeysens, M. (1988). "Studies of the cellulolytic system of *Trichoderma reesei* QM9414—Analysis of domain function in two cellobiohydrolases by limited proteolysis," *European Journal of Biochemistry* 170(3), 575-581.
- Vickerstaff, T. (1954). *The Physical Chemistry of Dyeing*, Oliver and Boyd, London.
- Wood, P. J., Fulcher, R. G., and Stone, B. A. (1983). "Studies on the specificity of interaction of cereal cell-wall components with Congo Red and Calcofluor: Specific detection and histochemistry of (1→4),(1→3),-β-D-glucan," *Journal of Cereal Science* 1(2), 95-110.
- Yang, Q., Goldstein, I. J., Mei, H.-Y., and Engelke, D. R. (1998). "DNA ligands that bind tightly and selectively to cellobiose," *Proceedings of the National Academy of Sciences of the United States of America* 95(10), 5462-5467.
- Yokota, S., Kitaoka, T., Sugiyama, J., and Wariishi, H. (2007). "Cellulose I nanolayers designed by self-assembly of its thiosemicarbazone on a gold substrate," *Advanced Materials* 19(20), 3368-3370.
- Yokota, S., Matsuo, K., Kitaoka, T., and Wariishi, H. (2008). "Specific interaction acting at a cellulose-binding domain/cellulose interface for papermaking application," *BioResources* 3(4), 1030-1041.
- Yokota S., Matsuo K., Kitaoka T., and Wariishi H. (2009). "Retention and paper-strength characteristics of anionic polyacrylamides conjugated with carbohydrate-binding modules," *BioResources* 4(1), 234-244.
- Yu, X., and Atalla, R. H. (1998). "A staining technique for evaluating the pore structure variations of microcrystalline cellulose powders," *Powder Technology* 98(2), 135-138.
- Yu, Y., Zhuang, Y.-Y., and Zou, Q.-M. (2001). "Interaction between organic flocculant PAN-DCD and dyes," *Chemosphere* 44(5), 1287-1292.
- Zhang, X., Beatson, R. P., Cai, Y. J., and Saddler, J. N. (1999). "Accumulation of specific dissolved and colloidal substances during white water recycling affects paper properties," *Journal of Pulp and Paper Science* 25(6), 206-210.

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