Development and Applications of Pyrene-Containing Fluorescent Probes for Monitoring the Photodegradation of Lignin-Rich Products

Klára G. Kónya and J. C. Scaiano*

Department of Chemistry, University of Ottawa, Ottawa, Ontario, Canada K1N 6N5

Received June 3, 1994. Revised Manuscript Received September 26, 1994[⊗]

o-Quinone moieties produced by irradiation of lignin-rich pulp and paper have been selectively derivatized with pyrene moieties following reaction with P(III) compounds. The results of spectroscopic and time resolved emission studies indicate that the tendency for quinone formation upon UV irradiation is enhanced by bleaching processes, specially by oxidative peroxide treatment. In steady state spectroscopic studies pulp fluorescence interferes with excimer emission. However, pulp fluorescence is a very short-lived process ($\tau \leq 2$ ns), and thus time-resolved spectroscopy can differentiate the two processes readily. These studies show some excimer fluorescence, even under conditions of very low pyrene incorporation. This suggests that *o*-quinone formation occurs in domains or islands, indicating that degradation occurs predominantly in some regions, or that oxidative degradation processes promote further degradation, perhaps by enhancing oxygen permeability. Fluorescence microscopy studies suggest preferential degradation in fines and fragmented fibers.

Introduction

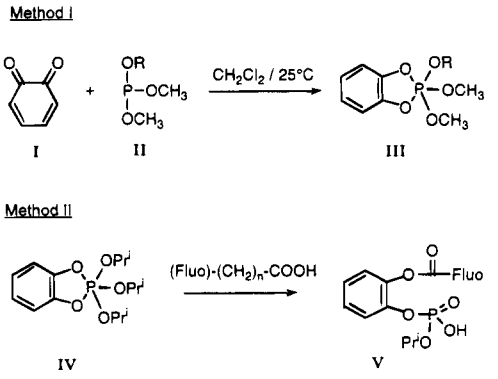
Lignin-rich papers such as those produced through thermomechanical pulping (TMP) acquire a yellow coloration quite rapidly upon exposure to near-UV light. The process is known to involve the photoinduced degradation of lignin and severely limits the value and applications of lignin-rich products. It has now been established that one of the primary sources of yellowing involves several free-radical and excited-state processes leading to the formation of phenoxyl radicals, especially, guaiacoxyl. Oxidative processes ultimately convert phenoxyl products to quinones which make a major contribution to color development.[1]

Over the past few years a number of studies on model compounds has led to a dramatic improvement in our understanding of the degradative pathways in lignin. However, studies on pulp and paper are more scarce, more difficult, and frequently less informative. The current study was undertaken in an attempt to develop highly sensitive techniques to monitor *o*-quinones in pulp and paper. We hope that this approach will eventually allow us to quantify the yields and map the location of *o*-quinones.

While *o*-quinones can be detected by UV spectroscopy, this approach is far from specific. Recently, Argyropoulos et al.[2] have derivatized *o*-quinones by reaction with P(III) compounds to yield cyclic oxyphosphorene adducts. These or their hydrolysis products were further characterized by ^{31}P NMR spectroscopy (Scheme 1).

Clearly the reactions of Scheme 1 provide a simple way of introducing fluorescent groups in *o*-quinone sites. Two different approaches have been employed in our work. In method I, one of the R groups in $P(OR)_3$ was replaced by a fluorescent moiety. In method II acid hydrolysis of **IV** was employed as an alternative approach for probe incorporation.

Scheme 1



An adequate selection of the fluorescent moiety should allow us to employ its emission characteristics as a sensor for the distribution of degradation sites and for the general characteristics (such as mobility, polarity, and proximity) at those sites.

The chromophore selected for our studies was pyrene, since we felt that its well established photochemistry and photophysics[3] should facilitate the analysis of the results. Further, the polarity dependence of its emission spectrum is well established[4,5] and the possibility of excimer formation should provide some information on the proximity between *o*-quinone moieties.[3] We note that an alternate approach to the incorporation of fluorescent probes is currently being developed by Gray and co-workers,[6–8] who have reacted *o*-quinones with o-phenylenediamine to generate fluorescent phenazines.

[⊗] Abstract published in *Advance ACS Abstracts,* November 1, 1994.

(1) Heitner, C.; Scaiano, J. C., Eds. *Photochemistry of Lignocellulosic Materials*; American Chemical Society: Washington, 1993; Vol. 531.
(2) Argyropoulos, D. S.; Heitner, C.; Morin, F. G. *Holzforschung* **1992**, *46*, 211.
(3) Winnik, F. M. *Chem. Rev.* **1993**, *93*, 587.
(4) Dong, D. C.; Winnik, M. A. *Photochem. Photobiol.* **1982**, *35*, 17.
(5) Dong, D. C.; Winnik, M. A. *Can. J. Chem.* **1984**, *62*, 2560.

0897-4756/94/2806-2369$04.50/0

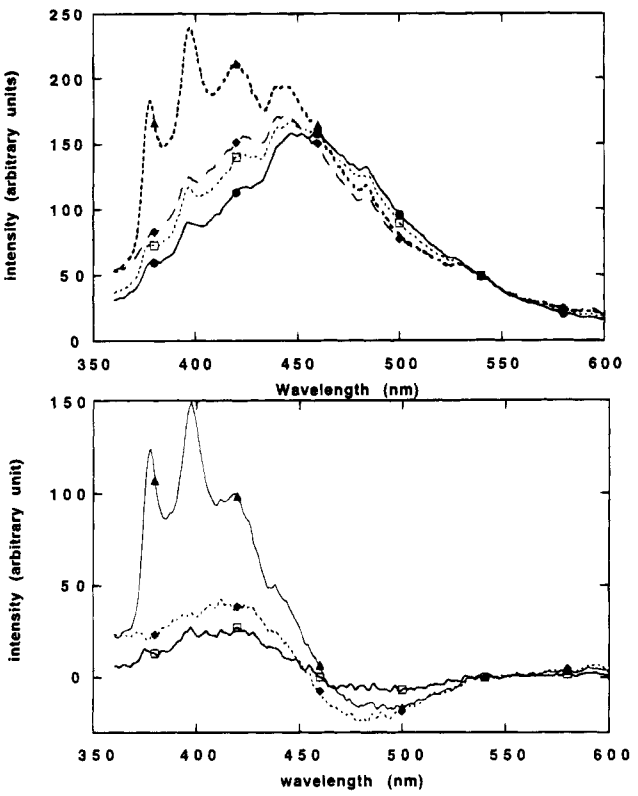
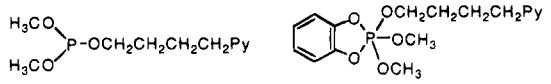


Figure 1. Front-face fluorescence spectra of paper at 330 nm excitation wavelength. Nonirradiated/derivatized (□), nonirradiated/untreated (●), and irradiated/derivatized (▲), irradiated/untreated (♦) samples. Bottom:. The difference spectra of the above samples. Nonirradiated/untreated sample as reference.

Results

As already noted, two methods were employed. In the first one the fluorescent moiety is incorporated as part of the phosphite. While this method appeared initially to offer the simplest approach to probe incorporation, it eventually proved to be the least specific of the two, and the one requiring more synthetic efforts.

Method I. The probe molecule in this case was 4-pyrenylbutyl dimethyl phosphite (**VI**), prepared by reaction of dimethyl chlorophosphite with pyrene butanol. In this case derivatization of *o*-quinones should yield the corresponding cyclic derivative (see Scheme 1), e.g.



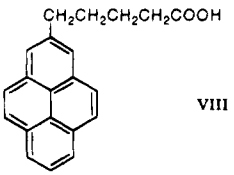
VI VII

where Py represents the pyrene moiety. Even secondary hydrolysis (as in method II in Scheme 1) should lead to retention of approximately one third (based on statistics) of the pyrene moieties.

Figure 1 shows the results of an experiment performed on thermomechanical pulp (TMP) under four different combinations of irradiated and non-irradiated, and treated or untreated with **VI**. Dry methylene chloride proved to be the best solvent for the reaction. The same figure (bottom) also includes a difference spectrum, where the nonirradiated/treated spectrum has been subtracted from that for the irradiated/treated sample. The emission spectrum is clearly that of the pyrene monomer, showing that quinone generation by UV photolysis of the pulp leads to enhanced incorporation of pyrene moieties. Under these conditions we did not detect any evidence of excimer emission, suggesting that at least those chromophores responsible for prompt emission do not diffuse sufficiently to find other pyrene moieties during the short singlet lifetime. It is also evident from Figure 1 that there is considerable interference from the luminescence from pulp. This emission has been recently characterized in considerable detail by Gray et al.[7,8]

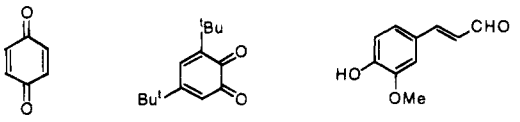
One of the main problems is that the reaction of **V** is specific not just to *o*-quinones but also to coniferylaldehyde, which is present or formed in irradiated pulps.[9] This led us to try our second approach, which proved to be more specific and easier to implement.

Method II. This approach is based on the acid hydrolysis of cyclic phosphorous compounds of the type **IV**. The molecule used as a probe is pyrene butyric acid, **VIII**. Probe molecule **VIII** is used to perform the reaction of method II in Scheme I.



VIII

NMR Reactivity Studies. To establish better the mechanism of the method II and selectivity of the reaction, the same reaction was examined by ^{13}C NMR using $CH_3{}^{13}COOH$ as a model reactant. Three model compounds, **IX**–**XI**, all of them structurally relevant to lignin chemistry were examined.



IX X XI

Other structural components, such as organic acids or anhydrides, may also react with phosphite, but these should already have been destroyed by base washing during the pretreatment of the pulp. Our experiments were aimed at establishing if under our experimental conditions *o*-quinones incorporated selectively the fluorescent probe.

Under our experimental conditions, coniferaldehyde (**XI**) was not reactive. The same applied to β-ionone, another α,β-unsaturated aldehyde tested briefly. In the case of *p*-benzoquinone (**IX**) reaction with triisopropyl phosphite occurred readily, as shown by the NMR spectra of Figure 2; however, addition of $CH_3{}^{13}COOH$ caused no change in the spectrum of the adduct, suggesting that the acid hydrolysis step does not work in this case.

(6) Zhu, J. H.; Olmstead, J. A.; Gray, D. G. *J. Wood Chem. Technol.*, submitted.
(7) Olmstead, J. A.; Gray, D. G. *J. Photochem. Photobiol. A: Chem.* **1993**, *73*, 59.
(8) Zhu, J. H.; Gray, D. G. *J. Photochem. Photobiol. A: Chem.* **1993**, *73*, 67.
(9) Ramirez, F. *Pure Appl. Chem.* **1964**, *9*, 337.

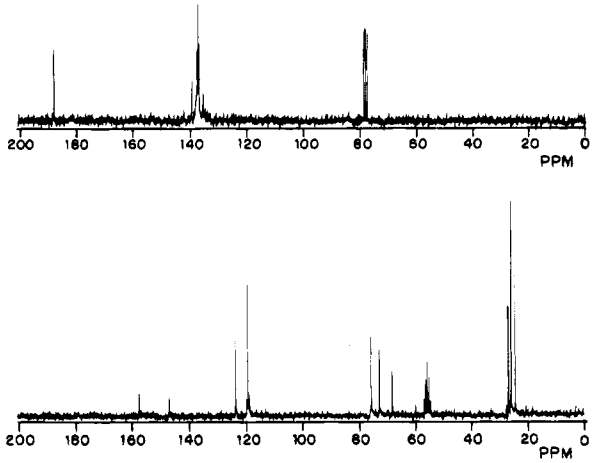


Figure 2. ^{13}C NMR spectrum of (top) **IX** (50 mg/mL) in $CDCl_3$, and (bottom) effect of addition of 1 equiv (119 μL) triisopropyl phosphite in CD_2Cl_2.

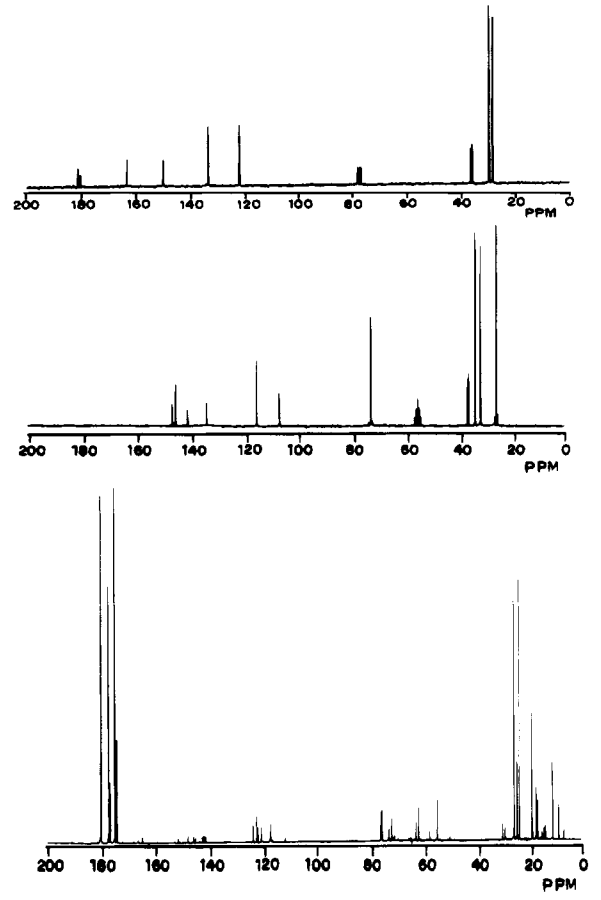


Figure 3. ^{13}C NMR spectrum of (top) **X** (100 mg/mL) in $CDCl_3$, (center) effect of addition 1 equiv (117 μL) triisopropyl phosphite in CD_2Cl_2, (bottom) product mixture of the hydrolysis with 2 equiv (60 μL) ^{13}C-labeled acetic acid.

In the case of *o*-quinone **X**, reaction with $P(OPr^i)_3$ occurs readily (see Figure 3) leading to the corresponding cyclic compound (see Scheme 1). Addition of $CH_3{}^{13}COOH$ gave unequivocal evidence for reaction with the cyclic phosphorous product, as illustrated in Figure 3. The spectra are somewhat complicated by the obvious formation of more than one product, as expected from the two possible modes of ring opening in this unsymmetric system. ^{31}P NMR (not shown) showed more doublet peaks, in the −2 to −4 ppm region (relative to 85% H_3PO_4), in agreement with the reported solid state spectrum for the open phosphite ester.[2] Further analysis of the NMR results of the region 178−179 ppm (acid carbon region) are neither easy nor essential to establish that acid hydrolysis occurs efficiently only in the case of **X**. However, in the region of the aromatic carbons (110−125 ppm), the spectrum shows more doublet peaks which correspond to the different products of ring opening.

Scheme 2

$$P + h\nu_0 \longrightarrow P^*$$
$$P^* \longrightarrow P + h\nu_1$$
$$P^* + P \longrightarrow P_2^*$$
$$P_2^* \longrightarrow 2P + h\nu_2$$

Steady-State Fluorescence Spectroscopy. Scheme 2 shows the key steps in the photophysics of pyrene and its derivatives. Monomer emission ($h\nu_1$) is characterized by well-resolved vibrational structure in the 370−400 nm region. The band positions and their relative intensities are sensitive to the environment. Typical lifetimes (τs) are around 400 ns in homogeneous nonpolar solvents. Pyrene is capable of forming a fluorescent excimer (Py_2^*), with a featureless emission in the 450−550 nm region ($h\nu_2$), with typical lifetimes of ~60 ns.[10] In a semirigid environment, excimer formation can be taken as evidence for proximity between pyrene chromophores. In our case this would reflect a high local quinone concentration prior to pyrene derivatization. Unfortunately, strong pulp fluorescence interferes with excimer emission under steady state conditions. Time-resolved spectroscopic experiments aimed at addressing this question are presented separately.

Experiments employing method II involved the same type of controls as already described for method I. Figure 4 illustrates a series of experiments performed on unbleached TMP. The spectra reveal quite clearly the incorporation of pyrene centers resulting from UV irradiation of the pulp. All other emission spectra presented from this point on have been corrected in a similar manner, and only the difference spectrum (as shown in the bottom boxes of Figures 1 and 4) will be given. Figure 5 illustrates the difference spectrum obtained from TMP paper, along with the diffuse reflectance spectra of samples treated and untreated with pyrene. The latter shows that the amount of pyrene incorporated is in fact small enough not to make a significant difference in the absorption (reflectance) spectrum of the sample. The fluorescence spectrum on paper is quite comparable with that from pulp, with characteristic pyrene bands at 375 and 397 nm. No evidence for excimer emission was obtained in these studies, although time resolved work (vide infra) tells a more complete story.

Several excitation wavelengths (e.g., 316, 330, and 345 nm) were employed in attempts to optimize the emission spectra. In general, longer excitation wavelengths lead to improved spectra, largely as a result of better discrimination in favor of pyrene rather than lignin absorption. The amount of pyrene that can be incorporated on the pulp depends on the surface area undergoing irradiation. The light used for irradiation can cause photodegradation only in the upper layer of

(10) Birks, J. B.; Dyson, D. J.; Munro, I. H. *Proc. R. Soc. (London)* **1963**, *275A*, 575.

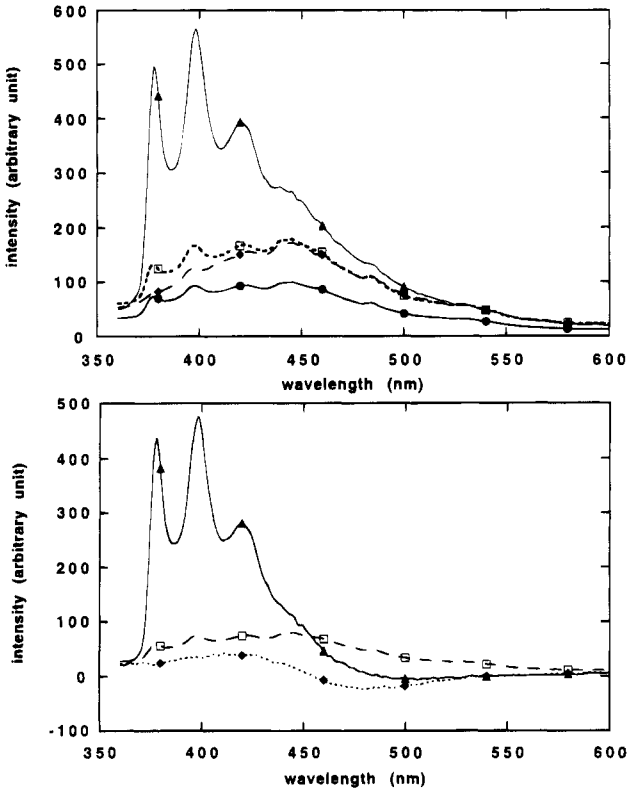


Figure 4. Front-face fluorescence spectra of TMP pulp at 330 nm excitation wavelength. Nonirradiated/derivatized (□), nonirradiated/untreated (●), and irradiated/derivatized (▲), irradiated/untreated (■) samples. Bottom:. The difference spectra of the above samples. Nonirradiated/untreated sample as reference.

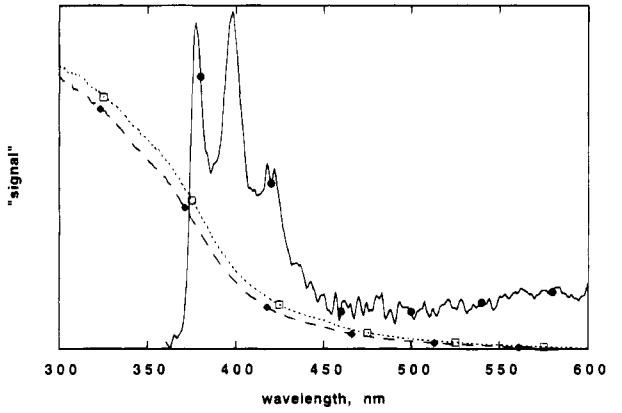


Figure 5. Fluorescence (●) and diffuse reflectance spectra of TMP treated (♦) and untreated (□) with pyrene using method II.

the surface. The amount of lignin in this layer determines therefore the amount of quinone units formed in the process of photodegradation.

Figure 6 shows the difference in pyrene incorporation following irradiation, for samples bleached with hydrogen peroxide or with sulfite (see Experimental Section for details of the experiments). Quite clearly, bleaching promotes the subsequent incorporation of pyrene following irradiation. The same observation was made when we used bleached TMP handsheets as opposed to the unbleached pulp. While both reductive and oxidative bleaching processes promote enhanced degradation during irradiation, it is apparent that peroxide treatment is most effective in causing increased degradation.

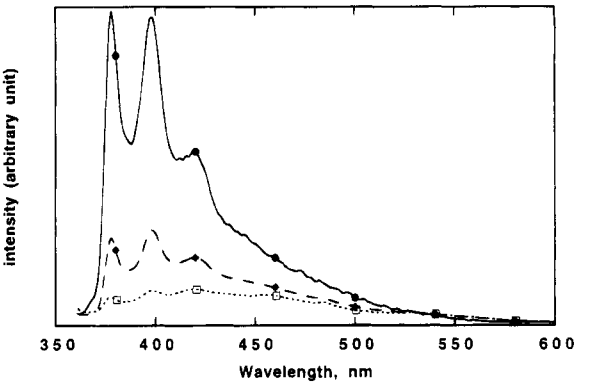


Figure 6. Front face fluorescence spectra of (□) unbleached TMP, (●) peroxide bleached TMP, (♦) hydrosulfite bleached TMP. Excitation wavelength 345 nm. Normalization is done at 520–580 nm

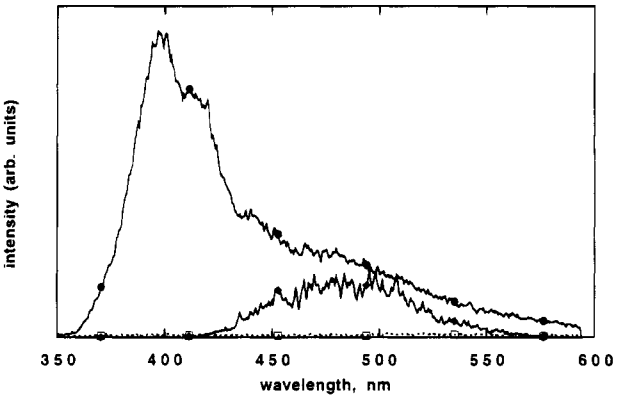


Figure 7. Gated spectra of irradiated TMP treated with low pyrenebutyric acid concentrated recorded between 53 and 115 ns after low excitation (●) and spectra for untreated pulp in the time windows of 0–25 ns (♦) and 53–115 ns (□). Excitation at 355 nm, under nitrogen, with 1 min integration time. Note that the untreated pulp was recorded at low gain, since its intensity at short time is quite high.

Since we have shown that in method II pyrene incorporation is a highly selective indicator of the formation of *o*-quinones, we conclude that bleaching enhances the tendency toward lignin photodegradation in this manner. Under extended irradiation (≥24 h) the amount of incorporated pyrene decreased. This supports the observation made by others that reaching the maximum, upon further irradiation, the quinone itself undergoes photodegradation.[11]

Time-Resolved Fluorescence Spectroscopy. We have noted earlier that the luminescence from pulp tends to interfere with pyrene emission, especially in the case of the pyrene excimer. Despite detailed spectroscopic work[7] no measurements of the emission lifetimes have been reported. Our early studies showed that this emission, centered around 480 nm was much shorter than the 355 nm pulse (~6 ns) from our nanosecond Nd: Yag laser. Given that pyrene emissions tend to be longer lived, this allows the straightforward differentiation of pulp and probe luminescence, by appropriate gating of the detection system. Figure 7 shows spectra of treated and untreated pulps. Note that untreated pulp shows no emission in the 53–115 ns time window.

(11) Argyropoulos, D. S.; Heitner, C.; Schmidt, J. A. Observation of Quinonoid Groups During the Light-Induced Yellowing of Softwood Mechanical Pulp; Report PGRL-565 from the Pulp and Paper Research Institute of Canada, Feb 1994.

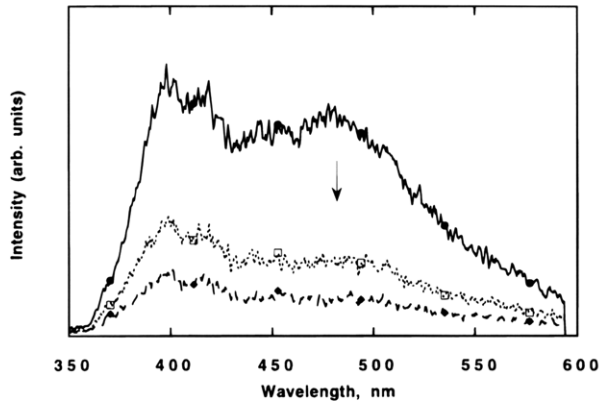


Figure 8. Time-resolved fluorescence spectrum of irradiated TMP treated under air at low pyrene–butyric acid concentration. Time window: (●) 51–134 ns, (□) 93–176 ns, (♦) 134–217 ns. Excitation: 355 nm, integration time: 1 min.

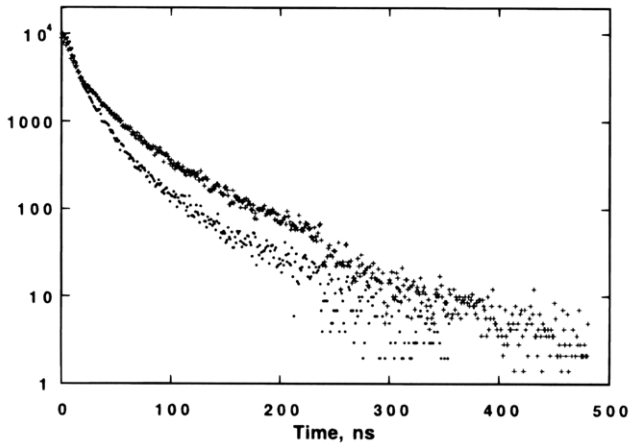


Figure 9. Decay kinetics of (●) the monomer and (+) excimer emission of TMP under air. The trigger of the detection system was delayed 30 ns to avoid interference from the intense pulp emission in the excimer region.

Pyrene incorporation experiments following irradiation were carried out for two different levels of pyrene incorporation. They are referred as "low" and "high" pyrene, and full details of the incorporation are provided in the Experimental Section.

Figure 7 shows the spectra recorded for the low pyrene sample under nitrogen. No change in spectra was recorded in a time window from 53 to 115 ns following the 6 ns, 355 nm laser pulse. The small shoulder in the 450–500 nm region is indicative of some excimer emission. Note also that the pyrene-free sample shows no detectable emission in the excimer region in the 53–115 ns window, indicating excellent discrimination between pyrene and pulp emission. Kinetic studies show that the decay is essentially insensitive to saturation with air. Biexponential analysis (starting 30 ns after the laser pulse) provides a reasonable fit to the data with lifetimes of ~16 and 57 ns.

The decay of pyrene emission in high load pulps is also comparable under nitrogen and under air, where a biexponential analysis led to lifetimes of 15 and 65 ns. In the excimer region, the lifetimes were ca. 13 and about 80 ns. The spectra recorded at different times following laser excitation are shown in Figure 8, and Figure 9 illustrates the fluorescence decay traces.

Fluorescence Microscopy. These experiments were carried out in order to map the formation of quinone centers, in the hope of establishing whether degradation occurred in specific regions of the fibers. These measurements were difficult because of the interference from pulp fluorescence. Unfortunately, these experiments do not have any temporal resolution and the type of discrimination shown in Figures 7 and 8 cannot be achieved in the microscopy experiments. Figure 10 illustrates the fluorescence and phase contrast photographs from an irradiated and treated (method II) sample. While the luminescence is evident, we found that it was difficult to draw any conclusions from examination of a small number of photographs because some intrinsic pulp fluorescence is always present. We tried to minimize this problem by using a rather long excitation wavelength (centered at 360 nm) and a cutoff filter at 420 nm. It would be impossible to include in this article a large number of photographs; however, such an examination leads to the conclusion that pyrene incorporation (and thus quinone formation) is enhanced in fines and fragmented fibers, while large intact fibers appear to be somewhat more resistant to degradation. Work in this area is currently being extended with the development of new probes, better suited (i.e., red emitting) for fluorescence microscopy work.

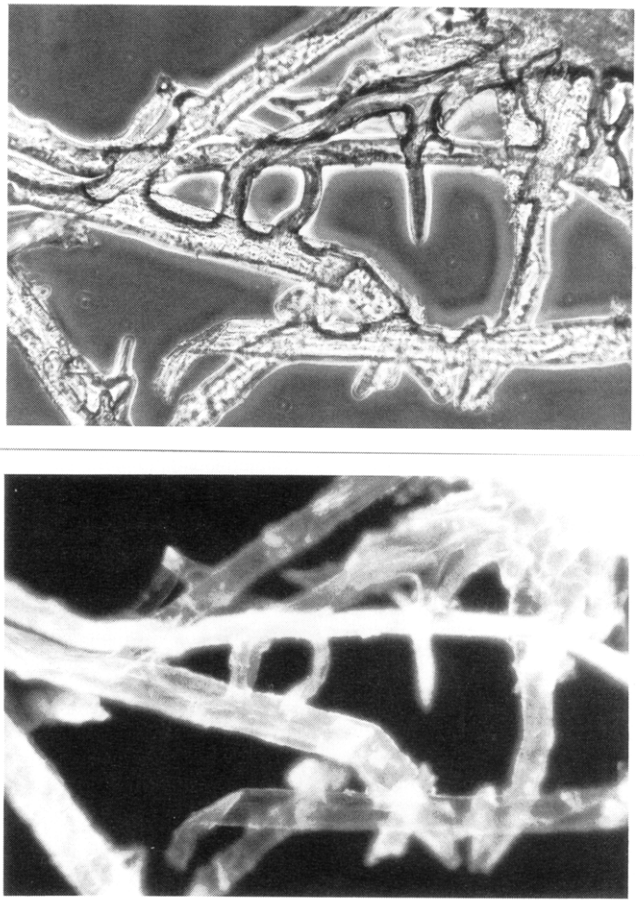


Figure 10. Fluorescence microscopy photographs of the same segment of treated (method II) and irradiated TMP. Top: Phase contrast photograph. Bottom: Fluorescence detection. Note the bright emission from the thin fibers, and at the top right corner, where the presence of small fragments resulted in high emission intensity.

Discussion

The results of our work demonstrate that it is possible to employ fluorescence spectroscopy as a tool to study

the photodegradation of pulp and paper. Pyrene probes, particularly when incorporated by method II are highly specific for the detection of *o*-quinones.

There are advantages and disadvantages related to the specific use of pyrene derivatives as probes, given that method II would also be applicable to other probes provided that an appropriate derivative attached to a carboxylic acid can be prepared. Pyrene has the advantage that its long fluorescence lifetime (even if considerably reduced in pulp) allows temporal discrimination from pulp emission. Further, excimer emission can provide a probe for chromophore proximity.

The vibrational structure of pyrene is well-known to be sensitive to the environment, and detailed studies of solvent effects[4,5,12] are frequently used as a *yardstick* to determine local polarity. This sensitivity is due to the Ham effect, which unfortunately is strongly symmetry dependent,[13] and therefore reported calibrations are not directly applicable to substituted pyrenes. However, examination of the emission intensity ratio for the III/I vibrational bands suggests a relatively polar environment. This is consistent with the presence of phenols, alcohols and water in the lignin-rich regions of the pulp.

Diffuse reflectance studies (see Figure 5) indicate that the actual amount of pyrene incorporated is very small. Despite this, our time-resolved work indicates some excimer emission, even in the case of low pyrene loading. At the same time, we failed to resolve any growth component in the long wavelength excimer emission. This observation suggests that those pyrene moieties responsible for excimer emission are already in close proximity at the time of excitation and that excimer formation requires none, or minimal diffusion. This, combined with the low pyrene loading, implies that quinone formation is not a random process but rather that chromophores are formed within *domains* or *islands* in the pulp. Whether this is due to the inhomogeneous nature of the distribution of quinone precursors or the result of a cooperative effect cannot be established from our experiments. A cooperative effect could result from increased accessibility of environmental oxygen to regions that have already undergone some degradation. The result is consistent with fluorescence microscopy data that suggest enhanced degradation in fines and fragmented fibers.

Time-resolved studies lead to pyrene emission lifetimes significantly shorter than those usually obtained in homogeneous solution, even when the pulp samples are saturated with a nitrogen atmosphere. This can be attributed to two origins, and most likely, to a combination of both. First, pyrene singlets usually have relatively shorter lifetimes in hydroxylic media, specially in water.[14] Even "dry" pulp probably contains enough moisture to influence the singlet lifetimes. Second, it is possible that some of the functionalities in pulp act as quenchers for pyrene singlets. Such groups may include substituted stilbenes. Pyrene is prone to electron transfer interactions and may interact with some of the abundant electron-rich moieties in pulp.

Finally, in the context of yellowing inhibition, our results suggest that given the nonhomogeneous distribution of the photodegradation process, it would be desirable to target those domains where degradation occurs with the development of inhibitors capable of seeking these regions. While this clearly poses a major scientific and technical challenge, it is also evident that such targeting would reduce the loading of inhibitor required, something that could obviously be economically desirable. The development of such domain-specific inhibitors would require further studies aimed at fully establishing the nature, polarity, and accessibility of degradation-prone regions of the fibers.

(12) Stahlberg, J.; Almgren, M. *Anal. Chem.* **1985**, *57*, 817.
(13) Koyanagi, M. *J. Mol. Spectrosc.* **1968**, *25*, 273.
(14) Lianos, P.; Lux, B.; Gerard, D. *J. Chim. Phys.* **1980**, *77*, 908.

Experimental Section

Materials. Triisopropyl phosphite, 1,6-benzoquinone, 3,5-di-*tert*-butyl-1,2-benzoquinone, 98%, coniferylaldehyde, and deuterated solvents were obtained from Aldrich, ^{13}C-labeled acetic acid was from Cambridge Isotopes, and pyrenebutyric acid was purchased from Fluorescent Probes Inc.

Dialkylpyrene phosphite was prepared as follows: 4.5 mL (50 mmol) of dimethyl phosphite was added to a vigorously stirred and cooled dispersion of 10 g (48 mmol) of phosphorus pentachloride in 100 mL of chloroform. After all the phosphorus pentachloride reacted, the reaction mixture was distilled first at atmospheric pressure to remove the solvent at 75 °C and then under reduced pressure (20 mmHg/65 °C). NMR of the starting material in $CDCl_3$ 6.2 ppm (dd), 7.25 (d). $J^1_{(P-H)} = 60$ Hz, $J^2_{(P-H)} = 12$ Hz, $J_{(H-H)} = 0.76$ Hz. NMR of the product in $CDCl_3$: 5.38 (d), 5.3(d). To a 5 mL chloroform solution of 0.1 mL of dimethyl chlorophosphite, a 5 mL solution of 100 mg of pyrenylbutanol was added dropwise. The mixture was stirred and refluxed for 3 h followed by washing with two portions with water and drying over $MgSO_4$. After this the solution was concentrated and cromatographed on a silica gel column with a chloroform–hexane mixture.

Pulp Origin and Pretreatment. In these experiments bleached handsheets and unbleached thermomechanical pulp were used. A measured amount of the dried pulp, was stirred for 2–3 h to separate fibers. After this, a thin sheet with a smooth surface was prepared, dried, and irradiated at 350 nm.

Alkaline peroxide bleaching and hydrosulfite bleaching were performed before making the handsheets. The bleaching of the pulp followed exactly the method described in the literature.[2]

The irradiation of the pulp was carried out at either 300 or 350 nm for 4 h employing a home built photochemical reactor fitted with 12 Rayonet lamps.

Pulp Postirradiation Treatment. The first step in this treatment is the neutralization of the carboxylic acid groups in the pulp. The pulp sample was dispersed in 0.1 M NaCl solution and the pH was adjusted to 10 by 0.1 M NaOH. The pulp was stirred for 2 h, followed by filtration and washing with water, ethanol and acetone.

Oxyphosphorylation of the Pulp. The pulp was dried in an oven and then cooled in a vacuum to remove as much of the adsorbed water as possible. The dry sample, generally 0.5 g, was reacted overnight in dry methylene dichloride with 1 mL triisopropyl phosphite. After the reaction was complete, the sample was washed with several portions of the solvent and reacted with pyrenebutyric acid overnight (method II). After several washings, the sample was dried under a nitrogen flow, and fluorescence spectra were taken. In some experiments, 4-pyrenylbutyl dimethyl phosphite (**VI**) was used directly instead of triisopropyl phosphite. In this case the second step was not necessary.

Preparation of the Samples for Time-Resolved Experiments. For these experiments 2 × 4 cm^2 rectangles of the TMP handsheets were used (~0.2 g). After performing the triisopropyl phosphite treatment mentioned above, they were reacted with 5 mg of pyrenylbutyric acid in the case of a low pyrene load and with 30 mg in the case of a high pyrene load.

General Techniques. UV–visible diffuse reflectance spectra were recorded using Cary 1E spectrophotometer. Steady-

state irradiation was performed in a photoreactor equipped with 12 RPR-3000 Å or RPR-3500 Å lamps. The temperature of the reactor was typically between 32 and 35 °C. Fluorescence spectra were recorded using front-face excitation, with a Perkin-Elmer LS-50 spectrofluorimeter equipped with a solid sample accessory. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Model Gemini-200 200 MHz spectrometer.

Time-Resolved Luminescence. Experiments were carried out using the third harmonic of a Surelite Nd:YAG laser (355 nm, ~6 ns, <50 mJ/pulse) for excitation, the emitted signal was collected by a Hamamatsu picosecond fluorescence lifetime measurement system using a Model C4334 streak scope.

Fluorescence Microscopy. These measurements were performed at the Health Canada laboratories in Ottawa using an Olympus BH2 reflected light fluorescence microscope. Fluorescence excitation was performed with a BP 405 UV excitation cube, and emission was monitored at $\lambda > 420$ nm.

Acknowledgment. Thanks are due to Drs. Phil Whiting (Abitibi Price) and to J. Schmidt (PAPRICAN) for providing the pulp and paper samples used in these studies. We are grateful to Dr M. Barra and Dr. F. Cozens for their help with the time resolved luminescence studies and to Dr. D. Gray for preprints of his luminescence articles.[7,8] Fluorescence microscopy work was carried out at the Health Canada laboratories in Ottawa with the help of Dr. J. McLean. This work has been supported by the Mechanical and Thermomechanical Pulps Network, a part of the Canadian Centres of Excellence Program.