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Fluorescence, Metal Ion Binding, and Viscosity Properties of Poly[2- and 4-vinylpyridine *N*-oxide]s in Aqueous Solution

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ABSTRACT: Poly(2-vinylpyridine *N*-oxide) (P2VPNO) and poly(4-vinylpyridine *N*-oxide) (P4VPNO) were synthesized by the oxidation of the corresponding poly(vinylpyridine)s. They are soluble in water and have a characteristic fluorescence in aqueous solutions. The fluorescence intensities of P2VPNO were over 3 times that of P4VPNO in 10^{-2} M aqueous solutions at ambient temperature, which was accounted for by the difference in the microenvironment of the pyridine *N*-oxide fluorophore. The *N*-oxide polymers formed strong complexes with Tb^{3+} in aqueous solution, and the fluorescence intensities of Tb^{3+} were greatly enhanced upon complex formation. The increase in fluorescence intensity is due both to the replacement of inner-coordinated water molecules of Tb^{3+} by *N*-oxide polymers and to a ligand-to-metal energy transfer. P4VPNO is a better complexing agent for Tb^{3+} than P2VPNO. The reduced viscosity of P4VPNO increased sharply at polymer concentrations smaller than 1.0 g/dL, as in polyelectrolytes. However, in the case of P2VPNO, the concentration dependence of the reduced viscosity was similar to that of nonionic polymers. These differences are due to the position of the N−O group. P2VPNO and P4VPNO have a charge separation across the $N^{+}-O^{-}$ bond with a large dipole moment. P4VPNO forms a partial complex with water which transforms the N−O group into a weak acid. The complex then behaves like a polyelectrolyte, and upon dilution, the reduced viscosity increases due to chain expansion. In the case of P2VPNO, the N−O group is close to the polymer backbone, which sterically decreases the formation of the water complex, and its reduced viscosity then behaves like that of nonionic polymers. Although P2VPNO does not behave like a polyelectrolyte, addition of Tb^{3+} reduces the solution viscosity due to a complexation of Tb^{3+} with the ligands.

Introduction

Poly(vinylpyridine *N*-oxide)s (Figure 1) were synthesized and characterized about 3 decades ago.[1] Poly(2-vinylpyridine *N*-oxide) (P2VPNO) was used as a chemotherapeutic agent against silicosis[2] and inhibits the fibrogenesis normally caused by quartz dust in the lungs of animals.[3] Poly(vinylpyridine *N*-oxide)s (PVPNO) form complexes with dichromates and were applied as versatile oxidizing agents for organic compounds.[4]

Recently, viscosity and light-scattering studies of poly(4-vinylpyridine N-oxide) (P4VPNO) in aqueous solution were reported.[5] However, the solution and fluorescence properties of P2VPNO and P4VPNO have not been investigated. We found that these polymers exhibit a characteristic fluorescence in aqueous solutions. Here we report the fluorescence, metal ion binding, and viscosity properties of two isomeric polymers (P2VPNO and P4VPNO) under the same conditions and discuss the results as reflecting the chemical structure of the polymers.

Experimental Section

Materials and Synthesis. Terbium chloride hexahydrate was purchased from Rhone-Poulenc Basic Chemicals Co. and used as received.

4-Methylpyridine *N*-oxide (4-MPNO) was purchased from Aldrich and purified by reprecipitating from the ethanol solution by adding ethyl ether.

Distilled and deionized water were used throughout.

P2VPNO and P4VPNO were synthesized as described in the literature.[1,5]

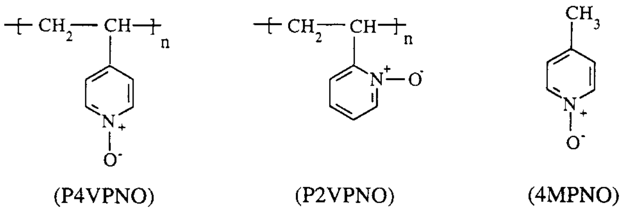

Figure 1. Chemical structures of poly[4-vinylpyridine *N*-oxide] (P4VPNO), poly[2-vinylpyridine *N*-oxide] (P2VPNO), and 4-methylpyridine *N*-oxide (4MPNO).

Poly(2- and 4-vinylpyridine)s. The monomers 2- and 4-vinylpyridines were purchased from Aldrich and vacuum distilled to remove inhibitor before use. Poly(2-vinylpyridine) (P2VP) and poly(4-vinylpyridine) (P4VP) were prepared by a free-radical polymerization in methanol. The monomer (20 g), 0.05 g of AIBN, and 50 mL of methanol were sealed into a 100-mL ampule under nitrogen. Polymerization proceeded at 60 °C for 7 h. The products (conversions of 43% and 51% for P2VP and P4VP, respectively) were reprecipitated three times from methanol into diethyl ether and finally freeze-dried from a dioxane solution. The dry materials were stored under nitrogen.

Poly(2- and 4-vinylpyridine *N*-oxide)s. A typical synthetic procedure for PVPNO is as follows. P2VP or P4VP (5 g) in glacial acetic acid (40 mL) was heated at 70−75 °C with 30% hydrogen peroxide (20 mL) for 24 h. The acetic acid was removed by distillation in a vacuum, and the residue was dissolved in water (60 mL) and poured through a column filled with Amberlite IRA-400 anion-exchange resin. The solution was freeze-dried, and the polymer was dissolved in absolute ethanol and precipitated into diethyl ether. Drying to constant mass gave yields of 72% and 80% for P2VPNO and P4VPNO, respectively.

Characterization. Intrinsic Viscosity. A Ubbelohde viscometer was used to determine the intrinsic viscosity [η] of

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poly(vinylpyridine)s and poly(vinylpyridine *N*-oxide)s in a thermostated water bath at 25 ± 0.1 °C. Ethanol and methanol were used as the solvents for P4VP and P2VP, respectively, while water was used for P2VPNO and P4VPNO.

Infrared Spectra. An Analect FX 6260 Fourier transform infrared spectrometer was used to identify the functional groups of the polymers.

Ultraviolet Spectra. A Cary 2300 UV−vis−near IR spectrometer was used to determine the absorption spectra of P2VPNO, P4VPNO, and 4MPNO in water.

Nuclear Magnetic Resonance Spectra. A General Electric GN-300 Fourier transform nuclear magnetic resonance spectrometer operating at 300 MHz was used to verify the structures of P2VPNO and P4VPNO in both the solid state and solution. ^{13}C NMR and ^{1}H NMR were examined at room temperature. The spectra of the ^{13}C and ^{1}H NMR and FTIR for poly(vinylpyridine *N*-oxide)s were in agreement with data reported in the literature[5,6] and indicated that the poly(vinylpyridine)s were quantitatively converted to *N*-oxide derivatives.

Steady-State Fluorescence Spectra. A Perkin-Elmer LS50B fluorescence spectrometer was used for steady-state fluorescence measurements at ambient temperature. The slit width was 5 nm for both excitation and emission monochromators, and the fluorescence intensity was reported in arbitrary units. The samples in a quartz cuvette (1 × 1 × 4 cm) were measured in the L-format arrangement. The fluorescence spectra were not corrected.

Time-Resolved Luminescence. The time-resolved luminescence decays in the millisecond time scale were measured by the Perkin-Elmer LS50B instrument in the phosphorescence mode. The Tb^{3+} luminescence intensity was measured at 20 different delay points following the excitation (xenon lamp equipped with chopper). The time-resolved fluorescence in the nanosecond time scale was measured using a FL 900 CDT fluorimeter (Edinburgh Analytical Instruments). This apparatus uses the time-correlated single photon counting method. The decays of fluorescence intensity were analyzed by deconvolution of the lamp pulse with the impulse response of the sample.[7]

Molar Weights of the Polymers. The relative molecular weights of P2VPNO and P4VPNO were estimated from the intrinsic viscosity measurements of the precursors. The intrinsic viscosities of P4VP in ethanol and P2VP in methanol were 0.776 and 0.766 dL/g, respectively. From the Mark−Houwink equation $[\eta] = kM_v^{a}$, where $k = 2.50 \times 10^{-2}$ mL/g and $a = 0.68$ for P4VP in ethanol[8] and $k = 1.13 \times 10^{-2}$ mL/g and $a = 0.73$ for P2VP in methanol,[9] the viscosity average of the molar weights was calculated as 1.36×10^{5} and 1.77×10^{5} for P4VP and P2VP, respectively. The viscosity averages of the molar weights of P4VPNO and P2VPNO were then 1.55×10^{5} and 2.02×10^{5}, respectively, assuming that all poly(vinylpyridine)s were transformed to the *N*-oxide without degradation.

Results and Discussion

Absorption and Fluorescence of the Ligands. P2VPNO and P4VPNO are soluble in water but do not dissolve in common organic solvents such as tetrahydrofuran, dimethylformamide, acetone, and chloroform. The absorption spectra of these polymers and the model compound, 4-methylpyridine *N*-oxide (4MPNO), are shown in Figure 2. The absorption bands of both polymers have a maximum at 255 nm, and the molar absorption coefficients of P2VPNO and P4VPNO are $\epsilon_{255} = 6550$ and 10 200 L mol^{-1} cm^{-1}, respectively. The molar absorption coefficient of 4MPNO at 252 nm is $\epsilon_{252} = 14\,200$ L mol^{-1} cm^{-1}.

Although the corresponding 4-methylpyridine and poly(vinylpyridine)s are not fluorescent, 4MPNO, P2VPNO, and P4VPNO exhibit a characteristic fluorescence (parts A, B, and C of Figure 3, respectively).

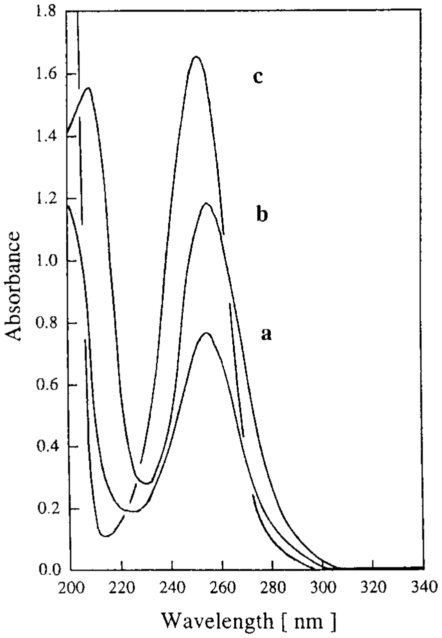

Figure 2. Absorption spectra of P2VPNO (a), P4VPNO (b), and 4MPNO (c) in water ([a] = [b] = [c] = 1.16×10^{-4} M, 1-cm cell).

With highly absorbing solutions, the inner-filter effect and the L-format arrangement lead to an emission intensity independent of the fluorophore concentration when the excitation wavelength is adjusted so as to keep a constant optical density. This emission intensity is then proportional to the fluorescence quantum yield. As seen in Figure 3A, this intensity is almost independent of the concentration of 4MPNO. Small differences may be due to the excitation slit width, which is not infinitesimally narrow. The maxima of the excitation spectra denote the wavelengths where the same optical densities for different samples were reached by scanning of the excitation (absorption) spectrum, and consequently, these maxima were shifted to longer wavelengths with increasing concentrations (Figure 3A, red shift e → a).

In the case of P2VPNO and P4VPNO, the fluorescence intensities in dilute water solution ($c \sim 10^{-4}$ M) are quite moderate, slightly higher for P2VPNO than for P4VPNO (Figure 3B and 3C). With increasing polymer concentration, the fluorescence intensity increases, and this increase is higher for P2VPNO. At a polymer concentration around 10^{-2} M, the fluorescence intensity of P2VPNO was found to be 3 times that of P4VPNO.

The fluorescence emission spectra of many fluorophores are sensitive to the polarity of their surrounding environment. If the emission spectrum of a probe such as ANS (1-anilino-8-naphthalenesulfonic acid) is examined in solvents of varying polarity, one finds that the emission spectrum shifts with increasing solvent polarity to longer wavelengths (red shifts), which is often accompanied by a decrease in the fluorescence quantum yield of the fluorophore.[10,11,13] The *N*-oxide polymers have no ionizable groups, but charge separation across the $N^{+}-O^{-}$ bond results in a large dipole moment.[12] The $N^{+}-O^{-}$ group in P4VPNO is located in the para position and separated from the backbone by the ring structure, whereas in P2VPNO, the $N^{+}-O^{-}$ group is close to its hydrophobic backbone. Consequently, the pyridine *N*-oxide fluorophores in P2VPNO are better shielded from the water medium by the polymer backbone than in

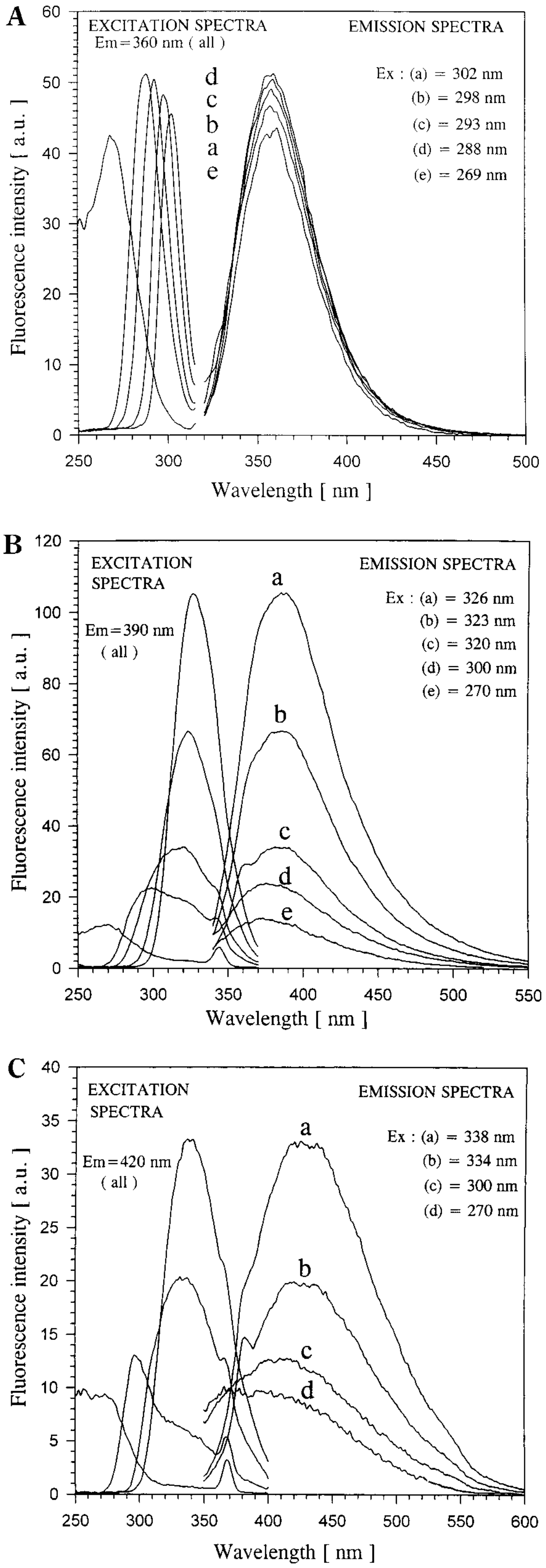

Figure 3. Fluorescence excitation and emission spectra for (A) 4MPNO, (B) P2VPNO, and (C) P4VPNO in water at different concentrations: [4MPNO] = 1.16×10^{-2} (a), 5.80×10^{-3} (b), 2.32×10^{-3} (c); 1.16×10^{-3} (d), and 1.16×10^{-4} M (e). [P2VPNO] = 1.16×10^{-2} (a), 5.80×10^{-3} (b), 2.32×10^{-3} (c), 1.16×10^{-3} (d), and 1.16×10^{-4} M (e). [P4VPNO] = 1.16×10^{-2} (a), 5.80×10^{-3} (b), 1.16×10^{-3} (c), and 1.16×10^{-4} M (d).

P4VPNO, and as a result, the fluorescence intensity for P2VPNO becomes higher than that of P4VPNO.

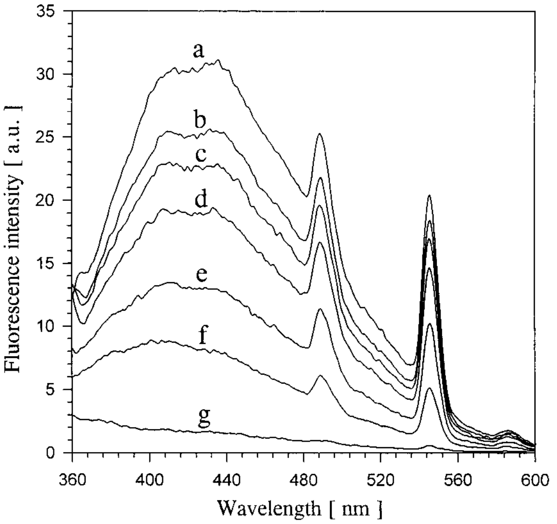

Figure 4. Fluorescence emission spectra of P4VPNO−Tb^{3+} complexes in water as a function of the ligand concentration ([P4VPNO] = 1.16×10^{-2} (a), 7.73×10^{-3} (b), 5.80×10^{-3} (c), 3.87×10^{-3} (d), 1.53×10^{-3} (e), 4.60×10^{-4} (f), and 0.00 M (g); [Tb^{3+}] = 7.0×10^{-4} M for all; ex = 322 (a), 320 (b), 319 (c), 318 (d), 314 (e), and 310 nm (f, g)).

The emission maximum was shifted from 390 nm for P2VPNO (Figure 3B) to 430 nm for P4VPNO (Figure 3C) at a concentration of around 1.0×10^{-2} M, and this supports the above interpretation in terms of the fluorophore microenvironment.

Metal Ion Binding: Tb^{3+} as a Fluorescence Probe. Pyridine *N*-oxide is a good ligand for many metal ions.[14] P2VPNO formed a stable complex with $HgCl_2$ in aqueous solution, whereas the Ca^{2+} cation complexed weakly with P2VPNO.[15] Lanthanide metal ions such as Tb^{3+} and Eu^{3+} exist as stable trivalent ions and exhibit their characteristic fluorescence in aqueous solution, which is known to be strongly influenced by their immediate coordinated environment.[16] The fluorescence intensity of the ions is normally quite weak in aqueous solution since the coordinated water molecules serve as efficient quenchers of the emission.[17] Recently we have observed[18,19] that fluorescence intensities of the Tb^{3+} ion are greatly enhanced upon binding to polycarboxylates. The results indicated that Tb^{3+} ions were strongly bound to carboxylate ions in aqueous solution and that some of their inner-coordinated water molecules were expelled upon binding.[19] We have investigated the fluorescence of Tb^{3+} complexes with P2VPNO and P4VPNO in aqueous solution.

The fluorescence emission spectra of [P4VPNO−Tb^{3+}] and [P2VPNO−Tb^{3+}] complexes in water are shown in Figures 4 and 5, as a function of the ligand concentration. The spectra were recorded using excitation at the maximum of the excitation spectrum. In the emission spectra, the peaks at 490 and 545 nm correspond to the $^5D_4 \rightarrow {}^7F_6$ and $^5D_4 \rightarrow {}^7F_5$ transitions of the Tb^{3+} ion, respectively,[20] and the ligand emission is in good agreement with those in Figure 3B and 3C for P2VPNO and P4VPNO, respectively. The fluorescence of Tb^{3+} increases with increasing ligand concentration. The increase of Tb^{3+} fluorescence intensity may be accounted for by two effects: a possible energy transfer from ligand to Tb^{3+} ion[21] and the replacement of some of the inner coordinated water molecules to the Tb^{3+} ion upon complex formation (see later discussion).

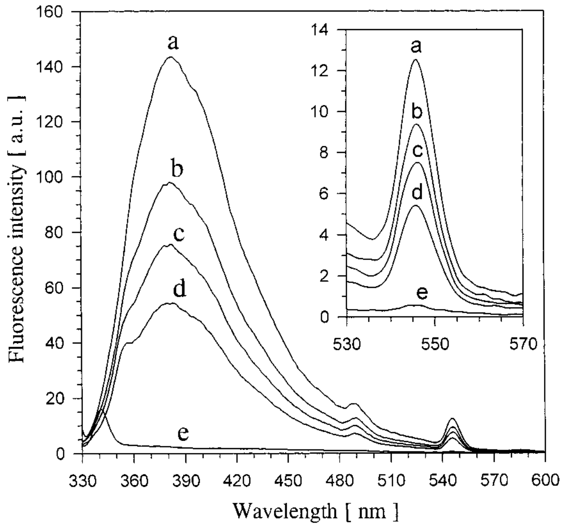

Figure 5. Fluorescence emission spectra of P2VPNO−Tb^{3+} complexes in water as a function of the ligand concentration ([P2VPNO] = 1.16×10^{-2} (a), 5.80×10^{-3} (b), 3.87×10^{-3} (c), 2.30×10^{-3} (d), and 0.00 M (e); [Tb^{3+}] = 7.0×10^{-4} M for all; ex = 321 (a), 318 (b), 316 (c, d), and 310 nm (e)).

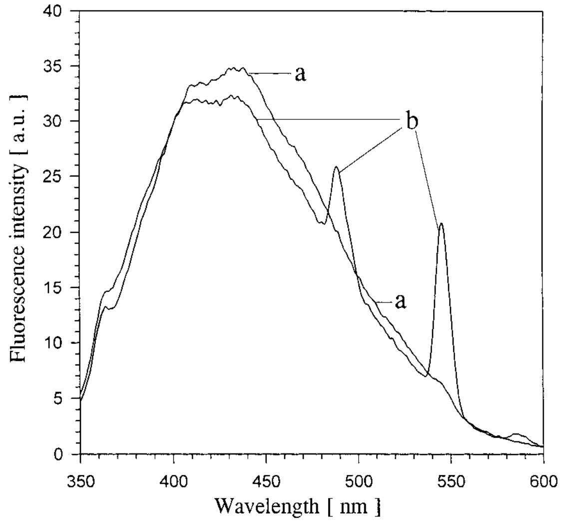

Figure 6. Fluorescence emission spectra of P4VPNO in the absence (a) and presence (b) of Tb^{3+} in water as a proof of an energy transfer ([P4VPNO] = 1.16×10^{-2} M, [Tb^{3+}] = 7.0×10^{-4} M, ex = 322 nm).

The emission spectra of poly(vinylpyridine *N*-oxide)s overlap the absorption spectrum of the Tb^{3+} ion. Thus, the fluorescence emission spectra (ex = 322 nm) of P4VPNO in the absence and presence of Tb^{3+} (Figure 6, spectra a and b, respectively) were taken in order to elucidate the ligand-to-metal energy transfer. At the same ligand concentration, the Tb^{3+} emissions at 490 and 545 nm are accompanied by a decrease in the fluorescence intensity of the donor (P4VPNO) at 430 nm, indicating that the energy absorbed by the ligand was partially transferred to the Tb^{3+} ion. A similar phenomenon was observed for the [P2VPNO−Tb^{3+}] complex; however, the effect was not as pronounced.

When the model fluorophore, 4-methylpyridine *N*-oxide (4MPNO), was added to the aqueous solution of TbCl$_3$, no increase in the fluorescence of Tb^{3+} was detected even when a large excess of 4MPNO was used ([4MPNO]/[Tb^{3+}] ~ 20). This showed that 4MPNO did not form a complex with Tb^{3+} in water solution. The result is attributed to the "polymer cooperative effect", i.e., the high local ligand concentration in the polymer domain.[22] Similar results were obtained in a solution of Tb^{3+} containing carboxylate ions;[18] the emission intensities of Tb^{3+} decrease in the order of polycarboxylate ≫ trimer > dimer > monomer.

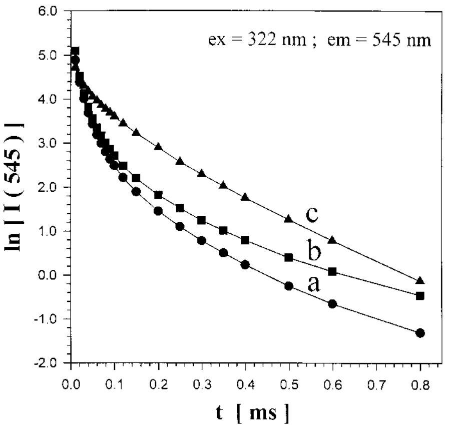

Figure 7. Luminescence decays of terbium at 545 nm for P4VPNO−Tb^{3+} in water (a) or deuterated water (b) and for P2VPNO−Tb^{3+} in water (c); semilogarithmic plot in the millisecond time scale ([ligand] = 1.16×10^{-2} M, [Tb^{3+}] = 1.0×10^{-3} M).

The fluorescence and viscosity (see later section) data show that the Tb^{3+} ion was bound strongly to the pyridine *N*-oxide, resulting in the replacement of some of the coordinated water molecules to the Tb^{3+} ion. The number of coordinated water molecules to Tb^{3+} can be calculated from the emission lifetimes of Tb^{3+} measured in water and deuterated water.[21] However, the luminescence decay of terbium in the systems P4VPNO−Tb^{3+} and P2VPNO−Tb^{3+} (ex = 322 nm, em = 545 nm) was found to be multiexponential both in water and in deuterated water (Figure 7). Several freshly prepared solutions with different samples behaved in a similar manner. The nonlinearity is more pronounced in the case of P4VPNO−Tb^{3+} than that of P2VPNO−Tb^{3+}. We measured the fluorescence lifetime of the ligand (PVPNO) in the nanosecond time scale in the presence and absence of Tb^{3+}. The fluorescence lifetime of the PVPNO at room temperature was found to be shorter than 0.2 ns (instrumental limitation of FL 900 CDT), and there was no sign of a longer lifetime component in the terbium-containing systems ([P2VPNO−Tb^{3+}], [P4VPNO−Tb^{3+}]). We cannot at present account for the multiexponential decay in the PVPNO−Tb^{3+} systems. Thus, the two effects for increasing the fluorescence intensity of Tb^{3+} upon complex formation with PVPNO, i.e., the energy transfer from ligand to Tb^{3+} and the replacement of coordinated water to Tb^{3+}, cannot be quantified at present.

Solution Viscosity. Aqueous solution viscosity data of P4VPNO are shown in Figure 8, where the reduced viscosities change only slightly with concentration above 1.0 g/dL but increase sharply upon further dilution. The shape of the curve is similar to that often found for polyelectrolytes. In the case of polyelectrolytes, an increased dilution reduces the shielding of the fixed charges, causing the polyion to expand. P4VPNO has a charge separation across the N^{+}−O^{-} bond but does not contain ionizable groups; hence, it is not a polyelectrolyte. The pH values of 5−10% purified P4VPNO (no trace amount of acetic acid remained) solutions in deionized water were measured. To our initial surprise, the solution was weakly acidic with a pH in the range

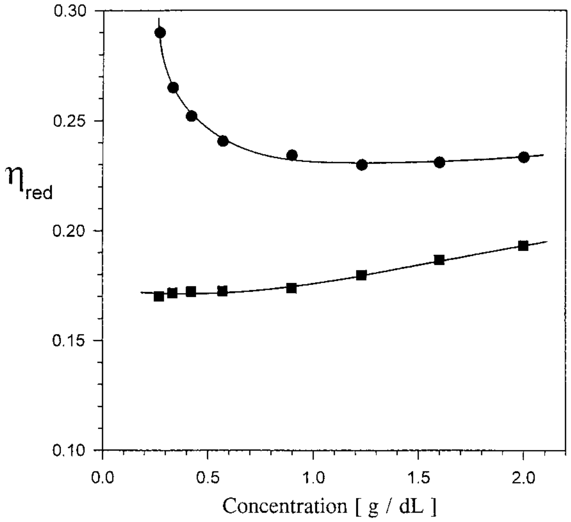

Figure 8. Reduced viscosity (η_{red}) of P4VPNO (M_v = 155 000) with and without Tb^{3+} in water at 25 °C as a function of concentration: P4VPNO (●); P4VPNO + Tb^{3+}, molar ratio 5:1 (■).

of 4.5. The measurements were repeated on different days with several freshly prepared samples and solutions. The results remained the same and confirmed the finding of Holt and co-workers,[6,23] who had investigated the relationship between pH and the viscosity of the aqueous PVPNO solutions. Even though they did not describe the ionic strength of the solutions, the reduced viscosity of the aqueous atactic P4VPNO solutions was found to increase sharply from pH 4 to 8. The increase was accounted for by the formation of a complex between P4VPNO and water. Whereas P4VPNO is amphoteric in aqueous solution, the polymer complexes partially with water.

The acidic hydrate is a polyelectrolyte; thus, upon dilution, the viscosity increases due to chain expansion (Figure 8). On the other hand, the concentration profile of the reduced viscosity for P2VPNO was similar to that of a nonionic polymer (Figure 9). This tendency was also observed by Holt and Lindsay[24] and Nasrallah.[15] The N−O group in P2VPNO is close to the polymer backbone, which sterically impedes the formation of the hydrate.

When Tb^{3+} was added to the aqueous solutions of P2VPNO and P4VPNO, the reduced viscosity decreased and followed a similar trend often found in salt-containing polyelectrolyte solutions. Since the P2VPNO does not behave as a polyelectrolyte, the decreased viscosity must be due to a complex formation causing a chain contraction.

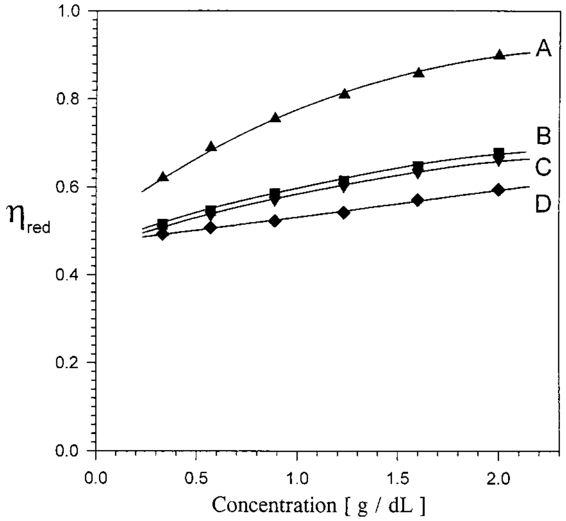

Figure 9. Reduced viscosity (η_{red}) of P2VPNO with and without Tb^{3+} in water at 25 °C as a function of concentration: P2VPNO, M_v = 202 000 (A); P2VPNO + Tb^{3+}, molar ratio 10:1 (B); P2VPNO + Tb^{3+}, molar ratio 5:1 (C); and P2VPNO + Tb^{3+}, molar ratio 3:1 (D).

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