Effect of the Molecular Weight and the Ionic Strength on the Photoluminescence Quenching of Water-Soluble Conjugated Polymer Sodium Poly[2-(3-thienyl)ethyloxy-4-butylsulfonate]

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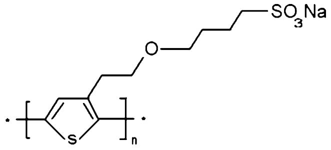
ABSTRACT: The photoluminescence (PL) quenching of sodium poly[2-(3-thienyl)ethyloxy-4-butylsulfonate)] (PTE-BS) by the cationic electron acceptor methyl viologen (MV^{2+}) was investigated in samples with high (HPTE-BS) and low (LPTE-BS) molecular weight. The Stern−Volmer constant decreases with decreasing the molecular weight from 8.4×10^5 M^{-1} down to 2.4×10^4 M^{-1}. This decrease is attributed to the lower concentration of the polyanion in the solution of LPTE-BS due to the lower solubility of this polymer when compared with HPTE-BS. Furthermore, we demonstrate that lowering HPTE-BS solubility by increasing the solution ionic strength affects the PL and the quenching mechanism. For the four salts investigated—NaCl, KCl, $MgCl_2$, and $CaCl_2$—the photoluminescence decreases with increasing the ionic strength, and the effect is conspicuous for the divalent cations investigated. The linear behavior characteristic of the quenching of the HPTE-BS luminescence by MV^{2+} disappears when the quenching is performed in the solution of $CaCl_2$ with ionic strength 0.3 M, which points to the formation of polymer aggregates due to the electrostatic bridging between monomers helped by the divalent cations introduced in the solution.

Introduction

The rationale for investigating the photoluminescence (PL) of water-soluble conjugated polymers is severalfold. First, they exhibit the remarkably property of having their fluorescence quenched by ultralow concentration of quencher, and this property makes them a very attractive materials for biosensors.[1−6] For example, the so-called superquenching, or amplified quenching effect, has been used for highly sensitive detection of proteins, nucleic acids, and enzymes.[1−4,6−10] Second, water-soluble conjugated polymers provide a new environment to investigate the properties of π-conjugated polymers that can lead to deeper understanding of the luminescence in these systems and to theoretical descriptions of charge-transfer reactions.[11] Third, the fluorescence methods are highly sensitive, easy to operate, and provide a tool to investigate how the electronic states of the luminescent polyelectrolyte are affected by the geometrical changes of the solvated photoluminescent polymer.[12,13]

Sodium poly[2-(3 thienyl)ethoxy-4-butylsulfonate] (PTE-BS) is a water-soluble luminescent conjugated polymer (Scheme 1). The photoluminescence of PTE-BS can be quenched by using low concentrations of methyl viologen (MV^{2+}), and this effect can be used for development of its applications in biosensors for ultra-analysis techniques. An understanding of how the photoluminescence is influenced by the molecular weight of the polymer and the ionic strength of the medium is important because these parameters may determine the yields and the stability of ultimate biosensor product.

Scheme 1. Molecular Structure of the Monomer Sodium [2-(3-Thienyl)ethyloxy-4-butylsulfonate)]



Nowadays, PTE-BS is commercially available (American Dye Source, Quebec) in two molecular weight forms: LPTE-BS ($M_n \approx 5000$, corresponding to ≈18 repeat units) and HPTE-BS ($M_n \approx 1\,000\,000$ corresponding to ≈3500 repeat units). In a previous study,[5] we have investigated several factors affecting the quenching behavior of LPTE-BS such as polymer concentration, oxygen in the solution, and the mixture with the conjugated polymer poly(2-methoxy-5-propyloxysulfonate phenylenevinylene) (MPS-PPV). In that study, we had commented on the low solubility of LPTE-BS in water which limited its applications. This problem disappears when using HPTE-BS which has the advantage of a much better solubility which facilitates its biosensor applications. Moreover, it has been reported that in cyanine dye pendant polylysine polyelectrolytes[14] the quenching sensitivity increases with the number of repeating units. Studies that span a reasonable range of polyelectrolytes and molecular weights are required for understanding to what extent this is a general phenomenon. We have undertaken this study in PTE-BS to investigate how the polymer PL quenching changes with the molecular weight.

The quantitative measure of PL quenching is given by the Stern−Volmer constant K_{SV} defined by

$$PL^0/PL = 1 + K_{SV}[Q] \qquad (1)$$

where PL^0 and PL are the intensity of fluorescence in absence and in the presence of the quencher respectively, [Q] is the concentration of quencher, and K_{SV} is the efficiency of quenching. In this work, we present a comparative study of the photoluminescence quenching by MV^{2+} of HPTE-BS and LPTE-BS. We found that the Stern−Volmer constant increases with increasing the

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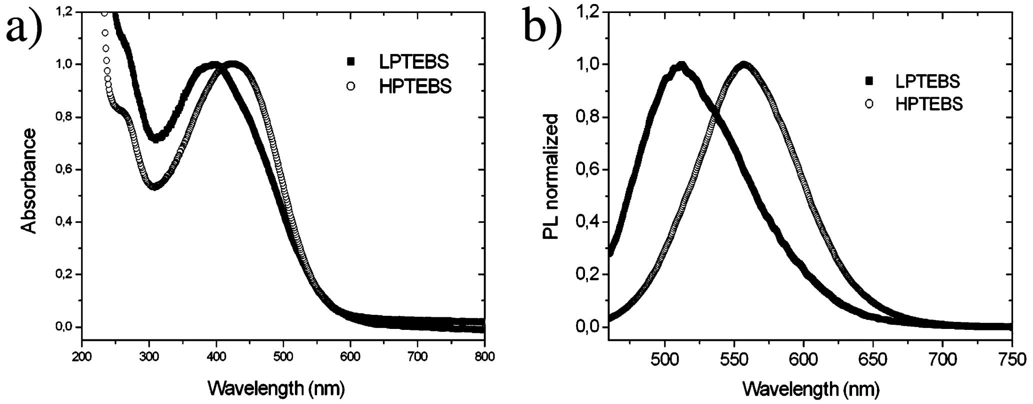

Figure 1. (a) Absorption spectra of low molecular weight (≈5 × 10^3) and high molecular weight (>10^6) PTE-BS in water (8 × 10^{-5} M). (b) Fluorescence spectra of LPTE-BS and HPTE-BS aqueous 8 × 10^{-5} M polymer solutions. The excitation wavelength was 400 nm for LPTE-BS and 438 nm for HPTE-BS. The normalization factor for the fluorescence spectra of HPTE-BS and LPTE-BS is 4.

molecular weight of PTE-BS, and this phenomenon is related to the solubility of the polyelectrolyte which can be varied by changing the ionic strength of the solution. We have studied the dependence of the PL on the amount of salt concentration in the solution, and interestingly the PL quenching is damped by the cation charge.

Materials and Sample Preparation

Sodium poly[2-(3-thienyl)ethoxy-4-butylsulfonate] with M_w ≈ 10^6 (HPTE-BS) and M_n ≈ 5 × 10^3 was purchased from American Dye Source. Polymer aqueous solutions were prepared, and PL and absorption measurements were performed according to previously reported methods.[5] Typically a stock solution of the polymer with concentration 1.89 × 10^{-3} M (concentrations are given in monomer repeat units) was prepared and purged with nitrogen for more than 3 h The solutions for the fluorescence and the absorption spectra measurements were made by diluting with nitrogen purged water the stock solution down to 1.89 × 10^{-4} M. The later concentration of HPTE-BS was used for all the experiments performed in this investigation. The ionic strength of the solution was adjusted to a given value by adding the required amount of salt. The solution was stirred for about 3 min before the PL measurement was performed, and a new amount of salt was added. The experiments were carried out at room temperature (≈23 °C).

Results and Discussion

Influence of the Molecular Weight in the Photoluminescence of PTE-BS. As the polymer molecular weight increases, both the absorption and fluorescence broad maximum evolve toward higher wavelengths. A red shift of the absorption maximum from 405 to 425 nm and of the fluorescence maximum from 512 to 557 nm can be observed in parts a and b of Figure 1, respectively.

We have investigated the photoluminescence quenching of HPTE-BS by MV^{2+}. In Figure 2 we present the fluorescence spectra from aqueous solution of HPTE-BS in the presence of a series of concentrations of MV^{2+} which range from 1.15 × 10^{-9} M at the top of the plot down to 1.13 × 10^{-5} M at the bottom of the plot when the quenching of HPTE-BS is around 93%. The Stern–Volmer plot for both HPTE-BS and LPT-BS is shown in Figure 3. The HPTE-BS value K_{SV} = 8.4 × 10^5 M^{-1} is considerably larger than the value 2.4 × 10^4 M^{-1} measured in LPTE-BS.[5] As is illustrated in Figure 3, the photoluminescence is quenched by MV^{2+} more efficiently in HPTE-BS than it was in LPTE-BS. The increase in K_{SV} with increasing the number repeating units in the polymer chain has been attributed[14] to the rise of the equilibrium constant for binding the quenchers and the amplified quenching of a delocalized exciton of around 100 polymer repeat units (3500 monomer units in HPTE-BS in contrast with the around 18 monomer units in LPTE-BS). It seems that increasing

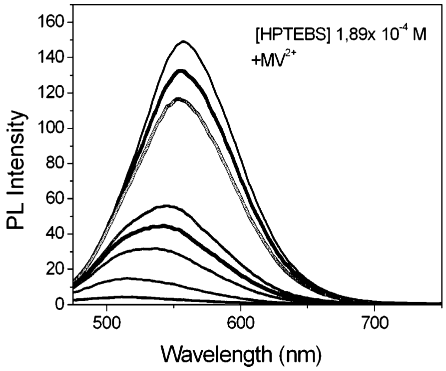

Figure 2. Emission quenching of HPTEBS (1.89 × 10^{-4} M) excited at 438 nm by MV^{2+} in water solutions. Oxygen was removed from the solution by nitrogen bubbling for 4 h prior to PL measurements. The concentration of MV^{2+} ranges from 1.15 × 10^{-9} M (second curve at the top of the plot) down to 1.13 × 10^{-5} M (curve at the bottom of the plot).

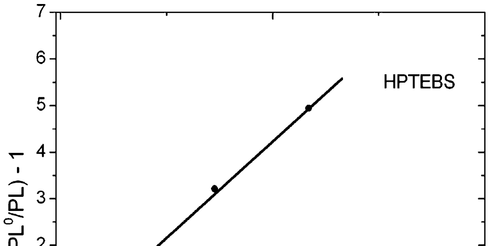

Figure 3. Stern–Volmer plot for fluorescence quenching by MV^{2+} for HPTE-BS and LPTE-BS aqueous solution of concentration 1.89 × 10^{-4} M. The emission wavelengths used to obtain the plot were 512 nm for LPTE-BS and 557 nm for HPTE-BS, and they correspond to the fluorescence maximum of the respective polymer solution without quencher added.

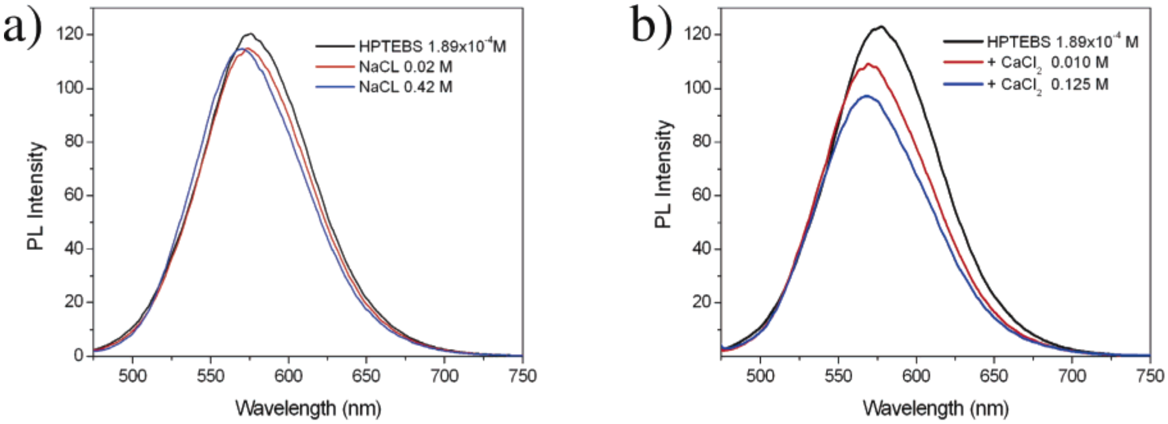

Figure 4. Evolution of fluorescence spectra (excited at 438 nm) of HPTEBS (1.89×10^{-4} M) in aqueous solutions of similar ionic strength: (a) as a function of the NaCl concentration; (b) evolution of fluorescence spectra as a function of the $CaCl_2$ concentration.

the M_w enhances the fluorescence of the polymer, and this factor is decisive for the quenching phenomena. Two different luminescent polymers with similar molecular weight (around 10^6 Da) such as MPS-PPV[5] and HPTE-BS present K_{SV} values of the same order of magnitude, 1.3×10^6 and 0.84×10^6 M^{-1}, respectively, whereas the same polymer but with smaller molecular weight, LHPE-BS, gives a K_{SV} value more than 300 times smaller, 2.4×10^4 M^{-1}, indicating that the structure of the polymer in the solution placed an important role in the quenching phenomena.

The luminescent polymer is a polyanion, and in pure aqueous solution there is equilibrium

$$\text{HPTE-BS} \overset{K_H}{\Leftrightarrow} \text{HPTE-B}[SO_3^{-}]_n + n\text{Na}^+ \qquad (2)$$

with equilibrium constant K_H. Following light absorption the HPTE-BS polyanion, HPTE-B$[SO_3^-]_n$, moves to the excited-state HPTE-B$[SO_3^-]_n^*$, and fluorescence emission results from the thermally equilibrated excited states. Since the solubility of LPTE-BS is smaller than that of HPTE-BS, the equilibrium constant $K_L < K_H$, and even though the concentration of the solution in monomer repeat units is similar, we do not have the same concentration of the monomer in the excited state. Furthermore, K_H can be expressed as a function of the activities as

$$K_H = a(\text{HPTBE}-[SO_3^{-}\,]_n)a(\text{Na}^+)^n = (\gamma_\pm)^{1/n+1}[\text{HPTBE}-[SO_3^{-}]_n]\ [\text{Na}]^n \quad (3)$$

where $\gamma_\pm$ is the mean activity coefficient. Increasing the molecular weight $(\gamma_\pm)^{1/n+1} \rightarrow 1$ and the solubility increases. Conversely, for small molecular weights the mean activity coefficient is smaller than 1, the solubility decreases, and the polymer aggregates, thus explaining the smaller PL of LPTE-BS in comparison with HPTE-BS. In this system, the formation of precipitates is a thermodynamically favored kinetic process that may start by the formation of micellar-like structures and may take some days. In fact, precipitates were observed at the bottom of the flash of the LPTE-BS solution after several days at rest in the laboratory.

Effect of Ionic Strength on the PL of HPTE-BS. In an attempt to further check the above interpretation and to find out whether the mean activity coefficient is a crucial parameter to understand the PL of the π-conjugated polyelectrolytes, we have investigated the influence of the ionic strength of the solution on the PL.

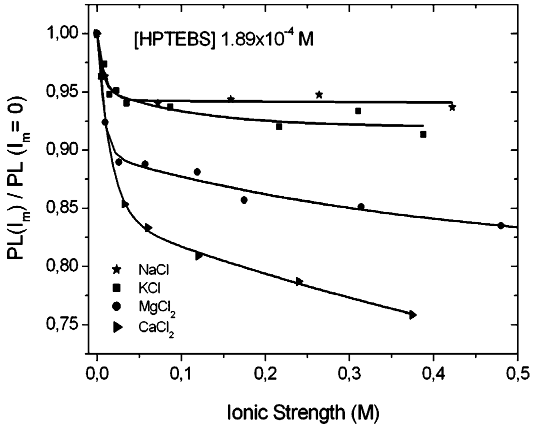

Figure 5. Normalized fluorescence intensity of HPTE-BS (excited at 438 nm) as a function of the ionic strength. The salts assayed were from top to bottom: NaCl, KCl, $MgCl_2$, and $CaCl_2$.

For this purpose we have prepared four solutions of HPTE-BS (10 mL each one), and we have added increasing amounts of the salts NaCl, KCL, $MgCl_2$, and CaCl.$_2$ In each solution, we have measured the PL as the amount of salt was being raised. To illustrate the effect of the salt on the PL, we show in Figure 4 the two extreme cases: the smallest effect for the monovalent cations and the strongest effect for the divalent cations. Figure 4a plots the evolution of the PL as a function of the NaCl concentration and Figure 4b of the $CaCl_2$ concentration. It can be seen that in all cases the PL decreases in response to salt concentration, and the higher the charge number of cation charge, the more efficiently the salt quenches the HPTE-BS fluorescence.

Properties of electrolyte solutions are better described as a function of the ionic strength ($I_m = \sum(z_i^2 m_i)/2$, where z_i is the charge number of an ion and m_i is its molality) which emphasizes the charges of the ions. Figure 5 shows the PL of HPTE-BS, normalized to its value at $I_m = 0$, as a function of I_m for the four salts investigated. As the amount of salt in the solution increases, the fluorescence decreases, and the effect is much more pronounced in the solutions prepared with divalent cations compared with the ones with monovalent cations. For example, at 0.4 M ionic strength the PL of the NaCl solution is around 0.94 the initial value whereas the PL of the $CaCl_2$ solution is only 0.75 the initial value.

In the range of the salt concentration investigated, the decrease of the PL with increasing the amount of salt can be thermodynamically interpreted as due to the decrease of the mean activity coefficient with increasing the ionic strength and therefore the solubility of HPTE-BS. Similarly, the different amount of decrease of the PL for the different salts would be a consequence of the behavior of $\gamma_{\pm}$ with the ionic strength, which for the four salts investigated parallels the behavior of the PL.[15] As it occurred for LPTE-BS, after some days a precipitate of fractal type appears at the bottom of the flasks containing the salt solutions of HPTE-BS, confirming the displacement of the equilibrium toward the left side in eq 2. This precipitate was not observed in the HPTE-BS free-salt solutions. In addition to the solubility effect, the specific interaction of the polymer with each particular ion also seems to play a role. The study of the interactions of alkali metal ions with aromatic groups[16] have demonstrated that the interaction with K^+ is sufficiently strong as to result in dehydration of the ion whereas there is no evidence of such a displacement in the case of Na^+. The differences observed in the PL quenching by salt solutions of NaCl and KCl could be related to the removal of water molecules, thus facilitating the energy transfer between the macromolecule and the quencher.

Let us next discuss the structural interpretation of the PL decrease with the ionic strength. It is known that polyelectrolyte size can be changed by the concentration and valence of counterions.[17−19] The effect of monovalent counterion is generally understood as due to electrostatic repulsion screening, and it has been shown that the larger the amount of the added monovalent salt, the closer the behavior of polyelectrolyte solution approaches that of uncharged polymer solution. It is also known[20,21] that multivalent counterions can condense polyelectrolytes because of the electrostatic attraction between the multivalent counterions and the charges along the polyelectrolyte molecule. In linear and flexible polyelectrolytes, phase separation that leads to precipitation may occur because of electrostatic bridging between monomers along the polymer chain helped by the multivalent counterion. The presence of divalent cations would modify the morphology and size of the HPTE-BS macromolecule in solution in a much deeper way than the monovalent cations; that is the behavior we have observed. Thus, the higher decrease of the PL in the HPTE-BS solution by presence of divalent cations could be attributed to the partial condensation of the polymer chain which buried most of the fluorophores.

To characterize the effect of the ionic strength on the quenching of HPTE-BS, we have investigated the quenching of the polymer's fluorescence by MV^{2+} in a $CaCl_2$ solution with $I_m = 0.3$ M in which the effect of the salt was most conspicuous. As is illustrated in Figure 6, MV^{2+} quenches the HPTE-BS fluorescence in a much more weak way as compared with the case when no salt was present. As can be seen in Figure 7, the Stern−Volmer plot shows a downward curvature which generally is an indication of two populations of fluorophores. In such case the representation of $PL^0/\Delta PL$ (where $\Delta PL = PL^0 - PL$) against $1/[MV^{2+}]$ should be linear.[5−22] However, as can be seen in Figure 8, the representation of $PL^0/\Delta PL$ against $1/[MV^{2+}]$ is only linear for small concentrations of quencher. For higher quencher concentrations $PL^0/\Delta PL$ becomes nearly constant, indicating that once the small amount of fluorophores available were quenched, the rest of the fluophores cannot be quenched.

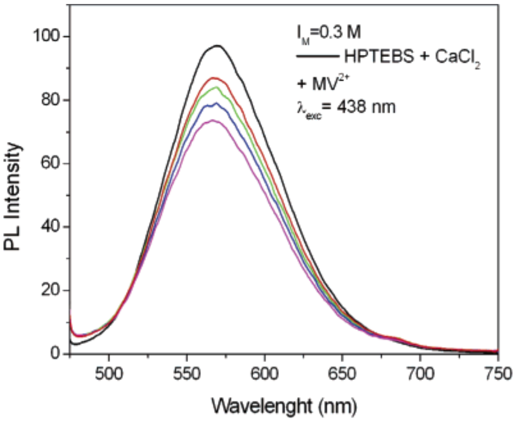

Figure 6. Fluorescence quenching (excited at 438 nm) by MV^{2+} of HPTEBS (1.89×10^{-4} M) in aqueous solutions 0.125 M of $CaCl_2$ (ionic strength 0.375). The concentration of MV^{2+} ranges from 3.5×10^{-6} M (second curve at the top of the plot) down to 7.0×10^{-5} M for the curve with lowest PL intensity.

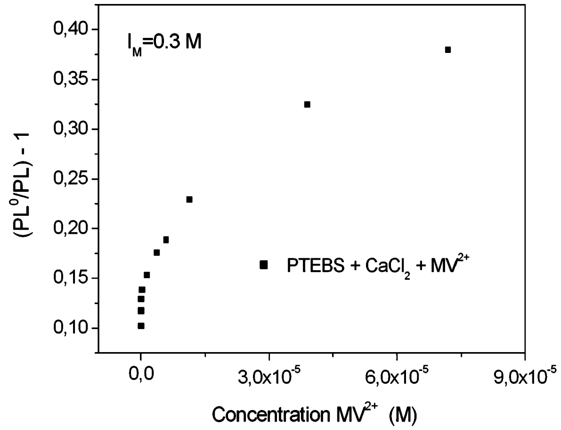

Figure 7. Stern−Volmer plot for quenching of the fluorescence by MV^{2+} for HPTE-BS (1.89×10^{-4} M) in aqueous solution of ionic strength 0.375. The excitation wavelength was 438 nm, and the emission wavelength was 577 nm.

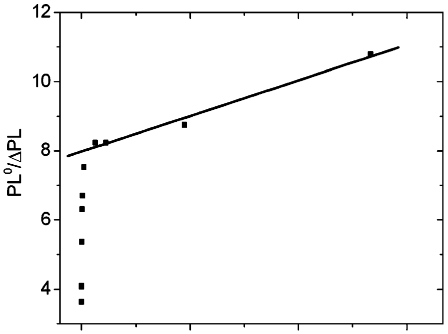

Figure 8. Representation of the quenching of HPTE-BS in aqueous solution of ionic strength 0.375 by MV^{2+} in a modified Stern−Volmer plot for two populations of fluorophores.

Conclusions

Our investigation confirms that the polymer molecular weight plays an important role in the fluorescence quenching, and for PTE-BS, the higher the molecular weight, the higher the K_{SV} observed. This PL increase is attributed to the better solubility of HPTE-BS which increases the equilibrium constant stabilizing the excited polymer form in the solution. Better polymer solubility would favor the deployment of the chromophores which can then be quenched more easily by the MV^{2+} molecules, and the opposite would occur under low-solubility conditions. The result reported in the present study seems to indicate that relatively large molecular weight polymers are required to obtain large values of the K_{SV}. In addition, the fluorescence can be partially quenched by the addition of salts thus controlling the solubility of the polymer. The fluorescence decreases with increasing the ionic strength and depends on the nature of the cation, being higher for the divalent cations investigated than for the monovalent ones. The difference in PL behavior observed between the monovalent and divalent cations are attributed to the screening effect of monovalent cations and to the bridging effect of the divalent cations. When using in biosensor application, this effect has to be taken into account since the presence of some amount of salts could modified the PL quenching effects.

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