Carboxylate Group Side-Chain Density Modulates the pH-Dependent Optical Properties of PPEs

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ABSTRACT: The synthesis of two new water-soluble, carboxylate-substituted poly(*p*-phenyleneethynylene)s (PPE) is reported. These PPEs differ in their concentration of free carboxylate groups per repeat (two aryleneethynylenes) unit. The photophysical properties of these two polymers are compared to each other and to that of the known dicarboxylate-PPE. An increasing concentration of carboxylate groups (on a per repeat basis) makes the polymers less fluorescent but also more sensitive toward quenching by mercury ions. The least carboxylate functionalized PPE shows the highest quantum yield (0.33). All three PPEs are pH-sensitive and show dramatic changes in absorption and emission at around pH 3.5, which is the pK_a of methoxyacetic acid, the common structural subunits in all of these PPEs.

Introduction

We report that the structurally simple polymers **5b**, **6**, and **9** with varying degrees of carboxylation show—pH-dependent—different photophysical properties in aqueous buffered solution. An increasing degree of carboxylation leads to quenching of emission when going into acidic pH (<4). Conjugated polyelectrolytes[1] have found spectacular applications in biosensory processes.[2,3] These materials are also useful as hybrid devices, representing a crossbreed of light-emitting electrochemical cell and light-emitting diode.[4] The main application, however, is in their promising sensory profiles, making the sensitive detection of metal ions, sugars, DNA, proteins, and bacteria possible.[5−11]

An important general issue is the concentration of the ionic groups per repeat unit, which in this case are carboxylates. Properties of conjugated polyelectrolytes are determined to a significant extent by the electric charges and the resulting electrostatic forces these charges exert. These forces will dominate aggregation, excimer formation, and analyte binding. To investigate these issues, we have prepared three carboxylate-substituted poly(*p*-phenyleneethynylene)s (PPE) in which 1, 2, or 3 carboxylate groups are present per aryleneethynylene unit.[10,11] The herein investigated PPEs feature the same conjugated backbone and all are derivatives of dialkoxy-PPEs. However, their pH-dependent absorption and emission spectra as well as their fluorescence quantum yields vary dramatically. The difference in behavior should be useful for the construction of biosensory platforms, where multivalent interactions, differential dye replacement, and assemblies with charged cofactors are exploited.[12−14] We have recently shown that specifically substituted gold nanoparticles loaded with negatively charged conjugated polymers unequivocally discern a set of proteins.[15] Assay strategies using conjugated polyelectrolytes will be increasingly useful in the detection of biologically important species, toxigenic materials, and bacteria. For effective replacement and quenching assays, groups of conjugated polyelectrolytes with tunable ratios of charge per repeat unit are in demand. Herein we report such a materials platform, PPEs with a varying degree of carboxylate functionalities per repeat unit, and investigate their photophysical properties.

Results and Discussion

Synthesis. Starting from **1**, saponification furnishes **2**, which after exposure to oxalic chloride is transformed into its double acid chloride. Treatment of this intermediate with diethyliminoacetate furnished monomer **3** in 46% yield and high purity. Heck−Cassar−Sonogashira−Hagihara[16−18] coupling of **3** to **4** afforded PPE **5a**. Saponification using sodium hydroxide in methanol rendered the desired hexacarboxylated **5b** as a water-soluble, yellow, and fluorescent material after precipitation from ethyl ether in almost quantitative yield (Scheme 1).

Polymer **9** was synthesized using a similar route. Here we started from the literature known diiodide **7**, which was coupled to TMS-acetylene and deprotected to afford monomer **8**.[16,17] This monomer was coupled to **1** under standard Heck−Cassar−Sonogashira−Hagihara conditions[18] using triethylamine as the solvent to yield polymer **9** after deprotection with a dilute solution of sodium hydroxide in methanol. The polymer was isolated in 60% yield after dialysis followed by lyophilization. The use of triethylamine instead of piperidine or morpholine was imperative to avoid transformation of the ester groups into amide functionalities by such secondary amines—a process undesired here. Polymer **6** was prepared according to the literature.[11] Polymers **5b**, **6**, and **9** have M_n's in the range of $(1-3) \times 10^4$ (see Table 1) and polydispersities between 1.8 and 4.3.

Dependence of Absorption and Emission Spectra on the pH. All three PPEs, **5b**, **6**, and **9**, are readily soluble in aqueous buffer at pH 7.2. They form yellowish solutions that are blue-turquois fluorescent. These solutions display dramatic effects in their absorption and emission upon change of the pH value (Figures 1−3 and Table 1). The introduction of the branched oligoethylene glycol substituent in **9** leads to a PPE with a significantly enhanced emission quantum yield in water, an effect reported in PPEs exclusively carrying branched oligoethylene glycol substituents.[16,17] The increase in quantum yield was originally thought to arise from steric shielding of the conjugated backbone by the branched oligoethylene glycol groups. However, as **9** has a quantum yield comparable to that

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Scheme 1. Synthesis of Polymer 5b

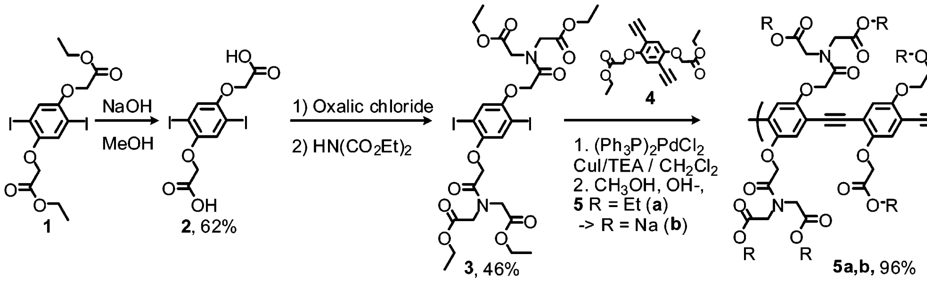


Table 1. Molecular Weight, Optical Properties, and Stern−Volmer Constants (K_{SV}) for Polymers 5, 6, and 9 at Different pH Values

polymer	5b	6	9
$\lambda_{\max\ \mathrm{abs}}$ pH 7 (nm)	419	408	425
$\lambda_{\max\ \mathrm{abs}}$ pH 4 (nm)	468	414	435
$\lambda_{\max\ \mathrm{abs}}$ pH 3 (nm)	472	463 sh	455
$\lambda_{\max\ \mathrm{emiss}}$ pH 7 (nm)	465	460	466
$\lambda_{\max\ \mathrm{emiss}}$ pH 3 (nm)	483 weak	438 weak	484
Φ pH 7 (nm)	0.08	0.08	0.33
K_{SV} Hg^{2+}	6.4×10^4 [a]	1.3×10^4 [a]	7.1×10^2 [a]
M_n	3.6×10^4	1.2×10^4	2.5×10^4
M_w/M_n	2.1	4.3	1.8

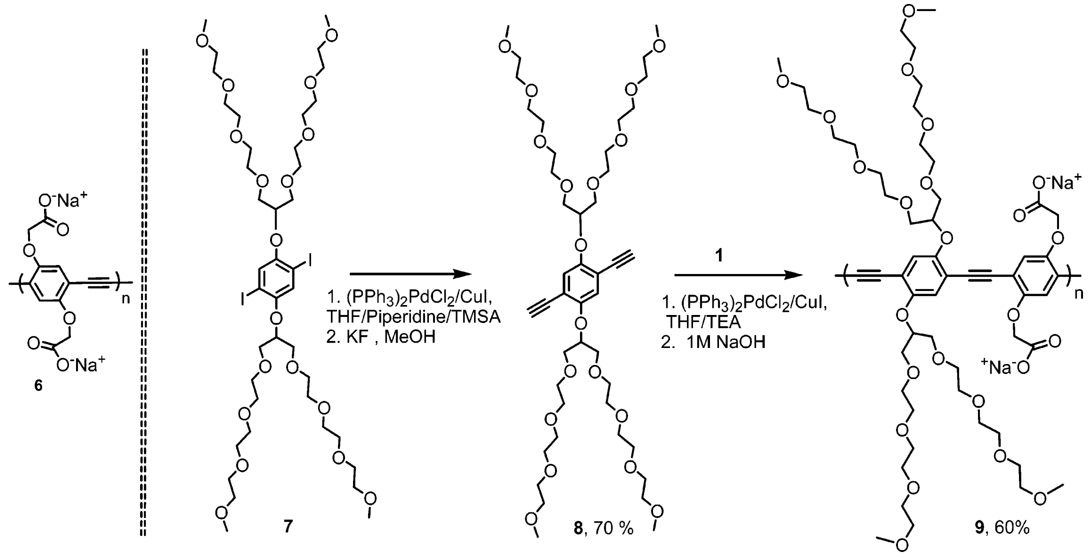
[a] The sensing of the mercury ions was performed in 0.1 M phosphate buffer at pH 7.2.

of the a PPE only carrying branched oligoethylene glycol substitutents, but is less sterically protected, there must be other additional factors operative that increase the quantum yield.

The introduction of more carboxylate groups on a per repeat unit basis as in **5b** does not influence the quantum yield when comparing to the quantum yield of PPE **6**. When going from pH 7.2 to pH 11, neither emission wavelength, intensity, nor the absorption spectra in any of the three polymers changed. However, when acidifying, the emission intensity of all three PPEs decreases substantially (Figures 1−3). The emission of **5b** and **6** is almost completely quenched while that of **9** is attenuated and also red-shifted due to some excimer/aggregate formation. When the pH is decreased further to pH 1, **5b**, **6**, as well as **9** begin to precipitate out of solution. The spectroscopic properties of the three PPEs at pH < 3, as long as they can be determined, are very similar to those recorded at pH 3. The most dramatic effects are observed in the absorption spectra, when going from a neutral to an acidic solution. Both **5b** and **6** show a red shift in their absorption maxima at a pH < 4. At pH 7.2, the three polymers show absorption maxima at 408−425 nm, *untypical* for dialkoxy-PPEs, as these absorb at around 456 nm when dissolved in a good solvent.[18a,b] We explain this blue shift by electrostatic effects inducing an increased twist between two adjacent aryleneethynylene units to minimize electrostatic repulsion.[19,20−23] In noncharged PPEs in good solvents, a model for the influence of the twist angle upon the electronic spectra has been developed by Berg and Yaron[23c,d] assuming a Boltzman distribution of twist angles and a low barrier of rotation. The introduction of repulsive negative charges will increase the barrier but will probably stabilize a more twisted conformation of adjacent phenyleneethynyle units as in those the negative charges are farther apart from each other. Upon protonation, **5b** and **6** show a significant red shift to 463 and 472 nm, respectively. This shift mirrors the red shift that is found in hydrophobic dialkoxy-PPEs, which show an absorption at 478 nm in their aggregated state.[20] While **6** only develops a shoulder in its absorption upon acidification, the polymer **5b** has a more pronounced response to pH. At pH 4, the absorption maximum is located at 468 nm, and upon going to pH 3 the absorption maximum shifts to 472 nm. We attribute the additional shift to the sequential protonation of the two types of carboxylate groups found in polymer **5b**. We assume that the carboxylate groups bound to the iminoacetic acid arms are protonated last, as methyliminodiacetic acid has two pK_a values (pK_a2 = 10.2, pK_a1 = 2.81), the first of which is somewhat below that of ethoxyacetic acid. Polymer **9** shows the least shift in absorption, and upon acidification to pH 3, **9** displays an absorption maximum at 455 nm.

The changes in absorption occur when the pH falls below 4, in line with the pK_a value of ethoxyacetic acid, with a pK_a = 3.53.[21] At pH 3, most of the carboxylate residues in the three

Scheme 2. Synthesis of Polymer 9 and Structure of the Known Dicarboxylate PPE 6



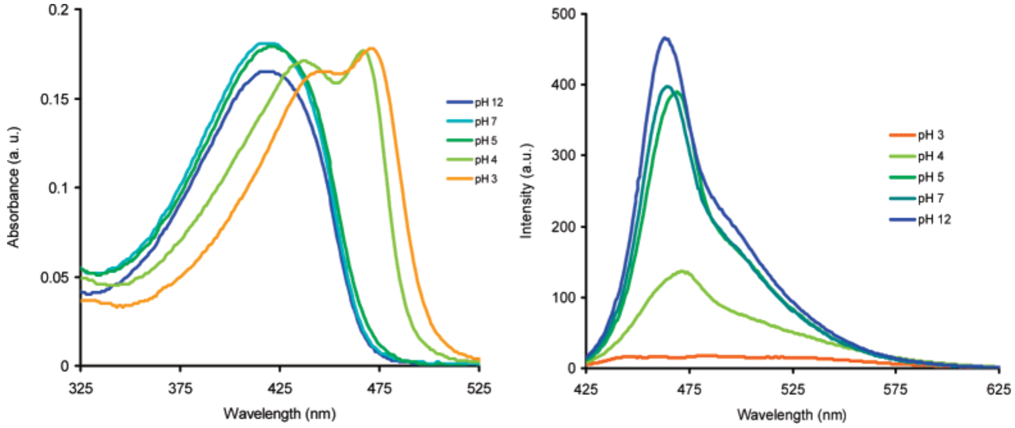


Figure 1. Absorption and emission spectra of **5b** at different pH values.

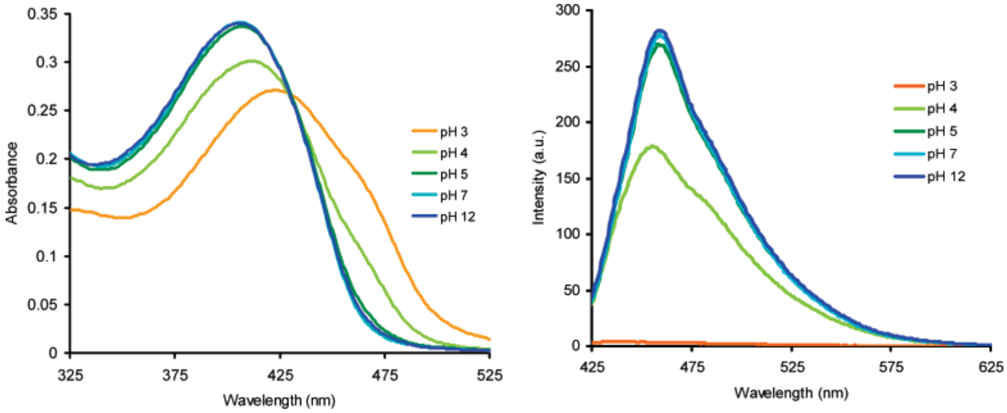


Figure 2. Absorption and emission spectra of **6** at different pH values.

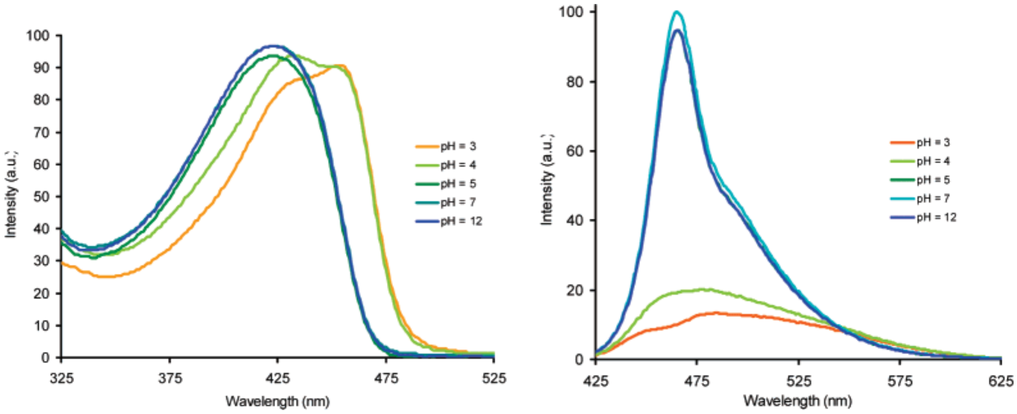


Figure 3. Absorption and emission spectra of **9** at different pH values.

investigated polymers are protonated. Not unexpectedly, the largest changes in absorption are visible around the pH value that corresponds to the pK_a value of the respective acids. Below pH 3, there is only minimal electrostatic repulsion because the polymer chains are now only weakly or not at all charged.

What is the mechanism for the observed red shifts in these PPEs? Conformational effects will play a major role.[22] In basic and neutral solutions, the neighboring aryleneethynylene groups are maximally twisted in **5b** and **6** to minimize electrostatic repulsion of the negatively charged carboxylate units. When decreasing the pH, planarization and desolvation of the now neutral and hydrophobic PPE become predominant. The change of optical properties reported here is similar to that observed in the aggregation of hydrophobic PPEs in methanol solution where it is governed by planarization of the main chains.[22,23]

The decrease in emission intensity of **5b**, **6**, and **9** upon protonation, and before precipitation, is mechanistically more difficult to explain, not well understood, but quite typical for conjugated polyelectrolytes upon charge neutralization in highly polar solvents. We contend that it is a combination of excimer formation and water-alleviated, nonradiative deactivation of the excited state of these polymers. If one is interested in the use of such polymers as a platform in biosensing applications, it is desirable that there is no interference from metal cations. We[14] and Schanze et al.[10] have shown that dicarboxylate-PPE **6** is quite sensitive toward the addition of calcium ions, inducing excimer formation with yellow emission; **6** is also sensitive toward mercury ions, which are efficient quenchers of fluorescence in **6**. Neither **5b** nor **9** is sensitive toward calcium ions in the millimolar range, nor does their emission characteristics change by the addition of other metal ions, with exception of mercury. To quantify the amount of mercury-induced quenching in **5b, 6**, and **9**, we have used the Stern−Volmer equation[24]

$$F_0/F_{[Q]} = K_{SV}[Q] + 1$$

in which $F_0/F_{[Q]}$ represents the fluorescence intensity of the PPE without (F_0) and at a concentration [Q] ($F_{[Q]}$) of the added quencher. The Stern−Volmer constant is abbreviated as K_{SV} and obtained as the slope of the plot of $F_0/F_{[Q]}$ vs the quencher concentration [Q]. Because of the short emission lifetime in PPEs (0.3−0.5 ns), the quenching process is static; i.e. K_{SV} represents an association constant. The Stern−Volmer formalism provides a way to quantify and compare the suceptibility of the three investigated PPEs toward quenching by mercury ions. The absolute numbers, i.e., the K_{SV} values, may not be very meaningful as association constants as there are multiple binding sites with different β values involved upon addition of mercury ions. However, the obtained composite number allows one to compare the sensitivity of the PPEs toward mercury ions. Table 1 shows that **5b** is efficiently quenched by mercury—by a factor of 5 more than **6**—while polymer **9** shows a Stern−Volmer constant that is very low and suggests that binding of **9** to mercury ions is weak as a consequence of the lesser concentration of the carboxylate groups. An increasing concentration of carboxylate groups makes these polymers therefore more suceptible toward mercury ions and potentially interesting as metal ion sensors.[25]

Conclusions

In conclusion, we have investigated three different PPEs in which the concentration of the ionic carboxylate units per repeat (two phenyleneethynylene units) unit is 6, 4, and 2. These PPEs show significantly different responses toward decreasing pH and mercury ions. For use as a platform in biosensory applications, we conclude that the mixed oligoethylene−carboxylate polymer **9** is the most attractive one, as it shows high emissive quantum yields in water and low interference with metal ions. If metal sensory properties are desired, polymers of the type **5b** will be more useful. In the future we will prepare PPEs in which the carboxylates are part of the conjugated systems, not attached by saturated bridges. The study of their metallochromic and pH-dependent photophysical behavior and their use in array-type detection schemes for proteins and bacteria is planned.

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Supporting Information Available: Synthetic details including NMR and UV−vis characterization of monomers and polymer **5b** and **9** and details of the quenching and photophysical experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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