Effect of Spacer Length and Solvent on the Concentration-Driven Aggregation of Cationic Hydrogen-Bonding Donor Polythiophenes

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ABSTRACT: Aggregation of cationic isothiouronium polythiophenes with alkoxy-spacers of different lengths at the 3-position of the thiophene ring was studied in solvents of different polarities. Hydrogen-bonding capacity was assessed by steady-state absorption and fluorescence spectroscopy, whereas the aggregation in aqueous solutions was studied by electron paramagnetic resonance spectroscopy, using paramagnetic probes of different polarities. The two polymers displayed similar features in respect to conformation, effect of cosolvents on aggregation, unstructured absorption—fluorescence spectra, Stokes shifts when aggregated, solvatochromic effect, and self-quenching concentration. However, these polymers also showed different specific interactions with water, Stokes shifts in water, effect of the solvent on the extent of dominant state of the S1 level, and also different inner cavities and hydrophobic—hydrophilic surface area in aqueous solution aggregates. Water maximized the difference between the polymers concerning the effect of specific increases in concentration, whereas the presence of 1,4-dioxane generated almost identical effects on both polymers.

INTRODUCTION

Molecular aggregation of π-conjugated molecules is relevant because the functional properties and electronic interactions of these systems are modulated by simply varying factors such as temperature, solvent polarity, and concentration.¹ Conjugated polyelectrolytes (CPEs) possess an electron delocalizing π-conjugated backbone, which confers these materials semiconducting, chromophoric, and fluorophoric properties, whereas functionalization with hydrophilic excess charge confers these molecules solubility in polar solvents (e.g., water). These materials possess the interaction driving forces of conjugated polymers (π–π stacking and hydrophobic interactions) and also those of polyelectrolytes, such as coordination through electrostatic forces² and hydrogen bonding (H-bonding),² with surrounding water molecules, allowing their aqueous solubilization.³ Recently, transmission electron microscopy studies demonstrated that the aggregation in solution of an ethylene oxide polythiophene is directed by the π–π interactions of backbones and also by the H-bonding capacity of the side chains.⁴

These phenomena guide the early stages of aggregation and ultimately the solid-state properties of these molecules because the conformation in solution obviously impact on the properties of films.

In regard to films of conjugation polymers (CPs) and CPEs, the lack of understanding of their solid-state photophysics arises from the inherent structural heterogeneity in bulk films, where various inter and intrachain morphologies coexist. Also, films present a relatively limited number of physical parameters to tune.⁵

A clear understanding about the impact that the polymeric conformation in solution has on the solid-state electronic and photophysical properties is still missing, and different approaches are being used, for example, by controlling molecular structure, effect of solvents, and surfactant deposition techniques at different temperatures, among others,⁷⁻¹¹ or new experimental setups to directly study single-chain motions.¹²

Among all of these possible approaches, aggregates suspended in solution have demonstrated to be a good model system to gain understanding on structure–property relations of CPs because they present a relatively simplified morphological heterogeneity compared to the bulk and also offer the possibility to probe the fundamental characteristics of excited states by simply changing the solvent.⁶

Supporting Information

**Figure:**

- **H-bonding:** Illustration of hydrogen bonding between molecules.
- **electrostatic:** Diagram showing electrostatic interactions.
- **pi-pi:** Representation of π–π interactions.

**Diagram:**

- ****H-bonding:** showing hydrogen bonding with water molecules.
- **electrostatic:** illustrating electrostatic interactions between molecules.
- **pi-pi:** Depicting π–π stacking interactions.

**Table:**

- | Solvent | Stokes Shifts |
- | --- | --- |
- | Water | 20 |
- | 1,4-dioxane | 15 |

**Graph:**

- ****H-bonding:** Graph showing the influence of solvent on H-bonding capacity.
- **electrostatic:** Chart illustrating the role of electrostatic forces.
- **pi-pi:** Plot showing π–π interactions.

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- **Supporting Information:** Additional data provided.

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The first CPEs were thiophene-based and have been used in solution as fluorescence-based sensors for measuring temperature, solvent polarity, pH, and also chemical and biochemical species. Different CPEs have been used in solid-state applications to build: (i) electroactive multilayer devices using layer-by-layer assembly of ionic species; (ii) electrode-buffer layers in optoelectronic devices; and (iii) active layers in hybrid organic solar cells, using anionic thiophene-CPEs. In recent years different CPEs have been “revisited” because of their interesting surface properties, which are linked to their charged nature, for example, their capability to show permanent dipoles when applied on top of different substrates.

Fluorescence is a particularly useful technique to study complex systems in solution (e.g., aggregation of polymers) because the properties of the excited state are often very sensitive to the immediate environment surrounding the molecules. For example, neutral and charged polythiophenes modify their fluorescence emission in response to different stimuli, such as temperature, solvent, surfactants, irradiation, other chemical species, and also by changing concentration.

Furthermore, the self-assembly of neutral polythiophenes is very sensitive to the placement and nature of the substituent chains attached to the thiophene ring because it modifies the conformational order in solution, which is known to impact their solid-state electrochemical and photovoltaic properties. For example, inert side chains reduce the light absorbing and semiconducting properties of copolymers, whereas alkyl or alkoxy groups raise the conformational entropy of the macromolecule in solution and disturb molecular packing. In this respect, a bulkier branched alkyl side chain reduces the tendency of the polymer to crystallize and generates a more twisted and less rigid backbone because of steric effects. In turn, these effects reduce the conjugation length and π-stacking. It is also known that the distance between the charged group and the conjugated polymer backbone has an impact on water solubility and critical solution temperature and the electronic properties of films of thiophene–zwitterions. On the other side, molecular dynamics simulations have shown that the spacer length of CPEs generates a fine balance between electrostatic and hydrophobic interactions with gemini surfactants.

It is possible to gain information on the optical properties of CPEs by studying the effect of increasing concentrations on their spectral features because such an approach reveals information on (i) the processes occurring during absorption and emission, (ii) efficient excitation energy transfer between chromophores, and (iii) nonradiative (internal conversion) fluorescence decay, such as aggregation-driven self-quenching of fluorescence. The elucidation of the physical—chemical mechanisms responsible for this phenomenon is a prerequisite for a fundamental understanding of the photochemistry of conjugated polymers because correlating changes in emission spectra and the degree of self-quenching provide information on the formation, number, and interaction pathways of chromophores. Concentration-based studies also provide indications on the type of aggregation in solution of conjugated charged biopolymers, synthetic conjugated polymers, and thiophene CPEs.

Solvent changes modify the Stokes shift, which provides information on possible specific solvent–fluorophore interactions. Solvent-polarity variations modify the relation between polarity and rigidity of both backbone and side chains of CPEs, inducing conformational changes. The use of a cosolvent changes the structure of copolymer CPEs in water, apparently because of the difference in the relative solubility between the solvent and copolymer. This approach also gives information on H-bonding because these interactions and solubility parameters in common solvents play an important role in the self-assembly structure of CPEs. For example, methanol–dimethylformamide (DMF) mixtures have provided information on the H-bonding interactions and dipole association during polymer intermolecular interactions; this is because the intermolecular complex between methanol and DMF interferes with the hydrogen-bonding effect that typically yields a nanoribbon morphology for such a molecule. Mixed solvents generate preferential solvation of certain parts of the polymer, such as backbone and attached functional group, in certain component of the binary mixture.

Also, besides fluorescence-based studies, the computer-aided analysis of the electron paramagnetic resonance (EPR) spectra of polymers able to self-aggregate in aqueous solutions has demonstrated to be a useful tool to obtain information on the aggregation behavior and the interactions occurring in solution.

In this context, isothioureas may be useful model molecules to study aggregation in solvents with different H-bonding capacities because the Y-shaped structure of the isothiourea functionality acts as a particularly strong hydrogen-bond donor, offering two complementary hydrogen-bonding points to solvents.

The amino group in the isothiourea protonates in water at pH 7, bearing then a positive charge, which allows electrostatic interactions and increasing solubility in water and in protic solvents. In fact, previously, a Y-shaped anionic carboxylate thiourea has been used to study the H-bond stabilization during self-assembly. The strong H-donor properties of these molecules are highly sensitive to the presence of a solvent capable of disrupting the hydrogen-bonding donor—acceptor ratio, such as 1,4-dioxane (DI), which is capable to accept two hydrogen bonds without donating any. The solid-state isothioureas are characterized by a surrounding electron density capable to participate in hydrogen bonds with indium tin oxide substrates. In general, the H-bonding ability of thioureas remains in the solid state for small molecules and also in polycyclic frameworks with one or more urea/thiourea fragments.

In our previous study, diluted solutions of isothiourea polythiophenes were characterized using steady-state absorption and fluorescence by changing alkoxy-spacer length and solvent. In the present study, the same polymer–solvent systems were accurately investigated as a function of concentration to follow the changes in the conformations, aggregation, and optical properties of these isothiourea polythiophenes and gain information on the role of hydrogen bonding in inter/intrapolymeric and polymer–solvent interactions.

Steady-state absorption and fluorescence were used to analyze samples with increasing concentrations in four solvents with different polarities and H-bonding capacities, namely, protic water, protic–protic water–isopropanol (IPA), protic–aprotic water–tetrahydrofuran (THF), and protic–nonpolar water–DI (all mixtures 0.5 v/v).

Aqueous solutions were studied by EPR spectroscopy using paramagnetic probes of different polarities. The analysis of the EPR spectra provided information on the aggregation process...
in solution and structural, dynamical properties and interacting ability of the polymers in water.

**MATERIALS AND METHODS**

**Materials.** Unless otherwise stated, deionized water was used in the experiments, while all used reagents and solvents were commercially available, analytical grade, and used as supplied (Sigma-Aldrich). Table S1 and S1-cont. in the Supporting Information show reported values of different physical—chemical parameters of all solvents used in this study, together with the H-bonding capacities of water and the pure cosolvents, (according to Burrell’s classification) and the hydrogen-bonding acceptor parameter (basicity parameter $b$) of the H-bond acceptors.

Scheme 1 shows the structure of the two cationic isothiouronium polythiophenes used in this study, that is, poly-$3$-$\left(N,N\text{-diethyl-S-iso-}t\text{hiouronium}\right)$ethylxy-4-methyl thiophene, termed PT1, and poly-$3$-$\left(N,N\text{-diethyl-S-iso-thiouronium}\right)$hexyloxy-4-methyl thiophene, termed PT2.

As discussed previously, poly-$3$-$\left(N,N\text{-diethyl-S-iso-thiouronium}\right)$ethylxy-4-methyl thiophene, termed PT1, and poly-$3$-$\left(N,N\text{-diethyl-S-iso-thiouronium}\right)$hexyloxy-4-methyl thiophene, termed PT2.

For the EPR study, after several attempts, the spin probes termed 4-tert-butyl-$\text{N,N-diethyl-S-iso-thiouronium}$hexyloxy-4-methyl thiophene, termed PT1, and poly-$3$-$\left(N,N\text{-diethyl-S-iso-thiouronium}\right)$hexyloxy-4-methyl thiophene, termed PT2.

As discussed previously, both polymers are assumed to have the same tacticity (mainly head-to-tail), degree of polymerization ($20$–$30$ repeating units), and dispersity ($D = M_w/M_n = 1$–$3$).

The polymer was dissolved in water at a concentration of $0.5$ mg/mL and stirred for $30$ min, generating solutions of PT1 and PT2 at $2.1$ mM (monomer base). Then, stock solutions in each solvent were obtained by duplicating the volume of polymer solutions and surfactants.

Polymeric Solutions. Each polymer was dissolved in water at a concentration of $0.5$ mg/mL and stirred for $30$ min, generating solutions of PT1 and PT2 at $2.1$ mM (monomer base). Then, stock solutions in each solvent were obtained by duplicating the volume of polymer solutions and surfactants.

Previous studies with methoxyethoxy polythiophenes showed that the conformational evolution as well as the aggregation process of polymer chains is a very slow process, probably related with their molecular weight. In the present study, fresh solutions obtained from the same stock solutions were used in all experiments because the absorbance—fluorescence spectroscopic features of aqueous solutions of both polymers were shown to be stable during at least $2$ months (results not shown).

Both polymers generate a pH value of $5$ at the highest concentration in water, whereas preliminary results, obtained by adding diluted HCl and NaOH solutions, showed that the absorption and fluorescence spectroscopic features of both polymers remain constant until pH $\approx 8$. Therefore, because in the present work all solvents used generate a pH $\leq 7$ (see Table S1 in Supporting Information), it was possible to avoid the use of pH buffers.

**Steady-State Absorption and Fluorescence.** Steady-state absorption (UV–vis) and excitation/fluorescence spectra were recorded using Hewlett-Packard 8453 and Cary Eclipse steady-state (Varian) spectrophotometers with slit widths of $5$ and $15$ nm, respectively. Both instruments have thermostated cell holders to perform the experiments at $25$ °C, and all measurements were performed using a Suprasil cuvette of $10$ mm path length.

Fluorescence spectra were obtained by testing excitation wavelengths ($\lambda_{\text{exc}}$) around the value of $\lambda_{\text{em}}$ to find the maximum fluorescence intensity ($I_{\text{em}}$). Four excitation wavelengths were tested ($\lambda_{\text{exc}} = 300$, $350$, $400$, and $450$ nm), and among them, $400$ and $450$ nm generated the largest intensities. This work presents data obtained from $\lambda_{\text{em}} = 400$ nm because the data obtained using $\lambda_{\text{em}} = 450$ nm generate similar trends (and therefore conclusions) for the two polymers (results now shown).

To minimize the possibility of fluorescence quenching by atmospheric O$_2$, the samples were purged with an N$_2$ flow before each measurement; strong bubbling was avoided to minimize the possibility of generating metastable aggregated solutions. Concentration versus $\lambda_{\text{em}}$ or $I_{\text{em}}$ was plotted for the four solvents, in a concentration range from $0.01$ to $1$ mg/mL.

All spectroscopic maxima were obtained from smoothed spectra, with the aid of the SpectraGryph optical spectroscopy software (Version 1.0.3).
The integrated absorption area was also estimated with aid of this software, by fixing the lower and upper limits of the absorption distribution ±60 nm around \( \lambda_{\text{max}} \) using only corrected baseline spectra.

**EPR Spectroscopy.** EPR spectra were recorded by means of an EMX-Bruker spectrometer operating at X band (9.5 GHz) and interfaced with a PC (software from Bruker for handling and recording the EPR spectra). The temperature was controlled by a Bruker ST3000 variable temperature assembly cooled with liquid nitrogen. The reproducibility was verified by repeating each experiment at least three times.

The concentration of 0.05 mM was selected for all probes (see Scheme 2) because it showed to be nonperturbative of the systems on the basis of the invariability of the spectral line shape by further decreasing this concentration.

The computation of the spectra was accomplished by means of the well-established procedure of Budil et al. The main parameters extracted from computation were (i) the \( A_{\alpha} \) components of the hyperfine coupling tensor \( A \) for the coupling between the electron spin and the nitrogen nuclear spin. These components measure the environmental polarity. Unless otherwise specified, for simplicity, the \( A_{\alpha} \) and \( A_{\beta} \) components were assumed constant (6 G), whereas only \( A_{\gamma} \) was changed. The accuracy of this parameter is ±0.01 G; (ii) the correlation time for the diffusional rotation motion of the probe \( (\tau) \), which measures the microviscosity around the probe, in turn monitoring the interactions occurring among the molecules. The accuracy in this parameter is ±1 ps.

**Dynamic Light Scattering.** Dynamic light scattering (DLS) measurements were carried out on a Zetasizer Nano-ZS device (Malvern Instruments Ltd.) at room temperature. To minimize the interference by dust, all sample solutions were filtered through 5–6 μm poly(tetrafluoroethylene) syringe filters and the scattered light was collected at 173° (i.e., backscatter detection). The device estimates the hydrodynamic radius \( (R_h) \) using the Stokes–Einstein equation.

**Density Functional Theory.** Density functional theory (DFT) calculations were performed to visualize the electrostatic potential surfaces of dimers of both polymers, optimizing at the B3LYP/6-31G** level (for details see ref 46). It is interesting that the simulated electrostatic potential is basically independent on the selected basis set of theory.

All calculations were carried out with the Gaussian 09 (G09) package, and the electrostatic potential maps were visualized using the software Avogadro, Open Babel Version 2.3.2.45.

### RESULTS AND DISCUSSION

**Steady-State Absorption and Fluorescence Spectro-photometry of the Polymers at Increasing Concentrations in Different Solvents.** Figure 1 shows raw and normalized absorption and fluorescence spectra (at 25 °C) of aqueous solutions of PT1 (continuous blue lines) and PT2 (dashed red lines) at concentrations of 0.084 mM (thin lines) and 1.05 mM (thick lines), respectively.

Figure 1 shows that in aqueous solutions, at concentrations of 0.084 and 1.05 mM, PT1 and PT2 display a single absorption and fluorescence band, which are related, respectively, to the \( \pi-\pi^* \) transition and to the efficiency in energy migration along the thiophene backbone. The same features as in Figure 1 were observed in the spectra obtained in the three cosolvent mixtures.

Not even the highest concentration did generate the appearance of new peaks, which has been reported when \( 10^{-3} \) mg/mL solutions of poly(3-hexylthiophene) (P3HT) in chloroform were exposed to hexane, at around 50 v/v %, and also in solutions of crystalline nanofibers of P3HT in organic solvents. In both cases, the 0–0 and 0–1 vibronic transitions were clearly seen. Such emergent absorption bands have been associated with a poor solubilization because they indicate an increase of effective conjugation length of the P3HT backbone and interchain \( \pi-\pi^* \) stacking. Conversely, a single band is related to well-dissolved, disordered states.

Therefore, because in the present work the emergence of new absorption bands was not observed in any case and even the highest concentrations generated structureless spectra, it is reasonable to assume that the aggregation observed is mainly related to changes in the conformation of the polymers due to aggregation and not to a loss of solubility.

The wavelengths of the maxima in absorption (\( \lambda_{\text{max}} \)) and fluorescence (\( \lambda_{\text{em}} \)) and their intensities were the main parameters analyzed in the present work. These wavelengths provided information on the ground and excited states (S0 and S1, respectively), whereas their shift due to the increase in concentration allowed us to compare the polymer–polymer and polymer–solvent interactions of both polymers. This is because a shift in the position of \( \lambda_{\text{max}} \) of small molecules and conjugated polymers is related to different types of aggregation modes, associated with the tilt angle between adjacent \( \pi \)-delocalized systems. This also applies for shifts of \( \lambda_{\text{em}} \) which are related to conformational changes in the poly-thiophene backbone of copolymers, and also to conformational changes, aggregation, and solvatochromic effects of semiconducting polymers.

Please notice that the maxima of the absorbance and photoluminescence (PL) spectra shown in Figure 1b, together with the maxima in fluorescence excitation, in all solvents, are conveniently resumed in Figure 3 (details ahead).

**Aggregation States.** A critical phenomenon is the change in the PL intensity due to variations of concentration because it provides information on the aggregation mechanisms.
However, most importantly, a decrease in PL intensity due to an increase in concentration is a criterion to identify the formation of aggregates because a nonlinear relationship of PL intensity with concentration indicates aggregation self-quenching. Also, the ratio between absorbance and PL intensity over a range of concentrations affords the fluorescence quantum yields, which provide information on charge-transfer states and radiative and nonradiative processes as a function of solvents.6,50,65

Figure 2 shows the effect of concentration on the PL intensity of both polymers (\(\lambda_{\text{ex}} = 400\) nm) in the four solvents.

Figure 2. Plots of PL intensity (\(\lambda_{\text{ex}} = 400\)) of PT1 and PT2 as a function of concentration in the concentration range 0.04–1.05 mM, in all solvents, at 25 °C. Vertical black arrows correspond to the three characteristic aggregation states: disaggregated (DIS), “middle” aggregation (CC), and maximum aggregation (AGG). The gray horizontal thick double arrow below the X-axis indicates the concentration range used in the DLS and EPR studies in aqueous solutions.

Figure 2 shows that, in all solvents, the PL intensities of both polymers display a linear increase until a concentration of around 0.1 mM. At higher concentrations, such linear behavior is lost, which indicates aggregation self-quenching. The arrows in Figure 2 indicate the three selected concentrations corresponding to characteristic aggregation states: disaggregated (DIS) at 0.084 mM, “middle” aggregation (CC) at 0.4 mM, and maximum aggregation (AGG) at 1.05 mM. The PL of both polymers at concentrations higher than CC is different for PT1 and PT2 showing increases and decreases, respectively. This indicates that at the highest aggregation tested, the role of the spacer length plays a role in the self-quenching generated by the aggregates.

In regard to absorbance, in Figure S1, it is observed that both polymers show a linear increase in concentration in every solvent, which allows for estimating their molar absorption coefficient (\(\epsilon\)), as stated before.46 This parameter indicates that PT2 has better absorption properties than PT1 because it has larger \(\epsilon\) values in every solvent.

The increases in both absorption and PL, as a function of polymer concentration, are presented in Figures S8 and S9, respectively, and are discussed ahead.

Maxima in Absorption, Excitation, and PL Emission. Figure 3 shows the wavelengths and intensities in the maxima of absorbance, excitation, and PL of both polymers in the four solvents and in the whole concentration range. The axes of the four plots in this figure have the same range for comparison purposes. Please notice that this representation allows visualizing changes of the maxima in the whole concentration range but does not present an explicit scale in regard to concentration; indeed, the distances between the points in the curves are a function of concentration implicitly and are a function of wavelength and intensity of \(\lambda_{\text{max}}, \lambda_{\text{ex}}, \text{and } \lambda_{\text{em}}\) explicitly (for the spectra of both polymers in water, at different concentrations, see Figure S2a,b).
Effect of Concentration and Solvent on $\lambda_{\text{max}}$ The data in Figure 3 show that, in all solvents, in the whole concentration range, $\lambda_{\text{maxPT2}}$ is bathochromically (red) shifted with respect to $\lambda_{\text{maxPT1}}$, especially in water. This seems to be mainly associated with a different ground state (S0) between these polymers.46 In regard to water, Figure 3a shows that at low concentrations (i.e., below CC), the difference in $\lambda_{\text{max}}$ between the polymers is particularly large, in comparison to the data from cosolvent mixtures (Figure 3b–d). This is apparently related to specific polymer–water interaction in the diluted solutions, causing a conformation of a higher energy.46

Figure 3a shows that $\lambda_{\text{maxPT1}}$ in water presents a large and sudden red shift when concentration increases to that of CC, whereas larger concentrations generate a behavior which is similar to that observed for both polymers in any of the solvents.

These results suggest that when PT1 aggregates in water, there is a clear transition in which the polymer–water interactions become weaker than polymer–polymer forces of interaction. On the other hand, Figure 3b in W–IPA shows that the wavelength $\lambda_{\text{maxPT2}}$ remains constant in the whole range of concentrations.

Self-association of polymers in solution or at the solid–liquid interface has been described using concepts such as J- and H-aggregation. A J-aggregate is a type of dye with an absorption band that shifts to a longer wavelength (bathochromic shift) of increasing sharpness (higher absorption coefficient) when it aggregates under the influence of a solvent or an additive or concentration as a result of supramolecular self-organization. Hypsochromically shifted H-bands arise from H-aggregates.

The dye molecules may aggregate in a parallel way (plane-to-plane stacking) to form a sandwich-type arrangement (H-dimer) or in a head-to-tail arrangement (end-to-end stacking) to form a J-dimer. This concept was extended to analyze the interaction. On the other hand, Figure 3b in W–IPA shows that the wavelength $\lambda_{\text{maxPT2}}$ remains constant in the whole range of concentrations.

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water, J-like aggregation is not the main mechanism because (1) in this solvent, both polymers display a null distortion of the excitation spectra due to concentration, suggesting that the driving force for aggregation is a mechanism different from J-like aggregation (i.e., H-like aggregation) and also (2) because in this solvent, a clear difference between the polymers in regard to the effect of concentration on the S0 state is observed, with concentration clearly modifying the S0 state of PT1 and not that of PT2.

For the aggregation in cosolvents, the mechanistic explanation proposed by Deng et al.\textsuperscript{41} seems reasonable to support our results: in water, some aromatic groups approach each other due to diffusion forming a stable J-aggregate with a low aggregation number because of strong π−π attraction. In such conditions, no π−π-aggregated chains and J-aggregates are in equilibrium, and their molar ratio does not change with increasing concentrations, as demonstrated by the invariance of the excitation spectra.

On the other hand, in the cosolvent mixtures, the number of J-aggregates increases because of an increased number of isolated aromatic groups forming J-aggregates. This causes a shrinking in the intensity of the excitation band at shorter wavelengths, whereas the bands at longer wavelengths are strengthening (i.e., a distortion in the excitation spectrum).

DFT studies have shown that charge delocalization plays a role in structuring hydrophilic ammonium fullerenes with different spacer lengths in aqueous solution, mainly because the cationic part of these fullerenes is delocalized far away from the atom(s) involving the electron deficiency; for longer spacers, the positively charged nitrogen in the ammonium group has no influence on the spherical fullerene.\textsuperscript{60} Therefore, it is possible that the electrostatic potential of PT1 and PT2 in water plays a role in the different behaviors of these polymers in disaggregated conditions.

Figure 4 shows the electrostatic potential surface maps of dimers of PT1 and PT2 in water (DT1 and DT2, respectively) optimized at the B3LYP/6-31G** level (for details see ref 46). It is interesting that the simulated electrostatic potential is basically independent on the selected basis set of theory.\textsuperscript{60}

Figure 4 shows that the cationic charge of the nitrogen in the isothiouronium group spreads in the backbone of DT1, whereas DT2 shows no charge at all in the backbone.

Therefore, the cationic isothiouronium functionality has effect on the electrostatic potential of the backbone, only for short spacers. Such an electrostatic interaction may in part explain why, in water, in disaggregated conditions, the spacer length generates a clearly different behavior of λ_{max} between the polymers, as observed in Figure 3.

On the other hand, in regard to the aggregated state, it is known that equally charged side chains generate electrostatic repulsion.\textsuperscript{60} Therefore, the results indicate that the isothiouronium groups in PT1 aggregates limit the extent of intermolecular thiophene−thiophene interactions between neighbor rings because of electrostatic repulsion, whereas the longer more flexible hexyloxy spacer of PT2 would allow other forces of interaction (e.g., intermolecular π−π interactions).

An experimental fact that supports this assumption is the water-nonsoluble character of a third isothiouronium polythiophene also tested in the present study, with a longer (octyloxy) spacer. This result indicates that when the isothiouronium functionality plays a secondary role in the interactions, π−π interactions dominates forming nonsoluble aggregates.

In regard to the effect of solvent, the data in Figure 3 show that at all concentrations and especially at the DIS state, the presence of cosolvents caused larger red shifts in λ_{maxPT1} than in λ_{maxPT2}. It is also observed that for λ_{maxPT1}, the red shift caused by W−DI in comparison with the other cosolvent mixtures shows a systematic increase with concentration. On the other hand, λ_{maxPT2} showed similar curves, regardless of the aggregation state. For a graphical representation of these trends, see Figure S5.

Therefore, the energy of the S0 level of the disaggregated chains and the aggregates of PT2 remained almost unchanged (<4 nm) because of a change in the polarity/H-bonding capacity of the solvent, whereas S0-PT1 showed a larger decrease after cosolvent addition, especially in the disaggregated state. This suggests that the aggregates of PT1 retained the propensity of this polymer to interact with water in a stronger way than PT2. This seems reasonable because it is known that molecules in aggregates largely retain their electronic and nuclear structure.\textsuperscript{66}

**Effect of Concentration and Solvent on the Wavelength of** λ_{em}. Figure 3 shows that, in water, the increase in concentration in the whole range causes λ_{emPT1} to have a total shift of 12 nm (from S25 at the lowest concentration to S37 nm at AGG), whereas λ_{emPT2} shifts barely of 5 nm.

This indicates that in water, the S1 → S0 relaxations of PT1 are larger than those of PT2, indicating that PT1 undergoes larger geometric distortion (and subsequent relaxation) due to the photons absorbed.

On the other hand, in every cosolvent mixture, both polymers show a similar total shift in λ_{em} of around 10 nm due to the concentration increase. This result indicates that when the content of water molecules decreases, the interactions of PT1 are not relevant, generating a smaller difference between polymers.

It has been suggested that the change from the S0 to the S1 state in polythiophenes implies conformational changes (e.g., from aromatic into quinoidal geometries)\textsuperscript{56} and that such changes depend on the rigidity of the conjugated backbone and also on the hydrogen bonding interactions between the charged side chains of the polymer and water.\textsuperscript{5,46}

Therefore, these results suggest that when the number of water molecules decreases, the S0 → S1 conformational changes are almost the same for the two polymers, regardless of the cosolvent mixture. Conversely, pure water seems to increase the S0 → S1 changes for PT1, whereas it reduces them for PT2.
Stokes Shift. To compare the relaxation energies, it is useful to analyze the Stokes shift ($\Delta \nu$), which points out the energy difference between $\lambda_{\text{max}}$ and $\lambda_{\text{em}}$. It is useful to consider that large Stokes shifts in oligo and polythiophenes are related with effective nonradiative relaxation pathways along but also between the chains. This is also observed in the values of molar absorptivities previously estimated and shown in Table S2.

Figure 5 shows the $\Delta \nu$ values of the two polymers in all solvents and aggregation states.

Figure 5 shows that for PT1, water generates the largest $\Delta \nu$, particularly in the DIS state as previously analyzed with the Lippert–Mataga plot. However, this method is not suitable for the analysis of aggregates. PT2 shows similar $\Delta \nu$ values of 9–10 eV in all aggregation states.

In the cosolvent mixtures, both polymers show similar trends in the DIS and CC states, with PT1 showing different values than PT2, may be due to a larger interaction of PT1 with the water molecules present in the solvent mixtures.

Also, in the cosolvent mixtures and in the AGG state, the $\Delta \nu$ values of both polymers are almost identical. This is probably because the hydrophobic–hydrophilic surface area of the aggregates at the maximum concentration becomes similarly available for interactions with the media, regardless of the length of the alkoxy spacer. However, in the solvent mixtures, the two polymers show similar $\Delta \nu$ values (the thickest lines almost overlap), whereas in water, they clearly differ from each other.

Absorbance and PL Intensities. The intensities at $\lambda_{\text{max}}$ and $\lambda_{\text{em}}$ reveal information about the oscillator strengths associated with the S0 $\rightarrow$ S1 and S1 $\rightarrow$ S0 transitions, respectively. The absorbance at $\lambda_{\text{max}}$ is related to the vertical excitation energy. This may occur in spite of having exactly the same S0 state and may be influenced by the medium. On the other hand, the PL intensity at $\lambda_{\text{em}}$ is related with the S1 $\rightarrow$ S0 transitions. This process depends on the molecular conformation. Furthermore, the properties of the excited state are very sensitive to the immediate environment surrounding the molecules.

Absorbance Values. Figure 3 shows that water generates the smallest absorbance (Abs, in arbitrary units) values for both polymers, whereas the cosolvent mixtures generate slightly larger Abs values. Regardless of the solvent, the absorbance of PT1 ($\text{Abs}_{\text{PT1}}$) is around 13% smaller than $\text{Abs}_{\text{PT2}}$ at all concentrations. This is also observed in the values of molar absorptivity previously estimated and shown in Table S2. These results indicate that water decreases the oscillator strength (i.e., the probability to absorb light) of both polymers. However, in each solvent, the oscillator strength of PT2 is larger than that of PT1.

In regard to the behavior of the absorbance spectra, Figure S1b shows the integrated area of the absorption bands of PT1 and PT2, integrated in wavelength ranges of $\lambda_{\text{max}} \pm 60$ nm, on corrected baseline spectra. In this figure, it is observed that both polymers show the same trends observed for absorbance, that is, there is no difference between the polymers in regard to the broadening of the absorption bands due to concentration.

To directly compare the relative effect of solvents, Figure S6 shows that the change in absorbance at $\lambda_{\text{max}}$ due to a decrease in solvent polarity/H-bonding capacity, in the DIS, CC, and AGG states, obtained by normalizing the Abs values with respect to the absorbance in water. This figure shows that regardless of the aggregation state, the two polymers display a similar behavior, with exception of the points at W–THF, which generates clearly smaller values of $\text{Abs}_{\text{PT1}}$ than those of $\text{Abs}_{\text{PT2}}$. It is also observed that aggregation reduces the increase in absorbance and also causes polymers to behave similarly with each other.

These results indicate that the presence of cosolvents provides similar increases in the oscillator strength of the polymers, specifically in the AGG state.

Effect of Concentration and Solvent on PL Intensities. In regard to the effect of concentration on the PL intensities, Figures 2 and 3 show that in all solvents, the PL intensity of PT1 (i.e., PL-PT1) either remains constant or increases with the increase of concentration. Conversely, the PL intensity of PT2 (PL-PT2) decreases at the highest concentrations. This indicates that the aggregates of PT2 have a larger extent of concentration self-quenching.

Figure 3 also shows that in the polar-protic solvents (water and W–IPA), the highest PL intensities for both polymers are similar to each other. On the other hand, in the mixtures with aprotic cosolvents, particularly in W–DI, the PL of PT2 is larger than that of PT1. It is known that larger polarities decrease the PL intensity; therefore, PT1 in W–DI shows an atypical behavior.

This atypical behavior does not seem to be related with self-absorption at shorter wavelengths because in W–DI, the polymers show similar curves of absorbance to each other. Therefore, these results suggest that in W–DI, PT1 shows an atypically low S1 $\rightarrow$ S0 relaxation.

To gain insight on the relative effect of solvents on PL intensity, Figure S7 shows the change in PL intensity at $\lambda_{\text{em}}$ due to a decrease in solvent polarity/H-bonding capacity, in the DIS, CC, and AGG states, by using the PL*/PL water ratio. This figure shows that the two polymers present basically the same trends at the three aggregation states. For both polymers, in the three aggregation states, W–DI causes the smaller increases in PL intensity. It is also observed that in the aggregated states, the presence of DI actually does not have any impact on PL-PT1.

In regard to the values of fluorescence quantum yield ($\Phi$), as stated previously, (2017 Dominguez), those of PT2 ($\Phi_{\text{PT2}}$) are larger than $\Phi_{\text{PT1}}$ in every solvent, especially when cosolvents are present. It is known that changes in $\Phi$ are associated with different $\pi$-conjugations and/or triplet formation of conjugated ring molecules (e.g., thiophenes). Therefore, it seems that the spacer length affects these $\pi$–$\pi$ interactions between the rings and also that cosolvents increase such difference, probably due to a better solvation.
Normalized Changes in PL Emission Due to Increasing Concentrations. The data from PL intensity shown in Figure 3 can be normalized using the ratio PL/PL₀, where PL₀ is the absorbance at the lowest concentration and PL is the absorbance at any higher concentration. This ratio indicates "how many times" the PL increased due to the same increase in concentration. This is basically the same approach used in the so-called Stern−Volmer plots, which normalize the changes in the PL intensity of a fluorophore in regard to a specific change in mass of a certain quencher. This allows the exploration of the kinetics of a photophysical intermolecular deactivation process, that is, how the presence of another chemical species can accelerate the decay rate of a certain chemical species in its excited state. Such an approach focuses on specific increases in the mass of a quencher but also allows comparing the interaction kinetics between one fluorophore and different quenchers or one quencher and different fluorophores. This holds regardless of whether these fluorophores present different S0 and S1 levels and oscillator strengths due to changes either in their molecular structure or in solvent and temperature.

Figure S8 shows the normalized plots of PL/PL₀ of both polymers in each of the solvents. The same scale is maintained for comparison purposes. This figure shows that in the disaggregated condition, the curves of the polymers are similar in all solvents, which means that the difference in the length of the alkoxy-spacer does not play a major role in the "kinetics" of photophysical intermolecular deactivation process.

However, Figure S8a shows that in aggregated conditions, water causes the PL/PL₀ value for PT1 to be larger than that of PT2, which means that in water, concentration has a larger relative effect on the oscillator strength of aggregated PT1.

On the other hand, Figure S8b-d shows that a decrease in the polarity/H-bonding capacity of the solvent (i.e., moving into the three cosolvent mixtures) provides a reduction of the difference between the curves of the two polymers with respect to the difference in water.

These results indicate that the alkoxy-spacer length improves the oscillator strength of the polymer aggregates, more for PT1 despite the smaller PL intensities, especially when polarity/H-bonding capacity is larger. Conversely, PT2 presents similar changes in PL for the different solvents due to specific changes in polymer mass.

However, the curves of PT2 are not exactly the same in all solvents, going to a maximum value of PL/PL₀ around 6 in water and 3 and 2 in the other solvents. Therefore, it seems that the similar behavior of the two polymers in W−DI is related to their similar aggregation ability and not because of a limitation related to the solvent.

Nevertheless, to clearly state this, it is useful to perform interaction studies between each polymer and suitable hydrophilic quenchers, in water and W−DI. Such a study is currently being performed by our research group.

EPR Study. A computer-aided EPR study was performed to obtain information on the aggregation behavior and the interacting ability of the polymers by means of selected spin probes at different polarities inserted in the systems.

The completely hydrophobic probe (SDSA) generated different results with respect to probes containing hydrophilic and hydrophobic groups (TOH, CAT8, and CAT16). Therefore, the results obtained with SDSA will be discussed separately.

Figure 6 shows, as an example, the experimental (298 K) spectrum of CAT16 at the concentration of 0.05 mM in solution with PT2 at the concentration of 0.1 mM. The spectral computation (red line) is also shown.

The main parameters obtained from computation (see Materials and Methods for the details) were the polarity parameters: $A_0 = 6, 6$, and 38.35 G and the microviscosity (interaction) parameter: $\tau = 45$ ns. These parameters indicated fast moving probes in a polar environment. On this basis, the interactions seem to be quite weak at the radical side.

However, the spectra showed some interesting variations changing the spin probe, the type of polymer, and its concentration.

Figure 7 shows the intensity variation (measured as double integral of the spectra) as a function of the polymer concentration for TOH (a), CAT8 (b), and CAT16 (c).

As Figure 7 shows, in all cases, a maximum of intensity was observed at about 0.25 mM of polymer concentration. Assuming that the intensity measures the probe solubility, this result indicated an increased probe solubility at this concentration. All probes contain a hydrophilic and a hydrophobic portion and solubilize better when they insert in aggregates where the hydrophobic part is protected from the hydrophilic one. Therefore, we may consider the intensity increase as a proof of the formation of micellar aggregates, where the hydrophobic parts of the polymers are condensing, surrounded by the hydrophilic parts.

This finding is in agreement with Bahri et al. who have indicated that the formation of micromicelles is accompanied by three different variations in spectral intensity: a linear increase, a nonlinear increase, and a decrease or constant intensity.

The higher increase in solubility of CAT16, containing a bigger hydrophobic portion, in comparison to the other probes, and the higher increase for PT2 with respect to PT1, when the surfactant probes CAT8 and CAT16 were used, were expected because PT2 contains a more significant hydrophobic portion if compared to PT1.

Figure 7a shows that the intensity of TOH (the only probe without hydrophobic alkyl chain) was almost identical for the two polymers, in the whole concentration range. This indicated that this probe interacted mainly with the cationic isothiouretonium group in both polymers, regardless of whether PT2 formed a slightly larger hydrophobic core with respect to PT1, because of the hydrophobic interactions between the packed hexyloxy chains within the π−π cores of the micelles.

These results suggest that at CC, larger spacers generate larger hydrophobic surface area in the aggregates and that the
aggregates possess similar hydrophilic surface area, regardless of the spacer.

Interestingly, Figure 7 also shows that at 0.05 mM, the intensities for the three probes were almost the same in both polymers. This suggests that at DIS conditions, both polymers possess similar hydrophobic and hydrophilic surface area.

Also, the achievement of a maximum indicated that high concentrations of polymers gave rise to less organized aggregates and therefore the intensity diminished at the highest concentrations.

Spectra computation showed that the polarity, measured by \( A_2 \) parameters, poorly changed from one to another sample for each of the three probes, TOH, CAT8, and CAT16, only indicating a small decrease in polarity when the aggregates were formed for the CAT probes, as expected on the basis of the insertion of the radicals into the polymer aggregates. Conversely, interesting variations from one to another sample occurred in respect of the microviscosity (interaction) parameter (\( \tau \)) as a function of the polymer concentration. These variations are summarized in Figure 8 for TOH (a), CAT8 (b), and CAT16 (c).

As for the intensity, also the microviscosity increased up to a maximum. The same trend as found for the intensity variation further showed an agreement with Bahri et al. about the formation of micromicelles. However, for the three probes, a higher microviscosity was observed for PT1 with respect to PT2.

This result is explained by considering that the PT1 polymer is characterized by a shorter chain length, and consequently, it forms aggregates where the stronger hydrophilic interactions with the probes prevail in respect to the weaker hydrophobic ones.

The results using TOH showed that the microviscosity continued to increase up to about 0.35 mM of PT2 concentration due to weak interactions of this probe with thiouronium groups. These interactions were quite independent on the aggregate formation but largely depended on the aggregate size. Conversely, CAT8 and CAT16 showed a maximum at a polymer concentration of 0.25 mM, when micromicelles were forming.

For polymer concentrations smaller than 0.25 mM, when the polymers were not aggregated, \( \tau \) for CAT16 was practically the same in both polymers, whereas for TOH, \( \tau_{PT1} \) values were larger than \( \tau_{PT2} \) ones.
The graphs in Figure 8 also indicated that the values of $\tau$ decreased at high concentrations mainly when the most hydrophobic probe CAT16 was used.

To verify if the phase separation due to the freezing of the solution was affected by the aggregation of the polymers, the spectral analysis was also performed as a function of temperature. Figure S10 in the Supporting Information shows, as an example, the first hyperfine line of the spectra of CAT8 in solution of PT1 and PT2 0.5 mM at 263 K. At this temperature, the system is still fluid when forming aggregates, but the decrease in intensity is related to the phase separation of a portion of the solution after freezing.

PT1 showed a significant decrease in intensity (up to 95%) if compared to PT2, mainly at the highest polymer concentrations. The logic explanation is that PT1 polymers formed smaller aggregates than PT2, the latter being able to create a protected region to host the probes. Therefore, PT2 aggregation avoided a phase separation of the probe from the solution. Conversely, PT1 formed aggregates which poorly hosted probes inside them and therefore could not avoid the separation of a large fraction of probes due to freezing of the solution.

However, by evaluating (from spectral computation) the microviscosity (interaction) parameter $\tau$ for CAT8 in the polymers solutions (0.5 mM) at 263 K, we verified that the probes showed a stronger interaction with PT1 ($\tau = 215$ ps) than with PT2 ($\tau = 180$ ps). The fraction of probes giving the EPR spectrum for PT1 at 263 K were the nonfrozen ones, which were trapped in few small aggregates in solution showing stronger interactions than in the more fluid and larger PT2 aggregates.

At 263 K, CAT16 probes distributed in three different regions of the polymer solutions, which corresponded to three different components in the EPR spectra shown in Figure 9, together with their computations. For PT1 (0.5 mM), the spectrum (Figure 9a) was constituted by 50% of a component, which, from the computation parameters, arose from a fluid region ($\tau = 52$ ps) at middle/low polarity ($A_h = 6, 6, 36.6$ G). This region probably was the interphase at the surface of the polymer aggregates; the other 50% of the spectrum for PT1 was characteristic of a little bit less fluid ($\tau = 74$ ps) but more polar ($A_h = 6, 6, 39.3$ G) region, which was probably the water solution trapped in the polar head groups of the polymers.

For PT2 the spectrum also contained a broad spectral component at a relative percentage of 15%. This component was extracted from the overall spectrum after subtraction of the spectrum for PT1 (Figure 9a). The experimental and computed broad spectral component is shown in Figure 9b. The parameters of computation indicated a middle/low polar ($A_h = 6, 6, 36.6$ G) region, quite microviscous ($\tau = 4200$ ps) and well-packed (line width = 12.5 G), formed inside the polymer aggregates.

In regard to the completely hydrophobic spin probe (5DSA), first, no spectrum was recorded for PT1 in the entire range of polymer concentration. Conversely, a low-intensity spectrum was recorded for PT2 at the highest concentrations, mainly above 0.25 mM.

In agreement with the results from the other probes, this result indicated that the short alkoxy chain in PT1 was not long enough to generate any interaction with the hydrophobic SDSA. This provided further evidence about the fact that the probe-PT1 interactions observed with the other probes were mainly hydrophilic.

Figure 9. (a) Experimental and computed spectra for CAT16 with PT1 at 0.5 mM (263 K). Computation was obtained by adding two spectral components at about 50%; (b) experimental and computed broad spectral component only present for PT2 (15%).

An example of the experimental spectrum recorded at 298 K for SDSA with PT2 at 0.5 mM and its computation is reported in the Supporting Information (Figure S11).

The spectrum of SDSA with PT2 aggregates in Figure 8 is very noisy because it was recorded in the same instrumental conditions as the spectrum in Figure 6, to demonstrate the different EPR intensities of CAT16 and SDSA due to the low solubility of SDSA in this system, because it only solubilizes into the hydrophobic region formed by polymer aggregates and it is repulsed by the charged polymer heads. The computation of the spectrum (red line in Figure 8) gave the main parameters $A_h = 6, 6, 35$ G, and $\tau = 1.3$ ns, which interestingly indicated that the radical group (at position 5 of the stearic chain) was located in a region at low polarity and quite high microviscosity, as expected for the hydrophobic core of a lipid aggregate.

Dynamic Light Scattering. Figure 10 shows the hydrodynamic radius ($R_h$) distributions of PT1 and PT2 water solutions at concentrations of 0.084, 0.2, and 0.4 mM.

Figure 10a shows that for both polymers, the highest concentration generates a broader distribution and lower intensity, keeping the hydrodynamic radius ($R_h$) value at 190 and 200 nm for PT1 and PT2, respectively. In this regard, the broader nature of the profiles at higher concentrations indicates the presence of smaller and larger aggregates, maintaining the average $R_h$ unchanged.

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CONCLUSIONS

As a brief resume of results, on one hand, regardless of the spacer length, the two polymers, PT1 and PT2, display several similarities, some of which have been widely associated to charged polythiophenes in solution in the literature. These correspondences are as follows: (i) a probable rodlike conformation; (ii) mainly weak, nonextontic, interchain interactions (unstructured absorption and emission spectra); (iii) solvantochromic features; (iv) formation of H–J-like aggregates; (v) increase of π–π interactions (increase in J-like aggregation) when cosolvents are present (change in shape of the excitation spectra); (vi) similar relaxation energies (Stokes shifts) when cosolvents are present; (vii) similar total red shift of $\lambda_{\text{max}}$ (decrease in the energy of the S0 state) due to concentration when cosolvents are present; (viii) increase in PL by increasing concentration in the aggregated state, regardless of the solvent; (ix) increase in PL by increasing concentration in the aggregated state, in the presence of DI; (x) maxim in EPR intensity at similar concentrations as in the PL spectra, indicating aggregate formation; (x) less structured aggregates at the maximum concentration; and (xi) the aggregates possess a fluid region at middle/low polarity which may be identified as the interphase at the polymer chain surface and a fluid polar region formed by the polar head groups of the polymers which is able to trap the water solution inside.

On the other hand, the two polymers display a clearly different behavior between each other, well-correlates with their different spacer lengths. The differences in their behavior are as follows: (1) regardless of the solvent, a longer spacer generates larger $\lambda_{\text{max}}$ (smaller energy in the S0 state), also in the aggregated state; (2) in water, a shorter spacer causes a sudden red shift of $\lambda_{\text{max}}$ due to aggregation, whereas the $\lambda_{\text{max}}$ is barely red-shifted; (3) in water, a shorter spacer causes higher Stokes shift values, regardless of the concentration; (4) when aggregated in water, a shorter spacer generates a larger increase in PL with the increase in concentration; (5) both EPR intensity and microviscosity indicate that a shorter spacer generates aggregates with smaller inner spaces; (6) EPR microviscosity suggests that a shorter spacer forms aggregates where hydrophilic interactions prevail with respect to the hydrophobic ones; (7) the polymer with longer spacer forms aggregates containing a low polar region, quite well-packed, able to trap low polar probes; and (8) a shorter spacer generates smaller $R_h$ values.

Therefore, it could be concluded that regardless of the spacer length, water seems to minimize π–π interactions during aggregation. Conversely, the higher energy of the S0 state of the polymer with a shorter spacer in water seems to be related to a twisting in the polymer backbone, probably due to the large degree of interaction of this polymer with water. The results about the red shift in water (e.g., the total red shift of $\lambda_{\text{max}}$ of the polymer with a longer spacer is more than twice that of the polymer with a longer spacer) suggest large geometry changes for the shorter-spacer polymer (PT1), which remains in the aggregated state. The fact that in this solvent aggregation causes a sudden decrease in the energy of the S0 level of the polymer with shorter spacer seems to indicate that when aggregation starts, there is a competition between the polymer–water and the dominance of polymer–polymer interactions. In water, the aggregates of the polymer with shorter spacer have smaller inner cavities (as also indicated by EPR results), suggesting that the water inside of the aggregate still has an effect on the twisting of the polymeric backbone, causing higher energy of the S0 state and relaxation energies (Stokes shifts). The PT1 aggregates also have larger hydrophilic interactions because their surface has smaller hydrophobic areas, which are larger in the aggregates of PT2 probably due to the exposure of the hexyloxy chains.

The presence of cosolvents increases the number of π–π interactions by increasing concentration and also ensures that $\lambda_{\text{max}}$ of both polymers is similar even in the disaggregated state. Also, the presence of cosolvents causes the polymers to have practically the same relaxation energies in the aggregated state. Finally, the cosolvent with smaller polarity/H-bonding capacity, DI, generates an equivalent increase in PL for the two polymers with the increase in concentration. These results suggest that a decrease in water content decreases the effect of the isothiouronium groups on aggregation, favored by π–π interactions.

These results confirm that H-bonding is maximized in water in the presence of a shorter spacer, with the electrostatic potential surface probably playing a role.

As future perspectives, it would be useful to compare several cateionic polythiophenes with short spacers to evaluate the effect of the isothiouronium group through detailed thermodynamic studies (e.g., van’t Hoff equation). Also, it would be useful to evaluate the effect of DI on the interactions of the polymers studied in this work with different anionic quenchers to evaluate if the same effect observed on the PL increments applies for such donor–acceptor pairs. This study could offer a platform to investigate H-bonding-mediated charge transfer.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.8b00808.

Figure 10. Hydrodynamic radius ($R_h$) distributions of PT1 and PT2 aqueous solutions at concentrations of 0.084, 0.2 and 0.4 mM, and 298 K.
fluorescence emission spectra of PT1 (left column) and PT2 at DIS, CC, and AGG states, in the four solvents, as labeled; normalized excitation spectra of PT1 (left column) and PT2 at DIS (black line), CC (orange line), and AGG (green line) states, in the four solvents, as labeled; shift of $\lambda_{max}$ due to a decrease in solvent polarity/H-bonding capacity at (a) DIS, (b) CC, and (c) AGG; Figure S6. Effect of decreasing the polarity on the absorbance of both polymers, at the DIS, CC and AGG states; effect of decreasing the polarity on the PL intensity of both polymers at the DIS, CC, and AGG states; plots of changes in PL intensity due to specific changes in the mass of PT1 (blue continuous lines) and PT2 (red dashed lines) in (a) water, (b) WIPA, (c) WTHF, and (d) WDI. The same scale is maintained for comparison purposes. The labels indicate the values of PL/PL0 obtained using the concentrations of DIS, CC, and AGG; plots of changes in absorbance due to specific changes in the mass of PT1 (blue continuous lines) and PT2 (red dashed lines) in (a) water, (b) WIPA, (c) WTHF, and (d) WDI. The same scale is maintained for comparison purposes. The labels indicate the values of PL/PL0 obtained using the concentrations of DIS, CC, and AGG; first hyperfine line of the spectra of CAT8 in solution of PT1 (thick line) and PT2 at 263 K; and experimental EPR spectrum (298 K) for SDSA (0.1 mM) with PT2 (0.5 mM) and its computation (PDF).

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