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Hg^{+2} Ion sensing application of Tri-armed Star Copolymer containing Pyrene side groups

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A pyrene-functionalized styrene polymer (P3) is synthesized via atom transfer radical addition; click reaction between the azide functional groups of the precursors and, 1-ethynylpyrene and finally nitroxy mediated polymerization reactions. T_g of the polymer increases remarkably upon the covalent connection of the pyrene groups, while the other thermal properties (Tonset, T_{max}) of the styrene polymers are not affected by the incorporation of pyrene units. P3 exhibits the characteristic pyrene monomer emission bands and, depict also the excimer emission bands of pyrene due to strong intermolecular π-stacking interactions of pyrene units in star-shaped polymer. The excimer emission of P3 is moderately to severe decreased by the addition of Hg^{+2} cation. The highest quenching efficiency (around 65%) was observed at 25 eq. addition of Hg^{+2}.

Keywords: *Sensors and actuators, polystyrene, sensing application, mercury ion, pyrene.*

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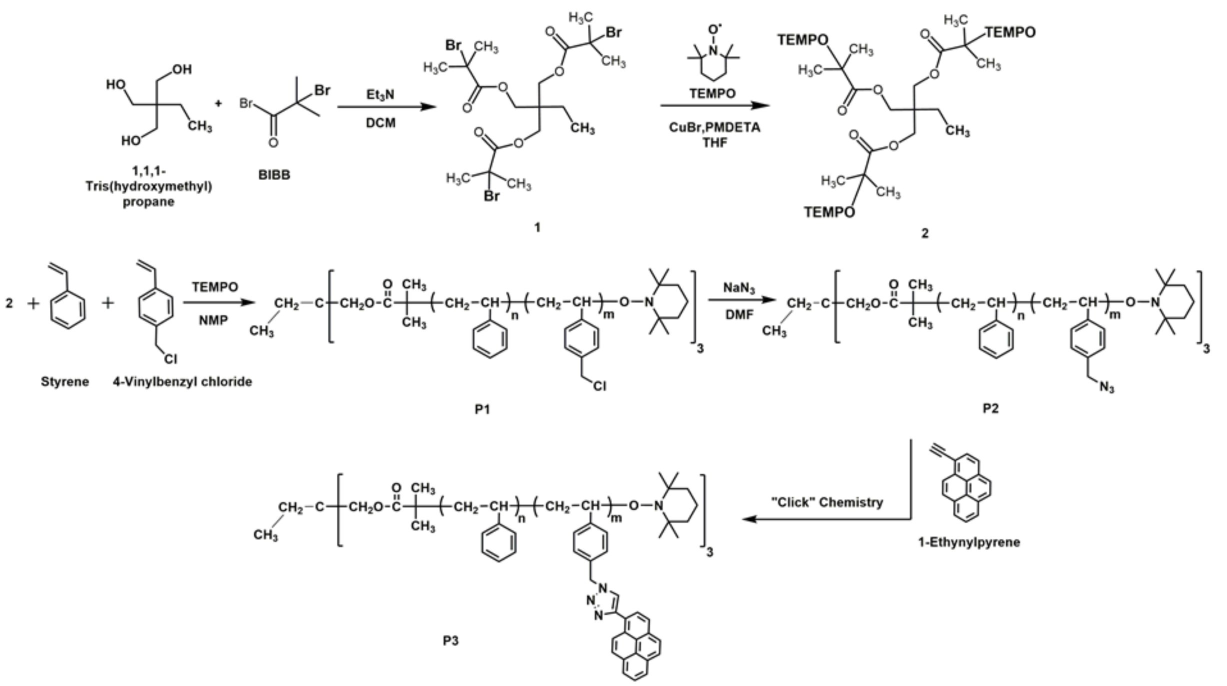
1. Introduction

Hg^{+2} is the most toxic heavy metal ion that affects nature and human health. When released into the water bodies, mercury is transformed by bacteria to its more toxic form organomercury derivative (CH_3HgX), which can accumulate in aquatic organisms. Intake of fish contaminated with this toxic metal can cause serious damage to human health like central nervous and endocrine systems, as well as it causes digestive and cardiac problems. Therefore, there is a necessity for molecular sensors for the fast, efficient and cost-effective sensing of mercury, especially in water media. Numerous works have been reported to detect mercury by using small organic molecules, polymers, and nanomaterials [1-3].

By detection of metal ions fuorescence spectroscopic method has significant advantages over other techniques since it shows excellent sensitivity, selectivity in short time, and the ability to do the detection in a non-destructive manner [4].

For fluorescent chemosensing systems, pyrene functional molecules have been used widely due to its excellent photostability, long fluorescence lifetime, high quantum yield, and relatively nontoxic nature [5] Pyrene functional small molecular compounds, dendrimers and polymers have been employed in chemical sensor applications based on fluorescence mechanism towards various anions, cations [6, 7].

In this study, we are reporting synthesis, characterization, and Hg^{+2} metal ion sensing application of tri-armed pyrene endcapped polystyrene star polymer (Scheme 1). P3 was prepared with a core-first approach nitroxy mediated polymerization procedure. Chemical structures of the obtained polymers (P1, P2, and P3) were confirmed by FTIR and ^{1}H-NMR spectroscopic techniques.



Schema 1. Synthetic route of pyrene functionalized star-shaped polymer (P3)

Then, the fluorescence responses of P3 in the presence of Hg^{+2} metal ion in the analytic media was investigated by fluorescence spectrophotometric method.

2. Experimental

2.1. Materials and reagents

Trifunctional initiator (1) was synthesized according to a previously reported procedure, with slight modifications [8]. The inhibitors of 4-vinylbenzyl chloride (VBC, Aldrich, 90%) and Styrene [St, Aldrich, 99.9%] was removed by running through from basic alumina column and were freshly used. Mercury(II) nitrate monohydrate (Aldrich, 98.5%), 2-Bromoisobutyryl bromide (Aldrich 98%), 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO, Acros, 98%), dimethylformamide (DMF, Merck, 99.8%), sodium azide (NaN_3, Aldrich 99.5%), N,N,N,N,N-pentamethyldiethylene-triamine (PMDETA, Aldrich 99%), copper(I) bromide (CuBr, Aldrich, 98%), 1-ethynylpyrene (Alfa Aesar, 96%), dichloromethane (DCM, Merck, 99.8%), and methanol (Merck 99.9%) were used as received. Triethylamine (99.5%) was distilled over potassium hydroxide before use. Tetrahydrofuran (THF, Aldrich 99%) was freshly distilled from sodium and benzophenone under argon atmosphere.

2.2. Instrumentation

Proton and carbon nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra were recorded on a Bruker Avance III HD 400 MHz Spectrometer in deuterated chloroform ($CDCl_3$). Fourier transform infrared (FT-IR) spectra were acquired on a JASCO FT/IR-6600. high-res. mass spectra were taken on an Agilent 6230 A LC–MS Spectrometer. UV–vis and fluorescence spectra were measured using quartz cuvettes (1 cm path length) on Shimadzu UV-2600 and Agilent Cary Eclipse spectrophotometers, respectively. Glass transition temperatures (T_g) of the star-shaped copolymers was investigated on a Hitachi DSC 7020 differential scanning calorimeter (DSC) under nitrogen flow (20 mL min^{-1}) at a heating rate of 200 ºC min^{-1}. Thermogravimetric analysis (TGA) was executed on a Seiko Exstar 6000 thermal analyzer from rt. up to 700 ºC at a heating rate of 10 ºC min^{-1} in under inert nitrogen atmosphere.

2.3. Synthesis of TEMPO-Capped Unimolecular NMP Initiator (2)

Under argon gas, THF (20 mL) was dissolved in TEMPO (1.74 g, 11.15 mmol) and Compound 1 (0.9 g, 1.23 mmol, 1 eq.) in a 50 mL one-necked round bottom flask. CuBr (0.69 g, 4.80 mmol) was then added, and the mixture underwent three cycles of freeze-pump-thaw deaeration. The solution turned a dark green color after the addition of PMDETA (1.66 g, 9.60 mmol), and the reaction was allowed to stir for 2 days at room temperature. After the reaction was complete, the solvent was drained, the raw material was dissolved in DCM, and it was purified by precipitating into a cold mixture of 1:3 methanol and water. The compound 2 was then dried under vacuum and collected as a white powdered product using a sintered glass filter (G4).

Yield: 0.63 (50%). m.p: 133-135 °C. ESI-HRMS (m/z): 810.59 $[M+H]^+$, MW_{theo}: 809.61 g/mol. FTIR (ATR, cm^{-1}): 3000 ve 2850 (-CH), 1730 (C=O), 1360 (N-O), 1130 (C-O). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.00 and 1.14 (s, 36H, CH_3 (TEMPO)), 1.46 (s, 18H, CCH_3), 1.40-1.70 (d, 20H, CH2), 4.08 (s, 6H, OCH_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 7.25 (s, CH_2-CH_3), 17.06 (s, CH_2CHCH_2), 20.47 and 24.60 (s, CH_3 (TEMPO)), 22.52 (s, -CH_2-CH_3), 33.49 (s, $OCCH_3$), 40.51 (s, $CH_2CH_2CH_2$), 41.05 (s, -CH_2-C-CH_2O)$_3$), 59.58 (s, -CH_2O), 63.76 (s, N-C-(CH_3)$_2$), 81.24 (s, C-(CH_3)$_2$), 175.80 (s, C=O).

2.4. Synthesis of Chloride Side-Functional Styrenic Star Copolymers (P1)

Through unimolecular nitroxide-mediated radical polymerization (NMP) of St and VBC (monomer feed ratios St/VBC=100/20 per arm), chloride side-functional styrenic star copolymer (P1) was produced. Compound 2 (0.046 g, 0.04 mmol), St (1.25 g, 12 mmol), VBC (0.366 g, 2.4 mmol), and TEMPO (0.08 g, 0.53 mmol) were added to a 25 mL Schlenk flask under argon and underwent three cycles of freeze-thaw degassing. The reaction mixture was agitated for 48 hours while the flask was submerged in an oil bath that was thermostat at 120 ºC. The polymerization was then quenched by fast cooling in a water-ice bath. After being dissolved in DCM (10 mL), the resulting crude polymerization product was refined by twice precipitating in cold methanol. P1 was purified by vacuum filtration using a sintered glass filter (G4), and it was then dried at 35 ºC.

Yield: 1.4 g (67%). FTIR (cm^{-1}): 3026-3080 (CH stretching, aromatic); 2848-2920 (CH stretching, aliphatic); 1601, 1493, and 1452 (C=C stretching, aromatic). ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 6.47-7.11 (C_6H_4 and C_6H_5); 4.52 ($C_6H_4CH_2Cl$); 1.44-2.05 (polymer backbone).

2.5. Synthesis of Azide Side-Functional Styrenic Star Copolymers (P2)

In a single necked round bottom flask with argon, anhydrous DMF (50 mL) was used to dissolve P1 (0.8 g, 0.5 mmol Cl units). One portion of NaN_3 (0.33 g, 5 mmol) was added to the mixture, which was then gently purged with Ar for 5 min while being stirred for two days at 80 oC. The organic layer was washed with brine (2 x 50 mL) and deionized water (50 mL) after the raw polymerization product was transferred to a separating funnel with DCM (100 mL) following the completion of the reaction. Azide functional star-shaped St copolymer was precipitated in cold methanol after the solvent was evaporated to 5 mL. P2 was separated by vacuum filtration on a sintered glass filter (G4) and dried under reduced pressure at 40 ºC until constant weight was attained.

Yield: 0.76 g (94.6%). FTIR (cm^{-1}): 3026-3080 (CH stretching, aromatic); 2848-2920 (CH stretching, aliphatic); 1601, 1493, and 1452 (C=C stretching, aromatic). ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 6.47-7.11 (C_6H_4 and C_6H_5); 4.21 ($C6H_4CH_2N_3$); 1.4422.05 (polymer backbone).

2.6. Synthesis of Pyrene Side-Functional Styrenic Star Copolymers (P3)

P3 was synthesized through the "Click" reaction between 1-ethinyl pyrene and polymer with -N_3 side groups (P2). DMF (20 mL) was used to dissolve P2 (0.5 g, 0.40 mmol of N_3 unit), 1-ethynylpyrene (0.27 g, 1.21 mmol), and PMDETA (0.69 g, 4.85 mmol), and the resultant solution was then purged with argon gas for five minutes. CuBr was added (0.84 g, 4.85 mmol) in one piece, and the mixture was then degassed once more with argon for five minutes before being stirred at room temperature for 48 hours. The solvent was then eliminated using a rotary evaporator, and the leftover material was dissolved in DCM (100 mL) and rinsed with water (2x100 mL). The organic phases were collected and dried over $MgSO_4$, and then P3 was purified by precipitating in cold methanol. P3 was collected on a sintered glass filter (G4) by vacuum filtration as a yellow product and dried under reduced pressure at ambient temperature until constant weight was attained.

Yield: 0.45 g (76%). FTIR (cm^{-1}): 3026-3080 (CH stretching, aromatic); 2848-2920 (CH stretching, aliphatic); 1601, 1493, and 1452 (C=C stretching, aromatic). ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 7.6828.70 (CH, pyrene); 6.5127.11 (C_6H_4 and C_6H_5); 5.47 ($C_6H_4CH_2$Pyr); 1.4422.05 (polymer backbone).

2.7. Detection of Hg+2 with Pyrene Functional Star-Shaped Styrenic Copolymer (P3)

Detection of Hg^{+2} by means of fluorescence spectroscopy of pyrene-functional star-shaped styrenic copolymer (P3) was performed as the following procedure: Definite amounts of P3 was dissolved in DMF at room temperature. Then, the fluorescent responses of this styrenic star copolymer upon the addition of prescribed.

3. Results

3.1. Synthesis and Structural Characterization

The tri-functional star-shaped styrenic copolymer having pyrene side groups (P3) was synthesized with core-first nitroxy mediated polymerization (NMP) technique

(Scheme 1). Moreover, this polymerization technique offers good control on the macromolecular design.ref. The tri-functional initiator (2) was synthesized via facile esterification and atom transfer radical addition reactions. First, 2-bromoisobutyryl bromide and 1,1,1-tris(hydroxymethyl)propane were combined, and the resulting product (1) was crystallized to remove impurities. Then, in the presence of a CuBr/PMDETA catalyst system, compound 1 was employed to generate transient carbon-centered radicals, which quickly interacted with 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) to produce a tri-TEMPO functional alkoxyamine unimolecular initiator compound (2). Next, a tri-armed styrenic star copolymer with chloride side-functional units was synthesized using the initiator (2) in the NMP of St and VBC (P1). Among controlled live radical polymerization procedures, the NMP method stands out because it does not require metal catalysts, which are exceedingly difficult to remove. Additionally, it can tolerate a wide range of functional groups and makes it simple to purify the polymerization products [9]. Then, the reaction of chloride side groups of (P1) with sodium azide in DMF gave azide functional copolymer (P2). In the final stage, Cu(I)-catalyzed 1,3-dipolar cycloaddition (click) reaction was used to synthesize P3. Azide functional units of (P2) were reacted with alkyne unit of 1-ethynylpyrene, providing pyrene-functional styrenic star copolymer (P3).

Chemical structures of the synthesized small molecular compounds and star-shaped styrenic copolymers were determined by FTIR and ^{1}H-NMR spectroscopies. In the FTIR spectra of compound 1 and 2, aliphatic CH and C=O stretching signals were observed around 2850–3000 and 1730 cm^{-1}, respectively. Besides, NO stretching frequencies of compound 2 were overlapped by those of germinal CH_3 deformations of TEMPO unit. ^{1}H-NMR spectra show the methylene ($CH_2OC=O$) and terminal CH_3 protons of 1 resonated at 4.35 and 1.96 ppm, respectively. On the other hand, the reaction of compound 1 with TEMPO, the signals of these protons shifted to higher magnetic field and were observed at 4.24 and 1.47 ppm, respectively, indicating completeness of the reaction. Also, some new peaks which were attributed to methyl and methylene protons of TEMPO unit were observed at 1.00–1.14 and 1.40–1.70 ppm.

Figure 1 shows the FTIR spectra of styrenic star copolymers. The star copolymers' aromatic and aliphatic CH stretching signals were visible in these spectra at around 3025–3080 cm^{-1} and 2850–2920 cm^{-1}, respectively. The signals at 1600, 1490, and 1450 cm^{-1} are assigned the aromatic C=C peaks. On the other hand, a new signal at 2100 cm^{-1} in Figure 1 was seen following the reaction of the chloride functional (P1) with sodium azide, indicating the existence of azide functional units in the chemical structure of the resulting polymer (P2). Then, after the "click" reaction between P2 and 1-ethynylpyrene, the complete disappearance of this peak demonstrated that azide-functional units were converted to pyrene groups, yielding P3.

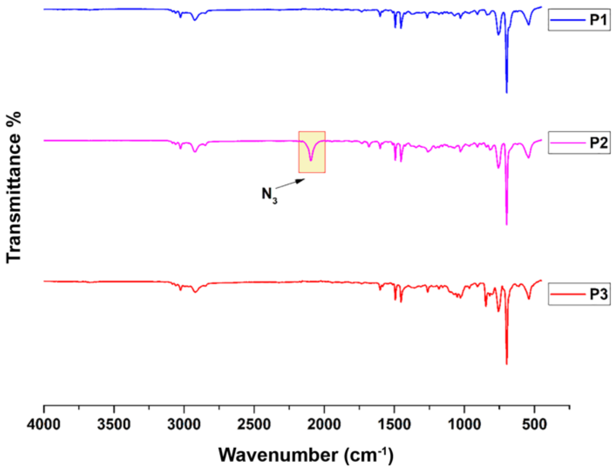


Figure 1. FTIR spectra of P1, P2 and P3.

^{1}H-NMR spectra of the styrenic star copolymers were given in Figure 2. In these spectra, the polymer main chain protons gave signals between 1.40 and 2.00 ppm, while aromatic CH protons (Ha,b) in St and vinylbenzyl repeating units produced resonances between 6.47 and 7.11 ppm. In the ^{1}H-NMR spectrum of P1 [Figure 2(a)] the signal of the methylene protons (Hc) adjacent to the benzene ring was observed at 4.50 ppm, and shifted to higher magnetic field (4.20 ppm) as a result of azidification [Figure 2(b)]. After "click" reaction between P2 with 1-ethynylpyrene, these signals moved to downfield and resonated at 5.47 ppm [Figure2(c)]. The pure shift of Hc protons on azidification and click reactions further proves the success of the reactions. Further, some new proton resonance signals which attributed to triazole methine protons (Hd, 8.70 ppm) and aromatic CH protons of pendant pyrene units (He, 7.68–8.68 ppm) were observed in the ^{1}H-NMR spectrum of P3 [Figure 2(c)].

Table 1. Thermal properties of star polymers with different functional groups

Polymers	T_g (°C) [a]	T_{onset} °C) [b]	T_{max} (°C) [c]	Char Yield (%) [d]
P1	104	280	405	6
P2	96	205	390	8
P3	125	335	415	20

[a] T_g is the glass transition temperature (The midpoint of the heat capacity change was taken) of the polymers in DSC experiments.
[b] T_{onset} is the onset decomposition temperature (3% mass fraction loss) of the polymers in TGA experiments.
[c] T_{max} is the temperature corresponding to the maximum rate of weight loss in TGA experiments.
[d] The percent of the mass remained at 700 °C in TGA experiments.

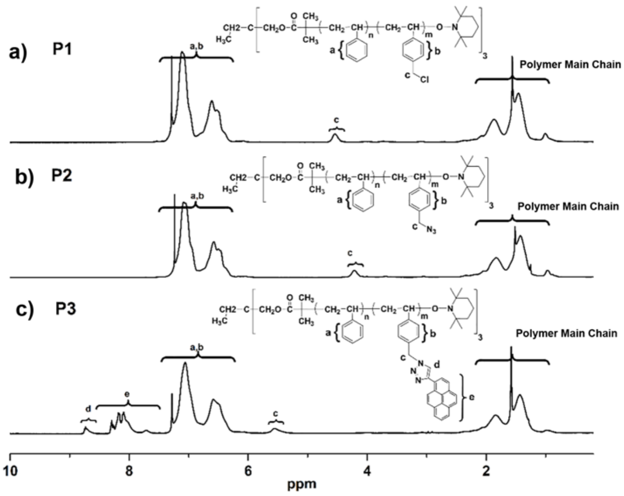


Figure 2. ^{1}H-NMR spectra of P1, P2 and P3.

3.2. Thermal Behavior of Polymers

Through TGA studies conducted in a N_2 atmosphere and heating the synthesized star polymers at a rate of 10 °C/min from ambient temperature to 700 oC, thermal stabilities of the materials were determined. Figure 3 displays the polymer percent weight loss against temperature curves. Additionally, summary of the TGA data is shown in Table 1. In these thermograms, the azide-functional star polymer displayed two-step breakdown profiles due to the evolution of nitrogen gas above 200 °C, while the chloride-functional star polymer degraded in a single step beginning at roughly 280 °C. Additionally, when the copolymer's azide content rose, a more striking quantity of disintegration in the first stage and a decline in the T_{onset} values of the star copolymers were observed. On the other hand, the addition of pyrene-functional units significantly increased the star copolymers' T_{onset} values as well as their char yields. The pyrene-functional star copolymer's (P3) relatively stiff structure with pyrene rings and the π-stacking interaction between these aromatic parts were linked to their very high onset decomposition temperatures and char yields.

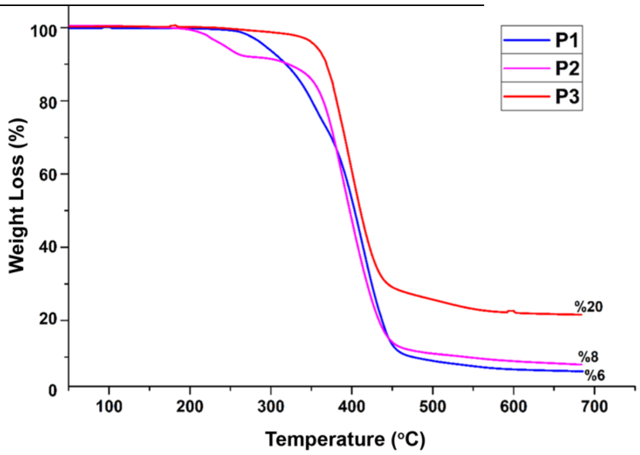


Figure 3. TGA thermograms of P1, P2 and P3.

DSC experiments were studied to determine thermal phase transitions of the synthesized polymers. The midpoint of the heat capacity change was taken as glass transition temperature (T_g) and the relevant data were presented in Table 1. DSC thermograms of polymers were demonstrated in Figure 4. In these thermograms, T_g of chloride functional polymer (P1) is found around 104 °C. Upon incorporation of azide functional units, only minor difference was seen in the T_g value of the polymer. Otherwise, binding of pyrene raised significantly the T_g of P3 by 21 °C and increase in T_g value of the pyrene-functional polymer. These results were attributed to the increase in the rigidity of the pyrene pendant star polymer due to π-stacking interaction between pyrene groups.

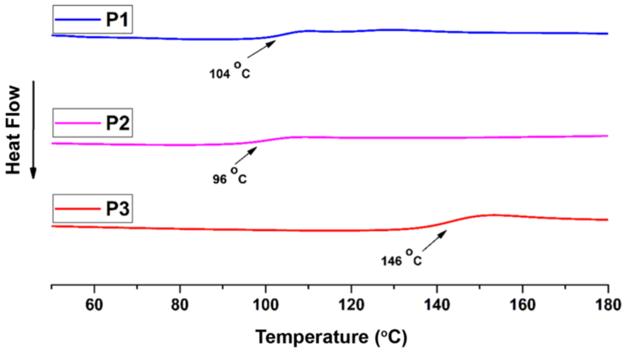


Figure 4. DSC thermograms of P1, P2 and P3.

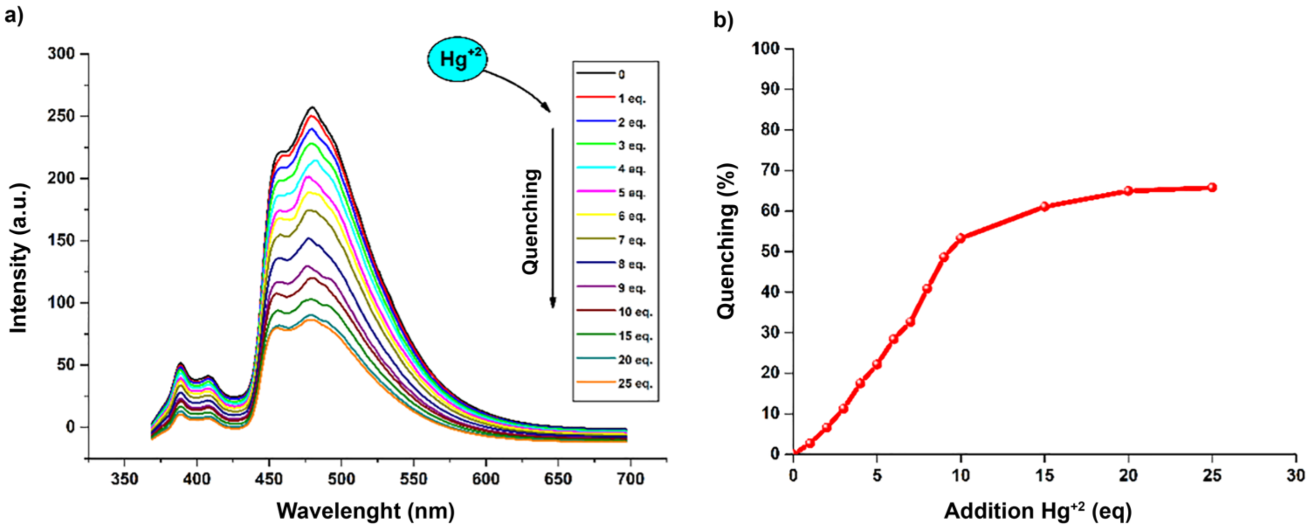


Figure 5. a) Fluorescence emission spectra and b) quenching ratios of P3 in the presence of increasing concentrations of Hg^{+2}.

3.3. Hg^{+2} Sensing Applications of Pyrene-Functional Styrenic Star Polymer (P3)

Pyrene itself emits monomer emissions (about 400 nm) in a variety of solvents at low concentrations, but excimer emissions require millimolar or higher concentrations (around 490 nm). Since pyrene excimer emissions are susceptible to polarity-related effects and are frequently used in turn-off fluorescence detection of different quencher molecules, to detect traces of targeted molecules in analytical medium, on the other hand, very low amounts of a chemical probe that turns off must be used. In this situation, increasing the local number of pyrene parts on a star polymer seems to offer the best chance of obtaining excimer emissions at extremely low concentrations without sacrificing the creation of excimer emissions. As shown in the emission spectra in Figure 5 (a), pyrene functional star-shaped polymer (P3) emits both pyrene monomer emission (about 400 nm) and excimer emission (around 490 nm). The presence of strong excimer emission indicates the presence of strong intermolecular π-stacking interactions of pyrene units.

Fluorescence spectrophotometric studies utilizing cuvettes with a 1 cm path length were used to determine the binding and recognition characteristics of the P3 toward the Hg^{+2} metal ion. Figure 5(a) shows the fluorescence emission spectra of P3 before and after titration with various concentrations of Hg^{+2} (1 to 25 equivalents to the number of moles of pyrene units), which were used in the metal ion sensing experiments of P3 in DMF solvent at 350 nm excitation wavelength at ambient temperature. Upon the addition of increasing concentrations of Hg^{+2}, these spectra demonstrate moderate to considerable and progressive changes in the emission intensity of P3. I_0 and I are the emission intensities before and after the addition of Hg^{+2}, respectively. QE= $[(I_0 / I)/I_0] \times 100$ was used to compute the quenching efficiency (QE) of Hg^{+2}. Figure 5(b) shows that following the addition of 10 equivalents of Hg^{+2}, P3 lost 50% of its initial fluorescence emission strength. When the Hg^{+2} concentration reached 25 equivalents ratio, quenching was seen to be approximately 65%. Hg^{+2} quenching appears to be slightly less in this sensitivity result when compared to prior investigations using pyrene-containing polymers [10, 11]. The measurement of selective photoluminescence with other metal ions was not examined in this study; subsequent research will look into the applicability of sensing to other ions.

4. Conclusion

Tri-armed styrenic star polymer having fluorescent-active pyrene side groups (P3) was prepared via atom transfer radical addition, click reaction and afterwards nitroxy mediated polymerization reactions. Data obtained from FTIR, and ^{1}H-NMR spectral analysis confirmed the successful synthesis of the polymers. P3 gave characteristic fluorescence emission bands for the pyrene fluorophore, indicating the presence of pyrene groups. Upon the addition of Hg^{+2} cation, fluorescence emission of P3 was mild to severe quenched. The highest quenching efficiency (around 65%) was observed at 25 eq. addition of Hg^{+2}. The potential of P3 to be used in the selective fluorogenic determination of other ions will be investigate further studies.

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