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Terpyridyl appended poly(metaphenylene-*alt*-fluorene) π-conjugated fluorescent polymers: Highly selective and sensitive *turn off* probes for the detection of Cu^{2+}

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Highly soluble and emissive poly(metaphenylene-*alt*-fluorene) π-conjugated alternative copolymers, with pendant terpyridyl binding sites conjugated to metaphenylene unit, have been synthesized through Pd(0)-catalyzed Suzuki coupling polymerization. For solubilizing in common organic solvents, octyl (**P1**) or 2-(2-(2-methoxyethoxy)ethoxy)ethyl (**P2**) side groups have been introduced at 9-position of fluorene. The well-defined copolymers have been characterized by multinuclear NMR and tetradetector GPC with excellent PDI values of 1.28 and 1.14 respectively. The blue emissive π-conjugated polymers show remarkable selectivity and sensitivity towards Cu^{2+} as *turn off* fluorescent probes without interference by other competing metal ions, as a result of molecular wire effect. The detailed photophysical and sensing studies have been demonstrated to understand the insight of polymer-metal ion interaction which is responsible for selective fluorescence quenching. Moreover, the polymeric sensors (**P1** and **P2**) are reversible, and can also act as secondary sensors for S^{2-}. The limit of detection (LOD) towards Cu^{2+} in ppb level with relatively high association constants of $4.1–3.2 \times 10^5\ M^{-1}$ has prompted us to explore as thin-film polymeric sensor for Cu^{2+} to develop solid state test kits for practical utility.

1. Introduction

Copper is the third most abundant transition metal found in all living organisms and is an essential trace element in redox chemistry playing a crucial role in biological, environmental and chemical systems. The copper cycle involves several enzymes and proteins in metabolism, respiration, and DNA synthesis, such as cytochrome oxidase, superoxide dismutase, ascorbate oxidase, and tyrosinase [1]. However, the imbalance of copper, both excess and deficiency, in living body consequences various diseases such as Wilson disease (WD), Alzheimer's disease, Haematological manifestation and Menkes disease (MD) [2]. WHO recommends the permissible level of 2 ppm for Cu in drinking water to avoid any harmful health effects [3]. Hence, it is highly important to develop an efficient and easy method for detecting and monitoring trace amount of copper (below ppm level) ion in biological and environmental samples.

Turn off or *turn on* fluorescent chemosensing [4] has become the mostly used and rapidly expanding method in the field of the chemosensing to afford instantaneous response owing to its high selectivity, sensitivity, operational simplicity, and cost-effectiveness over the other analytical methods such as atomic absorption spectra [5], cathodic stripping voltammetry [6], energy dispersive X-ray diffraction [7], inductive coupled plasma-mass microprobe [8], nuclear quadrupole resonance [9], liquid chromatographic mass spectrometry [10] and surface enhanced Raman spectroscopy [11]. Thereby, development of highly selective and sensitive chemosensory materials for the detection of toxic metal ions has attracted extensive scientific interest. Many small molecule fluorescence sensors based on coumarin, rhodamine, boron dipyrromethene (BODIPY), isoquinoline, 1,8-naphthalimide, quinolene, cyclen, helical imide, dansyl, imidazopyrene, imidazophenazine, imidazoquinoxalines and benzoxazole have been successfully designed and developed for the detection of heavy toxic metal ions (Zn^{2+}, Ni^{2+}, Pb^{2+}, Hg^{2+}, Cd^{2+} etc.) [12] including Cu^{2+} [13] by various groups.

In the recent years, π-conjugated polymer based chemosensory materials have received remarkable attention for the detection of metal ions in environmental and biological systems because of their high selectivity, sensitivity and ease of measurement

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[14]. The π-conjugated polymer is a prominent candidate for the improvement in fluorescence quenching processes than the small molecule based chemosensor due to the fast and facile exciton migration along the π-conjugated polymer chain, coined as *amplifying* effect resulting in optical signals for transduction of chemical or biological events [14a,n–o,15]. Functionalization of emissive π-conjugated polymers with pendant coordinating sites is an elegant strategy to design polymer probes for metal ions detection. The sensing efficiency of the π-conjugated fluorescent polymer is dependent on interaction between the receptor sites of the polymer and the analytes. Fluorescent polymeric sensors based on N-heterocycle ligand moieties such as 2,2′-bipyridine (bpy) [14d,p,16], and 2,2′:6′,2′′-terpyridine (*tpy*) [14i&j,17], dipyrido[3,2-a:2′,3′-c]phenazine (dppz) [18], 2,2′-biimidazole [14k], benzimidazolylpyridine (bip) [14l], benzodithieno-imidazole [14e,19], have been received considerable interest due to their high binding affinities towards many transition metal ions. In particular, π-conjugated polymer based chemosensory probes anchored with pendant conjugated terpyridyl units as recognition sites have received considerable attention due to its ease of synthesis and functionalization to take the advantage of tuning *molecular wire* effect.

Herein, we report the synthesis and characterization of π-conjugated poly(metaphenylene-*alt*-fluorene) copolymers having pendant terpyridyl groups aiming for developing an efficient and highly selective chemosensor for the detection of Cu^{2+}.

2. Experimental section

2.1. Materials and instruments

All the air and moisture sensitive reactions and manipulations were carried out under an atmosphere of pre-purified Ar or N_2 by using standard Schlenk techniques. The glasswares were oven-dried (at 180 °C) and cooled under vacuum. Tetrahydrofuran and diethylether were dried over Na/benzophenone. All chemicals were purchased from Sigma-Aldrich unless otherwise noted. All the analytical grade metal salts and Silica gel (60–120 mesh used for column chromatography) were purchased from Merck. TLC plates were visualized under UV light (254 nm) or in iodine chamber. 2,7-Dibromfluorene (**3**), 2,7-dibromo-9,9-dioctylfluorene (**4**), 2,7-dibromo-9,9-bis(2-(2-(2-methoxyethoxy)ethoxy)ethyl)fluorene (**5**), 2,7-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9,9-dioctyl fluorene (**6**) [20], 2,7-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9,9-bis(2-(2-(2-methoxyethoxy)ethoxy)ethyl)fluorene (**7**), [21] $Pd(PPh_3)_4$ [22] and $Pd(PPh_3)_2Cl_2$ [23] were prepared as previously reported literature procedure (SI).

^1H (600 MHz, 400 MHz), $^{13}C\{^1H\}$ (150 MHz, 100 MHz) NMR spectra were obtained from Bruker Lambda spectrometer using $CDCl_3$ unless otherwise mentioned. Spectra were internally referenced to residual solvent peaks ($\delta = 7.26$ ppm for proton and $\delta = 77.23$ ppm for carbon (middle peak) in $CDCl_3$). All coupling constants (J) are given in Hz. The absorption and fluorescence spectra were collected using a Shimadzu (Model UV-2450) spectrophotometer and a Hitachi (Model F-7000) spectrofluorimeter, respectively. FTIR spectroscopy was recorded in Spectrum-BX (Perkin Elmer). Solid state PL spectra were recorded in Flurolog Horiba (Model FL-1016, Spectracq). Molecular weights and polydispersity indices ($PDI = M_w/M_n$) of the polymers were obtained by Tetradetector Gel Permeation Chromatography (GPC) using a Viscotek VE 2001 equipped with automatic sampler, pump, injector, inline degasser, column oven (30 °C), styrene/divinylbenzene columns with pore sizes of 500 Å and 100,000 Å, VE 3580 refractometer, four-capillary differential viscometer and 90° angle laser, low angle laser (7°) light scattering detector (VE 3210 & VE270) including UV–vis detector. HPLC grade THF was used as the chromatography eluent, at a flow rate of 1.0 mL/min. Samples were dissolved in the eluent (1 mg/mL) and filtered with a PTFE syringe filter (polytetrafluoroethylene membrane of 0.20 μm pore size) before analysis. Calibration of all four detectors (refractive index, laser light scattering, UV–vis and viscometry) was performed using polystyrene standards (Viscotek). This equipment allows the absolute measurement of homopolymer molecular weights and PDIs. FESEM and EDX data were recorded in Supra 40, Carl Zeiss microscope. Cyclic voltammetric studies were performed on a BASi Epsilon electrochemical workstation. CV of thin film polymer sample was measured by drop casting of the polymer solution on an ITO coated glass plate (coating thickness: 1500 Å, resistivity: 15–20 Ω cm) which was used as a working electrode. The electrochemical study was conducted in CH_3CN with 0.1 M *tetra-n*-butylammonium hexafluorophosphate ($TBAPF_6$) as the supporting electrolyte. The solution state CV of the polymers was conducted in dichloromethane using Pt disc working electrode. The reference electrode was Ag/AgCl and the auxiliary electrode was a Pt wire. The ferrocene/ferrocenium couple occurs at $E_{1/2} = +0.51$ (70) V versus Ag/AgCl under the same experimental conditions. Thermo gravimetric analysis (TGA) was carried out using a Perkin Elmer Pyris Diamond TG/DTA instrument by measuring their weight losses while heating at a rate of 10 °C/min. Differential scanning calorimetry (DSC) was performed on a TA DSC Q20 unit operated at a heating and cooling rates of 10 °C/min. MALDI-TOF MS study was performed with DHB (2,5-dihydroxy benzoic acid) matrix by using Bruker UltrafleXtreme instrument. Electron paramagnetic resonance (EPR) spectra were recorded at 9.64 GHz with a Bruker ELEXSYS 580 X-band EPR spectrometer at room temperature (298 K). DLS data was recorded in Malvern Zetasizer Nano equipment.

2.2. Synthesis and characterization

2.2.1. Synthesis of polymers

Polymer P1: Monomer **2** (0.25 g, 0.53 mmol) and **6** (0.34 gm, 0.53 mmol) were dissolved in 7 mL of distilled THF in a 100 mL Schlenk flask under argon atmosphere. The reaction flask was degassed three times by *freeze-pump-thaw* technique. In an another 100 mL Schlenk flask, K_2CO_3 (0.74 g, 5.35 mmol) was dissolved in 4 mL of mili-Q water and degassed by purging Ar through a niddle for about 30 min. After that, the K_2CO_3 solution was transferred to the reaction mixture through a cannula. Next, $Pd(PPh_3)_4$ (0.035 gm, 0.016 mmol, 3 mol%) was added to it and the reaction mixture was heated to 65 °C for 48 h. The colour of the reaction turned to dark brown during the course of the reaction. After cooling to room temperature, the solvent was concentrated to minimum volume and polymer was precipitated to a stirring methanol solution. After the complete precipitation the methanol was removed and the brown colour polymer was washed another two times with methanol. Then it was dried under vacuum. Next the polymer was purified through Soxhlet extraction using distilled hexanes, methanol and at last collected in dry and distilled chloroform. The chloroform part was evaporated to get the orange-red polymer. The polymer was finally purified by fractional precipitation in cold distilled hexanes from a concentrated dichloromethane solution to achieve well-defined **P1**. Yield: 0.30 g (78%); ^1H NMR (600 MHz, $CDCl_3$) δ 8.94 (s, 2H, $H_{3',5'\text{-tpy}}$), 8.92–8.76 (m, 4H, $H_{6'6''\text{-}tpy}$ and $H_{3,3''\text{-}tpy}$), 8.18 (s, 2H, $H_{5,5''\text{-}tpy}$), 7.96–7.80 (m, 8H, H_{fluorene} and $H_{4,4''\text{-}tpy}$), 7.41–7.39 (m, 3H, H_{Ar}), 2.24–2.03 (m, 4H, octyl CH_2 attached to fluorene), 1.29–1.07 (m, 20H, H_{octyl}), 0.92–0.71 (m, 10H, H_{octyl}); $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$) δ 156.5, 156.3, 152.2 (*tpy*), 151.0, 149.4, 143.4, 140.7, 140.2, 137.1, 127.3, 126.8, 125.5, 124.1, 122.1, 121.7, 120.5, 119.6 (*tpy* and fluorene), 55.8 (carbon