

2,4-Diamino-6-Hydroxypyrimidine Based Poly(azomethine-Urethane): Synthesis and Application as a Fluorescent Probe for Detection of Cu^{2+} in Aqueous Solution

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Abstract A novel poly(azomethine-urethane)-based 2,4-diamino-6-hydroxypyrimidine was synthesized with chemical reaction and it designed as fluorescence probe for determination of Cu^{2+} in aqueous solution. The photoluminescence (PL) characteristic of the prepared Schiff base (HPAMP) and its poly(azomethine-urethane) (P-HPAMP) derivative were investigated in different polarity solvents such as MeOH, THF and DMF. PL measurements showed that both HPAMP and P-HPAMP have higher emission intensity and Stoke's shift value ($\Delta\lambda_{\text{ST}}$) in THF than the other solvents. Also, the proposed probe exhibited a specific fluorescent on response to Cu^{2+} over the other tested transition metal ions in aqueous solution. The sensor gave highly selective and sensitive response against Cu^{2+} as increasing a new emission peak at 341 nm, and possible interference and quenching effect of the other tested transition metal ions were found too low. Detection limit of Cu^{2+} sensor was also calculated as $7.87 \times 10^{-6} \text{ mol L}^{-1}$ in THF/deionized water (1:2, v:v).

Keywords Poly(azomethine-urethane) · Fluorescent sensor · Cu(II) sensor · Copper · Schiff base

Introduction

Over the last few decades, design and synthesis of selective and sensitive chemosensors for determination of transition metal ions such as Fe^{3+} , Cu^{2+} and Zn^{2+} etc. have gained enormous attention due to metabolic and biological importance of these transition metal ions [1–3]. Among the various kinds of chemical sensors, fluorescent sensors or probes for detection of metal ions have received increasing interest due to their high sensitivity, inherent simplicity, fast analysis with spatial resolution, and its sample/cell non-destructive nature [4–6].

Additionally, design of highly sensitive and selective fluorescent sensor or probe for detection of Cu^{2+} have attracted considerable attention due to this metal ions is one of the most important trace elements existing in animals, plants and human body [7]. As the third most abundant trace element in human body behind iron and zinc, copper plays an important role in many fundamental metabolic and physiological processes such as co-factor for electron transport, as a catalyst in some oxidation–reduction reactions, mitochondrial respiration, bone metabolism, wound healing, connective tissue formation, and the mobilization and uptake of iron [8–12]. On the other hand, high amount of this metal ion is harmful to biological systems and excess concentration of Cu^{2+} can cause serious neurodegenerative diseases such as Wilson's disease, Alzheimer's disease, prion-induced diseases, Wilson disease and Parkinson's disease [13–15]. Due to importance of Cu^{2+} , a method for selective, rapid and sensitive recognition of this transition metal ion in biological, water samples and aqueous solution is of great significance.

In this paper presents design and synthesis of a novel poly(azomethine-urethane) fluorescence probe for determination of Cu^{2+} in aqueous medium. As known, simply structured azomethine group ($-\text{N}=\text{CH}$) or Schiff base and their derivatives are potentially capable of forming stable complexes with

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metal ions due to nitrogen atom of azomethine ($-N=CH$) double bond in Schiff base structure [16, 17] and they have special coordination ability with transition metal ions [18]. Characterization of these prepared compounds were carried out by FT-IR, 1H , ^{13}C -NMR and SEC techniques. Photophysical behavior of Schiff base and its poly(azomethine-urethane) derivative was studied in different solvents such as DMF, MeOH or THF, and in presence of different metal ions focussing the attention on their emission properties using fluorescence spectroscopy. Until now, a lot of Schiff base [19] and polymer nanoparticle [20, 21] based fluorescence sensor for detection Cu^{2+} in aqueous media were developed and reported to literature. On the other hand, to the best of our knowledge, there are only three paper in literature about poly(azomethine-urethane)-based fluorescent sensor detection of Mn^{2+} [22], Cd^{2+} [23] and Zn^{2+} [24] in aqueous medium. Moreover, the proposed poly(azomethine-urethane)-based fluorescence sensor for detection of Cu^{2+} has

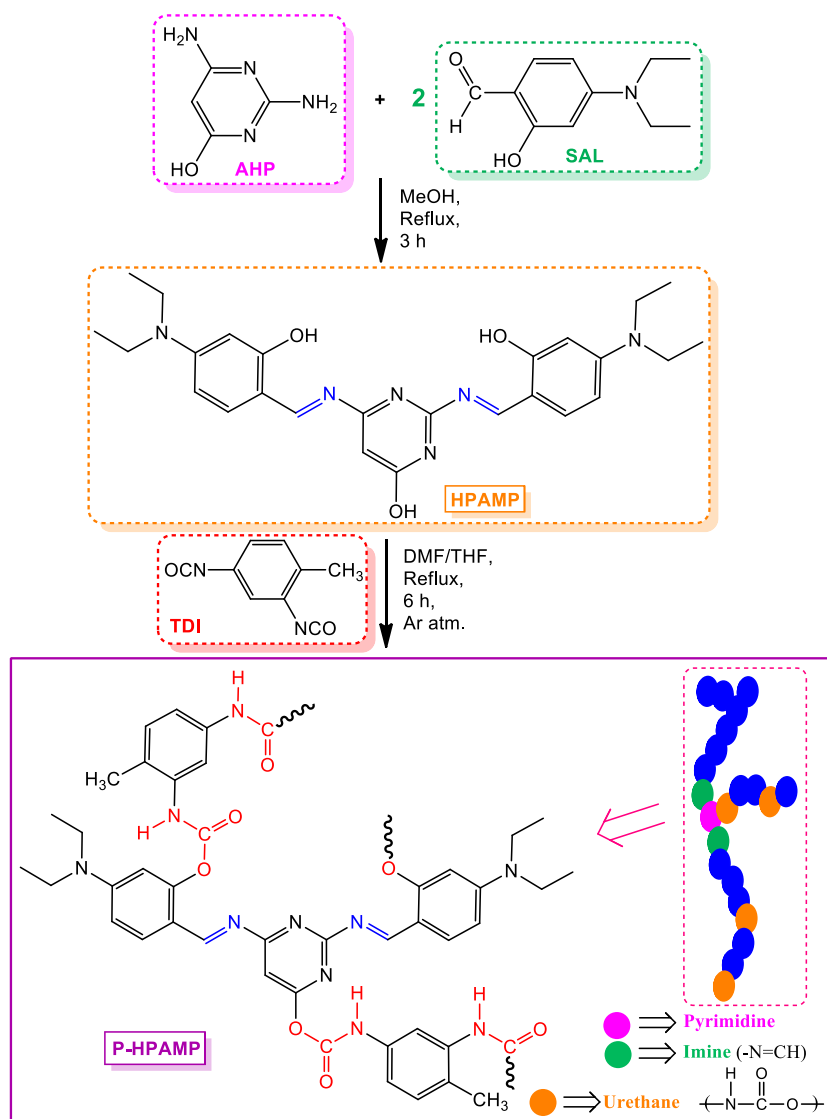
higher detection limit of than these poly(azomethine-urethane)-based sensor.

Experimental

Materials

All solvents and reagents were obtained commercially and used as received. 2,4-diamino-6-hydroxypyrimidine (AHP), 4-(diethylamino)salicylaldehyde (SAL), toluene diisocyanate (TDI), acetone, acetonitrile (MeCN), carbon tetrachloride (CCl_4), chloroform ($CHCl_3$), dimethylformamide (DMF), ethanol (EtOH), ethyl acetate, methanol (MeOH), n-hexane, tetrahydrofuran (THF), toluene, $Mn(CH_3COO)_2 \cdot H_2O$ and $Pb(CH_3COO)_2 \cdot 3H_2O$ were supplied from Merck Chemical Co. (Germany), $Cd(CH_3COO)_2 \cdot 2H_2O$, $Co(CH_3COO)_2 \cdot 4H_2O$, $Cu(CH_3COO)_2 \cdot H_2O$, $Ni(CH_3COO)_2 \cdot 4H_2O$ and

Scheme 1 Synthesis scheme of HPAMP and P-HPAMP



Zn(CH₃COO)₂·2H₂O were supplied from Fluka. CrCl₃ and ZrCl₄ were also supplied from Riedel Dehaen.

Instruments

The structure of the prepared compounds was characterized using infrared (FT-IR) and NMR spectra. FT-IR analysis were carried out by PerkinElmer FT-IR Spectrum one using the universal ATR sampling accessory (4000–550 cm⁻¹). ¹H and ¹³C-NMR spectra (Bruker AC FT-NMR spectrometer operating at 400 and 100.6 MHz, respectively) were recorded in deuterated DMSO-d₆ at 25 °C. Tetramethylsilane (TMS) was used as internal standard. The number average molecular weight (M_n), weight average molecular weight (M_w) and polydispersity index (PDI) were determined by Gel Permeation Chromatography-Light Scattering (GPC-LS) device of Malvern Viscotek GPC Dual 270 max. For GPC investigations a medium 300x8.00 mm Dual column. Addition, 1 g/L of lithium bromide in DMF (1 mL/min) was used as solvent. Light Scattering Detector (LS) and a refractive index detector (RID) were used to analyze the products at 55 °C.

Preparation of HPAMP

HPAMP (6,6'-((1E,1'E)-((6-hydroxy pyrimidine-2,4-diyl) bis(azanylylidene)) bis(methanylylidene)) bis(3-(pentan-3-yl)phenol)) was prepared by condensation reaction of AHP (1.380 g, 10 mmol) with SAL (3.865 g, 20 mmol) in MeOH (20 mL). The mixture was refluxed for 3 h at 70 °C (Scheme 1). The precipitated HPAMP was filtered, recrystallized from MeCN (2×50 mL) and dried in a vacuum oven at 60 °C for 24 h [25] (Yield: 84 %).

Preparation of P-HPAMP

P-HPAMP [Poly(6-((E)-(2-(((5-acetamido-2-methyl phenyl) carbamoyl) oxy)-4-(diethylamino) benzylidene) amino)-2-((E)-(4-(diethylamino)-2-hydroxybenzylidene) amino) pyrimidin-4-yl (4-acetamido-2-methylphenyl)carbamate)] was prepared by the step-polymerization reaction of HPAMP (0.949 g, 2 mmol) in 60 mL DMF/THF mixture (1/3) with TDI (3.865 g, 2 mmol) in 20 mL THF (Scheme 1). Reaction was maintained for 6 h under Argon atmosphere, cooled at the room temperature, and kept for 24 h. The obtained polyurethane was washed by MeOH (2×50 mL) and MeCN (2×50 mL) to remove the unreacted components. The product was dried in a vacuum oven at 75 °C for 24 h [26] (Yield: 78 %. SEC: Mn: 19900, Mw: 29500, PDI: 1.482).

Preparation of Stock Solutions

1.00×10⁻¹ M stock solution of metal ions including Cd²⁺, Co²⁺, Cr³⁺, Cu²⁺, Mn²⁺, Ni²⁺, Pb²⁺, Zn²⁺ or Zr⁴⁺ was

prepared in 20 mL deionized water. Stock solution of Cd²⁺, Co²⁺, Cu²⁺, Mn²⁺, Ni²⁺, Pb²⁺ or Zn²⁺ was prepared from their acetate salts while this solution of Cr³⁺ or Zr²⁺ was prepared from their chloride salts. Additionally, 1.00×10⁻³ M stock solution of P-HPAMP (4.13×10⁻² g, 5.00×10⁻⁵ moles) was prepared in different polarity solvents such as 50 mL DMF, MeOH or THF and these prepared stock solutions were also used in all measurements.

Fluorescence Measurements

To explore the photophysical properties, the fluorescence spectra of HPAMP (1×10⁻³ M) and P-HPAMP (1×10⁻³ M) in THF, MeOH and DMF were measured by Shimadzu RF-5301PC spectrofluorophotometer. Excitation and emission spectra of these compounds were obtained due to determine the optimal emission and excitation wavelengths in these used solvents. Also, transition metal ions effect on P-HPAMP was investigated by monitoring the fluorescence spectral behavior upon addition of several metal ions such as Cd²⁺, Co²⁺, Cr³⁺,

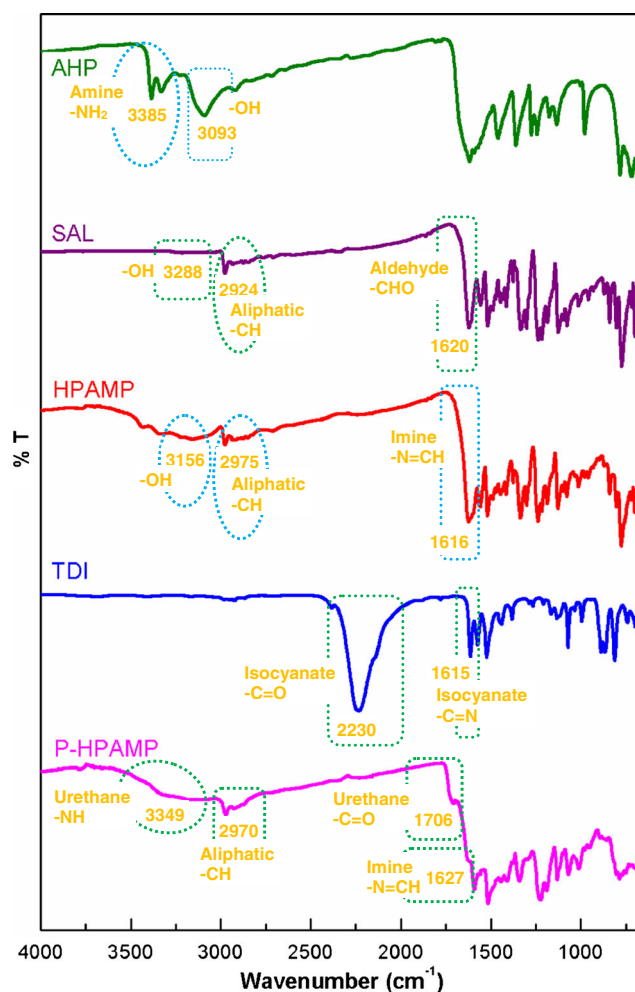


Fig. 1 FT-IR spectra of AHP, SAL, HPAMP, TDI and P-HPAMP

Cu^{2+} , Mn^{2+} , Ni^{2+} , Pb^{2+} , Zn^{2+} and Zr^{4+} in THF/deionized water solution (1:2, v:v). Polymer and transition metal ion concentrations were adjusted in these measurements as 1×10^{-3} M and 1×10^{-1} M, respectively. Concentration effect of Cu^{2+} ion on P-HPAMP was determined using a series of different concentrated metal solutions (in the range 1.20×10^{-7} to 1.00×10^{-3} M) in THF/deionized water solution (1:2, v:v). Slit width was adjusted as 5 nm in the mentioned experiments. This concentration range was also used in detection limit experiments. In these experiments excitation slit width was adjusted as 10 nm while the emission slit was kept as 5 nm due to the intensity differences more detectable due to the increasing separation of emission peaks from each other. Moreover, fluorescence quantum yield (Φ_F) was calculated by comparative methods using rhodamine 6G solution in ethanol as in the literature [27].

Morphological Properties

Atomic force microscopy (AFM) measurement of P-HPAMP was performed with WITec Alpha 300A AC mode (cantilever 42 N/m 285 kHz). The measurement was carried out at room temperature. The system covered with an acoustic chamber to prevent electromagnetic impacts, which may disturb the measurement.

Results and Discussion

Solubility and Characterization

The prepared HPAMP and P-HPAMP have red and black colored-powder compounds, respectively. P-HPAMP is completely soluble in THF and DMF, partly soluble in MeOH

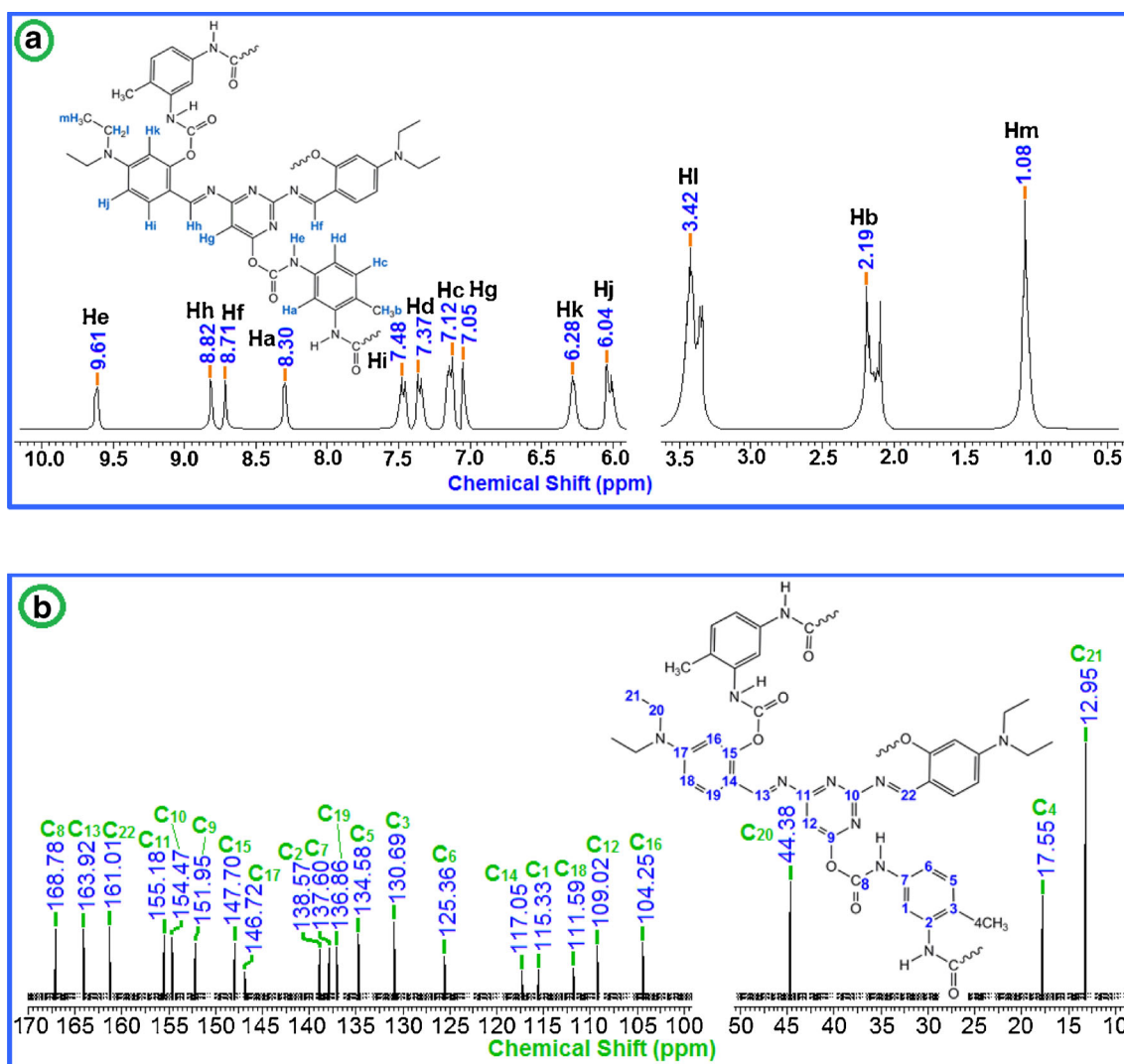
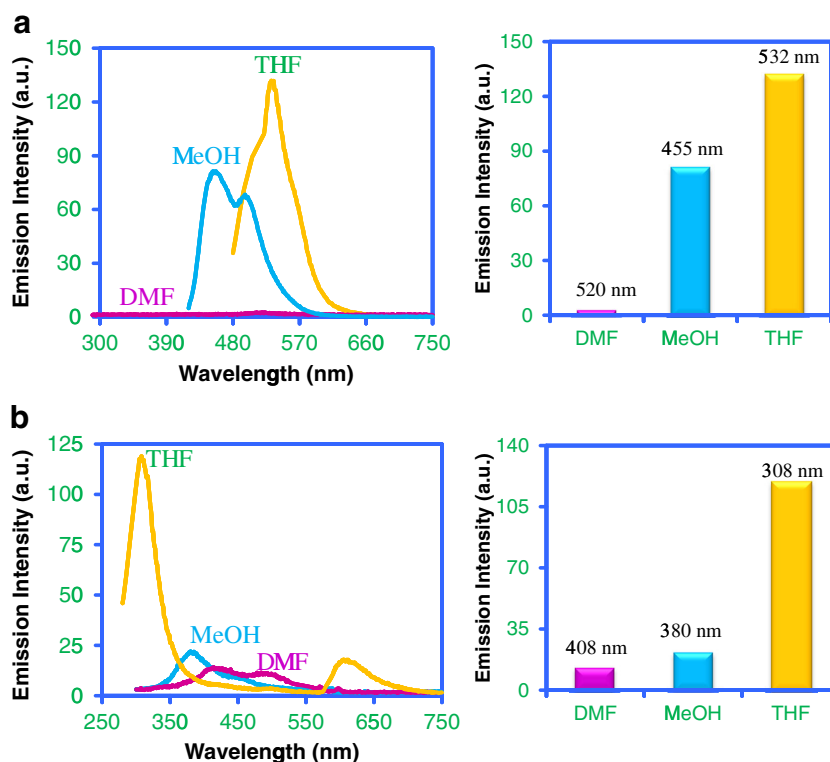


Fig. 2 ^1H (a) and ^{13}C -NMR (b) spectra of P-HPAMP

Fig. 3 Solvent effect on HPAMP (a) and P-HPAMP (b)



and EtOH while insoluble in MeCN, acetone, toluene, n-hexane, ethyl acetate, CHCl₃ and CCl₄.

Figure 1 shows FT-IR spectra of AHP, SAL, HPAMP, TDI and P-HPAMP. As can be seen in Fig. 1, characteristic amine (–NH₂) and hydroxyl (–OH) stretch vibrations of AHP are observed at 3385 and 3093 cm^{–1}, respectively. According to Fig. 1, characteristic hydroxyl (–OH), aldehyde (–CHO) and aliphatic –CH stretch vibrations of SAL are observed at 3288,

1620 and 2924 cm^{–1}, respectively. In the FT-IR spectrum of HPAMP, aldehyde (–CHO) stretch vibration of SAL and amine (–NH₂) stretch vibration of AHP disappear and imine (–N=CH) stretch vibration (at 1616 cm^{–1}) appears instead of these stretch vibrations. Hydroxyl (–OH) and aliphatic –CH stretch vibrations of HPAMP are also observed at 3156 and 2975 cm^{–1}, respectively. According to FT-IR spectrum of TDI, characteristic isocyanate –C=O and –C=N stretch vibrations of this compounds are observed at 2230 and 1615 cm^{–1}, respectively [28]. As can be seen in FT-IR spectrum of P-HPAMP, these characteristic isocyanate (–C=O and –C=N) stretch vibrations of TDI and hydroxyl (–OH) stretch

Table 1 Fluorescence measurements data of HPAMP and P-HPAMP

Solvent	^a λ _{Ex}	^b λ _{Em}	^c λ _{max(Ex.)}	^d λ _{max(Em.)}	^e I _{Ex.}	^f I _{Em.}	^g Δλ _{ST}
MeOH ^h	394	438	409	457	72	81	48
THF ^h	451	516	464	532	118	132	68
DMF ^h	470	504	486	523	2	3	37
MeOH ⁱ	321	367	334	385	18	22	51
THF ⁱ	229	295	235	308	106	119	73
DMF ⁱ	358	394	386	428	11	13	42

^a Excitation wavelength for emission

^b Emission wavelength for excitation

^c Maximum excitation wavelength

^d Maximum emission wavelength

^e Maximum excitation intensity

^f Maximum emission intensity

^g Stokes Shift value : [λ_{max (Em.)}– λ_{max (Ex.)}]

^h Compound: HPAMP

ⁱ Compound: P-HPAMP

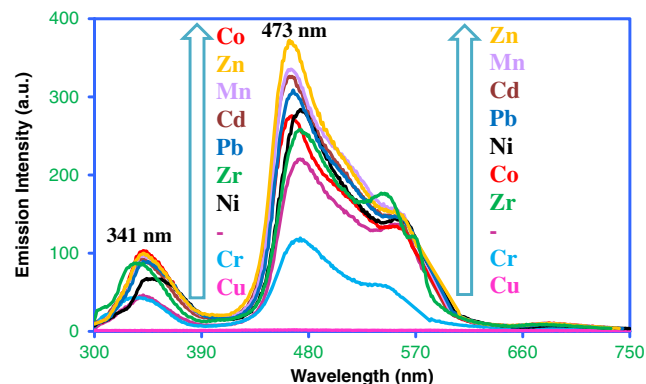


Fig. 4 Fluorescence spectra of P-HPAMP (1×10^{–3} M) in presence of 1×10^{–1} M concentration of different transition metal ions at room temperature in THF/deionized water (1:2, v:v) (λ_{Ex.}=350 nm, Slit: Ex: 5 nm, Em: 5 nm)

Table 2 Emission spectral data of P-HPAMP in presence of different transition metal ions

Metal Ions	Co ²⁺	Zn ²⁺	Mn ²⁺	Cd ²⁺	Pb ²⁺	Zr ⁴⁺	Ni ²⁺	Cr ³⁺	Cu ²⁺
^a I _{em} (341 nm)	103	100	98	93	89	87	65	43	2
^b I _{em} (473 nm)	258	346	318	309	297	255	284	118	1

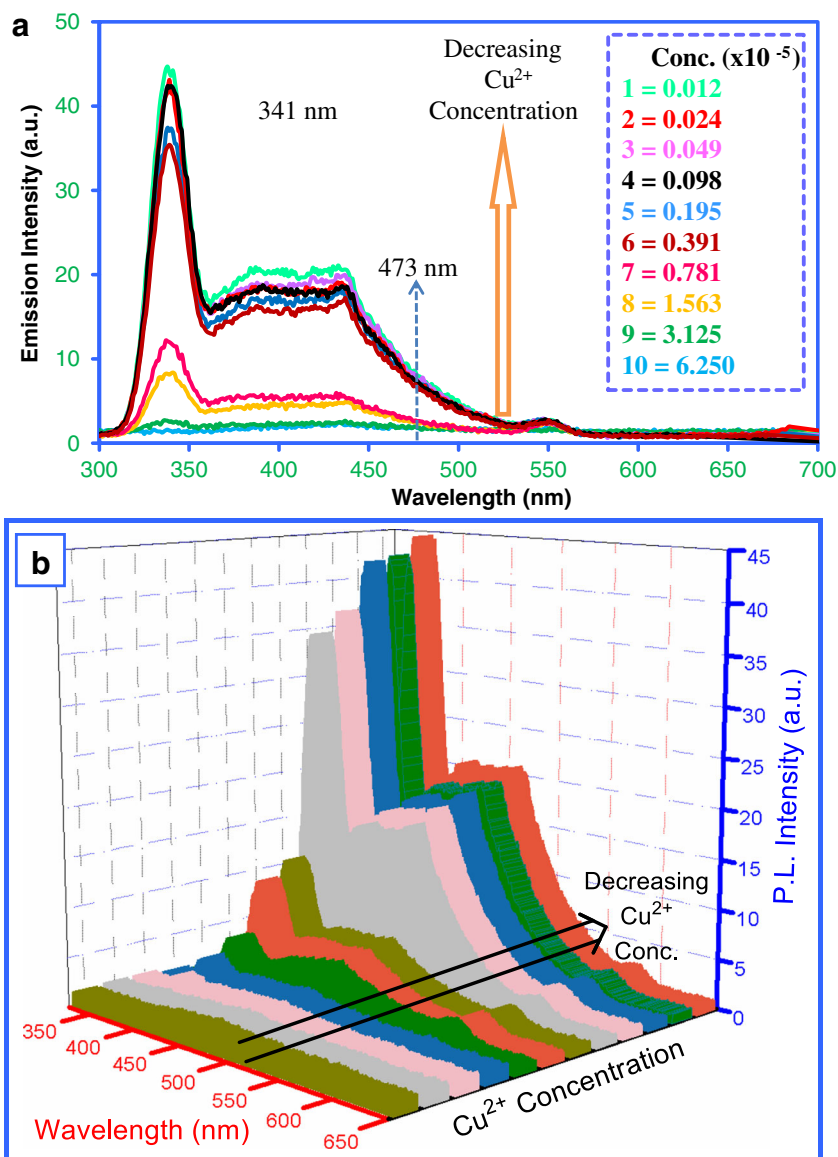
^a Emission intensity at 341 nm^b Emission intensity at 473 nm

vibration of HPAMP disappear and the new stretch vibrations such as urethane (–C=O and –NH) are observed instead of these stretch vibrations due to urethane formation. According to FT-IR spectrum of P-HPAMP, the urethane –NH and –C=O stretch vibrations are observed at 3349 and 1706 cm⁻¹, respectively. Imine (–N=CH) and aliphatic –CH stretch vibrations of P-HPAMP are also observed at 2970 and 1627 cm⁻¹,

respectively. These spectral data clearly confirm the formation of the proposed structure of HPAMP and P-HPAMP in Scheme 1.

Figure 2a and b show ¹H and ¹³C-NMR spectra of P-HPAMP. The structures of HPAMP and P-HPAMP are also confirm by these spectra and spectral data given below. NMR spectral data are summarized as follows:

Fig. 5 Fluorescence emission spectra of P-HPAMP in the presence Cu²⁺ ion with different concentration. (Conditions: Slit: λ_{EX}=5 nm, λ_{EM}=5 nm, λ_{EX}=229 nm, and concentration of P-HPAMP=1 × 10⁻³ M) (a) and 3-D images of decreasing Cu²⁺ concentration (b)



$^1\text{H-NMR}$ (DMSO- d_6 , δ_{ppm}): 10.11 ($-\text{OH}$), 8.78 and 8.63 (imine ($-\text{N}=\text{CH}$)), 7.78, 7.32, 6.35 and 6.14 (aromatic protons), 3.40 ($-\text{CH}_2-\text{CH}_3$) and 1.12 ($-\text{CH}_2-\text{CH}_3$) for HPAMP, 9.61 (s, Urethane $-\text{NH}$, He), 8.82 (s, Imine $-\text{N}=\text{CH}$, Hh), 8.71 (s, Imine $-\text{N}=\text{CH}$, Hf), 8.30 (s, Ha), 7.48 (d, Hi), 7.37 (d, Hd), 7.12 (d, Hc), 7.05 (s, Hg), 6.28 (s, Hk), 6.04 (d, Hj), 3.42 (d, $-\text{CH}_2-\text{CH}_3$, Hl), 2.19 (d, $-\text{CH}_2-\text{CH}_3$, Hb) and 1.08 (s, $-\text{CH}_2-\text{CH}_3$, Hm) for P-HPAMP.

$^{13}\text{C-NMR}$ (DMSO- d_6 , δ_{ppm}): 159.64 ($-\text{C}-\text{OH}$), 156.74 and 154.83 (Imine ($-\text{N}=\text{CH}$)), 149.65, 145.08, 135.94, 116.54, 111.32, 108.85 and 103.98 (aromatic carbons), 44.35 ($-\text{CH}_2-\text{CH}_3$) and 12.92 ($-\text{CH}_2-\text{CH}_3$) for HPAMP 168.78 (Urethane $-\text{C}=\text{O}$, C₈), 163.92 (Imine ($-\text{N}=\text{CH}$), C₁₃), 161.01 (Imine ($-\text{N}=\text{CH}$), C₂₂), 155.18 (ipso-C₁₁), 154.47 (ipso-C₁₀), 151.95 (ipso-C₉), 147.70 (ipso-C₁₅), 146.72 (ipso-C₁₇), 138.57 (ipso-C₂), 137.60 (ipso-C₇), 136.86 (C₁₉), 134.58 (C₅), 130.69 (ipso-C₃), 125.36 (C₆), 117.05 (C₁₄), 115.23 (C₁), 111.59 (C₁₈), 109.02 (C₁₂), 104.25 (C₁₆), 44.38 ($-\text{CH}_2-\text{CH}_3$, C₂₀), 17.55 ($-\text{CH}_3$, C₄) and 12.95 ($-\text{CH}_2-\text{CH}_3$, C₂₁) for P-HPAMP.

Fluorescence Properties of HPAMP and P-HPAMP

To investigate of the solvent effect on HPAMP and P-HPAMP, the emission and excitation spectra of these compounds were recorded in different polarity solvents. For this reason, these spectra of Schiff base and its poly(azomethine-urethane) derivative are recorded in MeOH, THF, and DMF (polarity order: DMF > MeOH > THF) [29, 30]. In these measurements both Schiff base and polymer concentration were adjusted as 1×10^{-3} M. The obtained emission spectrum of HPAMP and P-HPAMP is given in Fig. 3 and spectral data are also summarized in Table 1. Emission spectrum of these compounds showed that both HPAMP and P-HPAMP have higher emission intensity in THF than DMF and MeOH. This could be probably polarities of solvents. Similar tendency shown at Stokes shift ($\Delta\lambda_{\text{ST}}$) value of solvents and Stokes shift value of HPAMP and P-HPAMP is determined as 48 and 51 nm in MeOH, 68 and 73 nm in THF and, 37 and 42 nm in DMF, respectively. These results showed that both HPAMP and P-HPAMP have higher $\Delta\lambda_{\text{ST}}$ in THF than the other solvents. As known, Stoke's shift value is important for a fluorescence sensor or probe due to it supplies very low background signals. Resultantly, allows the usage of the material in construction of a fluorescence sensor [22]. In addition, the quantum yield (Φ_{F}) of P-HPAMP- Cu^{2+} solution and in the absence of Cu^{2+} ions found to be 3.4 % and 0.03 %, respectively.

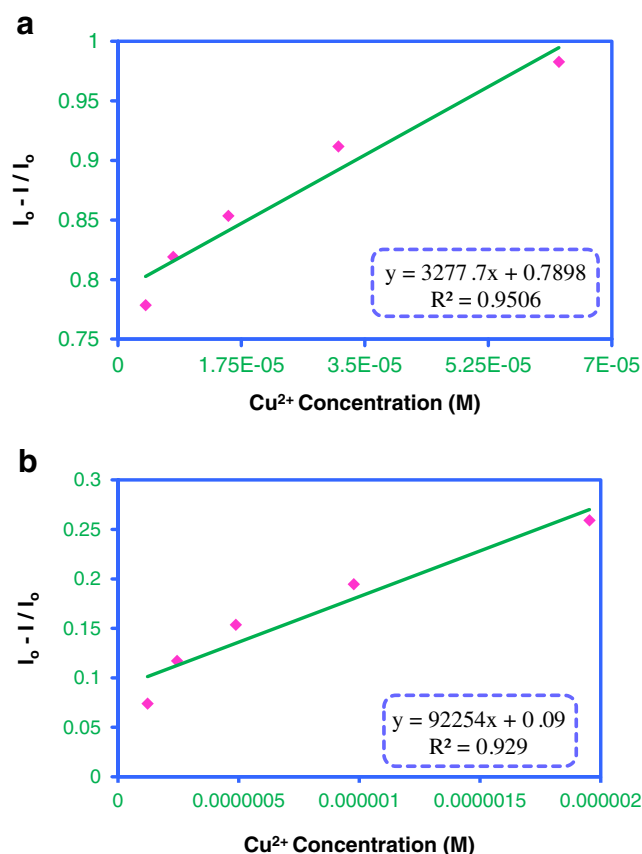


Fig. 6 A plot of the relationship between Zn^{2+} concentration and relative emission intensities

Selectivity of P-HPAMP

Selectivity of a fluorescence probe is very important parameter to evaluate the performance. The selectivity experiments of poly(azomethine-urethane) were carried out by fixing P-HPAMP with 1.00×10^{-1} M a number of transition metal ions such as Cd^{2+} , Co^{2+} , Cr^{3+} , Cu^{2+} , Mn^{2+} , Ni^{2+} , Pb^{2+} , Zn^{2+} and Zr^{4+} in THF/deionized water solution (1:2, v/v). In these selectivity experiments, poly(azomethine-urethane)

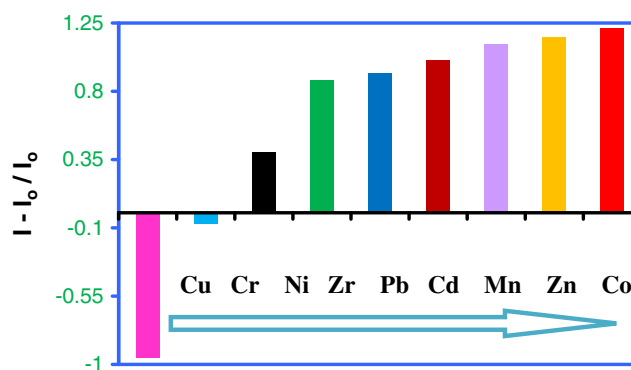


Fig. 7 Change in the relative intensity of P-HPAMP upon addition of various transition metal ions in THF/deionized water (1:2, v/v) at 341 nm

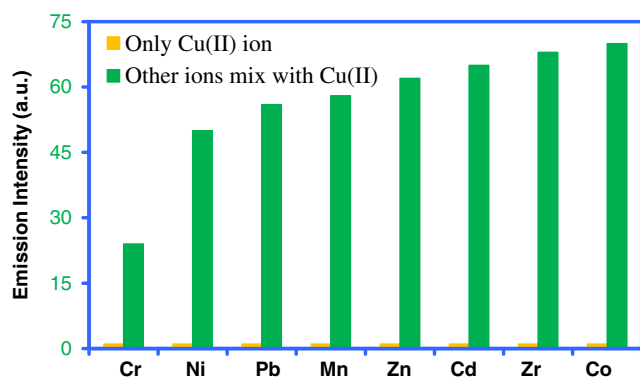


Fig. 8 Quenching ion effect on the proposed fluorescent sensor

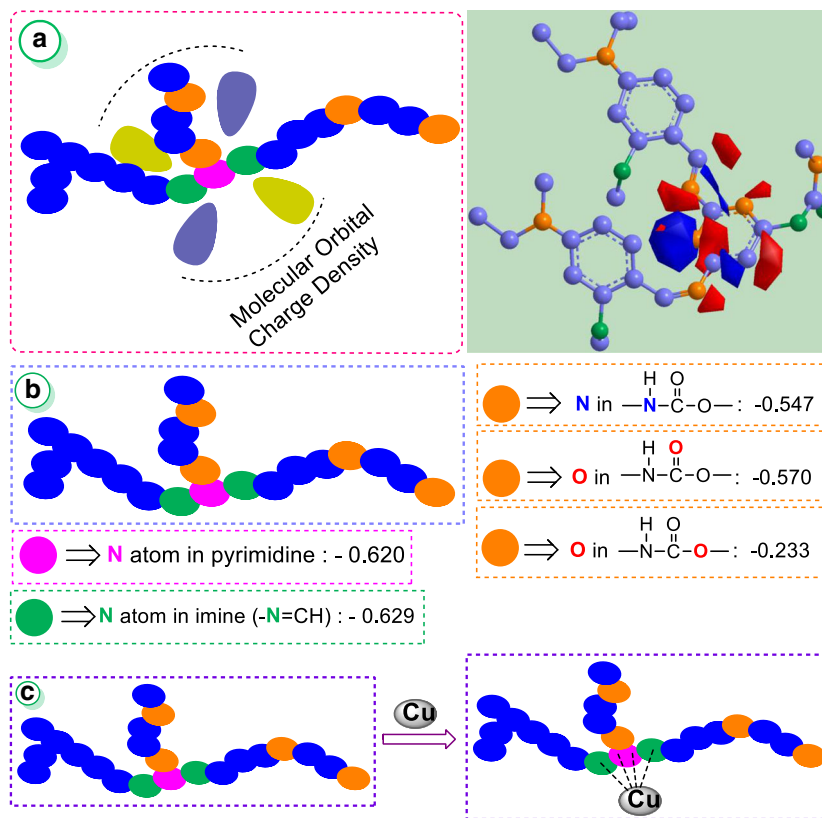
concentration was adjusted as 1.00×10^{-3} M due to minimize PL intensity of metal-free polymer solution in working range. Moreover, the change in fluorescence emission spectra of P-HPAMP in presence of different metal ions was given in Fig. 4 and the obtained results also summarized in Table 2. As can be seen in Table 2, emission intensity of metal-free polymer solution, Co^{2+} , Zn^{2+} , Mn^{2+} , Cd^{2+} , Pb^{2+} , Zr^{4+} , Ni^{2+} , Cr^{3+} and Cu^{2+} is found as 46–221, 103–258, 100–346, 98–318, 93–309, 89–297, 87–255, 65–284, 43–118 and 2–1 at 341 and 473 nm, respectively. These results clearly showed that the emission intensity of Co^{2+} , Mn^{2+} , Pb^{2+} , Cd^{2+} , Ni^{2+} , Zr^{4+} and Zn^{2+} is increased at 341 nm compared to the emission

intensity of metal-free polymer solution while emission intensity of Cu^{2+} and Cr^{3+} is decreased. Similar tendency in emission intensity of transition metal ions is observed at 473 nm. These results demonstrated that P-HPAMP has an excellent selectivity toward Cu^{2+} over the other transition metal ions and it could be used as Cu^{2+} sensor in aqueous solution due to only Cu^{2+} caused a significant fluorescence increasing.

Concentration Effect of Cu(II) on P-HPAMP

To evaluate the influence of Cu^{2+} concentration on the proposed probe, the fluorescence properties of P-HPAMP were studied in THF/deionized water solution (1:2, v/v) and the obtained spectra given in Fig. 5. Fluorescence spectrum and emission intensity of the proposed sensor are not changed when added Cu^{2+} ion between 1×10^{-3} and 1.25×10^{-4} M concentration range. As can be seen in Fig. 5, with the decrease of Cu^{2+} ion concentration the emission intensity continuously increased in the range 6.25×10^{-5} to 1.22×10^{-7} M Cu^{2+} ion concentration at 341 nm. On the other hand, a similar trend is not observed at 473 nm and the emission intensity of the proposed sensor is not significantly changed at 473 nm with the decreasing of Cu^{2+} ion concentration. Because of this property at 473 nm, the proposed sensor is not suitable

Fig. 9 Molecular orbital charge density of atoms in P-HPAMP (a) (purple : carbon atoms, orange : nitrogen atoms and green : oxygen atoms in right diagram, hydrogen atoms are not shown), the calculated charges of atoms in P-HPAMP (b) and proposed binding mode of P-HPAMP with Cu^{2+} (c)



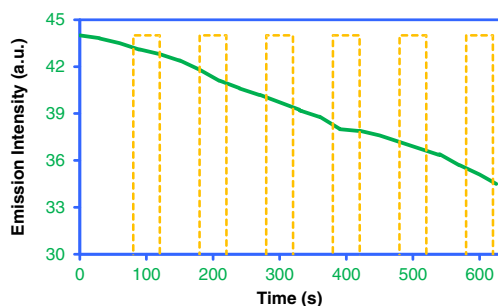


Fig. 10 The time-resolved photoluminescence spectrum of the proposed sensor presence of P-HPAMP (1×10^{-3} M) and Cu^{2+} (1×10^{-1} M) in THF/de-ionized water (1:2, v:v)

for determination of transition metal ions in aqueous solutions at this wavelength. Moreover, 3D image of decreasing Cu^{2+} concentration was shown in Fig. 5b.

To determine linearity relationship between Cu^{2+} ion concentration with relative emission intensity, regression equations and coefficients of the proposed Cu^{2+} sensor were calculated, as shown in Fig. 6. The calculation were performed using data the obtained from Fig. 5. Then, these intensities were plottes vs Cu^{2+} ion concentration in the range 6.25×10^{-5} to 1.22×10^{-7} M. Also, the obtained equations were given in Eqs. 1 and 2.

$$\frac{I_0 - I}{I_0} = 3277.7 [\text{Cu}^{2+}] + 0.7898 \quad (R_1 = 0.9506) \quad (1)$$

$$\frac{I_0 - I}{I_0} = 92254 [\text{Cu}^{2+}] + 0.09 \quad (R_2 = 0.929) \quad (2)$$

where I_0 is the emission intensity of metal-free polyurethane solution and I is the emission intensity of Cu^{2+} metal ion at 341 nm. According to these results, a good linearity relationship is obtained with regression coefficient $R_1=0.9506$ and $R_2=0.929$. According to the calculate regression equations and coefficients, it could be assumed that the novel fluorescent poly(azomethine-urethane) had potential prospects as a selective detector of Cu^{2+} ion in aqueous environment.

The detection limit of fluorescent sensor is another important parameter in sensor applications. For fluorescent probe P-HPAMP, a good linear relationship between the fluorescence intensity and the Cu^{2+} ion obtained in the 6.25×10^{-5} to 1.22×10^{-7} concentration range. The detection limit of the proposed sensor was calculated as in the literature [23] and it was determined as 7.87×10^{-6} mol L^{-1} .

Interference Study

Interference or anti-interference ability of the proposed Cu^{2+} sensor to the other transition metal ions such as Cd^{2+} , Co^{2+} , Cr^{3+} , Mn^{2+} , Ni^{2+} , Pb^{2+} , Zn^{2+} or Zr^{4+} is also important as well as the high selectivity of probe to the target metal ion. Thus, interference study of the proposed sensor was carried out using 1×10^{-1} M metal ions and 1×10^{-3} M stock polyurethane solution at 341 nm in THF/deionized water solution (1:1:2, v:v) (Fig. 7). As shown in Fig. 7, the tested transition metal ions were not induced significant fluorescence change except Cu^{2+} metal ion at 341 nm.

Quenching Ion Effect

Quenching ion effect on the proposed fluorescent sensor was investigated using 1×10^{-3} M P-HPAMP, 1×10^{-1} M Cu^{2+} and 1 mg $\text{Cd}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$, $\text{Co}(\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$, CrCl_3 , $\text{Mn}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$, $\text{Ni}(\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$, $\text{Pb}(\text{CH}_3\text{COO})_2 \cdot 3\text{H}_2\text{O}$, $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ or ZrCl_4 in THF/deionized water (1:2, v/v) (Fig. 8). As can be seen in Fig. 8, the presence of some transitional metal ions such as Cr^{3+} , Ni^{2+} , Pb^{2+} , Mn^{2+} , Zn^{2+} , Cd^{2+} , Zr^{4+} or Co^{2+} were not significantly quenched. They constituted only slight changes in the emission spectra of the proposed fluorescent probe.

Binding Model and Responsive Mechanism

To determine binding model between Cu^{2+} and P-HPAMP, charge density and molecular orbital charge density of atoms in the structure of the proposed probe were calculated using Huckel calculation method [23]. As can be seen in Scheme 1, nitrogen atoms in pyrimidine ring, imine ($-\text{N}=\text{CH}$) bonding and urethane ($-\text{NH}$, $-\text{C}=\text{O}$ and $-\text{O}$) groups composed a conjugated chromophore moiety in the proposed sensor.

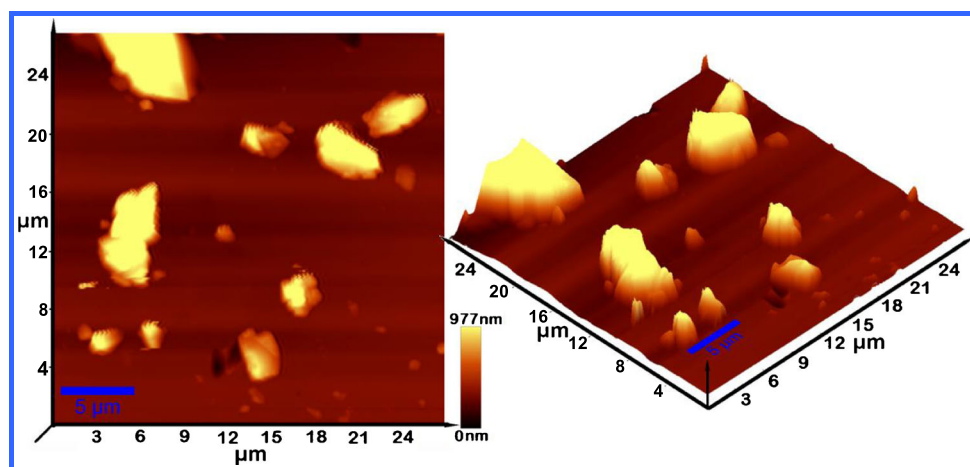
Figure 9a shows molecular orbital charge density of the proposed probe. As can be seen in Fig. 9a, pyrimidine ring and imine bonding have higher molecular orbital charge density than oxygen atom in carbonyl group, nitrogen and oxygen atoms in urethane group. This result indicated that the possible complexation between polymer and Cu^{2+} ion could be mainly carried out between these nitrogen atoms.

The calculated charge of atoms in the structure of P-HPAMP is shown in Fig. 9b. As can be seen in Fig. 9b, charge

Table 3 The time-resolved data of P-HPAMP

Time (s)	0	60	120	180	240	300	360	420	480	540	600
Wavelength (nm)	44.00	43.50	42.80	41.80	40.60	39.70	38.80	37.90	37.20	36.40	35.10
Degradation (%)	–	1.14	2.73	5.00	7.73	9.77	11.82	13.86	15.45	17.27	20.22

Fig. 11 AFM images of P-HPAMP



of nitrogen atoms in pyrimidine ring and imine bonding ($-\text{N}=\text{CH}$), oxygen atom in carbonyl group, nitrogen and oxygen atoms in urethane linkage is calculated as -0.620 , -0.629 , -0.547 , -0.570 and -0.233 , respectively. These calculated results showed that nitrogen atoms in pyrimidine ring and imine bonding have quite negative charge than the other heteroatoms in the urethane group.

The proposed binding mode P-HPAMP with Cu^{2+} ion is given in Fig. 9c. According to the charge density and molecular orbital charge density of atoms, nitrogen atom in pyrimidine ring and imine bonding have both higher molecular orbital charge density and quite negative charge than the other heteroatoms in urethane linkage. These results indicated that the binding mode P-HPAMP with Cu^{2+} ion could be carried out between nitrogen atoms in pyrimidine ring and imine bonding.

The Time-Resolved Curve

The time resolved PL spectrum of P-HPAMP (1×10^{-3} M) and Cu^{2+} ion (1×10^{-1} M) complex in THF/deionized water (1:2, v/v) is shown in Fig. 10 and results are also summarized in Table 3. As can be seen in Fig. 10 and Table 3, the fluorescence lifetime datas of the proposed sensor were found as 1.14, 2.73, 5.00, 7.73, 9.77, 11.82, 13.86, 15.45, 17.27 and 20.22 % for 60, 120, 180, 240, 300, 360, 420, 480, 540 and 600 s, respectively. These obtained results showed that P-HPAMP and Cu^{2+} complex is quite stable.

Morphological Property

Morphological property of poly(azomethine-urethane) was investigated by using AFM, as shown in Fig. 11. This technique was used in order to evaluate the evolution of topography and the phase roughness of P-HPAMP. According to AFM images of poly(azomethine-urethane), the surface of this compound seems to dense with uniform dispersion.

Conclusions

A new 2,4-diamino-6-hydroxypyrimidine based poly(azomethine-urethane) successfully synthesized and designed as fluorescent probe for determination of Cu^{2+} ions in aqueous solution. Photophysical behavior of Schiff base and its poly(azomethine-urethane) derivative was studied in different polarity solvents such as DMF, MeOH or THF. PL results showed that the prepared Schiff base and its polyurethane derivative have higher emission intensity and Stoke's shift value ($\Delta\lambda_{\text{ST}}$) in THF than the other solvents. The proposed probe has highly sensitive and selectivity towards Cu^{2+} compared with other transition metal ions. Detection limit of the proposed sensor found as 7.87×10^{-6} mol L^{-1} in THF/deionized water (1:2, v:v).

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