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ORIGINAL PAPER

Supramolecular Polymeric Chemosensor for Biomedical Applications: Design and Synthesis of a Luminescent Zinc Metallopolymer as a Chemosensor for Adenine Detection

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Abstract Adenine is an important bio-molecule that plays many crucial roles in food safety and biomedical diagnostics. Differentiating adenine from a mixture of adenosine and other nucleic bases (guanine, thymine, cytosine, and uracil) is particularly important for both biological and clinical applications. A neutral Zn^{II} metallosupramolecular polymer based on acyl hydrazone derived coordination centres (**P1**) were generated through self-assembly polymerization. It is a linear coordination polymer that behaves like self-standing film. The synthesis, ^{1}H-NMR characterization, and spectroscopic properties of this supramolecular material are reported. **P1** was found to be a chemosensor specific to adenine, with a luminescent enhancement. The binding properties of **P1** with common nucleic bases and nucleosides reveal that this supramolecular polymer is very selective to adenine molecules (~20 to 420 times more selectivity than other nucleic bases). The formation constant (K) of **P1** to adenine was found to be log K=4.10±0.02. This polymeric chemosensor produces a specific response to adenine down to 90 ppb. Spectrofluorimetric and ^{1}H-NMR titration studies showed that the **P1** polymer allows each Zn^{II} coordination centre to bind to two adenine molecules through hydrogen bonding with their imine and hydrazone protons.

Keywords Chemosensor · Supramolecular polymer · Nucleic bases detection · Acyl hydrazone

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Introduction

The development of new optical chemosensors for biologically important analytes and food ingredients is an emerging field [1–13]. Adenine, which is also known as vitamin B4, is a member of the B-complex family. Foods such as brewer's yeast, whole grains (breads and cereals), raw unadulterated honey, bee pollen, royal jelly, and propolis are high in vitamin B4. Vitamin B4 deficiency can cause blood and skin disorders, constipation, nausea, gastrointestinal disturbances, muscle weakness, hypoglycemia, physical and mental depression, and a weakened immune system response.

Adenine is a purine nucleobase found in the DNA molecular structure that takes part in many vital functions in the biological processes of the human body [14–17]. In biological systems, adenine controls blood pressure, helps prevent cardiac dysrhythmia, regulates inhibitory neurotransmitters, and modulates adenylate cyclase [18–20]. The level of adenine in plasma has been linked to various human disorders and diseases, such as cancer, AIDS, and poor myocardial cellular energy status [21–27]. Elevation of the urinary concentration of adenine is associated with the genetic disease adenine phosphoribosyltransferase deficiency, which can cause renal failure [28–31].

As adenine is a metabolite of adenosine, differentiating adenine from a mixture of adenosine and other nucleic bases (guanine, thymine, cytosine, and uracil) is particularly important in biological applications (Fig. 1). Chromatographic determination is the most widely adopted way of detecting adenine in biological and food samples [32–42]. Mass spectrometry, [32] high-performance liquid chromatography, [33–36] ion-pair liquid chromatography, [37–39] capillary electrophoresis, [40] and voltammetry [41, 42] have also been developed to determine adenine. However, the analytical

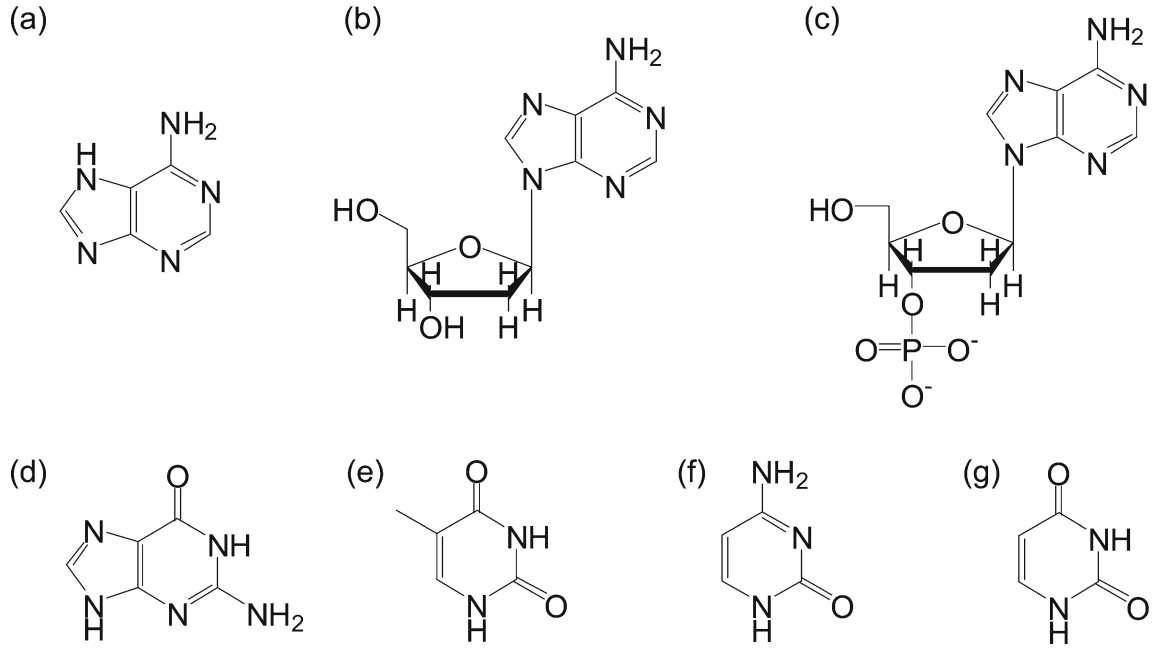


Fig. 1 Structures of **a** adenine, **b** adenosine, **c** adenosine-monophosphate, **d** guanine, **e** thymine, **f** cytosine, and **g** uracil

procedures involved in all of these processes are usually tedious, time-consuming, and ex-situ. Enzymatic biosensors, [43–45] molecularly imprinted polymers, [46] and nanoparticles [47, 48] have also been developed for adenine monitoring, but are restricted by their instability and intolerance to harsh environmental conditions. In this context, a small chemical "detector" that can selectively detect adenine to demonstrate the health status of patients in a visual way is highly desirable. However, there are few examples of such chemosensors for adenine.

Metallopolymeric chemosensing material that can selectively respond to biological analytes is an advanced field of research [49–55]. Metallopolymers are a special class of supramolecular polymer [49, 56–72] in which monomeric ligand components are linked via metal ion coordination. Metallopolymers have attracted much attention in the field of material science in recent years because of their properties as polymeric chemosensors, including charge, colour, luminescence, and macroscopic morphology, all of which can easily be tuned with the appropriate combination of ligands and metal ions [49–72]. Neutral charged metallopolymers [56, 57] are especially interesting, as they can be blended with conventional neutral polymers for the potential low-cost production of stable sensing elements. However, to the best of my knowledge, the use of metallopolymers for chemosensors is rare.

The Development of Acyl Hydrazone Based Metallopolymeric Chemosensors that are Capable of Recognizing Adenine and can Communicate the Recognition Event Through a Visual Signal is an Interesting Area In this work, the synthesis and characterization of a neutral metallopolymeric chemosensor based on the acyl hydrazone derived Zn^{II} coordination polymer (**P1**) is reported. This coordination polymer displays a particularly broad range of features: (1) a metal-ion coordination for luminescent properties; (2) an acidic acylhydrazone proton for host-guest interactions between adenine and the coordination centre; (3) a polydimethylsiloxane linkage for free-standing film formation qualities. The metallopolymeric chemosensor **P1** produces a luminescent response specifically to adenine in aqueous DMSO. The overall binding constant K between **P1** and adenine is $(1.25\pm0.15)\ x10^5\ M^{-1}$. A detection limit of 0.09 ppm of adenine in aqueous DMSO (pH 7.4) is achievable.

Experimental Section

Materials and General Procedures Hydrogen-terminated polydimethylsiloxane (MW_1 1,000–1,100 $gmol^{-1}$) and the Karstedt catalyst were obtained from ABCN. Bisacyl hydrazide polydimethylsiloxane (**BH1**) was prepared according to the method given in the literature [56, 57]. 8-Hydroxyquinoline-2-carboxyaldehyde, $Zn(acetate)\cdot 2H_2O$, ethyl-4-pentenoate, hydrazine monohydrate, and 4-hydroxymethylbenzoate were obtained from Aldrich. All of the solvents used were of analytical grade.

Physical Measurements and Instrumentation ^{1}H-NMR spectra were recorded using a Varian YH300 300 MHz NMR spectrometer. Electrospray mass spectra (ESI-MS)

were measured by a PE SCIEX API 365 LC/MS/MS system. Elementary analyses were performed on a Vario EL elementary analyzer. Thermal stability was measured on a TA Instruments thermogravimetry analyzer (TGA) Q50 from 30 to 1,000 °C at a heating rate of 10 °C/min under an N_2 atmosphere. UV–vis spectra were measured on a Hewlett-Packard 8452A ultraviolet visible diode array spectrophotometer. Emission spectra were recorded using a Horiba FluoroMax-3 spectrofluorometer with a 5 nm slit width and a 0.5 s integration time.

*Zn Metallopolymer Based on a Acyl Hydrazone Derived Ligand (***P1***)* A self-assembly reaction was performed by adding 8-hydroxyquinoline-2-carboxyaldehyde (0.3 mmol), bisacyl hydrazide polydimethylsiloxane (**BH1**; 0.15 mmol), Zn(acetate)·$2H_2O$ (0.15 mmol), and anhydrous Na_2SO_4 (3 mmol) to a mixture of MeOH and CH_2Cl_2 (1:1 v/v, 15.0 ml). The reaction mixture was stirred at room temperature under N_2 for 24 h. The solution was then evaporated to dryness, and CH_2Cl_2 was added to redissolve the mixture. Na_2SO_4 was filtered off. The polymer film was obtained by casting in a petri dish (2.5 cm in diameter), redissolving the polymer product in CH_2Cl_2, and then allowing the solution to slowly evaporate in an ambient atmosphere at 50 °C. Self-standing yellowish orange film was obtained: M_1~51,000 $gmol^{-1}$; ^1H NMR (25 mM, $CDCl_3$): δ=12.60 (s, 2H), 9.00 (s, 2H), 8.44 (br, 2H), 8.23 (br, 2H), 7.91 (m, 4H), 7.53 (m, 4H), 7.39 (br, 2H), 7.00 (br, 2H), 6.81 (br, 2H), 2.42 (t, 4H), 1.71–1.61 (m, 4H), 1.47–1.27 (m, 4H), 0.60–0.49 (m, 4H), 0.10 ppm (br, 90H). $T_{melting}$=60 °C; $T_{decomposition}$=215 °C.

Spectrofluorimetric Titrations All of the solvents used in the spectrofluorimetric titrations were of analytical grade. HEPES buffer used was 10 mM at pH 7.4. The spectrofluorimetric titrations were carried out in aqueous DMSO (5:95 v/v) (0.15 mL of aqueous HEPES buffer at pH 7.4+ 2.85 mL of DMSO). Measurements were taken after equilibrium had been reached between the receptor and the substrate. The 1:2 receptor-substrate interaction was analyzed according to the Benesi-Hildebrand equations [73–75] for spectrofluorimetric titration (Eq. (1)).

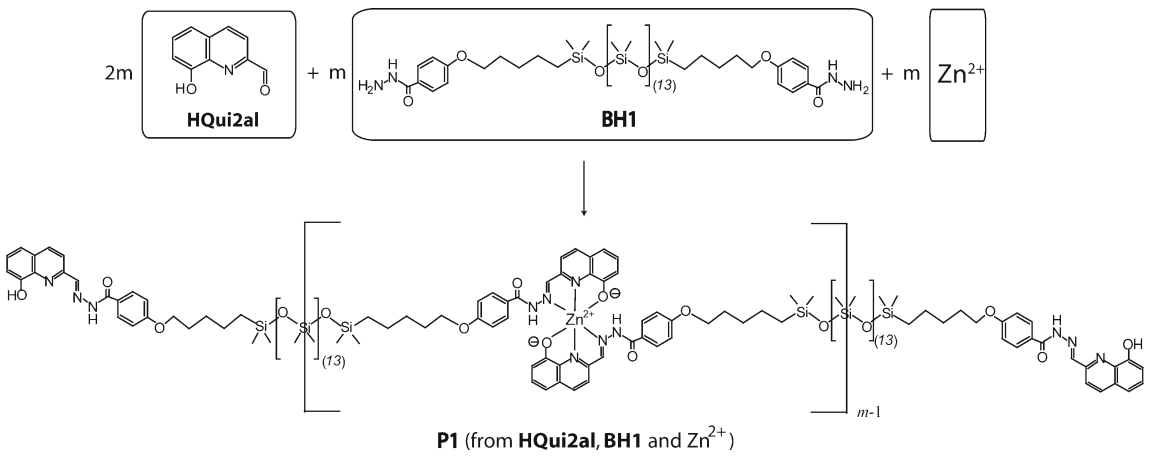
$$\frac{I_O}{I - I_O} = \left(\frac{a}{b-a}\right)^2 \left(\frac{1}{K[substrate^2]} + 1\right) \quad (1)$$

$$Detection\ Limit = t \times s.d. \quad (2)$$

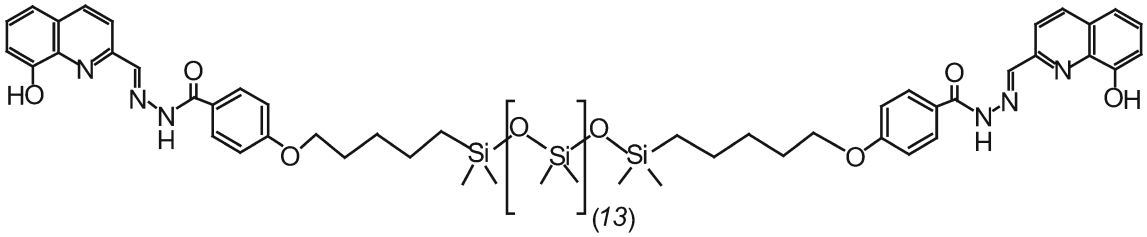
Formation constants (log K) were estimated from the ratio of the y-intercept and the slope of straight lines obtained by plotting $I_0/(I–I_0)$ vs. $[M]^{-2}$ depending on 1:2 receptor–substrate interaction, respectively. I_0 and I are luminescence intensities of the fluorogenic reagent in the absence and presence of the substrate; *a and b* are constants; [substrate] is the concentration of target analyte. t is the compensation factor from *Student's t Distribution Table* and *s.d.* is the standard deviation of the relative luminescence intensity [74, 75].

(a) **Mole Ratio of P1 towards Adenine – Mole Ratio Plot.** A series of adenine solutions (0 to 1.6×10^{-3} M) were mixed with **P1** (4.0×10^{-4} M). Spectral changes at 500 nm (I/I_o) of the resulting mixtures were plotted as a function of mole fraction of the adenine. The sharp turning point from the mole ratio plot revealed the mole ratio between donor and acceptor ensemble in ethanol (Fig. 4).

(b) **Formation Constants of P1 towards Various Analyte.** Spectrofluorimetric titrations of solutions: **P1** (8.0×10^{-5} M) by adenine: (0–6.4×10^{-4} M) were carried out in 5 % aqueous DMSO (5:95 v/v) at pH 7.4.



Scheme 1 Synthesis of a neutral zinc(II) metallosupramolecular polymer (**P1**) by self-assembly through multiple condensation-coordination-deprotonation reactions between bishydrazide **BH1** (0.15 mmol), aldehyde **HQui2al** (0.3 mmol), and Zn(acetate)·$2H_2O$ (0.15 mmol) in the presence of anhydrous Na_2SO_4 (3 mmol) in MeOH/CH_2Cl_2 (1:1 v/v, 15.0 ml) at room temperature



L1 (from **HQui2al** and **BH1**)

Scheme 2 Structures of **L1**

Spectrofluorimetric titrations of solutions: **P1** (4.0×10^{-4} M) by adenosine, thymine, cytosine, guanine and uracil: ($0–8\times10^{-3}$ M) were carried out in 5 % aqueous DMSO (5:95 v/v) at pH 7.4. Observed luminescent at 500 nm of the resultant mixtures were measured. Formation constants of the **P1** – analyte adducts were analyzed by fitting the titration curves with 1:2 Benesi-Hildebrand equation [Eq. (1)].

(c) **^{1}H-NMR titration of P1 towards Adenine.** ^{1}H-NMR titrations of solutions: **P1** (4.0×10^{-4} M) by adenine: ($0–1.6\times10^{-3}$ M) were carried out in d-DMSO. Observed H-NMR spectra were recorded.

(d) **Detection Limits of P1 towards Adenine.** A series of ten aqueous DMSO of **P1** (1.0×10^{-5} M) were added with a fixed known concentration of adenine. Spectroscopic changes of the resultant mixtures were recorded. The detection limits were calculated with Eq. 2.

Results and Discussion

Synthesis and Characterization

The free ligand **L1** incorporated in polymer **P1**, respectively, was prepared independently by simple condensation [76–82] and obtained as oily materials. Neutral metallopolymer, **P1**, which is connected through zinc(II) coordination centers, were obtained by a one-pot reaction of bis-hydrazide **BH1** with the carboxaldehyde **HQui2al** and Zn^{II}(acetate) at a 1:2:1 molar ratio in MeOH/CH_2Cl_2 (1:1 v/v). **P1** was generated from self-assembly polymerization involving three processes: 1) ligand subunit condensation to form the tridentate coordination moiety; 2) multiple metal-ligand coordination to connect the ligand monomers; and 3) simultaneous deprotonation to form neutral coordination centres (Schemes 1 and 2).

The polymers **P1** was a yellowish orange transparent film after the evaporation of the solutions in $CHCl_3$ (Fig. 2). Due to the presence of a polymeric dimethylsiloxane spacer in **P1,** the polymer is soluble in common chlorinated organic solvents such as chloroform and dichloromethane.

^{1}H NMR spectroscopy confirmed the formation of the metallopolymer [83]. The ^{1}H NMR signals of the non-coordinated imine protons in the free ligands **L1** were found at δ=8.61 ppm, and the corresponding signals of the zinc-coordinated imine protons in **P1** were found at δ=9.00 ppm. The linear supramolecular polymer structures were confirmed by the observation of the ^{1}H-NMR signals of uncomplexed imine end-groups. The integration of the ^{1}H NMR signals for the imine CH protons on free and complexed coordination sites gave an average molecular weight of 51,000 $gmol^{-1}$ (ca.27 repeating units) for **P1** (25 mM in $CDCl_3$). Polymer **P1** presented an emission at 500 nm under excitation at 365 nm due to the zinc(II) coordination centre, which contains acyl hydrazone residues. Viscoelasticity measurement revealed that the elastic modulus E' of **P1** was 4.5×10^7 Pa at 25 °C. The profile of the loss elastic modulus E'' gave the T_m of **P1** as 60 °C (Fig. S2). These results are in line with the hard film behaviour of **P1** as shown in Fig. 2.

The chemosensing properties of the neutral metallopolymer **P1** to adenine were demonstrated by spectrofluorometric

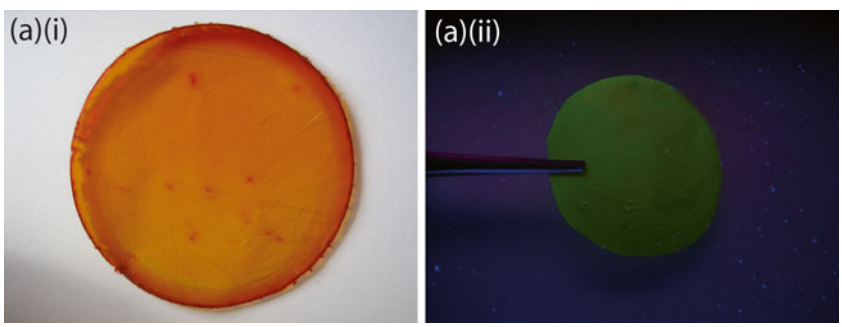


Fig. 2 Zinc(II)-based neutral metallosupramolecular polymers: yellowish orange transparent strong self-stand film **P1** with yellow emission The photographs of polymer fluorescence were taken under excitation at 365 nm

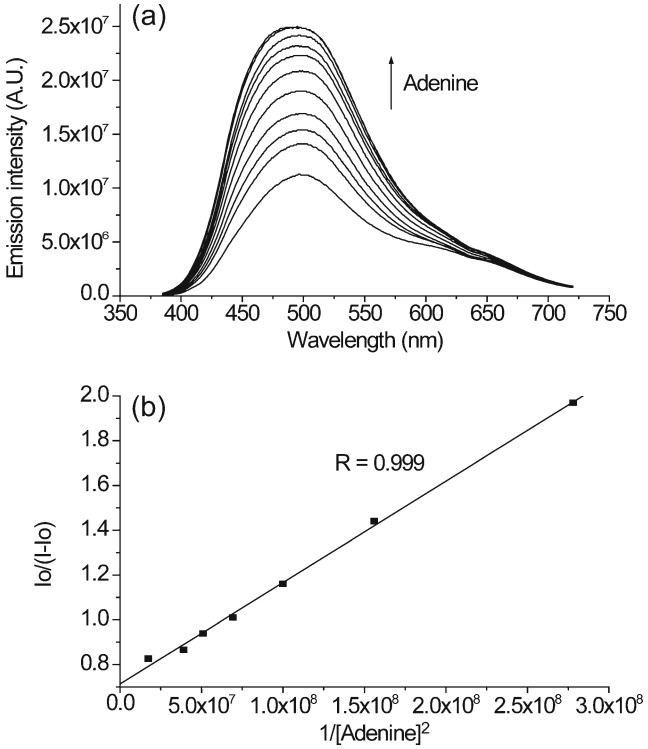


Fig. 3 **a** Spectrofluorimetric titrations of **P1** (8.0×10^{-5} M) with adenine (0 to 6.4×10^{-4} M). All of the titrations were carried out in 5 % aqueous DMSO (5:95 v/v) at pH 7.4 with excitation at 365 nm. The slope and y-intercept are 7.13×10^{-1} and 4.53×10^{-9} M^2, respectively, of the best fitted $I_o/(I\text{-}I_o)$ vs. $1/[adenine]^2$ plot with log K=4.10±0.02 at 500 nm

titration. Figure 3 shows the spectrofluorometric titrations of **P1** to adenine. Upon addition of adenine to the DMSO solutions of **P1**, the π-π* transitions of the Zn^{II} coordination centre remained at 500 nm with a significant enhancement in intensity (Fig. 3a). The slope and y-intercept were calculated as 7.13×10^{-1} and 4.53×10^{-9} M^2, respectively, by plotting the best fitted Io/(I-Io) vs. $1/[adenine]^2$ graph (Fig. 3b). The formation constant (log K) of **P1** to adenine was determined as 4.10±0.02 by fitting the titration curves with the 1:2

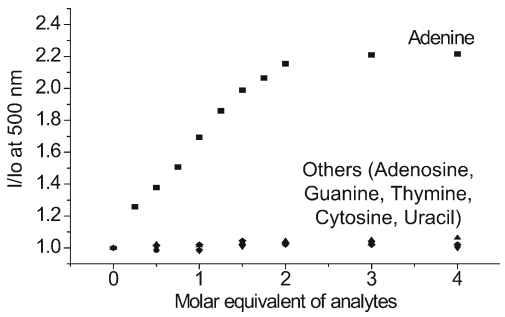


Fig. 4 Summary of spectrofluorometric titration (I/I_o at 500 nm) of **P1** (4.0×10^{-4} M) to adenine, adenosine, guanine, thymine, cytosine, and uracil monitored as a function of the increase in their concentration. All of the titrations were carried out in 5 % aqueous DMSO (5:95 v/v) at pH 7.4 with excitation at 365 nm

Table 1 Summary of the formation constants (log K) and detection limits of P1 with adenine, adenosine, guanine, thymine, cytosine, and uracil in aqueous DMSO (5:95 v/v) at pH 7.4

	log K^a
Adenine	4.10±0.02
Adenosine	2.50±0.08
Guanine	2.82±0.07
Thymine	1.48±0.03
Cytosine	–[b]
Uracil	–[b]

[a] The formation constants (log K) of P1 with various analytes were determined by plotting the best fitted $I_o/(I\text{-}I_o)$ vs. $1/[substrate]^2$ with the 1:2 Benesi-Hildebrand equation (Eq. (1))

[b] The formation constants (log K) were too small to be detected

Benesi-Hildebrand equation (Eq. (1)). Given the result, each Zn^{II} centre in polymer **P1** was expected to bind to two adenines.

Figure 4 summarizes the spectrofluorimetric titrations (mole ratio plot) of **P1** (1.0×10^{-4} M) with the common nucleic bases adenine, adenosine, guanine, thymine, cytosine, and uracil. Among the analytes, only adenine was able to induce a spectrofluorometric response. Structurally similar nucleic bases (such as guanine) and adenine-based nucleoside (adenosine) did not induce any observable spectrofluorometric changes.

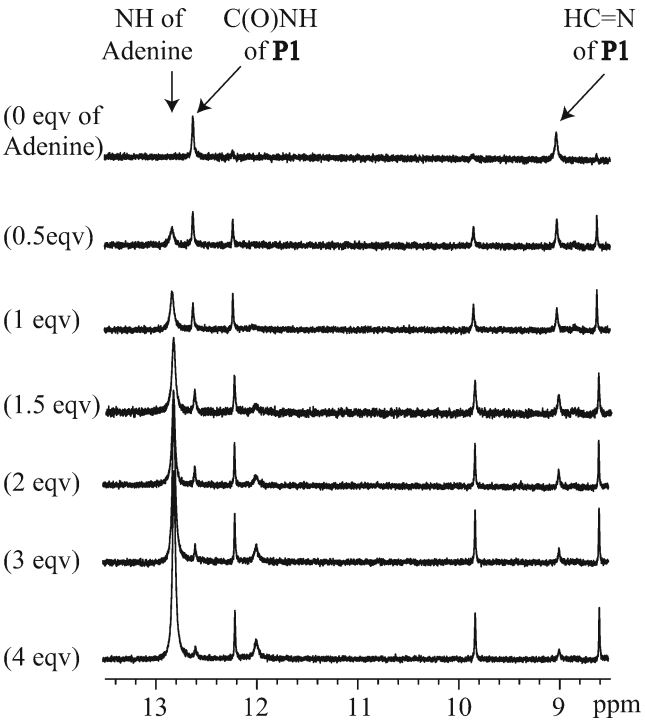


Fig. 5 ^{1}H-NMR titration study of **P1** with adenine in d-DMSO, [**P1**]= 4.0×10^{-4} M and [adenine]=0 to 1.6×10^{-3} M. The numbers in parentheses indicate the number of equivalent moles of adenine added

The mole ratio plot of **P1** to adenine reveals that the maximum response occurs at **P1**: adenine at a mole ratio of 1:2, which further confirms that each Zn^{II} coordination centre in **P1** binds with two adenine molecules. The sensitivity of **P1** to adenine in the luminescent mode of detection (as 3:1 signal-to-noise ratio) reached 90 ppb. It is worth noting that over 100 ppb of the plasma level of adenine was found in the patients with chronic renal failure [27]. Table 1 summarizes the log *K* of **P1** with adenine, adenosine, guanine, thymine, cytosine, and uracil in aqueous DMSO.

Specificity of P1 to Adenine

The binding specificity of the neutral metallopolymer, P1, to adenine was examined by ^{1}H-NMR titration. Upon addition of adenine (0 to 1.6×10^{-3} M) to **P1** (4.0×10^{-4} M) in d-DMSO, the imine (HC=N) two-proton singlet signal at 9.0 ppm split into two one-proton singlets at 8.6 and 9.8 ppm, while the hydrazone [C(O)NH] two-proton singlet signal at 12.6 ppm split into two one-proton singlets at 12.0 and 12.2 ppm (Fig. 5). These changes indicate that the **P1** polymer allows the Zn^{II} coordination centre to bind to adenine through hydrogen bonding with its imine and hydrazone protons [84–87]. However, when similar ^{1}H-NMR titrations were conducted with common nucleic bases (adenosine, guanine, thymine, cytosine and uracil), there was no evidence of any chemical shift in the NMR spectra. Figure 6 shows the proposed recognition mechanism of **P1** to adenine. The two donors, which are lone pairs of electrons at 3-N and 9-N in the adenine, are expected to be complementary to the acceptors (acidic protons) of the imine and hydrazone moieties in **P1**.

Conclusion

A new neutral Zn^{II} metallopolymer based on acyl hydrazone derived coordination centers, **P1**, has been synthesized and characterized. It is a linear coordination polymer with self-standing film-like properties. **P1** is the first luminescent polymeric chemosensor identified as being is selective to adenine, with a detection limit down to 90 ppb. The use of metallopolymeric chemosensing materials in which one

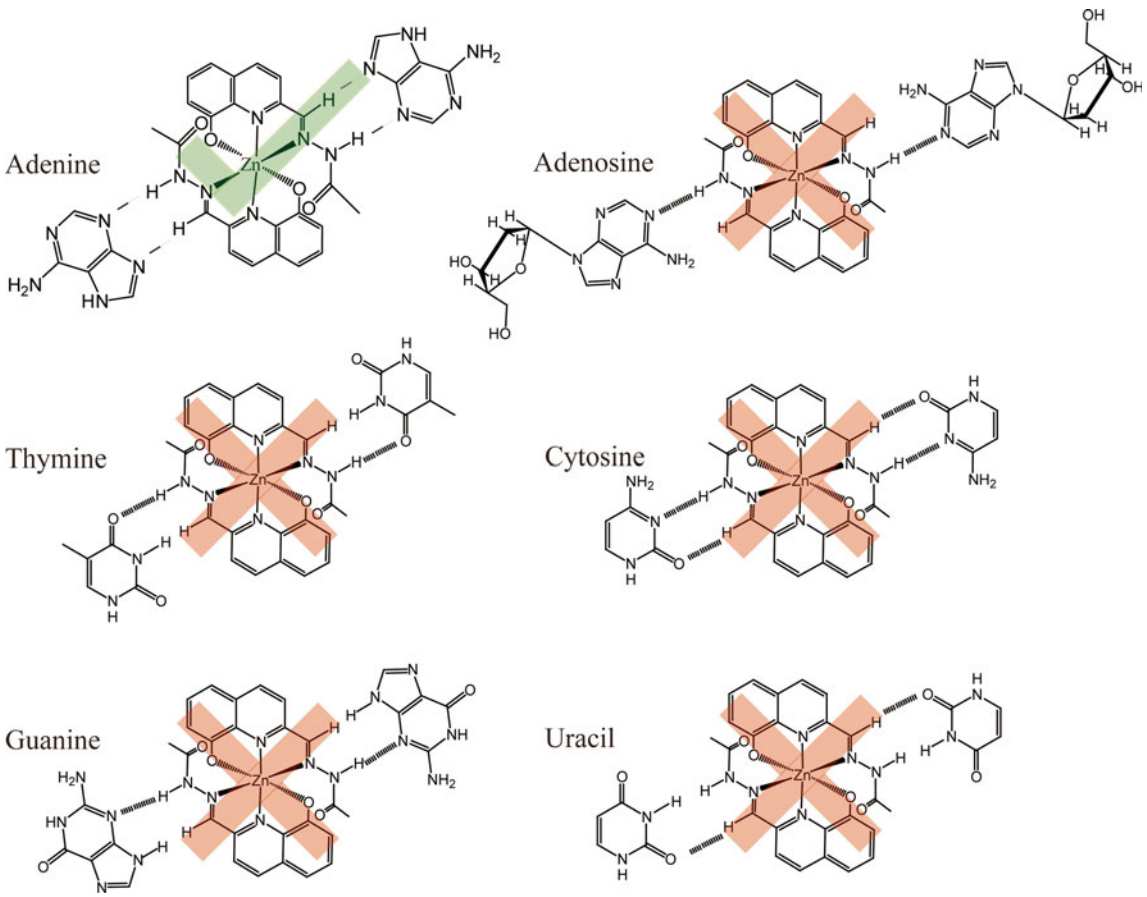


Fig. 6 Proposed host-guest interactions between the nucleic bases (adenine, adenosine, guanine, thymine, cytosine, and uracil) and the Zn^{II} coordination center in **P1** through hydrogen bonding by imine and hydrazone protons

metal center that acts as a functional specific chemosensing site is linked to the polydimethylsiloxane functionality responsible for polymeric morphology seems to be a versatile approach to the design of new supramolecular chemosensors.

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83. The ^{1}H-NMR spectroscopic data of metallosupramolecular polymer **P1** was described in the Supporting Information (Fig S1). The temperature dependence of the storage elastic modulus E' and the loss elastic modulus E'' of the film **P1** were described in the Supporting Information (Fig S2)
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