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PAPER

Pyridinium-based symmetrical diamides as chemosensors in visual sensing of citrate through indicator displacement assay (IDA) and gel formation†

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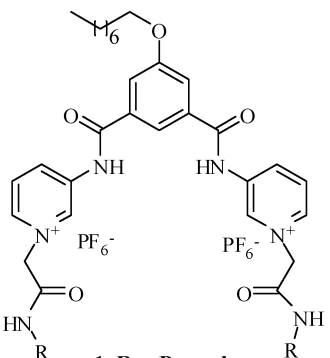
The design and synthesis of pyridinium-based symmetrical diamides **1** and **2** along with their anion binding studies through 'indicator-diplacement assay' are reported. Both the chemosensors effectively respond in in CH_3CN–H_2O (4 : 1 v/v) at pH = 6.3 for the selective naked-eye detection of citrate. Additionally, chemosensor **2** ($c = 6.29 \times 10^{-3}$ M) forms a stable gel only with citrate in CH_3CN, which validates its visual sensing.

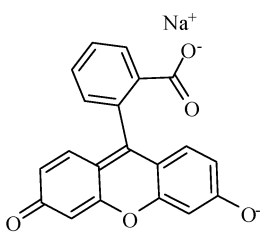
Introduction

The design and synthesis of molecular receptors which respond in an "Indicator-Displacement Assay" (IDA) for sensing of important analytes is an active area of research in supramolecular chemistry.[1] In this technique, an indicator is first allowed to bind reversibly to a receptor, and then a competitive analyte is introduced into the system causing the displacement of the indicator from the host, which in turn modulates the optical signal. Anslyn's group popularized this technique to such an extent that it is now widely used and recognized as a standard method for the development of sensors that are able to detect analytes of biological significance selectively.[1a] Based on this technique, the selective sensing of citrate,[2] tartarate,[3] phosphate,[4] heparin,[5] nitrate[6] *etc.*, is worth mentioning. Given the importance of carboxylic acids and carboxylates in biology,[7] interest in selective sensing of citrate is appealing. Citric acid is a tricarboxylic acid that plays an important role in the Krebs cycle to provide the vast majority of energy used by aerobic cells in human beings. Citrate is also the source of acetyl CoA, which is required for fatty acid and cholesterol synthesis which is essential for proliferating cells, tissue regeneration, embryogenesis and steroid hormone synthesis. Determination of its levels is important in clinical conditions and in normal cell metabolism. Diminished citrate levels in urine have been linked to various aspects of kidney dysfunction, for example in the pathogenesis of nephrolithiasis and nephrocalcinosis.[8] Several groups have reported different receptor systems for the recognition of citrate/citric acid.[2,9] Many of these are based on positively charged, hydrogen bond groups or unsaturated metal centers coordinated to 1,3,5-trialkylbenzene scaffolds which adopt a 'fly-trap' conformation.

During the course of our work on the recognition of neutral, cationic and anionic substrates by our artificial designed receptors,[10] we herein report simple and easy-to-make receptor modules **1** and **2** that respond efficiently in the IDA technique for naked-eye detection of citrate among the other different anions examined. In addition, receptor **2** formed a stable gel in CH_3CN in the presence of tetrabutylammonium citrate. This is considered to be worthy in the area of gel chemistry. In recent times, as a type of typical soft materials, gels have been of particular interest in materials science due to their intriguing properties between those of a solid and a liquid.[11] Especially, low molecular weight supramolecular gels which are formed due to interplay of weak noncovalent forces have received considerable attention due to their wide range of potential applications as soft materials in drug delivery, tissue engineering and chemical sensing.[12]



1. R = Propyl
2. R = Pyrene



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Results and discussion

The compounds **1** and **2** were obtained according to the synthesis shown in Scheme 1. Initially, the symmetrical diamide **4** was obtained by coupling of 3-aminopyridine with

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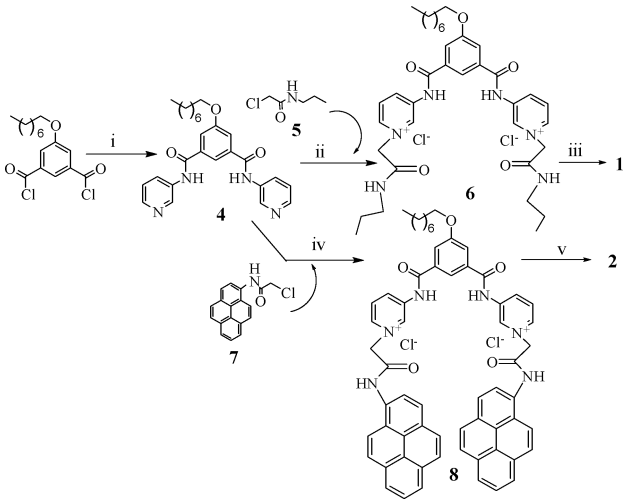
† Electronic supplementary information (ESI) available: Spectral data of receptor **1** and receptor **2**. Figures showing the change in selected fluorescence and UV-vis titration of receptor **1** and receptor **2** with various anions, Job plots of receptor **2** with anions from fluorescence and UV-vis. Selected binding constant curves of receptors **1** and **2** from fluorescence and UV-vis. Indicator displacement experiments, DFT optimized geometry of **1**. See DOI: 10.1039/c1ob05707c



Scheme 1 (i) 3-Aminopyridine, Et_3N, dry CH_2Cl_2; (ii) **5**, dry CH_3CN and DMF, reflux, 5 days; (iii) DMF/CH_3OH, aq. solution of NH_4PF_6; (iv) **7**, dry CH_3CN and DMF, reflux, 5 days; (v) DMF/CH_3OH, aq. solution of NH_4PF_6.

5-octyloxyisophthaloyl dichloride in dry CH_2Cl_2. Subsequent reaction of the diamide **4** with different chloroamides such as **5** and **7** in dry CH_3CN under refluxing conditions afforded the corresponding dichloride salts **6** and **8**, respectively. Finally, the chloride anions in **6** and **8** were exchanged with PF_6^- ions to give the desired compounds **1** and **2**, respectively. All the compounds were characterised by ^1H NMR, ^{13}C NMR, FTIR, mass and elemental analyses.

The interaction properties of both **1** and **2** towards a series of anions of different topologies were studied by fluorescence, UV-vis and ^1H NMR methods in CH_3CN–H_2O (4 : 1 v/v) at pH = 6.3 (10 mM Tris HCl buffer). Compound **1**, having no fluorophore, was titrated with a number of anionic guests (taken as their tetrabutylammonium salts) by the UV method and the change in absorbance was noted. The change in absorbance of **1** was minor with the gradual addition of anions (see the ESI†) and the binding stoichiometries of the complexes were evaluated to be 1 : 1 as confirmed by the Job method[13] (Fig. 1). Analysis of the absorbance data gave the binding constant values[14] (Table 1). As

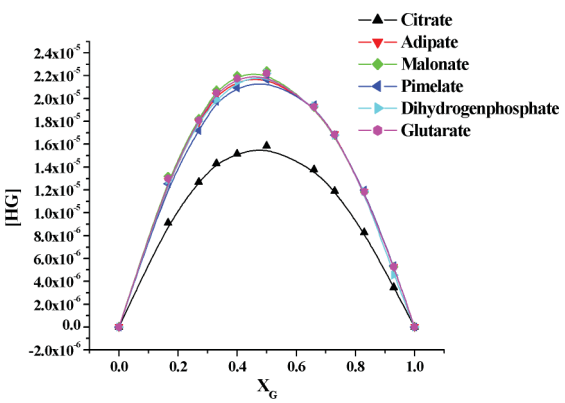


Fig. 1 UV-vis Job plots for **1** with tetrabutylammonium (a) citrate, (b) adipate, (c) malonate, (d) pimelate, (e) dihydrogenphosphate and (f) glutarate at 295 nm in CH_3CN–H_2O (4 : 1 v/v, pH = 6.3, 10 mM TrisHCl buffer), where [G] = [H] = 8.5×10^{-5} M at 25 °C.

Table 1 Association constants (K_a) for **1** based on UV-vis methods in CH_3CN:H_2O (4 : 1 v/v, pH = 6.3,10 mM Tris/HCl buffer)

Guest[b]	K_a/M^{-1}[a]
Citrate	$(2.34 \pm 0.01) \times 10^4$
Pimelate	$(4.05 \pm 0.05) \times 10^3$
Tartarate	[c]
Malate	[c]
Adipate	$(1.05 \pm 0.01) \times 10^3$
Glutarate	$(1.17 \pm 0.02) \times 10^3$
Succinate	$(1.62 \pm 0.01) \times 10^3$
Malonate	$(1.02 \pm 0.01) \times 10^3$
N-Ts glutamate	$(4.99 \pm 0.09) \times 10^2$
F^-	$(1.12 \pm 0.01) \times 10^3$
Cl^-	$(9.94 \pm 0.07) \times 10^2$
Br^-	$(8.98 \pm 0.07) \times 10^2$
I^-	$(9.96 \pm 0.07) \times 10^2$
$H_2PO_4^-$	$(1.01 \pm 0.03) \times 10^3$
AcO^-	$(1.61 \pm 0.02) \times 10^3$

[a] Determined by UV-vis method at the wavelength 295 nm. [b] All guests are tetrabutylammonium salts. [c] Association constants were not determined either due to minimal change or being difficult to determine.

can be seen, the receptor **1** shows a greater affinity for citrate over the other anions.

In a similar way, UV-vis and fluorescence titrations of **2** with the same anionic substrates were conducted to understand its interaction ability under similar conditions. The chemosensor **2** showed monomer emission at 385 nm upon excitation at 340 nm in aqueous CH_3CN (CH_3CN–H_2O = 4 : 1 v/v) at pH 6.3 (10 mM Tris HCl buffer). Upon complexation of citrate, pimelate and $H_2PO_4^-$ anions, the monomer emission of **2** was significantly perturbed (see the ESI†). For instance, Fig. 2 represents the change in emission of **2** upon titration with citrate.

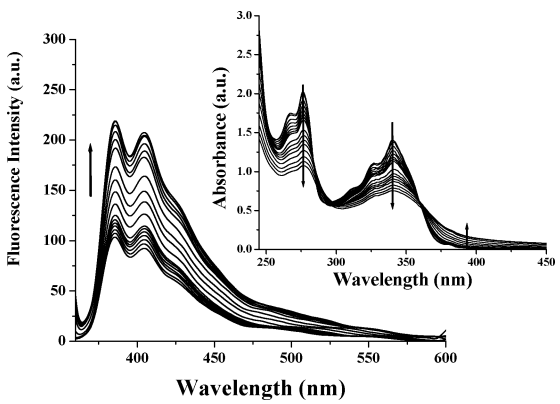


Fig. 2 Fluorescence titration spectra of **2** ($c = 2.5 \times 10^{-5}$ M) in CH_3CN–H_2O (4 : 1 v/v, pH = 6.3, 10 mM TrisHCl buffer) with the addition of citrate. Inset: UV titration spectra of **2** ($c = 2.5 \times 10^{-5}$ M) in CH_3CN–H_2O (4 : 1 v/v, pH = 6.3, 10 mM TrisHCl buffer) with citrate.

It is of note that the receptor **2** exhibited a strong excimer emission at 504 nm along with the monomer emissions at 388 and 408 nm in 5% CH_3CN in $CHCl_3$. Upon addition of a small amount of citrate the excimer emission initially increased and then decreased on progression of the titration (see the ESI†). The monomer emission weakly changed in an irregular fashion. Fig. 3 is the combined profile for the change in fluorescence ratio of **2** at 385 nm upon addition of 10 equivalent amounts of each anion in aqueous CH_3CN (CH_3CN–H_2O = 4 : 1 v/v) at pH 6.3. However,

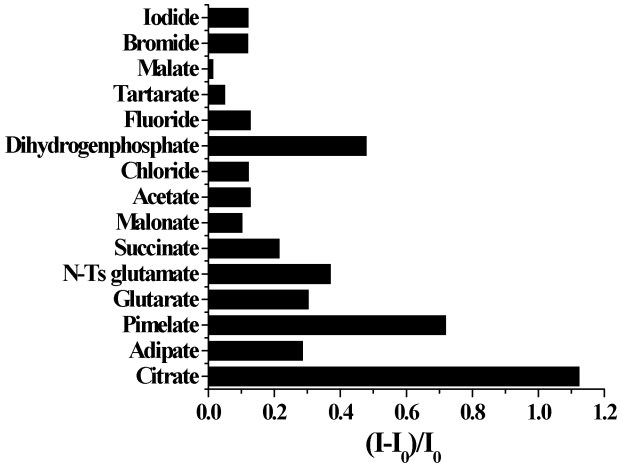


Fig. 3 Fluorescence ratio $[(I - I_0)/I_0]$ of **2** ($c = 6.27 \times 10^{-5}$ M) at 385 nm upon addition of 10 equiv. of a particular anion in CH_3CN–H_2O (4 : 1 v/v, pH 6.3, 10 mM Tris HCl buffer).

the change in absorbance upon titration was also appreciable (see the ESI†), particularly with citrate (inset of Fig. 2).

Analysis of both emission and absorbance data gave the binding constant values[14] (Table 2). Like **1**, the receptor **2** provided a greater binding constant with citrate over the other anions studied, attaining a 1 : 1 binding stoichiometry (Table 2).

Selectivity in the binding process was established by observing the emission behavior of **2** upon addition of citrate to the solution of **2** in aqueous CH_3CN (CH_3CN–H_2O = 4 : 1 v/v) at pH 6.3 containing other interfering anions. In this context, Fig. 4 shows the change in emission behavior when establishing the selectivity for citrate. It is evident from Fig. 4 that the anions studied negligibly interfere in the binding of citrate.

Furthermore, we studied the interaction of **2** with the anions at pH 7.3. Here, the change in emission of **2** was found to be considerably less than at pH 6.3 (Fig. 5). This suggested the inefficiency of **2** in slightly alkaline medium.

Table 2 Association constants (K_a) for **2** based on UV-vis methods in CH_3CN–H_2O (4 : 1 v/v, pH = 6.3,10 mM Tris/HCl buffer)

Guest[a]	K_a/M^{-1}[b]	K_a/M^{-1}[c]
Citrate	$(9.05 \pm 0.06) \times 10^4$	$(7.02 \pm 0.7) \times 10^4$
Tartarate	[d]	[d]
Malate	[d]	[d]
Pimelate	$(3.97 \pm 0.05) \times 10^3$	$(1.62 \pm 0.04) \times 10^3$
Adipate	[d]	$(1.50 \pm 0.02) \times 10^3$
Glutarate	$(1.8 \pm 0.02) \times 10^3$	$(1.49 \pm 0.02) \times 10^3$
Succinate	[d]	$(1.54 \pm 0.01) \times 10^3$
Malonate	[d]	$(6.24 \pm 0.08) \times 10^2$
N-Ts glutamate	[d]	$(6.88 \pm 0.1) \times 10^3$
F^-	[d]	$(1.18 \pm 0.01) \times 10^3$
Cl^-	[d]	$(1.07 \pm 0.02) \times 10^3$
Br^-	[d]	[d]
I^-	[d]	[d]
$H_2PO_4^-$	$(3.47 \pm 0.01) \times 10^3$	$(1.81 \pm 0.03) \times 10^3$
AcO^-	[d]	$(8.21 \pm 0.02) \times 10^3$

[a] All guests are tetrabutylammonium salts. [b] Determined by fluorescence method at the wavelength 385 nm. [c] Determined by UV-vis method at the wavelength 340 nm. [d] Association constants were not determined either due to minimal change or being difficult to determine.

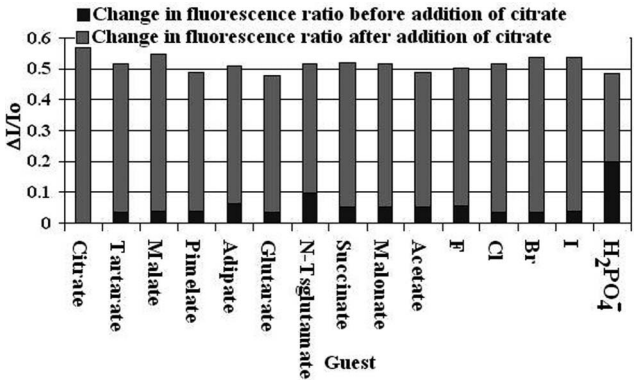


Fig. 4 Change in fluorescence ratio ($\Delta I/I_0$) of **2** ($c = 6.27 \times 10^{-5}$ M) upon addition of 3 equiv. amounts of tetrabutylammonium citrate in the presence of other anions (3 equiv.) in CH_3CN–H_2O (4 : 1 v/v 10 mM Tris HCl buffer at pH = 6.3) at 385 nm.

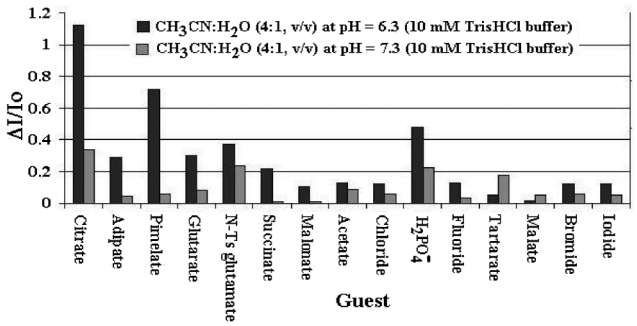


Fig. 5 Fluorescence ratio $[(I - I_0)/I_0]$ of **2** ($c = 6.27 \times 10^{-5}$ M) at 385 nm upon addition of 10 equiv. of a particular anion in CH_3CN–H_2O (4 : 1 v/v 10 mM Tris HCl buffer) at pHs 6.3 and 7.3.

Once the binding selectivities of **1** and **2** toward citrate were established, we investigated the naked-eye detection of citrate following the indicator displacement assay with the dye fluorescein (uranine) **3**. Dye **3**, an aromatic di-anion, was found to interact with both **1** and **2**. The absorption and emission properties of **3** were exploited to check its binding affinity for both **1** and **2**. Upon addition of **1** ($c = 4.2 \times 10^{-4}$ M) to a solution of **3** ($c = 8.0 \times 10^{-5}$ M) in aqueous CH_3CN (CH_3CN–H_2O = 4 : 1 v/v, 10 mM Tris HCl buffer, pH = 6.3) both the absorbance and emission of **3** were significantly reduced (see the ESI†) and the color of the resulting solution became colorless (Fig. 6).

Fig. 6 (1) Dye **3**, (2) receptor **1** + dye **3** (1 : 1) = **A**. **A** with 1 equiv. amount of (3) Cl^-, (4) pimelate, (5) glutarate, (6) $H_2PO_4^-$, (7) tartarate, (8) malate, (9) adipate, (10) *N*-Ts glutamate, (11) malonate, (12) acetate, (13) glutarate, (14) citrate; [**3**] = 8.0×10^{-5} M, [**1**] = 4.2×10^{-4} M, [G] = 4.62×10^{-3} M.

Gradual addition of **2** ($c = 4.2 \times 10^{-5}$ M) to a solution of **3** ($c = 8.5 \times 10^{-5}$ M) in CH_3CN–H_2O (4 : 1 v/v, 10 mM Tris HCl buffer, pH = 6.3) also resulted in similar changes in absorption and emission characteristics of **3** to that of **1** with **3**. Upon interaction with **2**, the solution of **3** became colorless (Fig. 7).

Fig. 7 (1) Dye **3**, (2) receptor **2** + dye **3** (1 : 1) = **B**. **B** with 1 equiv. amount of (3) Cl^-, (4) pimelate, (5) succinate, (6) $H_2PO_4^-$, (7) tartarate, (8) malate, (9) adipate, (10) *N*-Ts glutamate, (11) malonate, (12) acetate, (13) glutarate, (14) citrate; [**3**] = 8.5×10^{-5} M, [**2**] = 4.2×10^{-4} M, [G] = 2.0×10^{-3} M in CH_3CN–H_2O (4 : 1 v/v, 10 mM Tris HCl buffer, pH = 6.3).

The 1 : 1 stoichiometries for **1** and **2** with the dye **3** were established using Job's method of continuous variation[13] (Fig. 8). Analysis of absorption titration data in both cases gave the binding constant values[14] $(1.08 \pm 0.001) \times 10^4$ M^{-1} and $(3.89 \pm 0.1) \times 10^4$ M^{-1} for **1** and **2** with the dye **3**, respectively (see the ESI†). Moreover, fluorometric titration resulted in the binding constant value $(7.59 \pm 0.3) \times 10^4$ M^{-1} for **2** with **3**. However, these values are close in magnitude to the binding constant values for **1** and **2** with citrate, and thus the combinations of **1**/**3** and **2**/**3** will be ideal ensembles for the sensing of citrate. The selectivity of an indicator displacement assay is meaningful when the receptor has the same or slightly lower affinity than the analyte of choice, but a larger affinity than other competing analytes.[15]

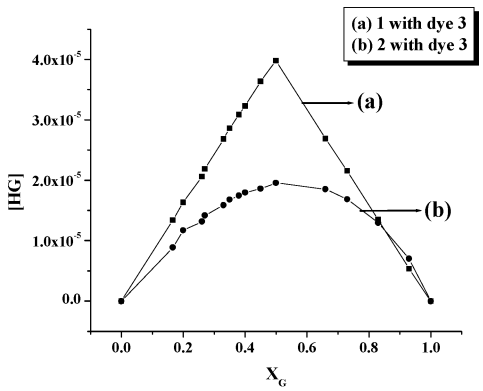


Fig. 8 Job plots of dye **3** ($c = 8.0 \times 10^{-5}$ M) with (a) **1** ($c = 8.0 \times 10^{-5}$ M) and (b) **2** ($c = 8.0 \times 10^{-5}$ M) at 502 nm from UV-vis in CH_3CN–H_2O (4 : 1 v/v, pH = 6.3, 10 mM Tris/HCl buffer) at 25 °C.

Upon addition of citrate to the ensemble of **1**/**3**, dye **3** is displaced from the binding cavity and both its absorbance and fluorescence are restored. In this regard, for example, while Fig. 9a shows the gradual reduction in emission of **3** (λ_{ex} = 480 nm and λ_{em} = 530 nm) upon successive addition of **1**, Fig. 9b demonstrates the change in emission of **3**, which is retrieved upon gradual addition of citrate to the ensemble of **1**/**3**.

The color of the solution turned into the original color of the dye (Fig. 6). The case with the ensemble of **2**/**3** was similar (see the ESI†). Anions that are less efficiently bound by both **1** and **2** than **3** were not capable of displacing dye **3**. Even substrates such as malate, tartrate *etc.*, which are closely related to citrate, did not restore the original greenish yellow color of the dye solution. They were also unable to restore absorbance as well as fluorescence. Figs. 6 and 7 corroborate the naked eye detection of citrate *via* the IDA approach by both **1** and **2**, respectively. Importantly, both **1** and **2** did not respond in naked eye detection of citrate *via* the IDA approach at pH 7.3 (see the ESI†).

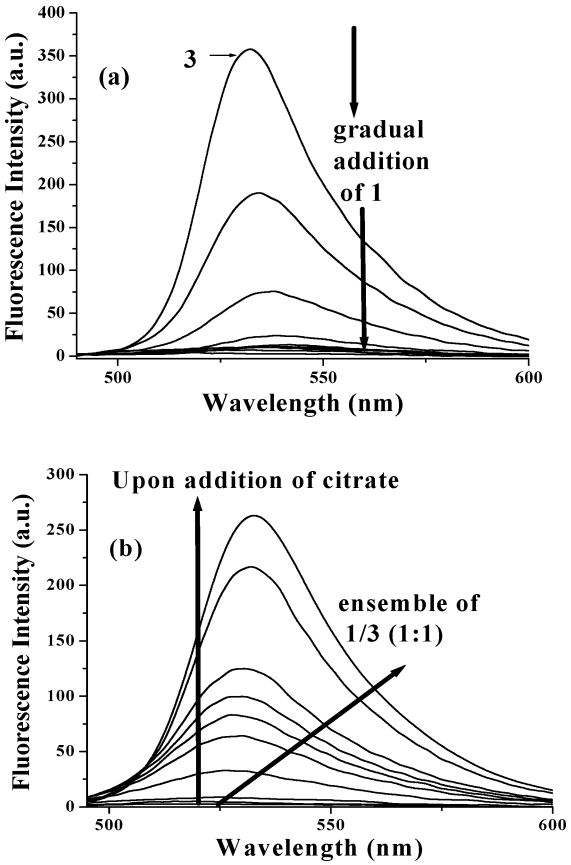


Fig. 9 (a) Change in emission of dye **3** ($c = 8.0 \times 10^{-5}$ M) upon increasing addition of **1** ($c = 4.2 \times 10^{-4}$ M) and (b) retrieval of emission upon increasing addition of citrate ($c = 4.62 \times 10^{-3}$ M) to the ensemble **1**/**3** in CH_3CN–H_2O (4 : 1, v/v, pH 6.3, 10 mM Tris/HCl buffer).

The strong binding of citrate to **1** and **2** was further understood from the ^1H NMR. During titration of **1** ($c = 6.29 \times 10^{-3}$ M) with citrate, the amide protons H_a and H_b moved in the downfield direction significantly. While the pyridinium protons $H_{o'}$ underwent downfield shift, the H_o protons showed upfield chemical shift, and thereby we suggested a binding structure where the pyridinium groups are involved in binding in a tilted fashion (Fig. 10). The non planar structure of the binding site is also evident from the DFT optimized structure of **1**[16] (see the ESI†).

A similar titration experiment was applied to **2** ($c = 6.29 \times 10^{-3}$ M) with citrate, but we failed to complete the titration (Fig. 11) due to the formation of gel in the NMR tube. In this case, the amide protons as well as the pyridinium H_o protons moved in the downfield direction with significant broadening.

Due to strong interaction, chemosensor **2** formed a gel in the presence of citrate in CH_3CN in the concentration range ~10^{-3} M. Thus, citrate in the present example acts as a chemical stimulus in gel formation. However, under identical conditions, **1** did not produce a gel with citrate, thereby suggesting a role of pyrene in maintaining a hydrophobic/hydrophilic balance in **2**. A survey of the literature reveals that citrate-selective gel formation by a suitable abiotic receptor which can be useful in visual sensing of citrate by the naked eye is unknown in the literature. To our belief, the present example is the first report on visual recognition of citrate through gelation of an organic molecule. Receptor **2**

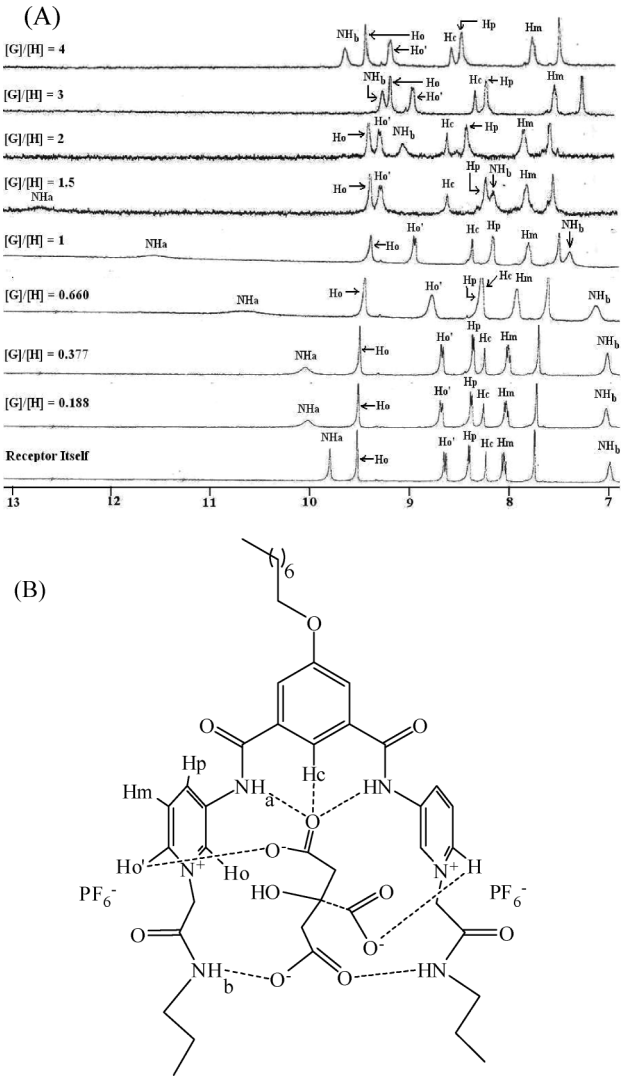


Fig. 10 (A) Change in ^{1}H NMR of **1** ($c = 6.29 \times 10^{-3}$ M) upon addition of citrate ($c = 2.17 \times 10^{-2}$ M). (B) Suggested hydrogen bonding structure of **1** with citrate.

($c = 6.29 \times 10^{-3}$ M) on mixing with tetrabutylammonium citrate in CH_3CN produced a gel (Fig. 12) which underwent a gel-to-sol phase transition at 92 °C. On cooling the sol below 92 °C, gel formation again took place. Under similar conditions no such anion binding-induced gel formation took place in presence of the other anions.

Importantly, the gel formation took place only in CH_3CN and not in other solvent combinations such as $CH_3CN/CHCl_3$, CH_3CN/H_2O. In this respect, Table 3 describes the gelation study of **2** in various solvents at 25 °C. The gel was characterized by recording SEM images (Fig. 13). The SEM images showed the presence of densely coiled fibers and also globular particles. The different pictures based on resolution are represented in Fig. 13.

Conclusion

In conclusion, we have presented here two new sensors, **1** and **2**, for the naked eye detection of citrate over a series of other anions in a semi-aqueous system through the IDA technique.

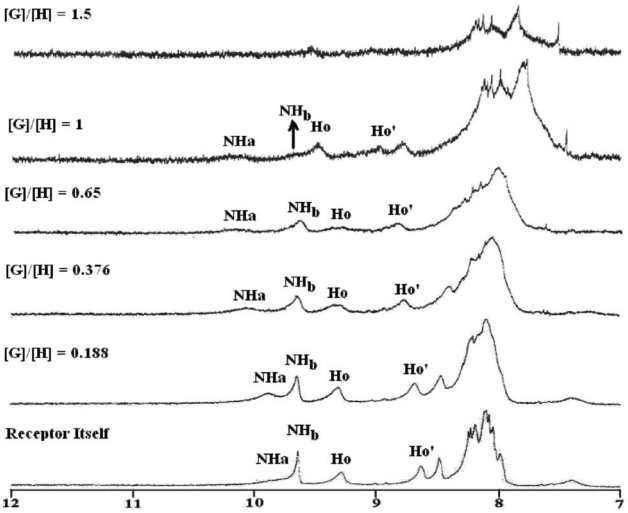


Fig. 11 Change in ^{1}H NMR of **2** ($c = 6.29 \times 10^{-3}$ M) upon addition of citrate ($c = 2.17 \times 10^{-2}$ M).

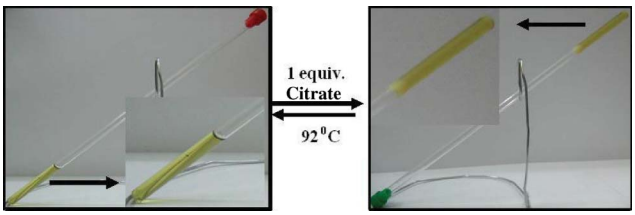


Fig. 12 Photographs showing phase changes for a gel of **2** ($c = 6.29 \times 10^{-3}$ M) with citrate in CH_3CN.

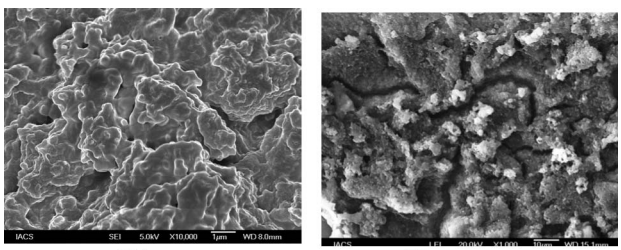


Fig. 13 SEM image of dried gel; scale bar: (A) 1 μm, (B) 10 μm.

Furthermore, it is established for the first time that sensor **2** is also capable of sensing citrate visually through gel formation in CH_3CN solvent. The conventional N–H···O, and unconventional C–H···O hydrogen bondings and charge–charge interaction are the weak forces that interplay cooperatively into the clefts of **1** and

Table 3 Gelatination study of **2** with tetrabutylammonium citrate in various solvents at 25 °C

Solvent	mgc/mg ml^{-1}	Gel status
CH_3CN	7.9	formed (opaque gel)
5% CH_3CN in $CHCl_3$	7.9	not formed (ppt)
1% DMSO in CH_3CN	7.9	not formed (s)
10% CH_3CN in $CHCl_3$	7.9	not formed (ppt)
CH_3CN–H_2O 4 : 1 (v/v)	7.9	not formed (ppt)

mgc = minimum gelatination concentration, s = soluble, ppt = precipitate.

2 to make them selective towards citrate ion. Further progress in this direction is underway in our laboratory.

Experimental

General methods

Materials were obtained from commercial suppliers and were used without further purification. Thin layer chromatography (TLC) was carried out using Merck 60 F254 plates with a thickness of 0.25 mm. Melting points were determined on a hot-plate melting point apparatus in an open-mouth capillary and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz instrument. For NMR spectra, $CDCl_3$, CD_3CN and d_6-DMSO were used as solvents, using TMS as an internal standard. IR spectra were recorded on a Perkin Elmer Spectrum One, using KBr discs. Fluorescence spectra were recorded on a Perkin Elmer Model LS 55 spectrophotometer and UV-vis spectra were recorded on a Perkin Elmer Model Lambda 25.

5-(Octyloxy)-*N*1, *N*3-di(pyridin-3-yl)isophthalamide (4). The bisamide **4** was obtained by reaction of 3-aminopyridine (0.59 g, 6.34 mmol) with 5-(octyloxy)isophthaloyl dichloride (0.7 g, 2.11 mmol) in 60 mL dry CH_2Cl_2 in the presence of Et_3N (1 mL) under nitrogen atmosphere. The reaction mixture was stirred overnight. After completion of the reaction, the solvent was removed under vacuum. The residual mass was extracted with a $CHCl_3$/CH_3OH mixture solvent (3 × 30 mL). The organic layer was washed with $NaHCO_3$ solution (3 × 20 mL) and dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure. The residual mass was purified by column chromatography over silica gel using ethyl acetate–hexane (3 : 2 v/v) as eluent to afford the desired compound **4** (0.820 mg, 86% yield, mp 92 °C). ^{1}H NMR ($CDCl_3$ containing few drops of d_6-DMSO, 400 MHz): δ 9.85 (s, 2H, amide NH), 8.90 (d, 2H, J = 4 Hz), 8.37–8.34 (m, 4H), 8.18 (s, 1H), 7.73 (s, 2H), 7.32 (t, 2H, J = 8 Hz), 4.09 (t, 2H, J = 8 Hz), 1.86–1.79 (m, 2H), 1.49–1.44 (m, 2H), 1.35–1.25 (m, 8H), 0.91 (t, 2H, J = 8 Hz); ^{13}C NMR (d_6DMSO, 100 MHz): δ 165.0, 158.7, 144.8, 142.1, 135.9, 135.6, 127.4, 123.5, 119.4, 116.8, 68.2, 31.2, 28.7, 28.7, 28.6, 25.5, 22.1, 13.9; FTIR (KBr, ν in cm^{-1}): 3349, 2914, 2850, 1668, 1543, 1486, 1426, 1294, 1240, 802; Mass (LCMS): 447.2 (M + 1)$^+$, 240.2, 224.2. Anal cal. $C_{26}H_{30}N_4O_3$: C, 69.93; H, 6.77; N, 12.55; Found: C, 69.81; H, 6.72; N, 12.49.

2-Chloro-*N*-propylacetamide (5). To a stirred solution of n-propylamine (2 g, 33.84 mmol) in $CHCl_3$ (30 mL), chloroacetyl chloride (4.2 g, 37.2 mmol) was added dropwise at 0 °C. Then water (10 mL) was added to the reaction mixture followed by addition of K_2CO_3 (7.01 g, 50.76 mmol). A catalytic amount of tetrabutylammonium hydrogensulphate was added to the reaction mixture and stirred for 4 h. After completion of the reaction, as monitored by TLC, the organic layer was separated and dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure. The residual mass was purified by silica gel column chromatography using 1% CH_3OH in $CHCl_3$ as eluent to afford pure compound **5** (3.65 g, 95%), bp. 88 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.84 (s, 1H), 4.05 (s, 2H), 3.27 (q, 2H, J = 8 Hz), 1.62–1.53 (m, 2H), 0.95 (t, 2H, J = 8 Hz); FTIR (KBr, ν in cm^{-1}): 3423, 3300, 2967, 2937, 1662, 1545; Mass (ESI): 136.1 (M + 1)$^+$.

2-Chloro-*N*-(4, 6-dihydropyren-1-yl)acetamide (7). To a stirred solution of 1-aminopyrene (0.57 g, 2.31 mmol) in $CHCl_3$ (30 mL), chloroacetyl chloride (0.29 g, 2.54 mmol) was added dropwise at 0 °C. Then water (10 mL) was added to the reaction mixture followed by addition of K_2CO_3 (0.48 g, 3.46 mmol). A catalytic amount of tetrabutylammonium hydrogensulphate was added to the reaction mixture and the mixture was stirred for 4 h. After completion of the reaction, as monitored by TLC, the organic layer was separated and dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure. The residual mass was purified by silica gel column chromatography using 1% CH_3OH in $CHCl_3$ as eluent to afford pure compound **7** (0.630 g, 92%), mp. 204 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.05 (s, 1H, amide NH), 8.44 (d, 1H, J = 8 Hz), 8.22–8.14 (m, 4H), 8.07–8.01 (m, 4H), 4.44 (s, 2H); ^{13}C NMR [d_6-DMSO (for homogeneity of the solution), 100 MHz]: δ 165.8, 130.9, 130.7, 130.4, 128.6, 127.3, 127.1, 126.8, 126.4, 125.3, 125.0, 124.9, 124.3, 124.0, 123.7, 123.3, 122.0, 57.4; FTIR (KBr, ν in cm^{-1}): 3234, 3114, 3021, 2957, 1663, 1598, 1525, 840; Mass (LCMS): 295.2 (M + 2)$^+$, 294.2 (M + 1)$^+$.

Synthesis of receptor (1). To a solution of **4** (0.100 g, 0.223 mmol) in dry CH_3CN (10 mL) containing dry DMF (2 mL), compound **5** (0.091 g, 0.672 mmol) in dry CH_3CN (20 mL) was added. The reaction mixture was refluxed with stirring for 5 days under nitrogen atmosphere. On cooling the reaction mixture, precipitate appeared. The precipitate was filtered off and washed with CH_3CN several times and then with diethyl ether to give pure dichloride salt **6** (0.130 g, 81%). The pure dichloride salt **6** (0.130 g, 0.181 mmol) was dissolved in 5 mL hot CH_3OH containing a few drops of DMF and the volume was reduced to 2 mL. Then aqueous solution of NH_4PF_6 (0.089 g, 0.54 mmol) was added in one portion to carry out the anion exchange reaction. After stirring the reaction mixture for 35 min, precipitate appeared. Filtration of the precipitate followed by thorough washing with diethyl ether afforded the receptor **1** in 65% yield (0.102 g), mp 116 °C. ^{1}H NMR (CD_3CN, 400 MHz): δ 9.80 (s, 2H, amide NH), 9.53 (s, 2H), 8.65 (d, 2H, J = 8 Hz), 8.41 (d, 2H, J = 8 Hz), 8.23 (s, 1H), 8.07–8.03 (m, 2H), 7.75 (s, 2H), 6.99 (br s, 2H, amide NH), 5.27 (s, 4H), 4.21 (t, 2H, J = 8 Hz), 3.25–3.21 (m, 4H), 1.95–1.83 (m, 2H), 1.60–1.49 (m, 6H), 1.40–1.24 (m, 8H), 0.97–0.91 (m, 9H); ^{13}C NMR ($CDCl_3$, containing few drops of d_6-DMSO, 100 MHz): 165.88, 163.37, 159.51, 140.01, 139.80, 136.73, 135.50, 134.68, 127.55, 119.24, 118.21, 68.75, 62.70, 41.74, 31.64, 29.18, 29.09, 28.98, 25.85, 22.48, 22.2, 14.0, 11.32; FTIR (KBr, ν in cm^{-1}): 3419, 2966, 2933, 2878, 1680, 1594, 1557, 1462, 838; Mass (ESI): 790.9 (M – PF_6–1)$^+$, 645.1 (M – 2PF_6–1)$^+$; Anal cal. $C_{36}H_{50}N_6O_5(PF_6)_2$: C, 46.16; H, 5.38; N, 8.97; Found: C, 46.12; H, 5.26; N, 8.83.

Synthesis of receptor (2). To a solution of **4** (0.08 g, 0.18 mmol) in dry CH_3CN (10 mL) containing dry DMF (2 mL), compound **7** (0.174 g, 0.59 mmol) dissolved in CH_3CN–DMF (1 : 1 v/v, 20 mL) was added. A catalytic amount of tetrabutylammonium iodide was added to the reaction mixture and the mixture was refluxed for 5 days under nitrogen atmosphere. On cooling the reaction mixture, precipitate appeared. The precipitate was filtered off and washed with CH_3CN several times and then with diethyl ether to give pure dichloride salt **8** (0.147 g, 79%). The dichloride salt **8** (0.100 g, 0.09 mmol) was next dissolved in 5 mL hot CH_3OH containing a few drops of DMF and the volume was reduced to 2 mL.

Subsequently, aqueous solution of NH_4PF_6 (0.048 g, 0.29 mmol) was added in one portion to carry out the anion exchange. After stirring the reaction mixture for 35 min, precipitate appeared. Filtration of the precipitate followed by thorough washing with diethyl ether afforded the receptor **2** in 89% yield (0.102 g); mp. 188 °C (decomposition). ^{1}H NMR (d_6-DMSO, 400 MHz): δ 11.48 (s, 2H, amide NH), 11.06 (s, 2H, amide NH), 9.75 (s, 2H), 8.96 (br s, 2H), 8.79 (d, 2H, J = 8 Hz), 8.48 (d, 2H, J = 8 Hz), 8.33–8.27 (m, 14H), 8.18 (br s, 3H), 8.10 (t, 2H, J = 8 Hz), 7.95 (s, 2H), 6.0 (s, 4H), 4.17 (t, 2H, J = 6.4 Hz), 1.81–1.79 (m, 2H), 1.46–1.44 (m, 2H), 1.40–1.26 (m, 8H), 0.91 (t, 3H, J = 6.4 Hz); ^{13}C NMR (d_6-DMSO, 100 MHz): δ 165.39, 164.40, 162.30, 158.80, 141.50, 137.61, 136.08, 134.98, 130.76, 130.48, 130.40, 128.66, 127.60, 127.39, 127.14, 126.95, 126.55, 125.48, 125.15, 125.00, 124.31, 123.89, 123.73, 122.96, 122.17, 119.86, 117.63, 68.34, 62.77, 31.2, 28.69, 28.63, 28.53, 25.47, 22.04, 13.91; FTIR (KBr, ν in cm^{-1}): 3383, 2927, 2855, 1688, 1593, 1508, 1459, 1340; Mass (ESI): 1107.5 $(M - PF_6)^+$, 961.7 $(M - 2PF_6 - 1)^+$, 704.6, 481.6; Anal cal. $C_{62}H_{54}N_6O_5(PF_6)_2$: C, 59.43; H, 4.34; N, 6.71; Found: C, 59.53; H, 4.42; N, 6.63.

General procedure for fluorescence and UV-vis titrations

Stock solutions of the receptors were prepared in 4 : 1(v/v) CH_3CN–H_2O containing 10 mM Tris/HCl buffer pH = 6.3 in the concentration range ~10^{-5} M. 2.5 ml of the receptor solution was taken in the cuvette. Stock solutions of guests in the concentration range ~10^{-4} M, were prepared in the same solvents and were individually added in different amounts to the receptor solution. Upon addition of guests, the change in emission of the receptor was noted. The same stock solutions for receptors and guests were used to perform the UV-vis titration experiment. Guest solution was successively added in different amounts to the receptor solution (2.5 mL) taken in the cuvette and the absorption spectra were recorded. Both fluorescence and UV-vis titration experiments were carried out at 25 °C. All the experiments were repeated thrice to check the reproducibility.

Job plots

The stoichiometry was determined by the continuous variation method (Job Plot).[13] In this method, solutions of host and guests of equal concentrations were prepared in the solvents used in the experiment. Then host and guest solutions were mixed in different proportions maintaining a total volume of 3 mL of the mixture. All the prepared solutions were kept for 1 h with occasional shaking at room temperature. Then emission and absorbance of the solutions of different compositions were recorded. The concentration of the complex, *i.e.*, [HG], was calculated using the equation $[HG] = \Delta I/I_0 \times [H]$ or $[HG] = \Delta A/A_0 \times [H]$ where $\Delta I/I_0$ and $\Delta A/A_0$ indicate the relative emission and absorbance intensities. [H] corresponds to the concentration of pure host. Mole fraction of the host (X_H) was plotted against concentration of the complex [HG]. In the plot, the mole fraction of the host at which the concentration of the host–guest complex concentration [HG] is maximum, gives the stoichiometry of the complex.

Determination of binding constants

Binding constant values were determined by fluorescence and absorption methods using eqn (1) and 2.[14]

$$I = (I_0 + IKC_G)/(1 + KC_G) \ldots \quad (1)$$

$$I = I_0 + ((I - I_0)/(2C_H))(C_G + C_H + 1/K - ((C_G + C_H + 1/K)^2 - 4C_GC_H)^{0.5}) \ldots \quad (2)$$

Where I represents fluorescence intensity; I_0 represents the intensity of pure host; C_H and C_G are the corresponding concentrations of host and the guest; K is the association constant (this equation also works in absorption). The association constants and correlation coefficients (R) were obtained by a non-linear least-square analysis of I *vs.* C_G for eqn (1) and the in case of eqn (2) the association constant and correlation coefficients (R) were obtained by a non-linear least-square analysis of I *vs.* C_H and C_G.

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