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Detection of NaCN in aqueous media using calixarene-based fluoroionophore containing ruthenium(II)-bipyridine as fluorogenic unit†

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A new molecular sensor containing calixarene and ruthenium(II)-bipyridine as fluorophore bridged by amide moiety has been synthesised, characterized and its anion binding property has been investigated. It selectively detects cyanide in 95:5 water-acetonitrile when sodium salts of various anions such as F^-, Cl^-, Br^-, I^-, PO_4^{2-}, IO_4^- , BO_3^-, CH_3COO^-, CN^- and SO_4^{2-} were used for investigation. The recognition event was monitored by fluorescence spectroscopy and the lower detection limit found is 70 ppb. However, when tetrabutylammonium salts of the same anions were used, then in addition to CN^-, CH_3COO^- was also detected under similar experimental conditions. Interestingly, CN^- exhibited substantial quenching, whereas CH_3COO^- showed enhancement in emission intensity. The interaction of anions with the fluoroionophore was also monitored by electrochemical technique and the result obtained is consistent to that found by fluorogenic method. Binding constants were determined from emission titration, composition of the anion-complexes formed were determined from mass and emission titration data, mechanistic aspects of the interaction has been discussed with the aid of NMR data and the role of cations in the contrast fluorescent *off and on* behaviour has also been discussed. This sensor has also been used to estimate cyanide in real samples and the result obtained is satisfactory.

Introduction

Selective detection of CN^- has received considerable interest because of its toxic effect towards mammalians result for its ability to interfere with the electron transfer process.[1] Cyanide toxicity lies by inactivation of cytochrome oxidase and inhibition of cellular respiration and consequent cellular anoxia.[2] Hence humans and animals cardiovascular, respiratory and central nervous systems are highly prone to affect by acute cyanide poisoning.[1] Despite its toxic nature, cyanides are industrially made in large quantities, and used in electroplating, raw materials for synthetic fibres, resins, herbicides and gold extraction.[3,4] Various industries produce worldwide as much as 1,400,000 tons of toxic cyanide per year.[5] Because of its acute toxicity, human beings as well as aquatic life have very low tolerance limit for CN^-. The U.S.EPA regulates cyanide content at very low levels of 0.2 ppm and 0.005 mg/L for drinking water and environmental primary standards, respectively.[6]

Several techniques have been developed for the detection of CN^- in water as well as non-aqueous media. These techniques include chromatography, potentiometric method, amperiometry etc.[7-10] However, these techniques are not user friendly and difficult to apply for physiological monitoring or for monitoring of drinking water. Significant work has been done for development of fluorometric and colorimetric based chemosensor for detection of CN^-.[11] However, simple and efficient method to monitor cyanide contamination in water, soil and biological fluids (blood, urine, saliva) is highly demanding.

For designing of molecular sensors for anions, especially for CN^-, metal complex based sensor has been extensively used as the positive charge on the metal ion facilitates interaction with negatively charged ions.[11-19] Apart from metal complexes, other chemosensors based on quantum dots, organic dyes and

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†Electronic supplementary information (ESI) available: Figs. S1 – S10 (Mass, NMR, fluorescence spectra and CV and DPV).

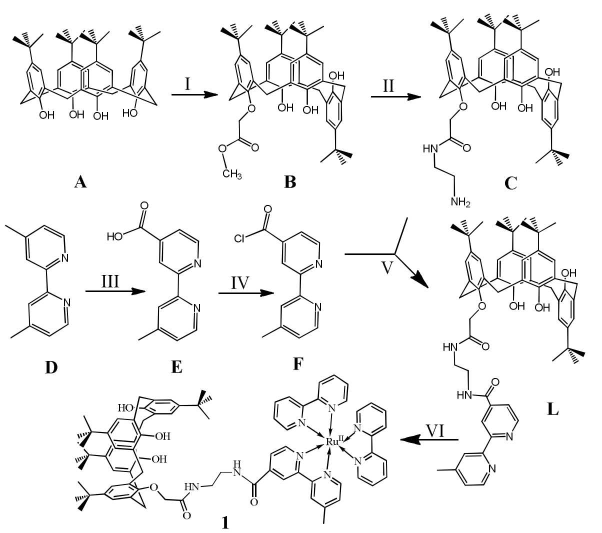
other protic chromophores have also been reported for detection of CN$^-$.[20-25] Chromophores containing NH and OH has advantage as it can make strong hydrogen-bonding interaction with the anions.[26-28] However, some of the CN$^-$ receptors reported also exhibit limitations such as interference from other anions, especially from F$^-$ and CH$_3$CO$_2^-$, basic pH, non-aqueous medium, high detection limit, risk of releasing HCN during experiment etc.[11,12,20,22,24,29,30]

Keeping the reported limitations in mind, we have designed a fluoroionophore containing photoactive Ru(II)-bipyridine unit as fluorogenic unit and amide containing calixarene moiety as binding site for anions. As receptor, calixarenes are found to be very attractive because modifications of calixarene gives rise to a large variety of derivatives with various functional groups, which provide a highly preorganized architecture for the assembling of converging binding sites.[31-35] As mentioned above, incorporation of metal ion has some advantages, the positive charge on it is expected to assist strong hydrogen bonding interaction between anions and NH and OH proton of the calix moiety. Moreover the spectroscopic and electrochemical properties of metal ion can also be used for monitoring of the recognition event.[36-38] This new fluoroionophore selectively detects CN$^-$ of NaCN in aqueous media in presence of various other anions including most interfering anions, F$^-$ and CH$_3$COO$^-$ and binding of CN$^-$ is also reversible. The performance of this CN$^-$ sensor has also been tested successfully in real samples. Herein we report synthesis and characterization of this new fluoroionophore and details of its performance as anion sensor in aqueous media.

Result and discussion

Synthesis and characterization of the fluoroionophore (1)

The route followed for the synthesis of **L** and its Ru(II) complex (**1**) is shown in scheme 1 and details of the experimental procedure is given in the Experimental Section. Compound **L** was synthesized from compound **C** and **F** (scheme 1) in dry acetonitrile using Et$_3$N as base under inert atmosphere. The acid chloride (**F**), obtained from corresponding acid (**E**), was directly used for the next step without isolation. Elemental analysis (C, H and N), IR, ESMS and ^{1}H NMR spectral data for the intermediate compounds, ligand **L** and complex **1** are given in the Experimental Section. The elemental analysis and mass data are in excellent agreement with the calculated value. The mass spectra for **L** and **1** are submitted as ESI (Figs. S1 and S2). It may be noted that the *m/z* value of the compound **L** corresponds to the H$^+$ adduct, which is a well-known phenomenon when LC-MS is used for the measurement of mass.[32-34,36]



Scheme 1. Route followed for synthesis of **L** and **1** is shown. Reagents and conditions: (I) CH$_2$BrCO$_2$Me, K$_2$CO$_3$, ACN; (II) NH$_2$CH$_2$CH$_2$NH$_2$, MeOH; (III) SeO$_2$, dioxane ; Ag$_2$O, dioxane/EtOH; (IV) SOCl$_2$, toluene; (V) ACN, Et$_3$N; (VI) Ru(bpy)$_2$Cl$_2$, CAN.

The IR spectra of L exhibited bands of moderate intensity at 3545 cm^{-1} and 3049 cm^{-1}, which are due to ν(OH) of phenolic group of calixarene and ν(NH) of the amide moiety, respectively. The strong bands, which appeared at 1677 cm^{-1} is assigned to ν(C=O). In the ^{1}H NMR spectrum of **L** (Fig. S3, ESI), the meta- protons (with respect to the OH substituent) of the calixarene moiety appeared at very closely spaced three singlets in the region δ 7.13-6.95, which is expected due to partial cone conformation of the calix moiety, as shown in scheme 1.[39] Three phenolic –OH appeared at δ 10.17, 9.58 and 9.48, as confirmed by D$_2$O exchange experiment. Bipyridine moiety exhibited five distinct signals in the region δ 8.72-7.61, another signal probably overlapped with the signals from calix moiety. The protons of the amide moieties –CONH- are overlapped with the multiplets due to aromatic moiety around δ 8.5. The methylene protons of ArO-CH_2-CO- appeared as a singlet at δ 4.63 and the Ar-CH_2-Ar protons of the calixarene moiety appeared as two closely spaced doublets in the region δ 3.49-3.31 and one singlet at δ 3.86, which suggest that calixarene moiety exists in a paco conformation.[39] The -CH$_2$ of ethylene chain appeared as a triplet at δ 4.12. The Ru(II) complex of **L** was synthesized by the reaction of cis-[Ru(bpy)$_2$Cl$_2$]$_2$H$_2$O and **L** in refluxing ethanol-water, isolated with PF$_6^-$ counter anion and purified by column chromatography. This complex gave satisfactory C, H and N analysis and the mass data (*e/m*) (Fig. S2, ESI) are in excellent agreement with the calculated value 1503.55. The IR spectra of **1** exhibited a broad band at 3433 cm^{-1}, which is due to ν(O-H). The bands at 1676 and 842 cm^{-1} are assigned to ν(C=O) and PF$_6^-$, respectively. The NMR spectrum (Fig. S4), the data of which are given in the Experimental Section, is consistent to the structure shown in scheme 1.

Anion binding study using fluorescence spectroscopy

The photophysical and electrochemical properties of the ruthenium(II)-bipyridine complexes is well known and these properties can be used as a tool to monitor ion-recognition event. Complex **1**, upon excitation the MLCT absorption band at 460 nm, exhibited a strong emission band at 634 nm, which has been used to monitor its interaction with anions. Upon addition of the Na$^+$ salts of the anions such as F$^-$, Cl$^-$, Br$^-$, I$^-$,

PO_4^{2-}, IO_4^- , BO_3^-, CH_3COO^-, CN^- and SO_4^{2-} in 95:5 H_2O-CH_3CN medium (details of the procedure is given in the Experimental Section), the intensity of the emission band for CN^- anion substantially quenched with slight blue shift, whereas for other anions no significant change is noted (Fig. S5). Bar diagram showing the plot of the relative change in intensity of the emission band with respect to various anions used in this study is shown in Fig. 1. The diagram suggests that the fluoroionophore **1** selectively interacts with CN^- in aqueous media.

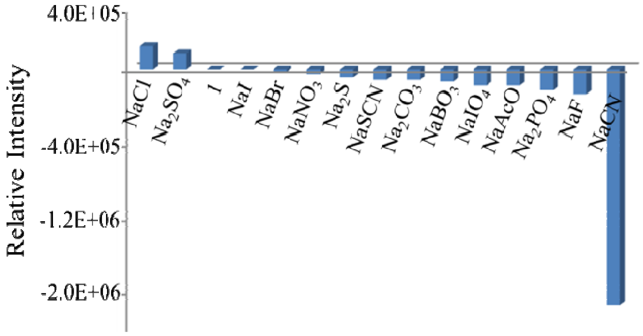


Fig. 1 Bar diagram showing the plot of the relative change in emission intensity for **1** (2×10^{-6} M) in presence of sodium salts of various anions (2×10^{-4} M) in H_2O:CH_3CN (95:5). Excitation wavelength: 461 nm.

Effect of pH

The emission spectrum of **1** was recorded with and without addition of CN^- in aqueous media with variation of pH. It may be noted that complex **1** exhibits its luminescence property in the pH range 2 to 12; however the intensity of the emission band increased at the lower pH (Fig. 2). After addition of NaCN, the intensity of the emission band is quenched but the extent of quenching is low at lower pH, similar to that found in absence of CN^- (Fig. 2). In acidic pH, the –$CONH_2$ can easily get protonated, which expected to lead reverse PET effect resulting increase in emission intensity of the Ru-bypridine moiety.[40]

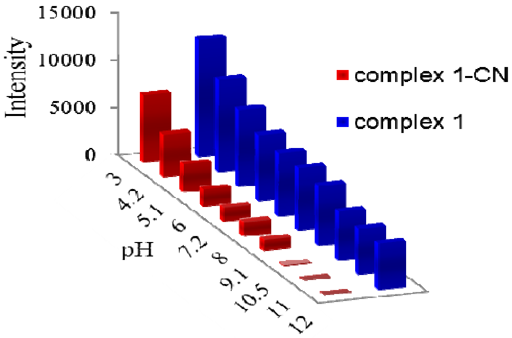


Fig. 2 Bar diagram showing the relative change in emission intensity of complex **1** before (blue bar) and after (red bar) addition of NaCN (25 equivalent) with different pH in water-acetonitrile (95:5)

Interference study

The interferences of other anions for the detection of CN^- was also investigated by recording emission spectra of **1** upon addition of a mixture of 10% of cyanide and 90% of other anions and the plot of the fraction of quenching in emission intensity as a function of mixture of different anions is shown in Fig. 3. It may be noted that no significant interference from any other anion is observed.

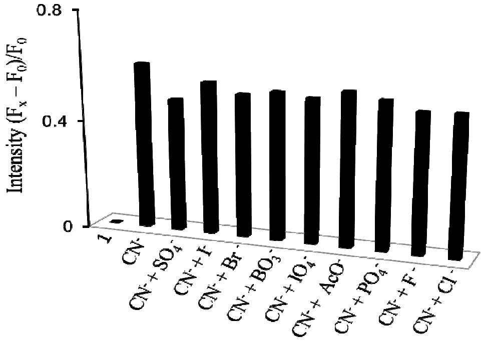


Fig. 3. Bar diagram showing the fraction of quenching in emission intensity for 1 at 642 nm upon addition of CN^- and mixture of anions containing 10% of CN^- (1.2×10^{-6} M) and 90% of other anions (1.08×10^{-5}M) recorded in H_2O-CH_3CN (95:5) solvent

Reversible binding of CN^-

The reversible binding of CN^- with **1** was also investigated using $Cu(ClO_4)_2$, as it can subtract CN^- from the complex forming insoluble CuCN.[34] It may be noted that after addition of 1 eqivalent of $Cu(ClO_4)_2$ with respect to NaCN added into the solution of **1**, the emission intensity is regained and it is close to that observed for **1** before addition of CN^-. To this solution, after further addition of same equivalent amount of NaCN, similar quenching as noted earlier was observed (Fig. S6, ESI). The observation thus indicates that the binding of CN^- with complex **1** is reversible in aqueous medium.

Determination of binding Constant

Binding constant of CN^- with complex **1** in aqueous solution (H_2O:CH_3CN, 95:5) was determined by fluorescence titration method.[36,41] Detail of the method has given in the Experimental Section and the emission spectral change upon incremental addition of CN^- is shown in Fig. 4. Binding constant was calculated from the titration data using the equation $(F_0 - F_x)/(F_x - F) = ([M]/K_{diss})^n$. The binding constant ($Ks$) was obtained by plotting $\log[(F_0 - F)/(F - F_\infty)]$ *vs.* $\log[M]$, the double logarithm plot, which exhibits linearity in the range 5.03×10^{-5} to 1.12×10^{-4} M ($R^2 = 0.969$), shown as inset in Fig.4, the slope of this line (1.86115) gave the binding constant, 2.5×10^4 M^{-1} and the value of n (2.2), which suggests 1:2 stoichiometry. The lower limit of detection of NaCN was also calculated from the emission spectral change and it is found to be 70 ppb in the same solvent system.

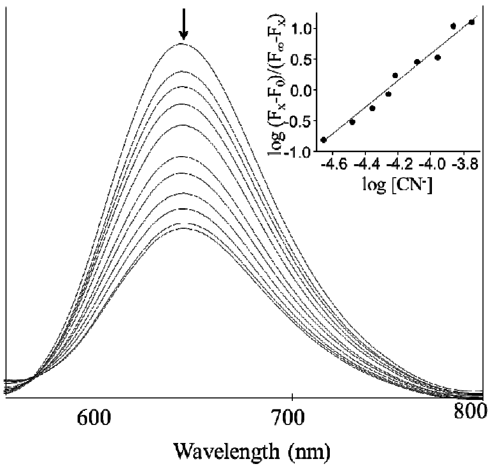


Fig.4 Emission spectral changes of 1(1 x 10^{-6} M) upon addition of increasing concentration of NaCN. Excitation wavelength: 458 nm. Inset: linear regression fit (double-logarithmic plot) of the titration data as a function of concentration of CN^- ion

Anion-sensing study using tetrabutylammonium salts

Anion-binding study when conducted with tetrabutylammonium (TBA) salt under similar experimental conditions as used for NaCN, then selectivity pattern was changed. Like NaCN salt, the TBA salt of CN^- exhibited substantial quenching, however fluoride also showed some quenching but CH_3COO^- exhibited significant enhancement in emission intensity, whereas other anions did not show any considerable change (Fig. 5). The observation suggest that the cation also plays important role in sensing and in presence of TBA ion, like CN^-, CH_3COO^- also makes strong interaction with **1** and F^- also shows weak interaction. This cation dependence selectivity is probably related to interaction of cation with the calix moiety and also its conformation in solution. It is well established that alkali metal ions form complex with calixarene and metal-calix interaction takes place at the lower rim of the calix moiety because of the presence of OH groups and also due to enhanced π electron delocalization at that site.[42] Anions can interact with the hydrogen atoms of the amide moiety or OH groups of the calix, however in this case NMR study showed that (discussed in the mechanism section) anions made interaction with the amide hydrogen atoms. Therefore, if Na^+ is in the vicinity of the lower rim of the calix, then due to steric crowding it is difficult for the large size anions to enter in the loop made by the calix unit and Ru-bipyridine unit (structure of 1 in scheme 1) to make interaction with the amide hydrogen atom. The TBA cation being large size, it is difficult for it to enter in the loop to make any interaction and therefore anions are free to enter in the cage like space to interact with the amide. This is probably the reason why acetate interacts strongly when TBA cation is used but not in presence of Na^+ salt. We have also carried out study with 1,butyl-3-methylimmidazolium acetate (ionic liquid), which contains large size cation and observed that like TBA^+, it also exhibits significant enhancement in emission intensity of **1** (Fig. S7), which supports the speculation that large size cations do not enter in the loop to make any interaction and it facilitates entry of only anion into the loop to make interaction with the amide moiety. For a particular cation, the ability of anions to make interaction depends on the competitive hydrogen bonding/electron donating ability of the anions in a given solvent. In other words the basicity of the anions and the decreasing order of pK_b value of anions in aqueous medium is CN^- > AcO^- > F^- > other halides.[43] This is qualitatively consistent to the selectivity observed. The mode of binding of anions and the consequent effect on emission spectra are discussed below in the mechanism section.

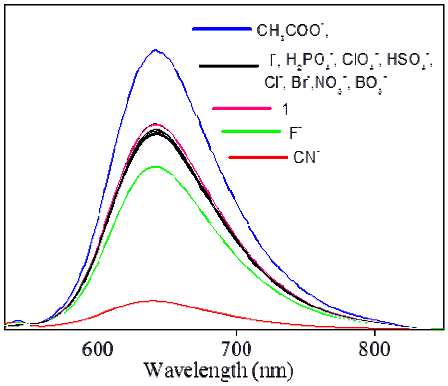


Fig. 5 Emission spectral changes of 1 (1 x 10-6 M) in presence of various anions in H2O: ACN (95:5) solvent. Excitation wavelength 461.

Binding constants for AcO^- and CN^- with TBA cation have also been calculated from emission titration data following the similar procedure as described above for NaCN. The spectral change for acetate is shown in Fig. 6 and that of CN^- is submitted as ESI (Fig. S8) and the binding constants obtained

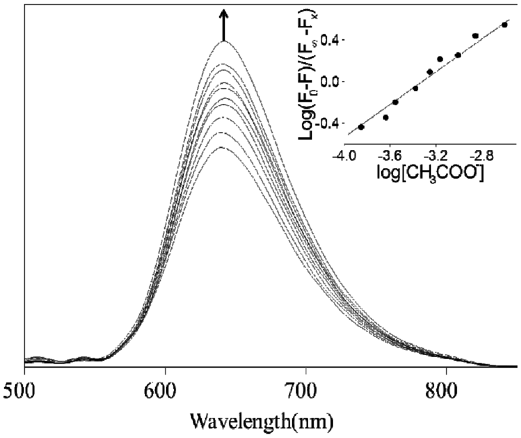


Fig.6 Emission spectral change of complex**1** (1 x 10^{-6} M) upon addition of increasing amount of AcO^- in H_2O-CH_3CN (95:5). Excitation wavelength:458 nm. Inset: linear regression fit (double-logarithmic plot) of the titration data as a function of concentration AcO^- ion.

are 8.4×10^4 M^{-1} and 2.4×10^3 M^{-1} for CN^- and AcO^-, respectively. Slight difference in binding constant for CN^- with Na^+ and TBA^+ ions is probably due to involvement of cation(s) in making interaction with the calix moiety.

Anion sensing by electrochemical method

For Ru(II)-bipyridine based molecular sensor, the redox property of the Ru(II) can be used for monitoring interaction of analite with the sensor molecule.[36-38] The redox property of Ru(II) of **1** was investigated by recording cyclic voltammetry (CV) as well as differential pulse voltammetry (DPV) in acetonitrile and the CV and DPV for the region 0.0 to +2.0 V are shown in Fig. S9. It may be noted that the CV of the complex **1** exhibited a quasireversible redox wave at the potential 1.35 V and the DPV also showed a oxidation wave at the same potential, which is attributed to Ru(II) → Ru(III) oxidation.[36,44,45] The CV and DPV of **1** was again recorded under similar experimental conditions in presence of excess amount (50 molar equivalent) of TBA salts of CN^- and AcO^-. The DPV of **1** before and after addition of CN^- and AcO^- are shown in Fig. 7. It is interesting to note that in presence of CN^-, the oxidation potential of Ru(II) is cathodically shifted from 1.35 to 1.1V and in presence of AcO^- the same oxidation potential has anodically shifted from 1.35 to 1.8 V. The observation suggests that both the CN^- and AcO^- anions interacted with the molecule and altered the redox potential of the metal ion significantly. However, the opposite shift of the redox potential indicates the interaction with anions resulted in opposite electronic effect on the metal ion. The CN^- ion having strong back bonding ability, enhanced the electron density on the metal ion resulting in oxidation of it at lower potential whereas the AcO^- ion has reduced the electron density on Ru(II) making it difficult to oxidize and hence oxidation occurred at higher potential. The observation is consistent to the finding noted in luminescence study, the CN^- with higher electron density on metal ion promoted intramolecular quenching whereas AcO^- with lower electron density on metal ion reduced intramolecular quenching, which in turn enhanced intensity in emission band.

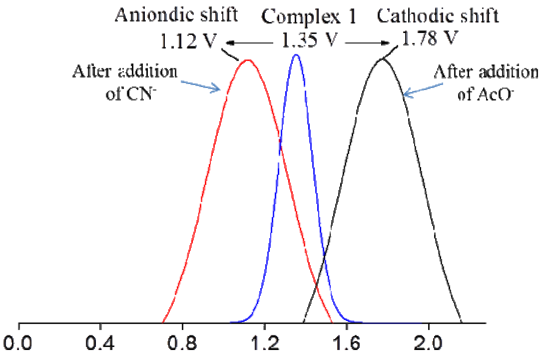


Fig. 7 Differential pulse voltammogram DPV) of **1** before and after addition of TBA salts of CN^- and AcO^- (50 molar equivalent excess) recorded in acetonitrile.

Mass spectrometry

In order to find out the composition of the complexes formed, mass spectra of **1** were recorded in acetonitrile/water upon addition of excess (10 molar equivalent) amount of tetrabutylammonium salts of AcO^- and CN^-. The relevant portions of the mass spectra of **1**, recorded with CN^- and AcO^- are shown in Fig. 8 and S10 (ESI). In the case of CN^-, the *e/m* value 1429.74 corresponds to the species $[\mathbf{1}\text{-}2PF_6^-{+}2CN^-{+}H_2O\text{-}H^+]^-$ (calculated value 1429.72), in which one of the OH is deprotonated to generate anionic species and the composition is consistent to the formation of 1:2 complex. For acetate ions, the observed *e/m* value 1764.43 corresponds to the composition $[\mathbf{1}{+}AcO^-{+}ACN{+}H_2O]^-$ (calculated value 1767.92), which is in agreement with the 1:1 complex formation, as found from emission titration.

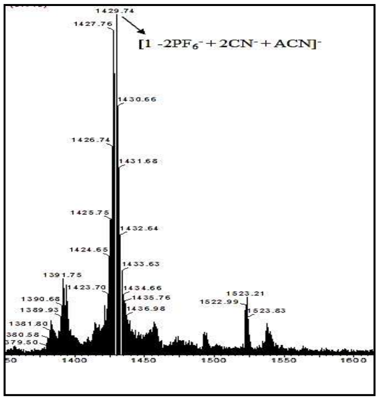


Fig. 8 Relevant portion of the mass spectra for **1** in presence of TBACN (10 equivalents) recorded in acetonitrile.

Mechanistic aspects of anion binding

To investigate the site of interaction by anions, ^1H NMR study was carried out. ^1H NMR spectrum of **1** was recorded in CD_3CN before addition of anion and then after incremental

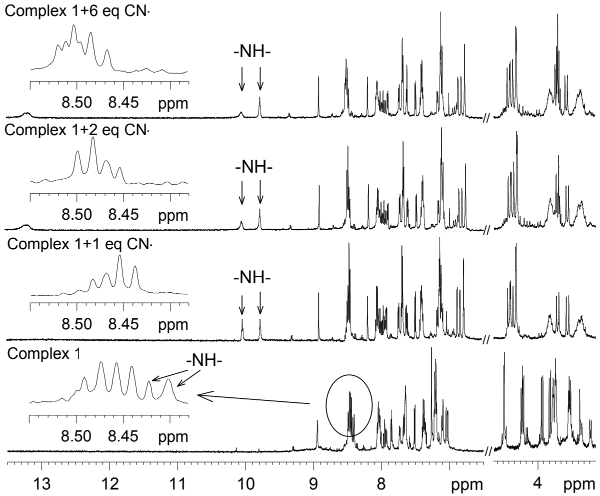


Fig. 9 Selected portion of the ^1H NMR spectra of complex 1 in CD_3CN with addition of increasing amount of CN^-.

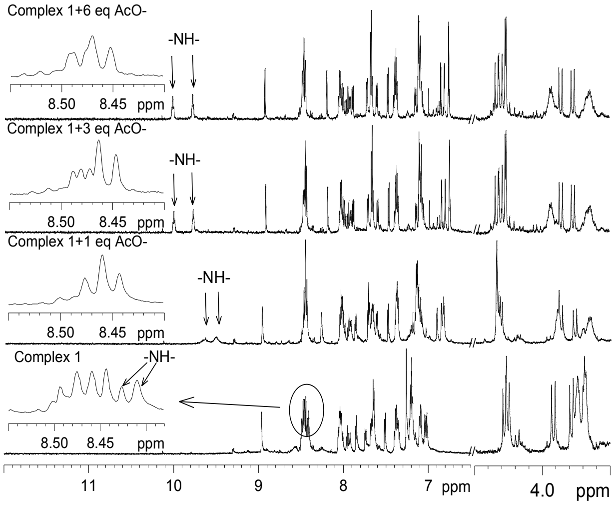


Fig. 10 Selected portion of the ^{1}H NMR spectra of complex 1 in CD_3CN with addition of increasing amount of AcO^-

addition of anions up to six molar equivalents. The spectra thus obtained for CN^- and AcO^- are shown in Figs. 9 and 10. It may be noted that two N-H signals, which were overlapped with the aromatic protons in the range δ 8.39-8.46, appeared as two distinct singlets and the significant down field shifts of the signals is due to strong interaction with anions. It may be noted that the intensity of one of these two signals reduced with the formation of a new broad signal at δ 13.20 upon addition of more than equimolar amount of CN^-. This broad signal observed at δ 13.2 suggests that one of the protons behaves differently and it may be due to very strong interaction with CN^- and also formation of HCN by deprotonation of amide N-H may not be ruled out.[24] For AcO^- anion, almost similar observation was noted (Fig. 10), however intensity of the new signal for N-H did not diminish and no new broad signal, as observed for CN^-, was noted. On the basis of this information, it is proposed that the two oxygen atoms of the AcO^- anion interacted with two N-H protons, as shown in Fig. 11. Probably the electron delocalization at the bidentate acetate anion pulls electron density towards itself from the adjacent amide groups reducing intramolecular luminescence quenching, which consequently enhanced intensity of the emission band. For CN^-, the push of electron density from two anions to the bipyridine ligand enhanced electron density causing enhancement of intramolecular quenching. It is also consistent to the observation noted in the electrochemical oxidation of metal ion in presence of CN^- and AcO^-.

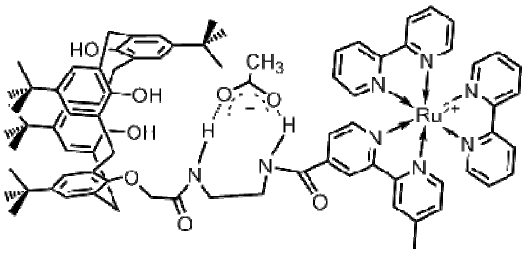


Fig. 11 Proposed structural drawing of the CH_3COO^- bound complex **1**.

Application in real sample

Complex **1** has been used for quantitative detection of cyanide in human saliva and drinking water. As the human saliva and drinking water is free from CN^-, known concentration (given in table) of sodium cyanide has been spiked. At first human saliva and drinking water samples collected were filtered through 0.45 μm GNWP nylon membranes to remove insoluble materials, if any, and 0.5 mL samples of both of them have been spiked with suitable amount (2 x 10^{-4} M) of sodium cyanide stock solution. Then the sensor has been used to estimate the concentration of NaCN (recovery) in human saliva and drinking water by using the standard double logarithm curve generated by estimating CN^- in various solution of known concentration (Fig. 12). The results obtained are summarized in Table 1, which shows satisfactory recovery of NaCN compared to the amount added. This experimental result thus demonstrated that this fluorometric assay has potential for detection of NaCN in various samples in aqueous media.

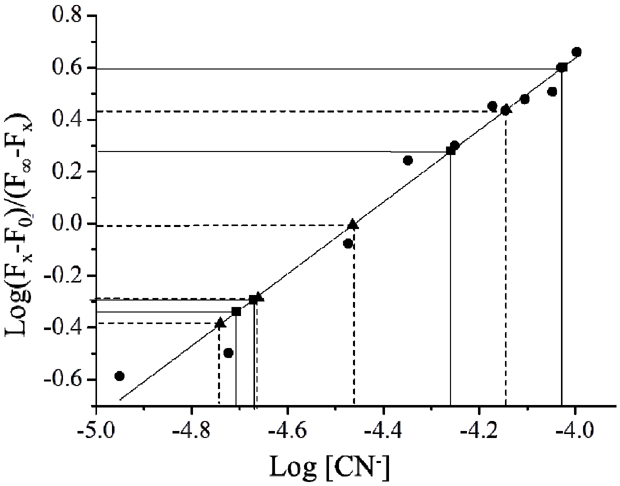


Fig. 12 Calibration line generated from standard solution of NaCN (•) in aqueous media and it has been used for estimation of CN^- in real sample. The values for spike of NaCN in drinking water (□) and human saliva (Δ) are shown and corresponding concentrations are calculated.

Table1. Determination of CN^- of NaCN in real samples

Drinking water	Added (ppm)	Found (ppm)	Recovery (%)	RSD% (n = 3)
D0	0	nda	-	-
D1	0.40	0.42	105	±4
D2	0.50	0.48	96	±7
D3	1.50	1.60	106	±2
D4	2.50	2.44	98	±6
Human Saliva				
S0	0	nd[a]	-	-
S1	0.40	0.38	95	±5
S2	0.50	0.47	94	±3
S3	1.00	1.10	110	±6
S4	2.00	2.10	104	±7

Conclusions

A molecular sensor comprising calix[4]arene as receptor and ruthenium(II)-bipyridine moiety as fluorophore connected by amide moiety has been synthesised and its anon binding property has been investigated. It selectively detects CN^- out of a large number of Na^+ salts of various anions in virtually aqueous media with lower detection limit 70 ppb, whereas with tetrabutylammonim salts, it detects CN^- as well as AcO^- under similar experimental conditions. The anion recognition event was monitored by fluorescence spectroscopy and electrochemical study. Composition of the anion bound complexes and their binding constants were determined. Mechanism of interaction and possible energy transfer process involved in the recognition event has been discussed with the aid of spectroscopic data. The sensor has also been tested successfully to estimate cyanide in real samples such as water from lake and saliva.

Experimental Section

Instrumentation

Elemental analysis (C, H, and N) were performed on a model Vario Micro CUBE elemental analyzer. Mass spectra were recorded on a Q-TOF MicroTM LC-MS instrument. Infrared spectra were recorded on a Perkin-Elmer spectrum GX FT-system as KBr pellets. NMR spectra were recorded on models DPX 200 and Avance II 500 Brucker FT-NMR instruments. The UV/Vis and luminescence spectra were recorded on a CARY 500 scan Varian spectrophotometer and model Fluorolog Horiba Jobin Yvon spectrofluorimeter at room temperature. Electrochemical measurements were carried out using PARASTA 2273 equipment.

Chemicals and reagent

The compounds 2, 2'-bipyridine, 4,4'dimethyl-2,2'-bipyridine, selenium dioxide, ammonium hexafluorophosphate, tetrabutylammonium salts of anions used in this study were purchased from Alfa Aesar (Johnson Matthey Company). Hydrated ruthenium trichloride was purchased from Arora Matthey. Neutral alumina and silica gel were obtained from the National Chemical Co. All other reagents including sodium salts of anions used in this study were purchased from S.D. Fine Chemicals. All organic solvents were analytical grade and were used as received for synthetic purpose. Solvents for spectral studies were freshly purified by standard procedures. The starting compounds p-tert-butylcalix[4]arene,[46] calix[4]arene,[47] calix[4]arene monoester,[48] 4'Methyl-(2,2'-bipyridine-4-caboxylic acid, cis-$[Ru(bpy)_2Cl_2].2H_2O$[49] were synthesized following the literature procedures.

Synthesis of the compound C

A mixture of compound B (0.72 g, 1mmol) and excess amount of ethylene diamine (0.5 mL, 7.5mmol) was refluxed in chloroform-methanol (1:3, 60 mL) for 12 h. To this solution, upon addition of water (1 mL) a white precipitate was formed. It was filtered-off and washed with water and methanol. The white product was then kept in high vacuum for overnight. Yield: 0.83 g (80 %). IR, ν_{max} (KBr pellet)/cm^{-1} 3398 $\nu_{(OH)}$, 1688 $\nu_{(C=O)}$; ^1H NMR (200 MHz, $CDCl_3$): δ 9.25 (br s, 1H, Ar-OH), 7.08-7.02 (overlapped signals, 8H, Ar-Hm), 4.6 (s, 2H, -OCH_2CO), 4.26-4.16 (m, 4H, $ArCH_2Ar$), 3.61-3.47 (m, 8H, $ArCH_2Ar$, -$CONHCH_2CH_2$-NH-), 1.22-1.18 (overlapped signals, 36H *J* = 13.4 Hz, - $C(CH_3)_3$). ESMS (m/z): found 748.31(100%) calcd for $[\mathbf{C} - H^+]^-$ 748.02. Anal. calcd. for $C_{48}H_{64}O_5N_2$; C, 76.96; H, 8.86; N, 3.73. Found: C, 75.58; H, 8.61; N, 3.78.

Synthesis of the compounds F and L

A mixture of E (0.428 g, 2 mmol) and thionyl chloride (3 mL) in 30 mL dry toluene was stirred and reflux under nitrogen for 6 h. The excess $SOCl_2$ and toluene was then removed by rotary evaporation. The resulting greenish-yellow solid mass (F) was used directly in the next step for the synthesis of L. In the next step, compound C (1.50 g, 2 mmol) was dissolved in dry ACN (50 mL) and triethylamine (1 mL) was added to this solution. Then the ACN solution (30 mL) of the acid chloride (F) obtained in the previous step was added dropwise to the reaction mixture over a period of 1h and the solution was then stirred at room temperature for another 2 h. Finally the reaction mixture was refluxed for 24 h under nitrogen atmosphere. The solution was then allowed to cool to room temperature and evaporated to dryness by rotary evaporation. The residue was dissolved in $CHCl_3$ (100 ml), and the organic layer was washed three times with water (70 ml each time), dried over Na_2SO_4 and evaporated in vacuo. The crude product was purified by column chromatography on activated neutral alumina by using 1% (v/v) MeOH/$CHCl_3$ as eluent. Yield: 0.592 g, (45 %). IR, ν_{max} (KBr pellet)/cm^{-1} 3545 (OH), 3049 (NH), 1677 (C=O). ^1H NMR (500 MHz, $CDCl_3$): δ = 10.13 (br-S,1H, -CONH), 9.58-9.48 (m, 3H, Ar-OH), 8.72 (s, 1H, ligand bipy-H), 8.62 (d, 1H, J = 5 Hz, ligand bipy-H), 8.46 (d, 1H, *J* = 4.8, ligand bipy-H), 8.17 (s, 1H, ligand bipy-H), 7.62 (d, *J* = 3.6, 2H, ligand bipy-H), 7.00-7.05 (m, 6H Ar-Hm), 6.95 (s, 2H, Ar-Hm), 4.63 (s, ArO-CH2-CO-), 4.12 (t, 4H, *J* = 12.6 Hz, CONH-CH_2-CH_2-CONH-),3.86 (s, 4H, Ar-CH_2-Ar), 3.49-3.31 (m, 4H, Ar-CH_2-Ar), 2.43 (s, 3H, bipy-CH_3), 1.19 (br-s, 27H, -$C(CH_3)_3$),1.15 (br-s, 9H, -$C(CH_3)_3$). ESMS (m/z): found 946.38 (100%) calcd for $[\mathbf{L} + H^+]^+$ 946.23. Anal. calcd. for $C_{60}H_{72}N_4O_6$: C, 76.24; H, 7.868; N, 5.93. Found: C, 76.04; H, 7.43; N, 5.77.

Synthesis of complex $[Ru(bpy)_2(L)](PF_6)_2$ (1)

A mixture of cis-$[Ru(bpy)_2Cl_2].2H_2O$ (0.13 g, 0.25 mmol) and compound L (0.24 g, 0.25 mmol) in ethanol-water (2:1, 60 mL) was refluxed for 10 h. The reaction mixture was then allowed to cool to room temperature; volume was reduced to ca. 20 mL by rotary evaporation, filtered and to the filtrate was added solid NH_4PF_6 (0.82 g, 5 mmol). The precipitate thus separated was collected by filtration washed with water and diethyl ether. The complex was purified by column chromatography using a column packed with deactivated (2% water) alumina and acetonitrile-toluene (1:1) as eluent. The small first fraction was discarded; the large orange-red colored second fraction gave the desired complex. After removing solvent, the residue was again dissolved in acetonitrile and was precipitated by vapor diffusion method using diethyl ether. Yield: 60-70%. IR, ν_{max} (KBr pellet)/cm^{-1} 3433 (OH), 1653 (C=O). ^{1}H NMR [CD$_3$CN] δ = 8.90 (s, 3H, Ar-OH), 8.39-8.49 (m, 6H, bipy-H, -CONH), 8.06-8.08 (m, 3H bipy-H), 7.94 (t, *J* = 4.0, 2H, bipy-H), 7.86 (1H, *J* = 6, ligand bipy-H), 7.71 (d, *J* = 6.0 1H, ligand bipy-H), 7.64-7.68 (m, 4H, bipy-H), 7.51 (d, *J* = 5.5, 1H, ligand bipy-H), 7.40-7.38 (m, 3H, ligand bipy-H, bipy-H), 7.27 (s, 2H, ligand bipy-H), 7.20-7.25 (m, 4H, Ar-Hm), 7.11 (d, 2H, *J* = 5.5, Ar-Hm), 7.05 (d, *J* = 7.75, 2H, Ar-Hm), 4.59 (s, 2H, ArO-CH$_2$-CO-), 3.91 (t, *J* = 11.5, -CH$_2$-CH$_2$-CONH-), 3.44-3.48 (m, 4H, CONH-CH$_2$-CH$_2$-CONH-), 3.80 (d, *J* = 13.5, Ar-CH$_2$-Ar), 3.75 (d, *J* = 20.5, 1H), 3.72-3.62 (m, 4H, Ar-CH$_2$-Ar), 3.28 (d, *J* = 13.5, 1H, Ar-CH$_2$-Ar), 3.12 (d, *J* = 13.5, 1H Ar-CH$_2$-Ar), 2.34 (s, 3H, bipy-CH$_3$), 1.15-1.17 (m, 36H, -C(CH$_3$)$_3$), MS (m/z): found, 1503.57 (65%) calcd for [**1** - PF$_6^-$]$^+$, 1503.55. Anal. calcd. for $C_{80}H_{90}RuN_8O_6P_2F_{12}.2H_2O$; C, 57.03; H, 5.50; N, 6.66. Found: C, 56.83; H, 5.63; N, 6.66. UV-Vis: λ_{max} (CH$_3$CN)/nm (ε/dm^3 mol^{-1} cm^{-1}): 461 (10730), 289 (25600).

Ion-binding Study

Selectivity of 1 with anions was examined by fluorescence study. The luminescence spectrum of complex 1 (1×10^{-6} M) was recorded with excitation at the absorption maxima (λ_{max}) of the MLCT band, which is 461 nm, then luminescence spectra of 1 upon addition of anions (100 fold excess, 1×10^{-4} M) were recorded. These spectra were compared with that of the compound 1 to ascertain the interactions of the anions with the ionophore. For the determination of binding constant with strongly interacting anions, emission titration was performed. For this purpose, same stock solution of the complex was used and the solutions of the anions with desired concentrations were prepared by diluting the concentrated standard stock solution (2×10^{-3} M). Then, 2 mL of each solution was mixed in a 5 mL volumetric flask and the luminescence spectra of the resulting solutions were recorded. The binding constants were calculated following the literature procedure.[41,50] The binding constant and stoichiometry of the complex formation were calculated following literature procedure describe in result and discussion section. The stock solution of (1×10^{-4} M) was also used to study UV/ Vis spectral changes. For NMR study, 2 mg of complex was dissolved in 0.5 ml of [D$_3$] acetonitrile and ^{1}H spectrum of the resulting solution was recorded. The tetra butyl ammonium salt of cyanide and acetated (1-10 equivalent) was added to the solution and the spectrum of the solutions were recorded.

Electrochemical study

Cyclic and differential pulse voltammetry (CV and DPV) studies were carried out in a three-electrode cell consisting of a platinum working electrode, a platinum-wire auxiliary electrode, and an SCE reference electrode. Solutions of the complexes in purified acetonitrile containing 0.1M tetrabutylammoniumtetrafluoroborate as supporting electrolyte were deaerated by bubbling nitrogen for 10 min prior to each experiment.

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