Research Article

Journal of Molecular Recognition

Received: 20 September 2012, Revised: 31 December 2012, Accepted: 29 January 2013, Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jmr.2268

# Simple azo-based salicylaldimine as colorimetric and fluorescent probe for detecting anions in semi-aqueous medium

**Sivalingam Suganya and Sivan Velmathi***

**A series of novel, highly sensitive, and selective azo-based anion sensors 1–3 have been designed and synthesized from the condensation reaction between 4-amino azo benzene and three different aldehydes. The structure of the sensors 1–3 were confirmed by IR, HRMS, $^{1}$H NMR, and $^{13}$C NMR spectroscopic methods. Colorimetric naked-eye analysis revealed the anion detection by receptors 2 and 3 as color changes from yellow to pink and yellow to orange, respectively. Anion sensing ability of all receptors was further investigated by $^{1}$H NMR titration, UV-vis experiment, and fluorescence titration. UV-vis measurements highly indicate the selective recognition of fluoride and acetate ions in 9:1 dimethyl sulfoxide–$H_2O$ (v/v) for receptors 2 and 3. Binding constant value showed among all receptors, receptor 3 has strong affinity toward $F^-$ and $AcO^-$ in semi-aqueous medium, which is due to the presence of chromogenic signaling unit in it. The $F^-$ ion detection property of receptor 2 in organic medium was also extended in the real sample like toothpaste. Copyright © 2013 John Wiley & Sons, Ltd.**
*Supporting information may be found in the online version of this paper*

**Keywords:** chromogenic sensor; Azo derivative; $F^-$and $AcO^-$ recognition; aqueous medium; fluorescence

## INTRODUCTION

The construction of chemical sensor toward various anions (Suksai and Tuntulani, 2003) is an extensive area in the field of supra molecular chemistry (Gale *et al*., 2008; Gale and Quesada, 2006) due to their vital roles in biological system and environmental and clinical applications. Anions play an important role in a wide range of industrial (Lehn, 1985; Hijji *et al*., 2009; Zuhair *et al*., 2006) and biological processes (Nguyen and Kim, 2009; Mizuno *et al*., 2002; Yu *et al*., 2007; Beer and Gale, 2001), and considerable attention has been focused for the recognition of anion species selectively through visible, electrochemical, and optical responses. Among all anions, fluoride and acetate are of particular interest because of their wide range of biological and environmental application. For example, $F^-$ ion is used for the prevention of dental problems and treatment of osteoporosis, and excess fluoride causes fluoride toxicity, namely, "fluorosis" (Xu and Tarr, 2004; Sole and Gabbai, 2004; Lee *et al*., 2004; Cho *et al*., 2003). $AcO^-$ have abundant application in metabolic processes of enzymes and antibodies through carboxylate ion, and $AcO^-$ production has been used as an indicator of organic decomposition (Ho *et al*., 2002; Gupta *et al*., 2006; Nie *et al*., 2004). Therefore, much attention has been paid to design anion sensor for the detection of $F^-$ and $AcO^-$ ions.

The common binding sites for hydrogen bonding formation used in fluorogenic anion sensors are imines (Suganya *et al*., 2011; Udhayakumari *et al*., 2011), urea (Cho *et al*., 2005), thiourea (V´azquez *et al*., 2004), amide (Kang *et al*., 2006), phenol (Libra and Scott, 2006), –OH (Lee *et al*., 2001), and pyrrole (NH) subunits (Velmathi *et al*., 2012). The advantage of producing aqueous sensing receptor is the determination of water content in a given substrate (Zhang *et al*., 2009), and also the contact between anions and organic molecules in biological and environmental systems will occur in aqueous medium. However, the difficulty in making the anion sensor in aqueous medium is that strong hydration stops the sensors from detection of anions through hydrogen bonding. Still now, only a few sensors have been designed for anion recognition in the aqueous phase (Wang *et al*., 2005; Gunnlaugsson *et al*., 2006; Kim *et al*., 2009; Chaicham *et al*., 2010). This diversity made an interest to develop anion sensors **2** and **3** that work in dimethyl sulfoxide (DMSO) and in DMSO/$H_2O$ binary solutions by colorimetric titration and UV-vis spectroscopy.

In this article, we described the synthesis and anion sensing behavior of sensors **1–3** bearing phenol O–H and imine group. Sensor **1** was reported by Peker and Serin (2004) for the preparation of metal complex. To the best of our knowledge, for the first time, we are reporting the synthesis of sensors **2** and **3** and anion sensing studies of sensors **1–3**. Sensors **2** and **3** showed colorimetric sensitivity for $F^-$ and $AcO^-$ in DMSO/$H_2O$ (9:1 v/v) solutions. The detection of anions in aqueous environment is the most interesting target in chemosensor field. Water plays an important role in the analysis of real samples in industries, and the detection of anions in biological system needs an aqueous environment.

* *Correspondence to: Sivan Velmathi, Organic and Polymer Synthesis Laboratory, Department of Chemistry, National Institute of Technology, Tiruchirappalli-620 015, India.*
*E-mail: velmathis@nitt.edu*

*S. Suganya, S. Velmathi*
*Organic and Polymer Synthesis Laboratory, Department of Chemistry, National Institute of Technology, Tiruchirappalli 620 015, India*

## EXPERIMENTS

### Materials

All solvents such as dimethyl sulphoxide (DMSO), acetonitrile (ACN), ethanol (EtOH), and reagents used for spectroscopic experiments and synthesis were received commercially and used as such without any further purification. All anions used for titration experiment were added in the form of tetrabutylammonium (TBA) salts, which were purchased from Sigma-Aldrich Chemical, stored in a vacuum desiccator containing self-indicating silica, and dried fully before using.

### Apparatus

$^1$H NMR spectra were obtained on a BRUKER AV III 500-MHz spectrometer using DMSO-$d_6$ as solvent. IR spectra were recorded on NICOLET IS5 instrument using KBr plates. UV-vis spectra were recorded on a Shimadzu UV-2600 spectrophotometer with a quartz cuvette (path length = 1 cm) at room temperature (r.t). Fluorescence spectra were recorded on Shimadzu RF-5301 PC spectrophotometer. A $2.5 \times 10^{-5}$ M solution of receptors **1–3** and $1.5 \times 10^{-3}$ M TBA salts of the respective anions in DMSO and ACN were prepared and used for all spectroscopic studies.

### General experimental procedure for the synthesis of the receptors

One millimole of 4-amino azobenzene (0.197 g) and 1 mmol of salicylaldehyde (0.1221 g) were dissolved in 10 ml of EtOH, and the resulting mixture was allowed to reflux for 2–3 h at 70 °C. After cooling to room temperature, the precipitate formed was filtered, washed with cold EtOH, recrystallized from EtOH, and dried in a vacuum oven (receptor **1**). Following the previous procedure, receptors **2** and **3** were synthesized (Figure 1). The results were as follows:

Receptor 1. Yield, 80%; mp, 160 °C. $^1$H NMR (500 MHz, DMSO-$d_6$ δ ppm): 12.83 (s, 1H, OH), 9.04 (s, 1H, CH = N), 8.00 (d, 2H, Ar), 7.98 (d, 2H, Ar), 7.70 (dd, 1H, Ar), 7.58–7.59 (m, 5H, Ar), 7.40–7.45 (m, 1H, Ar), and 6.9–7.0 (m, 2H, Ar).$^{13}$C NMR (125 MHz, DMSO-$d_6$ δ ppm): 164.7, 160.8, 152.4, 151.3, 150.8, 134.2, 133.2, 132, 129.9, 124.4, 123, 119.9, 119.8, 117.2. IR (KBr plates, cm$^{-1}$): 3449, 3057, 1619, 1560.

Receptor 2. Yield, 84%; mp, 204 °C. $^1$H NMR (500 MHz, DMSO-$d_6$ δ ppm): δ 12.69 (s, 1H, OH), 9.04 (s, 1H, CH = N), 8.01–8.10 (m, 2H, Ar), 7.91–7.92 (m, 2H, Ar), 7.82 (d, 1H, Ar), 7.58–7.60 (dd, 4H, Ar), 7.43–7.44 (m, 1H, Ar), 7.33–7.35 (m, 1H, Ar), and 7.05 (d, 1H, Ar). IR (KBr plates, cm$^{-1}$): 3440, 3059, 1610, and 1560. Mass: calculated, 335.7944; found, 335.9604

Receptor 3. Yield, 86%; mp, 230 °C. $^1$H NMR (500 MHz, DMSO-$d_6$ δ ppm): δ 13.91 (s, 1H, OH), 9.22 (s, 1H, CH = N), 8.72 (s, 1H, Ar), 8.29–8.30 (m, 2H, Ar), 7.97 (dd, 4H, Ar), 7.62–7.65 (m, 2H, Ar), 7.17 (d, 1H, Ar), 6.66 (d, 1H, Ar), and 6.08 (s, 1H, Ar). IR (KBr plates, (cm$^{-1}$): 3493, 3096, 1627, and 1584. mass: calculated: 346.342, found: 347.1144.

## RESULTS AND DISCUSSION

Single crystal of receptor 2 was obtained by slow evaporation method in chloroform and its structure was analyzed by X-ray diffraction (Enraf Nonius CAD4-MV31 Bruker Kappa APEXII instrument). The crystal system of receptor **2** was found as orthorhombic with Pca21 space group. Cell parameters

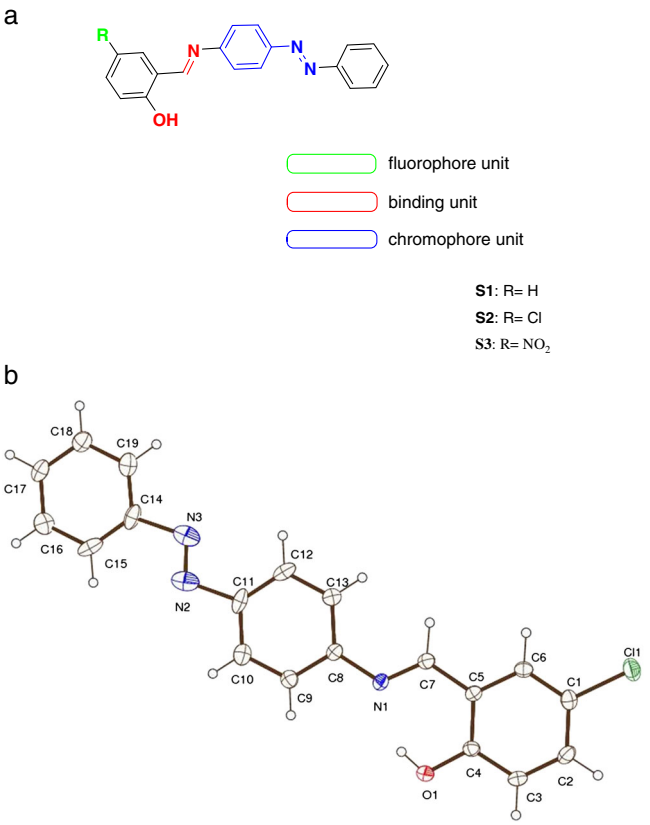



**Figure 1**. (a) Structure of receptors **1**, **2**, and **3**; (b) ORTEP of the receptor 2.

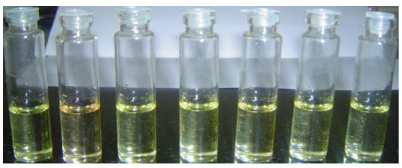
are a = 12.2215(3) A °, b = 4.5174(9) A °, c = 27.775(2) A ° along with R value of 0.0374. The intra molecular hydrogen bonding (2.593 A°) present within the receptor 2 was confirmed from the ORTEP diagram of receptor 2 (Fig.1b). Intra and intermolecular hydrogen bonding and other parameters present in receptor c are given in (Suppo. info Figure. 11).

### Colorimetric naked-eye analysis

The binding event of anions by receptor molecules **1–3** was monitored by "naked eye" through color changes. The variation in color changes can affect the electronic properties of the receptor molecule and lead to the formation of the complex between anion and receptor molecule through hydrogen bonding. All the three receptors are expected to be in transform, so that they can bind with anions effectively through charge transfer mechanism. Also, the *cis* form of azo-based receptors is inactive for anion sensing (Thangadurai *et al.*, 2011). The sensing action of receptors **1–3** were precisely investigated by colorimetric studies, UV-vis, and fluorescence spectroscopy via the addition of 4 Eq of different anions $F^-$, $Cl^-$, $Br^-$, $AcO^-$, $H_2PO_4^-$, and $OH^-$ ($1.5 \times 10^{-3}$ M) to sensors **1–3** ($2.5 \times 10^{-5}$ M), respectively. When 4 Eq of anions ($1.5 \times 10^{-3}$ M in DMSO) was introduced to the ($2.5 \times 10^{-5}$ M in DMSO) solution of sensors **1–2**, sensor **1** responded with slight color changes from yellow to light pink only for $F^-$ ion (Figure 2a) and sensor **2** showed dramatic color change from yellow to pink for $F^-$ and $AcO^-$ ions (Figure 2b). The visible color changes are mainly because of the detection of anions by the receptor molecules through hydrogen bonding. The same titrations were repeated with other anions under same conditions. But no color changes were observed

Journal of Molecular Recognition

with other anions. To verify the sensing ability of receptor **2** and $F^-$/$AcO^-$ in aqueous medium, the anions were prepared in DMSO/$H_2O$ (v/v) (9:1, 7.5:2.5, and 5:5) with different ratios. When the content of $H_2O$ was increased, the pink color of the anion–receptor complex turned into orange (Supplementary Figure 1a) and then yellow, indicating the regeneration of the receptor molecule.



a. Color Changes observed upon the addition of 4 eq. of various anions (1.5 x $10^{-3}$ M) to S1 (2.5 x $10^{-5}$ M) (from left to the right : Free S1, $F^-$, $Cl^-$, $Br^-$, $AcO^-$, $H_2PO_4^-$, $OH^-$)

b. Color Changes observed upon the addition of 4 eq. of various anions (1.5 x $10^{-3}$ M) to S2 (2.5 x $10^{-5}$ M) (from left to the right : Free S2, $F^-$, $Cl^-$, $Br^-$, $AcO^-$, $H_2PO_4^-$, $OH^-$)

c. Color Changes observed upon the addition of 4 eq. of various anions (1.5 x $10^{-3}$ M) to S3 (2.5 x $10^{-5}$ M) (from left to the right : Free S3, $F^-$, $Cl^-$, $Br^-$, $AcO^-$, $H_2PO_4^-$, $OH^-$)

**Figure 2**. (a) Color changes observed on the addition of 4 Eq of various anions ($1.5 \times 10^{-3}$ M) to S1 ($2.5 \times 10^{-5}$ M) (from left to the right: free S1, $F^-$, $Cl^-$, $Br^-$, $AcO^-$, $H_2PO_4^-$, and $OH^-$). (b) Color changes observed on the addition of 4 Eq of various anions ($1.5 \times 10^{-3}$ M) to S2 ($2.5 \times 10^{-5}$ M) (from left to the right: free S2, $F^-$, $Cl^-$, $Br^-$, $AcO^-$, $H_2PO_4^-$, and $OH^-$). (c) Color changes observed on the addition of 4 Eq of various anions ($1.5 \times 10^{-3}$ M) to S3 ($2.5 \times 10^{-5}$ M) (from left to the right: free S3, $F^-$, $Cl^-$, $Br^-$, $AcO^-$, $H_2PO_4^-$, and $OH^-$).

Similarly, the same colorimetric titration was extended to receptor **3** in ACN medium with same concentration of both receptor and anions. The reason for choosing ACN as solvent is because of the presence of highly acidic hydroxyl proton. If polar DMSO is used, it will compete with receptor molecule and affect hydrogen bonding. Hence, visible color change and optical signal did not occurr. With the addition of 4 Eq of anions ($1.5 \times 10^{-3}$ M in ACN) to receptor **3** ($2.5 \times 10^{-5}$ M in ACN), the yellow color of the receptor was changed into orange for $F^-$, $AcO^-$, $H_2PO_4^-$, and $OH^-$ ions (Figure 2c). However, no color change was observed for $Cl^-$ and $Br^-$ ions. The more number of anion detection by receptor **3** is due to the presence of the chromogenic signaling unit ($-NO_2$) in it. Again, to check the sensing ability of receptor **3** in aqueous medium, the anions were prepared in DMSO/$H_2O$ (v/v) (9:1, 7.5:2.5, 5:5) with different ratios. For receptor **3** and $F^-$/$AcO^-$, the orange color of the anion-receptor **3** complex turned into yellow (Supplementary Figure 1b), resulting in the regeneration of the receptor molecule. Finally, the regeneration of receptors **2** and **3** were occurred at DMSO/$H_2O$ (7.5:2.5), which is due to the effect of water molecule in hydrogen bonding of anion complex of receptors **2** and **3**.

## $^1$H NMR titration

$^1$H NMR technique is a powerful technique used to study the molecular interaction between receptor **2** and anions. For the further understanding of the detection nature of anions by receptor **2**, $^1$H NMR titration was carried out using the solution of TBA salt of $F^-$ and $AcO^-$ ions to receptor **2** in DMSO-$d_6$ (Supplementary Figures 2 and 3). Commonly, two main effects will takes place while adding anions to receptor molecules, that is, downfield shifting of protons present with heteroatoms (OH, NH, and SH) due to hydrogen bonding and deprotonation with excess of anions. In the absence of $F^-$ or $AcO^-$ ion, receptor **2** showed singlet at 12.69 ppm for OH proton and at 9.02 ppm CH = N proton. Also, double doublet peak at 7.9–8.0 ppm was observed due to the phenyl ring proton of azo benzene. In the presence of 1 Eq $F^-$ and $AcO^-$ ions, the –OH signal at 12.69 ppm disappeared, suggesting that the OH proton was deprotonated by incoming anions. However, there was no shift in imine and aromatic protons observed with 1 Eq of $F^-$ and $AcO^-$ ions. In addition, the signal corresponding to imine

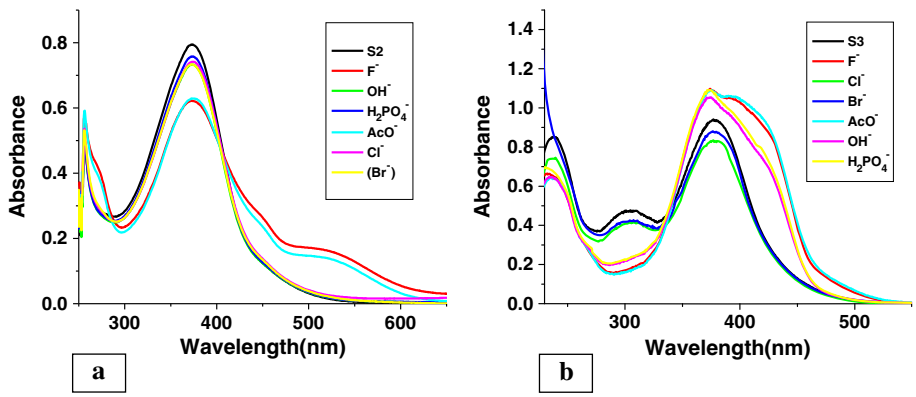



**Figure 3**. Absorption spectra of (a) receptor **2** in DMSO and (b) receptor **3** in ACN recorded ($2.5 \times 10^{-5}$ M) after the addition of 4 Eq of all anions ($1.5 \times 10^{-3}$ M).



(CH = N) proton at 9.00 ppm had a upfield shift to 8.82 ppm with the addition of 4 Eq of $F^-$ and $AcO^-$ ions. Similarly, aromatic rings proton at 7.9–8.0 ppm also got chemical shift in upfield to 7.87 with the addition of 4 Eq of $F^-$ or $AcO^-$ ions. $^1H$ NMR titration concluded that the disappearance of OH signal and chemical shifting of other protons present in receptor **2** system by $F^-$ and $AcO^-$ ions is mainly because of the detection property of anion by receptor **2** via the deprotonation of OH group of receptor **2**, which leads to the formation of phenolate ion. Hence, the electrodensity of aromatic system will be high and act as a π conjugation with aromatic ring.

### UV-vis titration

The anion detection was further confirmed by optical spectroscopy. The spectroscopic titrations were carried out in DMSO medium for receptors **1** and **2** and ACN for receptor **3**. In the absence of anion, both receptors **1** and **2** showed band at 375 nm, which is due to intramolecular hydrogen bonding present within the receptor molecule. With the addition of 4.0 Eq of $F^-$, $Cl^-$, $Br^-$, $AcO^-$, $OH^-$, and $H_2PO_4^-$ ($1.5 \times 10^{-3}$ M in DMSO) into the solution of receptors **1** and **2** ($2.5 \times 10^{-5}$ M in DMSO), receptor **1** showed a new band at 520 nm only with $F^-$ ion; and for receptor **2**, a new and strong absorption peak at 520 nm was observed for $F^-$ and $AcO^-$ ions (Figure 3a). The formation of new band at 520 nm is due to the breakage of intramolecular hydrogen bonding and the formation of new complex between anion and receptor molecule. On the incremental

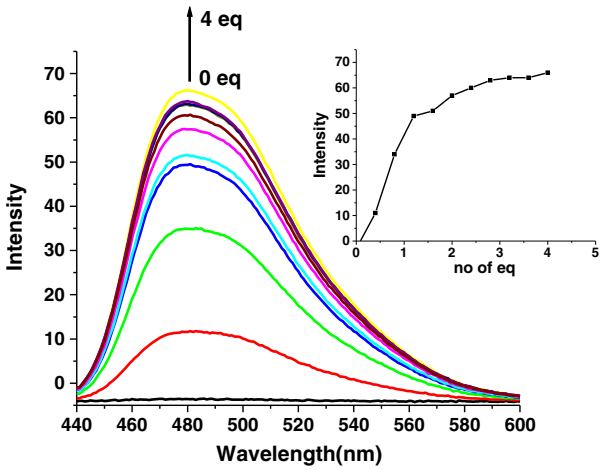



**Figure 6**. Emission spectra of receptor **1** recorded in DMSO ($2.5 \times 10^{-5}$ M) after the addition of 0–4 Eq of $F^-$ ($1.5 \times 10^{-3}$ M) with the excitation wavelength of 420 nm.

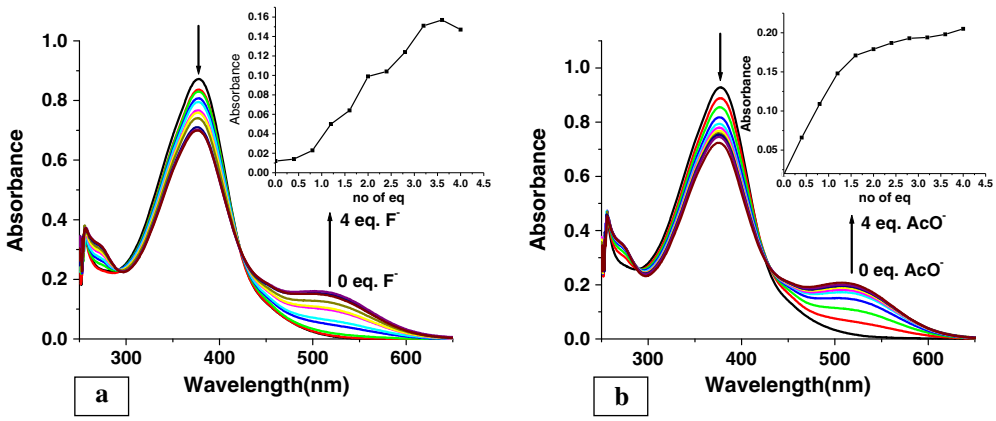



**Figure 4**. Absorption spectra of receptor **2** recorded in DMSO ($2.5 \times 10^{-5}$ M) after the addition of 0–4 Eq of (a) $F^-$ and (b) $AcO^-$ ($1.5 \times 10^{-3}$ M).

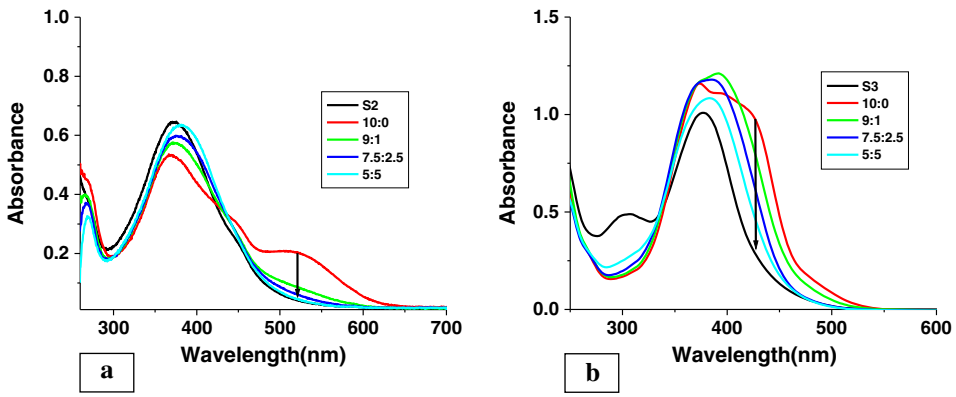



**Figure 5**. Absorption spectra of (a) receptor **2** in DMSO and (b) receptor **3** in ACN recorded ($2.5 \times 10^{-5}$ M) before and after the addition of 4 Eq of $F^-$ (10: 0, 9:1, 7.5:2.5, 5:5 DMSO/ACN/$H_2O$ $1.5 \times 10^{-3}$ M).

addition of 0–4 Eq of $F^-$ ion to receptor **1**, an increase in the intensity of the band at 520 nm (Supplementary Figure 4); and on the incremental addition of $F^-/AcO^-$ to receptor **2**, there is an increase in the intensity of band at 520 nm and a decrease in the intensity of the band at 375 nm with the isosbestic point at 422 nm is observed (Figures 4a and 4b).

**Table 1.** Binding constant values for receptors **1**, **2**, and **3** with corresponding anions by the Benesi–Hildebrand plot

| Receptors | Anions (DMSO/$H_2O$ v/v) | Binding constants($K_a$) |
|---|---|---|
| S1 | $F^-$(10:0) | $9.568 \times 10^3$ |
| S2 | $F^-$(10:0) | $1.709 \times 10^4$ |
| S2 | $F^-$(9:1) | $1.238 \times 10^4$ |
| S2 | $AcO^-$(10:0) | $1.49 \times 10^4$ |
| S2 | $AcO^-$(9:1) | $8.37 \times 10^3$ |
| S3 | $F^-$ | $2.484 \times 10^4$ |
| S3 | $AcO^-$ | $6.585 \times 10^4$ |
| S3 | $OH^-$ | $4.47 \times 10^3$ |
| S3 | $H_2PO_4^-$ | $7.94 \times 10^3$ |

The effect of water content in the anion complex of receptor **2** is also performed to monitor the sensitivity and stability of the receptor toward $F^-/AcO^-$ ions in aqueous medium. To find the sensing action of receptor **2** in aqueous medium, $H_2O$ content was adjusted from DMSO to 9:1 DMSO/$H_2O$ mixture. Then the yellow color of the receptor changed into orange for $F^-$ and $AcO^-$ ions. Accordingly, UV-vis titration showed the newly formed band at 520 nm only for $F^-$ and $AcO^-$ ions (Supplementary Figure 5). With the incremental addition of 0–4 Eq of $F^-/AcO^-$ ion (9:1 DMSO/$H_2O$), there is an increase in the intensity of the newly formed band at 520 nm, and the band at 375 nm is decreased (Supplementary Figures 6a and 6b). Further, the water content was adjusted from DMSO to 9:1, 7.5:2.5, and 5:5 (DMSO/$H_2O$) mixtures, and the intensity of the band at 375 nm is increased and at 520 nm is decreased and attains the receptor peak. This highly indicates that the water molecule will destroy the guest–host complex and re-generate the free receptor **2** species with the intramolecular hydrogen bonding as shown in (Figure 5a).

The similar titration was repeated for receptor **3** in ACN medium with same concentration. Receptor **3** shows bands at 370 nm in the absence of anion, which is corresponding to the intramolecular hydrogen bonding present within the molecule.

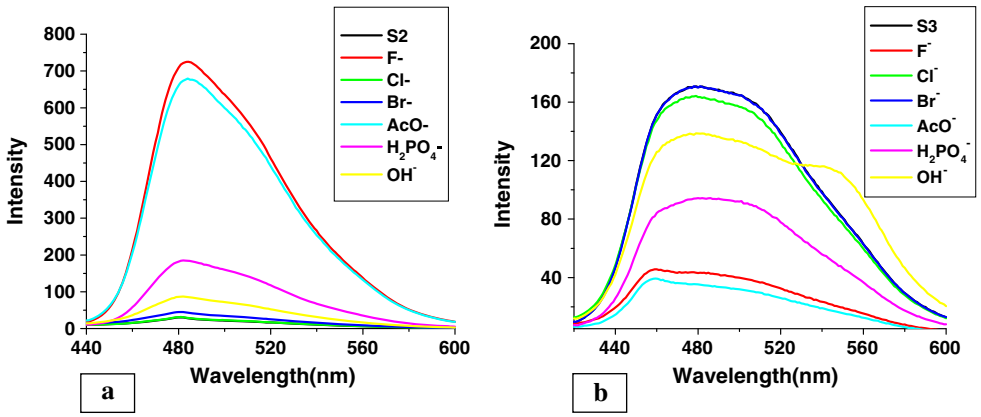



**Figure 7**. Emission spectra of (a) receptor **2** in DMSO and (b) receptor **3** in ACN recorded ($2.5 \times 10^{-5}$ M) after addition of 4 Eq of all anions ($1.5 \times 10^{-3}$ M).

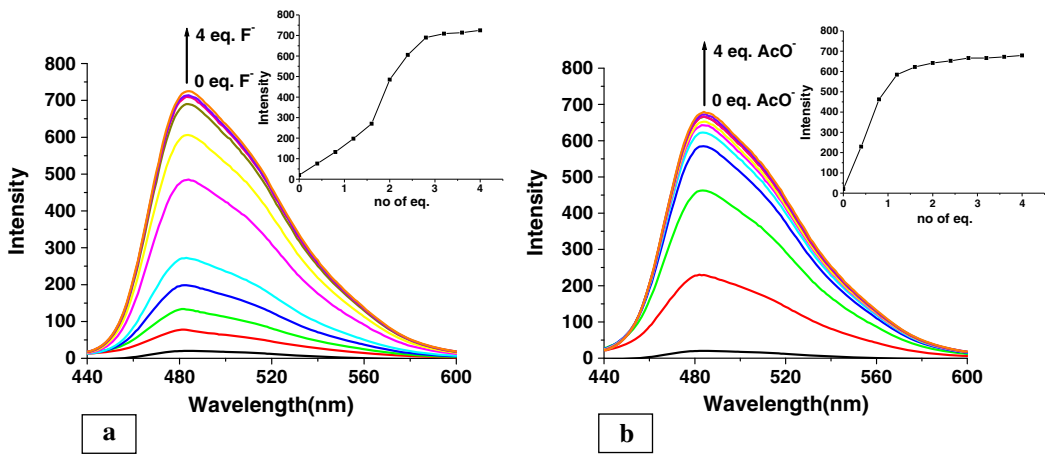



**Figure 8**. Emission spectra of receptor **2** recorded in DMSO ($2.5 \times 10^{-5}$ M) after the addition of 0–4 Eq of (a) $F^-$ and (b) $AcO^-$ ($1.5 \times 10^{-3}$ M) with the excitation wavelength of 420 nm.



While adding 4 Eq of anions to receptor **3**, a new band at 420 nm for $F^-$, $AcO^-$, $OH^-$, and $H_2PO_4^-$ was noticed (Figure 3b). However, in this case, during the incremental addition of 0–4 Eq anions to receptor **3**, the intensity of both band at 370 nm and the band at 420 nm is increased. The main reason for the enhancement in the intensity of the band at 370 nm is due to the formation of six-membered transition state complexes between receptor **3** and anions (Supplementary Figures 7a–7d).

When the same titration was performed in the 9:1 ACN/$H_2O$ medium, a selective recognition of $F^-$ and $AcO^-$ was observed (with the addition of 4 Eq) from light yellow to intense yellow, and optical changes are also noticed with the addition of 4 Eq of anions to receptor **3**. The intensity of both bands at 370 and 420 nm is increased (Supplementary Figure 8). As mentioned earlier, when the water content was adjusted from ACN to 9:1, 7.5:2.5, and 5:5 (ACN/$H_2O$) mixtures, the intensity of the band at 370 and 420 nm is decreased and attains the receptor peak. This suggests that the water molecule will destroy the guest–host complex and regenerate the free receptor **3** species with the intramolecular hydrogen bonding (Figure 5b).

The binding ability of sensors **1–3** toward various anions was studied using UV-vis titration experiments by nonlinear least squares fitting curve at 535, 520, and 420 nm for receptors **1**, **2**, and **3**, respectively. The association constants ($K_a$) were calculated using the Benesi–Hildebrand plots, which are given in the Table 1. Job's plot studies (Supplementary Figure 9) was performed to find out the stoichiometry of the complex formed between receptors **2** and **3** and anions, and it was found that the complex formed was in **1:1** stoichiometry.

### Emission spectral studies

Emission spectroscopy will give more information about the anion sensing properties of the receptors. Emission titration was carried

**Table 2.** Quantum yield and quenching constant values for receptors **1**, **2**, and **3** with corresponding anions

| Receptors | Quantum yield | Quenching constants ($K_{sv}$) |
|---|---|---|
| S1 | 0.009 | — |
| S1 + $F^-$ | 0.078 | — |
| S2 | 0.016 | — |
| S2 + $F^-$ | 0.65 | — |
| S2 + $AcO^-$ | 0.61 | — |
| S3 | 0.20 | — |
| S3 + $F^-$ | 0.05 | $3.029 \times 10^4$ |
| S3 + $AcO^-$ | 0.04 | $3.978 \times 10^4$ |
| S3 + $H_2PO_4^-$ | 0.123 | $9.776 \times 10^3$ |
| S3 + $OH^-$ | 0.18 | $1.841 \times 10^3$ |

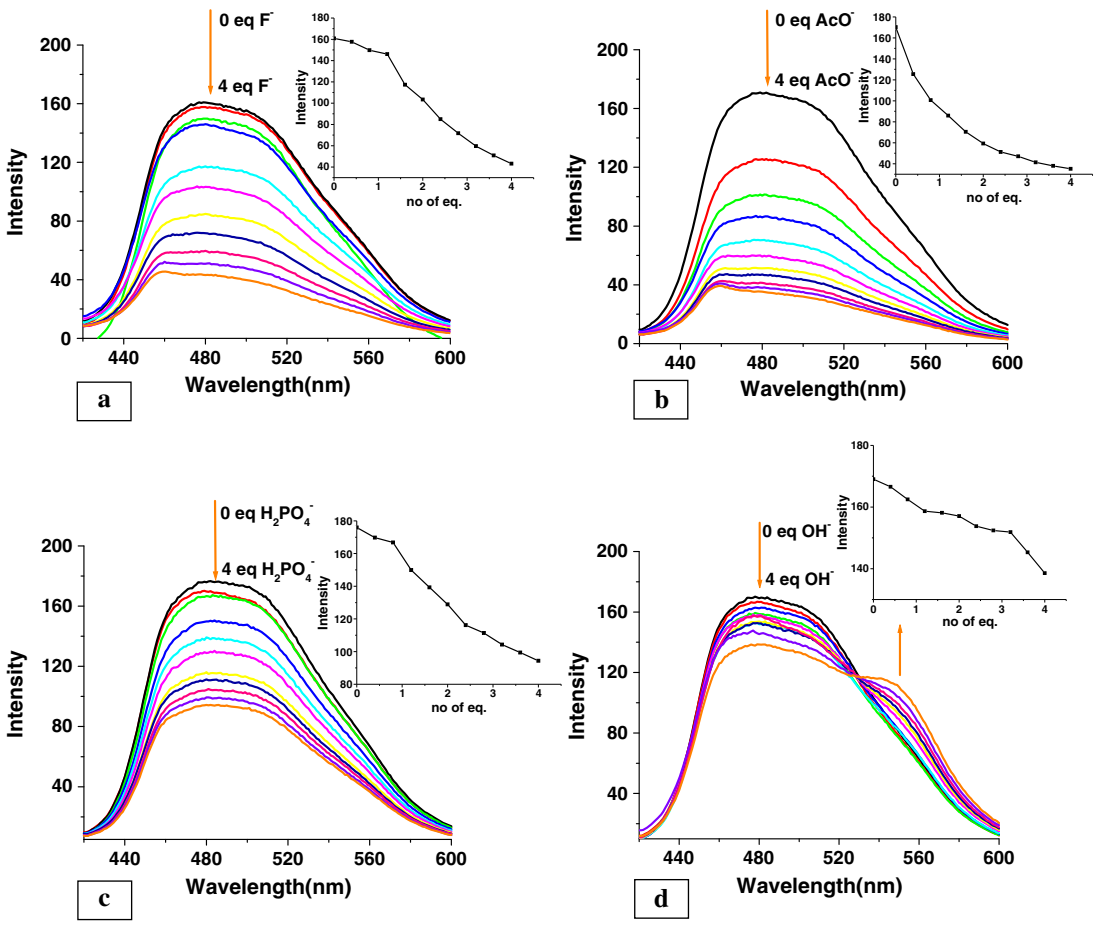



**Figure 9**. Emission spectra of receptor **3** recorded in ACN ($2.5 \times 10^{-5}$ M) after the addition of 4 Eq of (a) $F^-$, (b) $AcO^-$, (c) $H_2PO_4^-$, and (d) $OH^-$ ($1.5 \times 10^{-3}$ M) with the excitation wavelength of 400 nm.

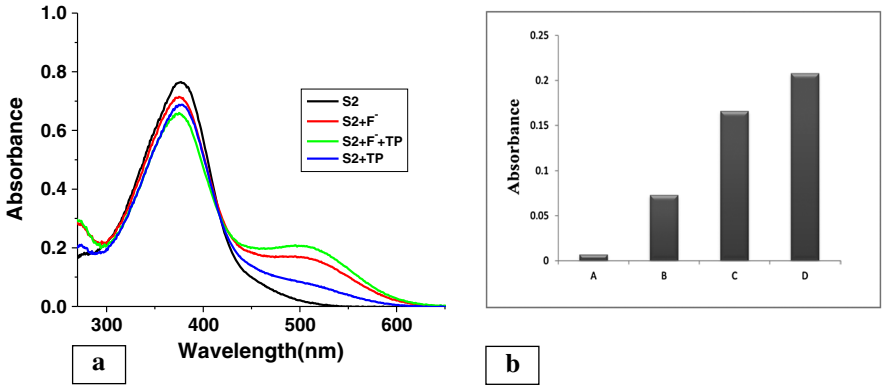



**Figure 10**. (a) UV-vis spectrum and (b) proof of F- ion detection in TP of receptor **2** ($2.5 \times 10^{-5}$ M in DMSO) (A), **2**+ TP (20 mg/ml in DMSO) (B), **2** + F- ion ($1.5 \times 10^{-3}$ M in DMSO) (C) and **2**+ F- ion+TP (D).

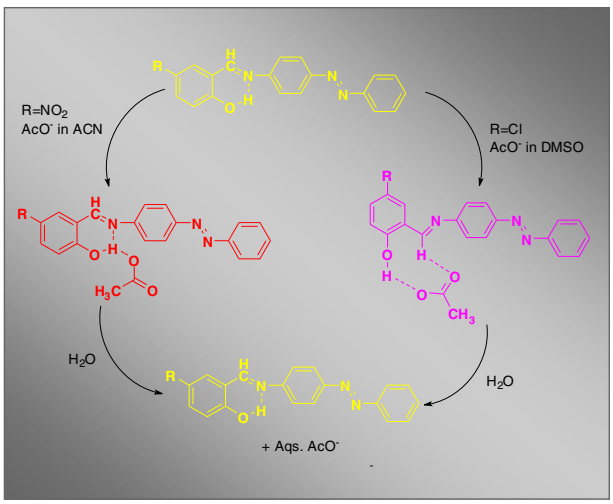



**Figure 11**. The proposed mechanism for the complex formed between receptors **2/3** and acetate anion.

out similar to UV-vis spectroscopy under same condition with excitation wavelength around 420 nm for receptors **1** and **2** and 400 nm for receptor **3**. On the addition of 4 Eq of anions ($1.5 \times 10^{-3}$ M) to receptors **1** and **2** ($2.5 \times 10^{-5}$ M), only $F^-$ ion shows emission maxima for receptor **1** at 480 nm (Figure 6); $F^-$ and $AcO^-$ ions show emission maxima for receptor **2** at 480 nm (Figure 7a). With the incremental addition of 0–4 Eq of $F^-$ ion to receptor **1**, enhancement in the fluorescence intensity was observed. Similarly, enhancement in the fluorescence intensity was noticed during the incremental addition of 0–4 Eq of $F^-$ and $AcO^-$ ions to receptor **2** (Figures 8a–8b). However, receptor **3** showed fluorescence quenching instead of enhancement toward $F^-$, $AcO^-$, $OH^-$, and $H_2PO_4^-$ ions at 480 nm (Figure 7b). This quenching mechanism of receptor 3 in the presence of anions is due to the presence of $-NO_2$ functionality as fluorescence quencher. When the titration of receptor **3** was carried out with the incremental addition of 0–4 Eq of $F^-$, $AcO^-$, $OH^-$, and $H_2PO_4^-$ ions, the intensity of the band at 480 nm is decreased (Figures 9a–9d). Simultaneously, by the addition of $OH^-$ ion to receptor **3**, a new band was created at 540 nm with fluorescence enhancement as well as the band at 480 nm with fluorescence quenching. This result confirms the deprotonation of highly acidic OH group by $OH^-$ ion (Equation (1)), and the formation of anion complexes of receptor **3** is taking place at equilibrium. The reason behind this enhancement and quenching in the fluorescence intensity may be due to the formation of anion complexes of receptor molecules **1–3** through intermolecular hydrogen bonding, which leads to nonradiative decay Internal Charge Transfer (ICT) from the excited state.

$$[LH] + OH^- \rightleftharpoons [L^-] + H_2O \rightarrow \quad (1)$$

The quantum yield of receptors **1–3** and their anion complexes were calculated using the emission titration method with rhodamine B as a standard. Quenching constants (Ksv) of receptor **3** and anion complexes of receptor **3** were also calculated using the Stern–Volmer equation (Eq 2) (Supplementary Figure 10), and their values are given in Table 2. The quenching rate constants were found higher for the $F^-$ and $AcO^-$ ions than for the $H_2PO_4^-$ and $OH^-$ ion complexes of receptor **3**.

$$F_0/F = 1 + Ksv[Q] \rightarrow \quad (2)$$

With this knowledge, we can design the biologically active fluorescence sensor containing OH binding site for the detection of biologically important anions. On the basis of the previously mentioned results, the mechanism behind the anion sensing property of receptors **2** and **3** in organic and semi-aqueous medium will be proposed as shown in Figure 10. On the basis of the binding constant values, the order of binding affinity of receptors toward anions is S3 > S2 > > S1 (Figure 11). The main reason for this order is the presence of Cl and $NO_2$ substituents in S2 and S3. Because of their electron withdrawing nature, it will enhance the acidity of the hydroxyl proton present in receptors **2** and **3** and form an effective hydrogen bond with the anions. By increasing the electron-withdrawing properties, we can be able to tune strong anion complexes with higher binding constants. The binding order of anions for receptors is as follows (in either DMSO/ACN or DMSO/ACN/water): $AcO^- > F^- > OH^-$, $H_2PO_4^- > Cl^-$, $Br^-$, and $I^-$.

### Qualitative detection of $F^-$ ion in toothpaste

To extend the molecular recognition properties of receptor **2** in real sample (Kumar *et al*., 2010), we have carried out the qualitative detection of $F^-$ ion experiment in commercially available toothpaste. The result was analyzed by UV-vis spectroscopic



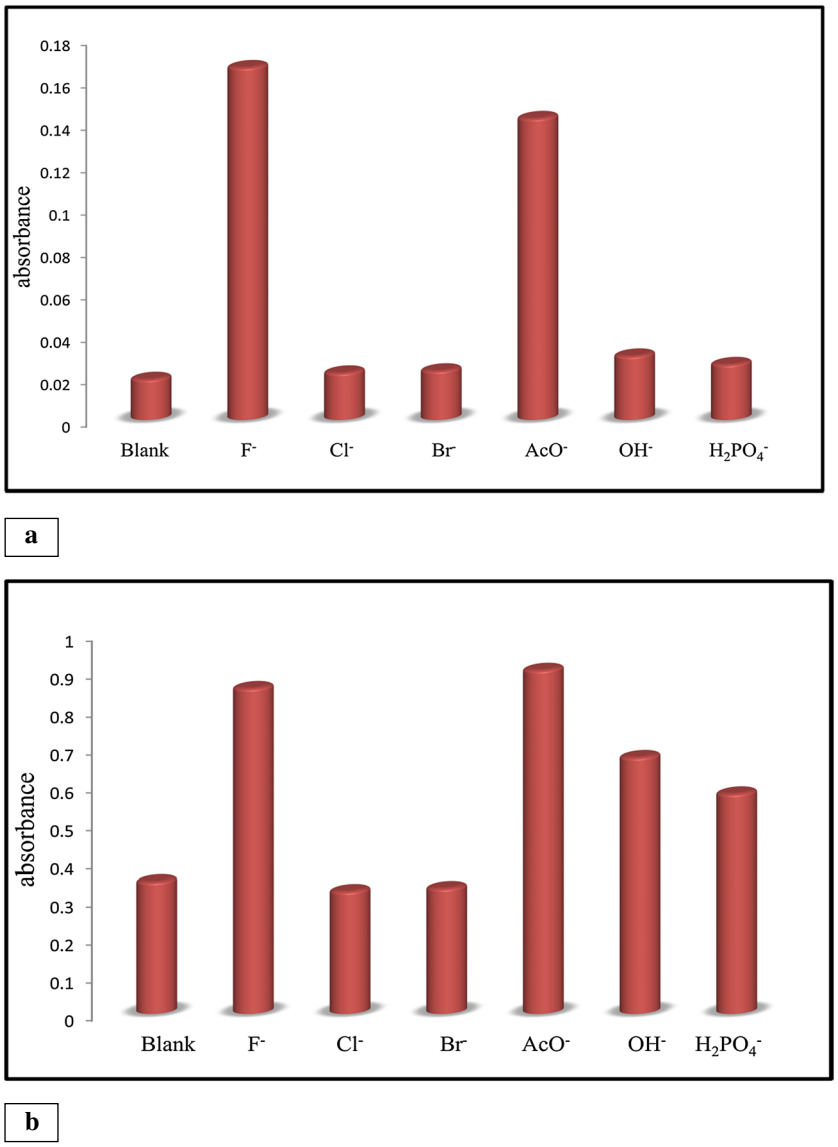



**Figure 12**. The absorbance at 520 nm for receptor (a) **2** and at 420 nm (b) **3** after the addition of all anions.

method. The samples used for UV-vis titration are toothpaste in DMSO (20 mg/ml), 4 Eq $F^-$ ($1.5 \times 10^{-3}$ M in DMSO), and receptor **2** ($2.5 \times 10^{-5}$ M in DMSO). The absorption measurements clearly ] show that the $F^-$ ion complex of receptor **2** has less absorbance than that of the $F^-$ ion complex of receptor **2** with toothpaste solution. Also, the formation of a new band at 520 nm in UV-vis spectrum only with the addition of toothpaste solution to receptor **2** indicates that the $F^-$ ion present in the toothpaste was detected by receptor **2** (Figure 12). To device a sensor system for the $F^-$ ion detection under aqueous medium is aimed for future research.

## CONCLUSION

In conclusion, we have developed a series of simple and novel colorimetric probes **1–3**, which are applied for the detection of various anions in organic and organic–aqueous mixture medium. Receptors **2** and **3** showed distinct color changes for $F^-$ and $AcO^-$ in 9:1 DMSO/$H_2O$ mixture. The strong affinity of receptors **2** and **3** toward $F^-$ and $AcO^-$ ions in competitive solvent mixture indicates the stability of the complex formed between receptor molecules and anions. In comparison, receptor **3** shows high affinity toward $F^-$ and $AcO^-$ ions with **1:1** stoichiometric ratio, which is proved by the higher binding constant values. This is mainly because receptor **3** contains highly acidic OH group through $NO_2$ substituent in it. Finally, receptor **2** was applied for the qualitative detection of $F^-$ ion in commercially available toothpaste sample.

## Acknowledgement

S.V. thanks Defence Research and Development Organization (DRDO) (grant no. EPIR/ER/1006004/M/01/1333) for a major sponsored project and for financial assistance.

Journal of Molecular Recognition

## REFERENCES


Beer PD, Gale PA. 2001. Anion recognition and sensing: the state of the art and future perspectives. *Angew. Chem. Int. Ed.* **40**: 486–516. DOI: 10.1002/1521-3773(20010202).

Chaicham A, Kulchat S, Tumcharern G, Tuntulani, T, Tomapatanaget B. 2010. Synthesis, photophysical properties and cyanide detection in aqueous solutions of $BF^{2-}$curcumin dyes. *Tetrahedron* **66**: 6217–6223. DOI: 10.1016/j.tet.2010.05.088.

Cho EJ, Moon JW, Ko SW, Lee JY, Kim SK, Yoon J, Nam KC. 2003. A new fluoride selective fluorescent as well as chromogenic chemosensor containing a naphthalene urea derivative. *J. Am. Chem. Soc.* **125**: 12376–12377. DOI: 10.1021/ja036248g.

Cho EJ, Ryu BJ, Lee YJ, Nam KC. 2005. Visible colorimetric fluoride ion sensors. *Org. Lett.* **7**: 2607–2609. DOI: 10.1021/ol0507470.

Gale PA, Quesada R. 2006. Anion coordination and anion-templated assembly: highlights from 2002 to 2004. *Coord. Chem. Rev.* **250**: 3219–3244. DOI:10.1016/j.ccr.2006.05.020.

Gale PA, Garcia-Garrido SE, Garric J. 2008. Anion receptors based on organic frameworks: highlights from 2005 and 2006. *Chem. Soc. Rev.* **37**: 151–190. DOI: 10.1039/B715825D.

Gunnlaugsson T, Glynn M, Tocci (n´ee Hussey) GM, Kruger PE, Pfeffer FM. 2006. Anion recognition and sensing in organic and aqueous media using luminescent and colorimetric sensors. *Coordn.Chem. Rev.* **250**: 3094–3117. DOI:10.1016/j.ccr.2006.08.017.

Gupta VK, Jain AK, Kumar P. 2006. PVC-based membranes of dicyclohexano-24-crown-8 as Cd(II) selective sensor. *Electrochim. Acta* **52**: 736–741. DOI:10.1016/j.electacta.2006.06.009.

Hijji YM, Barare B, Kennedy AP, Butcher R. 2009. Synthesis and photophysical characterization of a schiff base as anion sensor. *Sens. Actuators B* **136**: 297–302. DOI: 10.1016/j.snb.2008.11.045.

Ho TY, Scranton MI, Taylor GT, Varela R, Thunell RC, Karger FM. 2002. Acetate cycling in the water column of the cariaco basin: seasonal and vertical variability and implication for carbon cycling. *Limnol. Oceanogr.* **47**: 1119–1128. DOI: 10.4319/lo.2002.47.4.1119.

Kang SO, Begum RA, Bowman-James K. 2006. Amide-based ligands for anion coordination. *Angew. Chem. Int. Ed.* **45**: 7882–7894. DOI: 10.1002/anie.200602006.

Kim DS, Chung YM, Jun M, Ahn KH. 2009. Selective colorimetric sensing of anions in aqueous media through reversible covalent bonding. *J. Org. Chem.*, **74**: 4849–4854. DOI: 10.1021/jo900573v.

Kumar V, Kaushik MP, Srivastava AK, Pratap A, Thiruvenkatam V, Guru Row TN. 2010. Thiourea based novel chromogenic sensor for selective detection of fluoride and cyanide anions in organic and aqueous media. *Anal. Chim. Acta*, **663**: 77–84. DOI:10.1016/j.aca.2010.01.025.

Lee DH, Lee KH, Hong JI. 2001. An azophenol-based chromogenic anion sensor. *Org. Lett.* **3**: 5–8. DOI: 10.1021/ol006690t.

Lee JY, Cho EJ, Mukamel S, Nam KC. 2004. Efficient fluoride-selective fluorescent host: experiment and theory. *J. Org. Chem.* **69**: 943–950. DOI: 10.1021/jo0356457.

Lehn JM. 1985. Supramolecular chemistry: receptors, catalysts, and carriers. *Science* **227**: 4689. 849–856. DOI: 10.1126/science.227.4689.849.

Libra ER, Scott MJ. 2006. Metal salen complexes incorporating triphenoxymethanes: efficient, size selective anion binding by phenolic donors with a visual report. *Chem. Commun.* **14**: 1485–1487. DOI: 10.1039/B517944K.

Mizuno T, Wei WH, Eller LR, Sessler JL. 2002. Phenanthroline complexes bearing fused dipyrrolylquinoxaline anion recognition sites: efficient fluoride anion receptors. *J. Am. Chem. Soc.* **124**: 1134–1135. DOI: 10.1021/ja017298t.

Nguyen TNP, Kim KJ. 2009. Transformation of monohydrate into anhydrous form of risedronate monosodium in methanol – water mixture. *Ind. Eng. Chem. Res.*, **49**: 4842–4849. DOI: 10.1021/ie901677n.

Nie L, Li Z, Han J, Zhang X, Yang R, Liu WX, Jiang YB. 2004. Development of n-benzamidothioureas as a new generation of thiourea-based receptors for anion recognition and sensing. *J. Org. Chem.* **69**: 6449–6454. DOI: 10.1021/jo049088f.

Peker E, Serin S. 2004. Synthesis and characterization of some cobalt (II), copper (II), and nickel (II) complexes with new Schiff bases from the reaction of p-aminoazobenzene with salicylaldehyde. *Synthesis and reactivity in inorganic and metal-organic chemistry*. **34**: 859–872. DOI: 10.1081/SIM-120037512.

Sole S, Gabbai FP. 2004. A bidentate borane as colorimetric fluoride ion sensor. *Chem. Commun.* **11**: 1284–1285. DOI: 10.1039/B403596H.

Suganya S, Velmathi S, Sivakumar R, Anandan S. 2011. Selective binding of copper ion by salicylaldimine based schiff base chromogenic receptors. *Sensors. Lett.* **9**: 570–576. DOI:10.1166/sl.2011.1514.

Suksai C, Tuntulani T. 2003. Chromogenic anion sensors. *Top. Curr. Chem.* **255**: 163–198. DOI: 10.1007/b101166.

Thangadurai TD, Chung G, Kwon O, Jin D, Lee YI. 2011. Differentiation of cis- and trans-isomers of the novel napthalene-aza receptor by naked-eye colorimetric anion sensing. *Tetrahedron Lett.* **52**: 6465–6469. DOI:10.1016/j.tetlet.2011.09.103.

Udhayakumari D, Saravana Moorthy S, Ashok M, Velmathi S. 2011. Simple imine linked colorimetric and fluorescent receptor for sensing $Zn^{2+}$ ions in aqueous medium based on inhibition of ESIPT mechanism. *Tetahedron Lett.* **62**: 4631–4635. DOI: 10.1016/j.tetlet.2011.06.097.

V´azquez M, Fabbrizzi ML, Taglietti A, Pedrido RM, Gonz´alez- Noya AM, Bermejo MR. 2004. A colorimetric approach to anion sensing: a selective chemosensor of fluoride ions, in which color is generated by anion-enhanced π delocalization. *Angew. Chem. Int. Ed.* **43**: 1962–1965. DOI: 10.1002/anie.200353148.

Velmathi S, Reena V, Suganya S, Anandan S. 2012. Pyrrole based schiff bases as colorimetric and fluorescent chemosensors for fluoride and hydroxide anions. *J. Fluoresc.* **122**: 155–162. DOI: 10.1007/s10895-011-0942-z.

Wang Q, Li X, Wang L, Cheng Y, Xie G. 2005. Effect of water content on the kinetics of p-xylene liquid-phase catalytic oxidation to terephthalic acid. *Ind. Eng. Chem. Res.* **44**: 4518-4522. DOI: 10.1021/ie048755s.

Xu G, Tarr MA. 2004. A novel fluoride sensor based on fluorescence enhancement. *Chem. Commun.* **9**: 1050–1051. DOI: 10.1039/B316121H.

Yu M, Lin H, Zhao GH, Lin HK. 2007. A benzimidazole-based chromogenic anion receptor. *J. Mol. Recognit.* **20**: 69–73. DOI:10.1002/jmr.810.

Zhang YM, Lin Q, Wei TB, Wang DD, Yao H, Wang YL. 2009. Simple colorimetric sensors with high selectivity for acetate and chloride in aqueous solution. *Sens. Actuators B* **137**: 447–455. DOI:10.1016/j.snb.2009.01.015.

Zuhair SA, Jayaraman KV, Krishnan S, Chan WH. 2006. The effect of fatty acid concentration and water content on the production of biodiesel by lipase. *Biochem. Eng. J.* **30**: 212–217. DOI: 10.1016/j.bej.2006.04.007.

