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เซนเซอร์ที่เปลี่ยนสีได้จากคาร์บาโซลที่ต่อไนโตรเอโซเบนซีนที่มีความจำเพาะต่อการตรวจวัดไซยาไนด์และฟลูออไรด์

Selective Colorimetric Detection of Cyanide and Fluoride from Carbazole Possessing Nitro-azobenzene

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บทคัดย่อ

ทำการสังเคราะห์เซ็นเซอร์ที่เปลี่ยนสีได้จากคาร์บาโซลและไนโตรเอโซเบนซีน (**S1**) พบว่าในระบบตัวทำละลายอะซิโตน สามารถนำไปใช้ตรวจวัดไอออนลบที่มีความเป็นเบสสูงได้อย่างจำเพาะนั่นคือ ฟลูออไรด์ และไซยาไนด์ไอออน โดยสังเกตเห็นการเปลี่ยนสีจากสีเหลืองเป็นสีแดง การเปลี่ยนสีดังกล่าวสอดคล้องกับผลการวัดสเปกตรัมการดูดกลืนแสงของเซ็นเซอร์ **S1** ที่พบการเลื่อนตำแหน่งสเปกตรัมไปทางความยาวคลื่นที่มากขึ้นหลังการเติมฟลูออไรด์และไซยาไนด์ไอออน ผลจากการไทเทรตกับฟลูออไรด์และไซยาไนด์ไอออนด้วยเทคนิคโปรตอนเอ็นเอ็มอาร์พบว่าหมู่ NH-เอไมด์ของ **S1** ถูกไอออนลบดึงโปรตอน และสามารถคำนวณอัตราส่วนการจับกันระหว่างโฮสต์-เกสต์เป็นแบบ 1:2 การศึกษาเบื้องต้นในการหาค่าขีดจำกัดต่ำสุดของเซ็นเซอร์ **S1** ในการตรวจวัดไอออนลบด้วยการสังเกตการเปลี่ยนสีด้วยตาเปล่าพบว่าความเข้มข้นต่ำสุดของฟลูออไรด์และไซยาไนด์ไอออนวัดได้เท่ากับ 4x10^{-4} และ 2x10^{-4} โมลาร์ ตามลำดับ

Abstract

A colorimetric sensor based on carbazole and nitro-azobenzene (**1**) was successfully synthesized and was able to selectively detect the strong basic anions which are F$^-$ and CN$^-$ by a “naked-eye” color change from light yellow to red in acetone media. This change in color corresponded to a red shift observation of the sensor absorption band after F$^-$ and CN$^-$ addition. Result from ^{1}H-NMR titration revealed a deprotonation of sensor **1** at NH-amide binding unit. A host-guest complex formation of 1:2 stoichiometry was calculated. A preliminary study in a detection limit of **1** for anion sensing using the naked eye observation was obtained the values of 4x10^{-4} and 2x10^{-4}mol/L for F$^-$ and CN$^-$, respectively.

คำสำคัญ: คาร์บาโซล ไนโตรเอโซเบนซีน เซนเซอร์ที่เปลี่ยนสีได้ การเปลี่ยนสีที่เห็นได้ด้วยตาเปล่า

Keywords: Carbazole, Nitro-azobenzene, Colorimetric sensor, Naked-eye color chage

INTRODUCTION

A colorimetric sensing method is one of the most effective and selective method of the detection of many guests such as anions, cations and organic compounds (Chmielewski, 2004; Thangadurai, 2007; Shao, 2008; Danjou, 2012; Kyu, 2012; Mahapatra, 2012; Shang, 2012; Cheng, 2013; Helal, 2013; Mahapatra, 2013; Shi, 2013; Tang, 2014; Vedarajan, 2014; Wang, 2014; Zhu, 2015). Various colorimetric sensors such as anthracene (Gross, 2010), dansyl (Chen, 2004), imidazole (Goswami, 2011), anthraquinone (Batista, 2014) and indole (Lee, 2010) were designed to obtain their best construction for guest binding. The advantages of this method over other techniques are high sensitivity, simplicity, fast response, analysis in both quality and quantity, analysis using simple instruments and easily observation by color changes in the presence of target guests (Bissell, 1992; Beer, 2001). In the sensing of cyanide and fluoride, the selectivity and sensitivity of the sensor are challenging. These two basic anions show a benefit over other anions. Fluoride is the smallest and most electronegative anion. It is capable of forming very strong hydrogen bonds or acting as a base in the deprotonation with sensor binding units (Chen, 2004; Boiocchi, 2004; Goswami, 2011; Kigga, 2014; Mukherjee, 2014) Due to a strong nucleophile of cyanide, the nucleophilic attack to an electron deficient carbonyl group can achieve the naked eye detection (Niu, 2008; Odago, 2010; Batista, 2014; Gupta, 2014).

Fluoride is one of an essential trace element for health benefits when using in low concentration. If a high concentration of fluoride (above 2 ppm) is used, it will be toxic to human body causing diseases as silkworm and dental fluorosis. Fluoride is able to find in many oral care products, including drinking water. The standard concentration of fluoride in drinking water which is 1.5 mg/L determining by WHO was reported (Edition, 2011).Cyanide is well known as a highly toxic anions for environments and living organisms (Kulig, 1991). Although cyanide has a high toxicity, it is extensively used in industrial fields such as electroplating, gold mining, metallurgy and polymer synthesis (Young, 2001). Therefore, the determination of cyanide which contaminated to environments is very important. WHO has determined the standard for drinking water concentration of cyanide not more than 0.07 mg/L (Edition, 2011). The United States environmental protection agency (EPA) has also determined the concentration of cyanide not exceed 0.2 ppm.

In the sensing of cyanide and fluoride, the selectivity and sensitivity of the sensor are challenging. These two basic anions show a benefit over other anions. Fluoride is the smallest and most electronegative anion. It is capable of forming very strong hydrogen bonds or acting as a base in the deprotonation with sensor binding units (Kigga, 2014; Mukherjee, 2014). Due to a strong nucleophile of cyanide, the nucleophilic attack to an electron deficient carbonyl group can achieve the naked eye detection. During the past decade, a variety of synthetic colorimetric sensors for cyanide have been reported the utilizing of hydrogen bonding interactions (Miyaji, 2001), metal coordination (Chung, 2009; Zeng, 2008), deprotonation (Gimeno, 2008), luminescence measurement (Anzenbacher, 2002) and nucleophilic addition (Niu, 2008; Odago, 2010; Batista, 2014; Gupta, 2014). To the design of colorimetric sensors, these binding processes can be selected to obtain the selective recognition of fluoride or cyanide ions. Most cyanide sensor molecules were reported to work in organic media. A few reports were successful sensing in aqueous system (Niu, 2008; Xu, 2010; Batista, 2014).This research work reported the synthesis of a colorimetric sensor based on carbazole possessing azobenzene and used this sensor in the naked eye detection for cyanide and fluoride. Carbazole has been designed and worked as an efficient anion sensor for many years. It has a rigid structure which is easy to modify at 1, 3, 6, 8 and NH positions (Xu, 2014). Carbazole derivatives are known as high emission intensity compounds which are useful for optical sensing materials for example OLED, synthetic dyes and advanced polymer (Liu, 2006; Adhikari, 2009; Zhao, 2009). Azo dye is well known as a good chromophore because it has vivid colors especially red, orange and yellow (Ros-Lis, 2007; Niu, 2008; Kigga, 2014). Some azobenzene compounds are used as acid-base indicators due to the difference in color of their acid and salt forms. To the design of a sensor **S1**, nitro-azobenzene was designed as a sensory unit while amide and hydroxyl groups which incorporated to conjugated system of azobenzene were used as a binding unit. The sensor **S1** is capable of sensing the strong basic anions via deprotonation process at the sensor binding sites attributing to the intramolecular charge transfer (ICT) transition at the sensory unit to provide the naked eye color change from light yellow to red in organic solvent.

RESEARCH METHODOLOGY

1. Material and Instrumentations

Carbazole that purchased from SIGMA Life Science was used without purification. Reagents and solvents were analytical grade. All anions were used without any purification in the form of tetrabutylammonium salts and purchased from SIGMA-ALDRICH and Fluka. ^{1}H-NMR spectra was recorded on a Bruker Avance 400. FT-IR spectra were recorded on a Perkin Elmer Spectrum GX using KBr-disc method for sample preparation. UV/Vis spectra were recorded on a Jasco V-650 spectrophotometer. Elemental analysis was carried on Truspec Micro CHN analyzer. ESI-M S was performed with Agilent 6540 Q-TOF LC/MS.

2. Synthesis

Sensor **S1** was synthesized by following procedures as shown in **Scheme 1**.

2.1 Synthesis of 9-butylcarbazole (2)

Carbazole (1.00 g, 6.0 mmol) and NaOH (0.26 g, 6.5 mmol) were mixed with 20.0 mL dimethyl sulfoxide in a two-neck round bottom flask under stirring. The mixture was heated to 60 $^\circ$C for 60 min to dissolve all solids and then was gradually added with 1-bromobutane (1.45 g, 6.5 mmol). The reaction temperature was kept at 60 $^\circ$C for 6 h then poured into water. The precipitation was filtered and purified with silica gel column chromatography using dichloromethane : hexane. (3:7) as eluent.

Compound **2**; Yield: 51.48%; ^{1}H-NMR (400 MHz, CDCl$_3$): δ = 8.11 (d, 2H$_g$, J = 7.6 Hz), 7.45 (t, 2H$_f$, J = 8.0 Hz), 7.41 (d, 2H$_e$, J = 8.0 Hz), 7.25 (dd, 2H$_h$, J = 2.8, 7.6 Hz), 4.32 (t, 2H$_d$, J = 7.2 Hz), 1.87 (pentet, 2H$_c$, J = 7.2 Hz), 1.41 (sextet, 2H$_b$, J = 7.6 Hz) and 0.96 (t, 3H$_a$, J = 7.6 Hz). FT-IR: 3042.90 (=CH stretching), 2927.04, 2857.43 (-CH stretching), 1624.25 (C=C stretching) and 1482.92, 1450.88 (CH bending).

2.2) Synthesis of 9-butyl-3,6-dinitro carbazole (3)

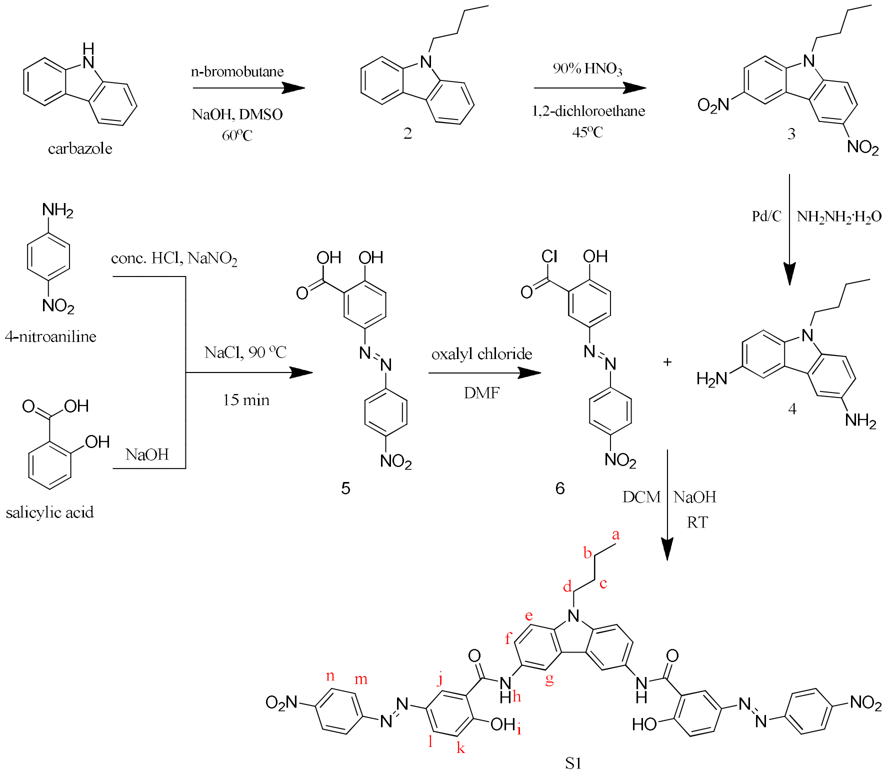
9-Butylcarbazole (0.67 g, 3.0 mmol) and 10 mL of 1,2-dichloroethane were added in a two-neck round bottom flask under stirring. The mixture was cooled at 10 $^\circ$C and a mixture of 90% HNO$_3$ 0.5775 mL (16.5 mmol) and 0.5 mL of 1,2-dichloroethane was added drop wise while stirring and temperature not exceed 10 $^\circ$C. Following the addition of 1/8 of the whole volume of HNO$_3$, the cooling bath was replaced with an oil bath. The mixture was heated to 45 °C and all volume of HNO_3 was added. This temperature was kept for 4 h before the reaction was poured into water to obtain a yellow precipitation. The yellow solid was filtered and purified by silica gel column chromatography using dichloromethane : hexane (3:2) as eluent.

Compound **3**; Yield: 77.06%; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 9.52 (d, $2H_g$, *J* = 2.4 Hz), 8.44 (dd, $2H_f$, *J* = 2.0, 8.8 Hz), 7.96 (d. $2H_e$, *J* = 9.2 Hz), 4.582 (t, $2H_d$, *J* = 7.2 Hz), 1.79 (pentet, $2H_c$, *J* = 7.2 Hz), 1.31 (sextet, $2H_b$, *J* = 7.6 Hz) and 0.88 (t, $3H_a$, *J* = 7.2 Hz). FT-IR: 3091.65 (=CH stretching), 2930.37, 2870.94 (CH stretching), 1632.41 (C=C stretching), 1479.13 (CH bending), 1514.28, 1335.34 (NO_2 stretching)

2.3) Synthesis of 9-butyl-3,6-diamino carbazole (4)

To a 250 mL two-neck round-bottomed flask, 9-butyl-3,6-dinitrocarbazole (0.1565 g, 5.0 mmol) and Pd/C were suspended in ethanol (50.0 mL). The mixture was refluxed and a solution of hydrazine (1.0 mL) in ethanol (5.0 mL) was added dropwise. After the addition was completed, the mixture was refluxed for 12 h until a color of the mixture was changed to colorless. Then, the hot suspension was filtered and washed with ethanol. The solvent in filtrate was removed to obtain anolive-greensolid which was recrystallized in ethanol.

Compound **4**; Yield: 48.05%; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 7.14 (d, $2H_g$, *J* = 8.8 Hz), 7.08 (d, $2H_f$, *J* = 1.6 Hz), 6.72 (d, $2H_e$, *J* = 2.0, 8.4 Hz), 4.58 (s, 4H, NH_2), 4.13 (t, $2H_d$, *J* = 7.2 Hz), 1.64 (pentet, $2H_c$, *J* = 7.2 Hz), 1.25 (sextet, $2H_b$, *J* = 8.4 Hz) and 0.86 (t, $3H_a$, *J* = 7.6 Hz). FT-IR: 3387.55, 3283.61 (NH stretching), 3180.44 (=CH stretching), 2956.11, 2859.29 (CH stretching), 1632.61 (C=C stretching), 1478.96 (CH bending)



Scheme 1 General synthetic route to the sensor **S1**

2.4 Synthesis of 2-hydroxy-5-((4-nitrophenyl)diazenyl)benzoic acid (5)

In a test tube, a mixture of conc. HCl (1.5 mL) and H_2O (1.5 mL) was cooled in an ice-bath. In a beaker, 4-nitroaniline (0.70 g, 5.0mmol), $NaNO_2$ (0.38 g, 5.0mmol) and H_2O (1.5 mL) were mixed and then cooled in an ice-bath. The cooled HCl was slowly added to the mixture in a beaker to obtain an olive-green solid. The mixture was filtered off the solid and collected yellow liquid for the next step reaction. Salicylic acid (0.74 g, 5.4mmol) was dissolved in 2.5 M NaOH (10.0 mL). This solution which was cooled in an ice-bath for 10 min was slowly added into the yellow solution. Then, conc. HCl (1.5 mL) was further added and then the mixture was heated to 100 °C. When the mixture was boiled, NaCl (1.0 g) was added and then hot-filtered to provide a red solid which was purified by silica gel column chromatography using ethanol: ethyl acetate (1:4) as eluent to provide compound **5** as a product.

Compound **5**; Yield: 43.01%; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 8.37 (d, $2H_n$, J = 8.4 Hz), 8.33 (d, $1H_j$, J = 2.8 Hz), 7.97 (d, $2H_m$, J = 8.4 Hz), 7.86 (d, $1H_l$, J = 2.8, 8.8 Hz) and 6.75 (d, $1H_k$, J = 8.8 Hz).

2.5 Synthesis of 2-hydroxy-5-((4-nitrophenyl)diazenyl)benzoyl chloride (6)

2-Hydroxy-5-((4-nitrophenyl)diazenyl)benzoic acid (0.10 g, 4.2 mmol) was dissolved in dichloromethane (10.0 mL) under nitrogen atmosphere. Oxalyl chloride (0.2 mL, 23.0 mmol) and few drops of DMF were added. The mixture was stirred for 4 h at room temperature. The solvent was evaporated and then purified the obtained product by silica gel column chromatography using dichloromethane as eluent.

Compound **6**; Yield: 70.24%; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 8.45 (d, $2H_n$, J = 9.6 Hz), 8.32 (s, $1H_j$), 8.14 (d, $2H_m$, J = 8.8 Hz), 8.10 (d, $1H_l$, J = 8.8 Hz), 7.83 (d, $1H_k$, J = 8.4 Hz) and 5.75 (s, $1H_i$).

2.6 Synthesis of sensor (S1)

9-Butyl-3,6-diaminocarbazole (0.10 g, 40.0 mmol), NaOH (a pellet) and 2-hydroxy-5-((4-nitro phenyl)diazenyl)benzoyl chloride (0.2410 g, 80.0 mmol) were mixed in dichloromethane (20.0 mL) in a two-neck round bottom flask under stirring and nitrogen atmosphere. The reaction was refluxed for 24 h. A reddish orange solid was precipitated and then was filtered and recrystallized in acetone.

Compound **S1**; Yield: 56.91%; ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 10.73 (s, $2H_h$), 8.59 (d, $2H_g$, J = 2.0 Hz), 8.46 (d, $4H_n$, J = 7.6 Hz), 8.21 (s, $2H_j$), 8.14 (d, $4H_m$, J = 7.2 Hz), 8.11, (d, $2H_l$, J = 8.8 Hz), 7.89 (dd, $2H_k$, J = 2.4, 8.4 Hz), 7.68 (d, $2H_f$, J = 8.8 Hz), 7.66 (d, $2H_e$, J = 8.8 Hz), 4.40 (t, $2H_d$, J= 6.8 Hz),1.66 (pentet, $2H_c$, J = 7.2 Hz),1.27 (sextet, $2H_b$, J= 7.6 Hz) 0.88 (t, $3H_a$, J = 7.2 Hz). ^{13}C-NMR (400 MHz, DMSO-d_6): δ = 13.73, 19.76, 30.73, 42.18, 109.61, 111.42, 119.34, 121.64, 122.76, 123.74, 125.18, 125.47, 130.66, 131.30, 134.29, 137.59, 138.28, 148.84, 150.13, 154.83 and 163.58. FT-IR: 3421.03 (OH stretching), 3245.89 (NH stretching), 2930.15 (CH stretching), 1650.76 (C=O stretching), 1590.37 (C=C stretching), 1523.66 and 1342.29 (NO_2 stretching), 1489.96 (CH bending). Mass (+ESI): Receptor **S1** + H^+ - (N_2-C_6H_4-NO_2) = 642.193, Anal. Calcd for $C_{42}H_{33}N_9O_8$+CH_2Cl_2: %C 58.90; %H 4.00; %N 14.38. Found: %C 59.12; %H 4.33; %N 13.49.

3. Investigation of anion sensing

Anion sensing by sensor **S1** was investigated using three techniques; a naked-eye observation, UV-Vis and ^{1}H-NMR. Experiments of naked-eye observation were studied a selectivity in anion sensing, solvent system optimization and a detection limit determination. A concentration of **S1** at 5x10^{-5} M in various organic solvent including DMSO, acetone, acetonitrile and ethyl acetate were determined in this experiment. In case of UV-Vis technique, the solution of **S1** with all anions was prepared in 0.01 M NBu_4PF_6 solution in acetone. To understand the binding of **S1** with anions, ^{1}H-NMR experiments were performed using DMSO-d_6. The concentration of **S1** solutions were $1x10^{-3}$ M and $1x10^{-5}$ M for ^{1}H-NMR and UV-Vis experiments, respectively. All anions were used in a form of tetrabutylammonium salts.

RESULTS AND DISCUSSION

1. Investigation of anion sensing by naked eye observation and UV-Vis spectrophotometry

The ability of the sensor **S1** for anion sensing was investigated by an observable change in color of **S1** upon the addition of various anions (OH^-, F^-, CN^-, AcO^-, $H_2PO_4^-$, NO_3^-, HSO_4^-, BzO^-. Cl^-, Br^-, I^- and ClO_4^-). Four organic solvents including acetone, DMSO, ethyl acetate and acetonitrile were used in the experiments considering from the solubility of **S1**. A solution of **S1** in organic solvent is pale yellow. This yellow color was turned to red color after the addition of strong base anions such as OH^-, CN^- and F^-. To observe the naked eye color change, the color of **S1** solution ($5x10^{-5}$ M) was compared before and after the addition 50 equiv of anions. The effect of solvent on the naked eye sensing was firstly studied as shown in Figure 1. It was found that a distinct color change from pale yellow to orange-red could be clearly seen by the naked eye when acetone and DMSO were used as solvents. This color change may be due to the deprotonation at the binding unit of the sensor. A selectivity for OH^- was obtained in DMSO media while a solution of **S1** in acetone showed the anion sensing ability with three strong base anions (OH^-, F^- and CN^-). Meanwhile, a solution of **S1** in ethyl acetate and acetonitrile did not observe any color change with all anions. The colorimetric sensing of **S1** was influenced from both polarity of the solvent and basicity of anions. The polarity of aprotic solvents is in the order of DMSO > acetonitrile > acetone >ethyl acetate. High polar solvents are highly appropriate media for a naked eye color change due to the deprotonation process (Batista, 2014). However, their high polarity properties allow them to dissolve anions very well. This high solvation perhaps interrupt the interaction between anion and sensor. From the preliminary result, acetone was an appropriate media because it was only one solvent which could attribute the naked eye sensing for CN^- and F^-.

UV-Vis absorption experiments (Figures 2a and 2b) were conducted using a solution of **S1** ($1x10^{-5}$ M) in acetone which showed a single absorption band at the maximum wavelength of 350 nm. This band could be assigned to the excitation of π- electrons in the carbazole system (Shang, 2012). The addition of 50 equiv of anions (F^- and CN^-) caused an appearance of a new absorption band at the wavelength around 500 nm while the absorbance of the band at 350 nm was decreased and shifted to 370 nm. Meanwhile, one isobestic point was observed at 375 nm, indicating the formation of a new species. The new band at 500 nm corresponding to the red color of the solution may be attributed to the deprotonated product from **S1**. The other anions such as AcO^-, $H_2PO_4^-$, NO_3^-, HSO_4^-, BzO^-. Cl^-, Br^-, I^- and ClO_4^-did not induce any color change or the new absorption band. Due to weaker basic anions as compared to F^- and CN^-, these anions could not deprotonate at the binding sites of the sensor **S1** (Park, 2014).

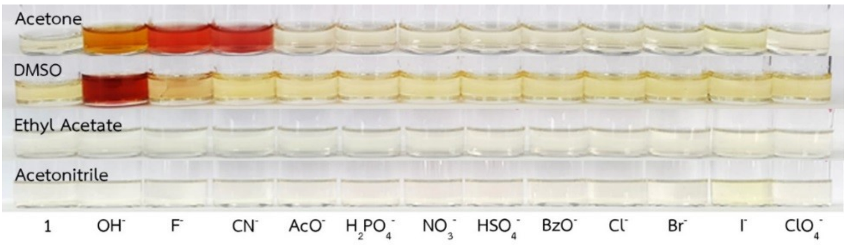


Figure 1 Color changes of **S1** ($5x10^{-5}$ M) in acetone, DMSO, ethyl acetate and acetonitrile in the absence and the presence of different anions (50 equiv).

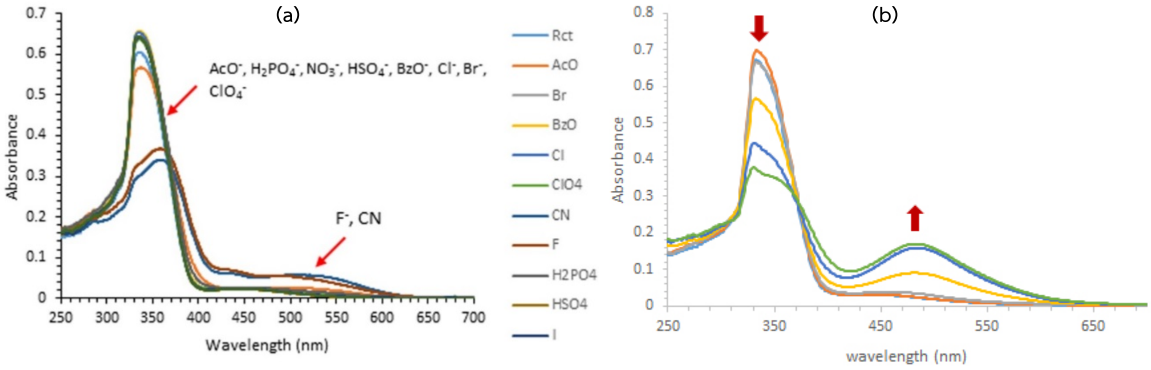


Figure 2 UV/Vis spectra of **S1** ($1x10^{-5}$M in acetone) (a) with various anions 50 equiv (b) upon titration with fluoride ion (0-125 equiv)

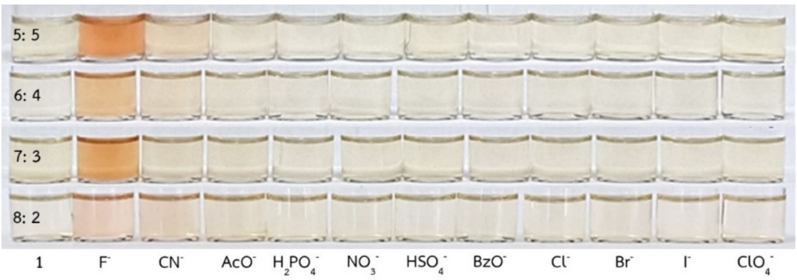


Figure 3 Color changes of **S1** ($5x10^{-5}$ M) in various ratios of acetonitrile : acetone after the addition of 50 equiv of various anions.

Figure 4 Color changes of **S1** ($5x10^{-5}$ M) in acetone with various concentrations of CN^-.

Figure 5 Color changes of **S1** ($5x10^{-5}$ M) with various concentrations of (a) F^-in acetone and (b) F^- in acetonitrile: acetone (7:3).

To develop the selectivity of **S1** for F^- or CN^- in acetone, a mixed solvent system which increased solvent polarity was studied under a hypothesis that only the strongest base anion can interact and deprotonate the sensor binding sites in a high polar solvent. The basicity order of anions in DMSO is $F^->$ $CN^->AcO^->BzO^->H_2PO_4^->Cl^->Br^-$ (Bordwell pK_b data). A replacement of various solvent ratios of acetonitrile: acetone such as 5:5, 6:4, 7:3 and 8:2 were carried out using the same amount of **S1** and anions (Figure 3). The selectivity for F^-only was achieved when the solvents were in the ratios of 5:5, 6:4 and 7:3. The existence of F^- in a solution of **S1** in these ratios caused a color change from pale yellow to orange.

The lowest concentration of F^- and CN^- that could be clearly observed by the naked eye from a solution of **S1** ($5x10^{-5}$ M) was examined in two solvent media which were acetone and acetonitrile : acetone (7:3). Different concentrations of F^- and CN^- were added into the **S1** solution. The naked eye color change from each experiment was shown in Figure 4 and 5. In acetone, the lowest concentrations of F^- and CN^- that could be detected by **S1** were $4x10^{-4}$ and $2x10^{-4}$ M, respectively. Results obtained from acetonitrile : acetone (7:3) showed a poorer in F^- detection than that obtained from acetone. The lowest concentration of F^- for a solution of **S1** in acetonitrile : acetone (7:3) was $2x10^{-3}$ M (Figure 5b). These results revealed that compound **S1** in acetone was able to detect cyanide and fluoride ions but this sensor could detect CN^- at lower concentration than F^-.

2. Investigation of host-guest binding by ^{1}H-NMR spectroscopy

To understand the anion binding interaction of sensor **S1** with F^- and CN^-, ^{1}H-NMR experiment of a solution of sensor **S1** in DMSO-d_6 was studied. It is well recognized that the interaction of anions with amide and phenol protons through hydrogen bond interactions affect a downfield shift and a broadening of the resonance of these protons with increasing anion concentrations (Chen, 2014; Mukherjee, 2014; Sen, 2016). A deprotonation at the sensor binding sites by anions can cause the disappearance of the related ^{1}H-NMR signal after a downfield shift and broadening of the proton peaks. (Ros-Lis, 2007; Goswami, 2011)

A ^{1}H-NMR titration spectra of sensor **S1** with F^- (0 – 2.0 equiv) revealed that the NH-amide proton at 10.75 ppm was shifted to downfield and broadened after the addition of F^- (0.3 – 0.5 equiv). The addition of F^- over 0.5 equiv, the NH-amide proton was completely disappeared. These results support a deprotonation at the amide binding sites by F^-. This mechanism was confirmed by the observation of a new triplet peak of 16.1 ppm (Figure 6, inset) at a higher concentration of F^- (4 equiv) addition. This peak was corresponded to F_2H^- ion, a deprotonation product from F^- (Kigga, 2014; Mukherjee, 2014). The most important result observed in ^{1}H-NMR titration was a broadening and a disappearance of azobenzene protons in a range of 7.8 – 8.5 ppm after the addition of F^- over 0.3 equiv. This result is due to the deprotonation at HO-phenol proton by F^-, inducing the intramolecular charge transfer (ICT) from donor to the acceptor substituent ($-NO_2$) of azobenzene. When the ICT process is in equilibrium, the broadening and subsequent disappearance of azobenzene protons was observed (Figure 8). Nitro-azobenzene has been reported to the occurrence of ICT by a negative charge nearby (Kigga, 2014; Odago, 2010). This phenomena result in a bathochromic shift in the absorption band as well as in color change of the sensor (Suksai, 2003). Corresponding to the change in UV-Vis experiments with F^- and CN^-, the appearance of a new band at 520 nm was due to the deprotonation of HO-phenol which initiated the ICT process at azobenzene moieties causing the color change to red of the sensor.

In case of a ^{1}H-NMR titration of sensor **S1** with CN^-, similar results in a change of ^{1}H-NMR signals were observed (Figure 7). Unlike F^-, a larger amount of CN^- must be used in the experiment to complete the deprotonation of NH-amide. Although 2.0 equiv of CN^- were added, the NH-amide proton became broadening and did not vanish. The complete disappearance of the amide proton was observed when 4.0 equiv of CN^- were added (Figure 9). Additionally, it was found that azobenzene protons were also broadened and disappeared after the addition of 4.0 equiv of CN^-. This observation indicated that excess amounts of CN^- was able to deprotonate at HO-phenol and then attributed the ICT at azobenzene moiety. Considering the nature of CN^-, this anion can act as a base in deprotonation or nucleophile in the nucleophilic addition with sensor binding units. To find out the interaction of **S1** with CN^-, the ^{1}H-NMR titration with Zn^{2+} into a solution of sensor **S1** ($5x10^{-4}$ M) + CN^- (4.0 equiv) in DMSO-d_6 was carried out. The ^{1}H-NMR titration results with Zn^{2+} as demonstrated in Figure 9 showed that after the addition of Zn^{2+} over 0.6 equiv, the NH-amide and azobenzene peaks were reemerged. All ^{1}H-NMR signals was changed to the initial position of **S1** after adding 1.0 equiv of Zn^{2+}. Not only the reappearance of the ^{1}H-NMR peak, but also an observable color change from red back to pale yellow was found. These results indicated that the interaction process between **S1** and $CN^-$is reversible. This demonstrates that CN^- is not bond-forming reaction by nucleophilic addition because this process is usually irreversible. The same experiment was reported in the proof of CN^- interaction mechanism in hydrazine based sensor (Cao, 2013). They explained that Zn^{2+} which is capable of strong complexing with CN^-, abstract CN^- from HCN and then free H^+ is released. This proton recombines with the deprotonated receptor to obtain the starting form of receptor. All ^{1}H-NMR results exhibited the deprotonation at the binding units of sensor **S1** by F^- and CN^-. This process occurred faster in case of F^- than CN^- because of the stronger base anion of F^-.

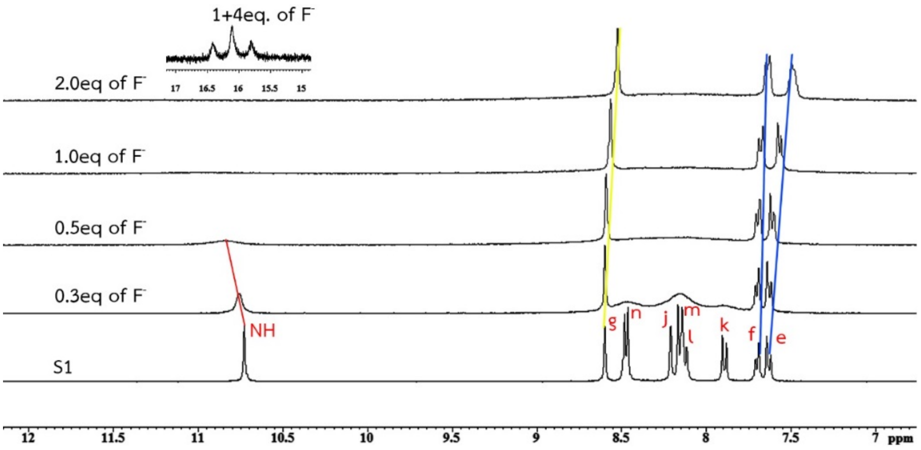


Figure 6 ^{1}H-NMR titration of **S1** ($1x10^{-3}$ M) in DMSO-d_6 upon the addition of fluoride ion (0-2 equiv) and a proposed mechanism in the binding of **S1** and F^-.

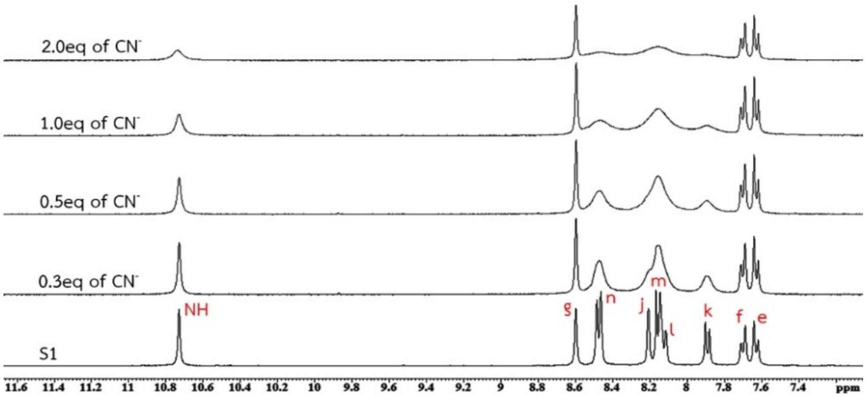


Figure 7 ^{1}H-NMR titration of **S1** (1x10^{-3} M) in DMSO-*d*$_6$ upon the addition of cyanide ion (0-2 equiv) and a proposed mechanism in the binding of **S1** and CN$^-$.

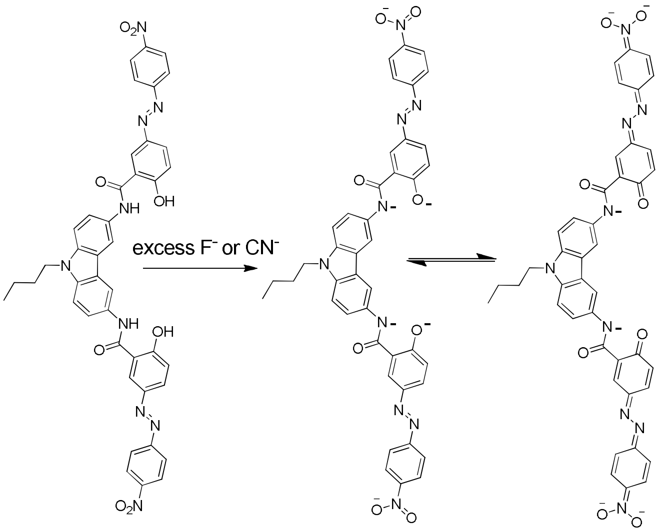


Figure 8 A proposed mechanism of sensor **S1** in the binding with fluoride and cyanide ions.

To compare the selectivity of F$^-$ and CN$^-$ with a relatively less responsive anions, ^{1}H-NMR spectrum of sensor **S1** was studied by the addition of 2.0 equiv of other anions such as AcO$^-$, $H_2PO_4^-$, HSO_4^-, NO_3^- and BzO$^-$ (Figure 10). It was found that an expected downfield shift of NH-amide proton in the case of AcO$^-$ and BzO$^-$ was observed. This shift indicated a hydrogen bonding interaction taken place in case of the less basic anions (Hijji, 2009). Interestingly, the addition of AcO$^-$, $H_2PO_4^-$, HSO_4^-, NO_3^- and BzO$^-$ did not affect any change of azobenzene protons. This result is agreed well with the naked eye observation which exhibited no change in color of sensor **S1**. Thus, results from all techniques support that the deprotonation of sensor **S1** by highly basic anions inducing ICT process at azobenzene lead to the naked eye distinct color changes of the sensor. Stoichiometry of **S1** with anions was studied using Job's plot from ^{1}H-NMR titration data. In case of AcO$^-$, BzO$^-$ and $H_2PO_4^-$ (large anion), 1:1 complex formation was obtained as shown in Figure 11a. Different stoichiometry of 1:2 host-guest complex was determined from the titration data with F$^-$ and CN$^-$ (small anion, Figure 11b).

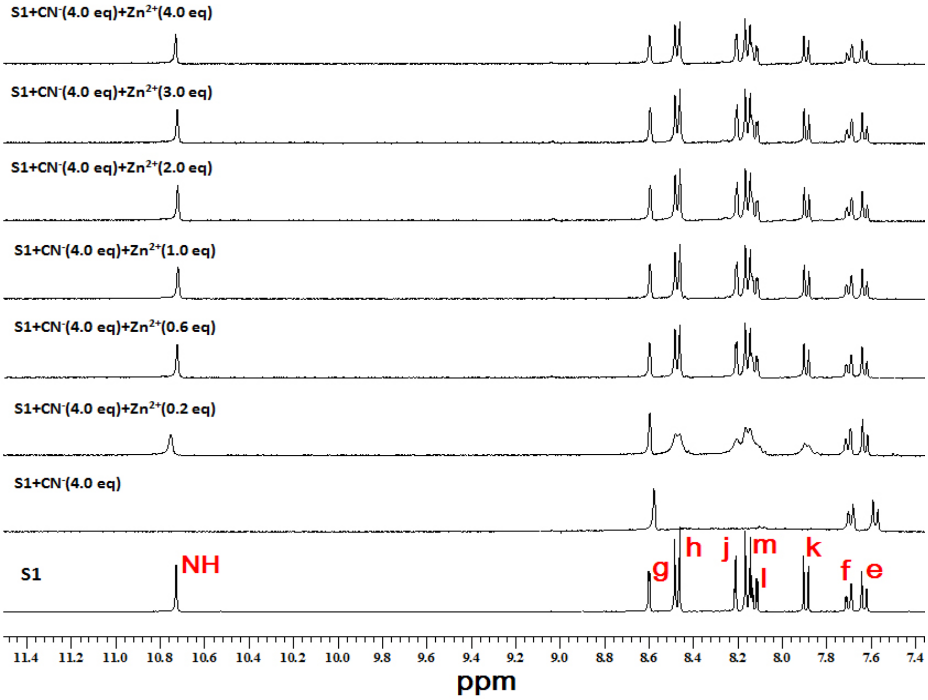


Figure 9 ^{1}H-NMR titration of **S1** + CN$^-$ (4.0 equiv) in DMSO-d_6 upon the addition of Zn^{2+} ion (0-4 equiv).

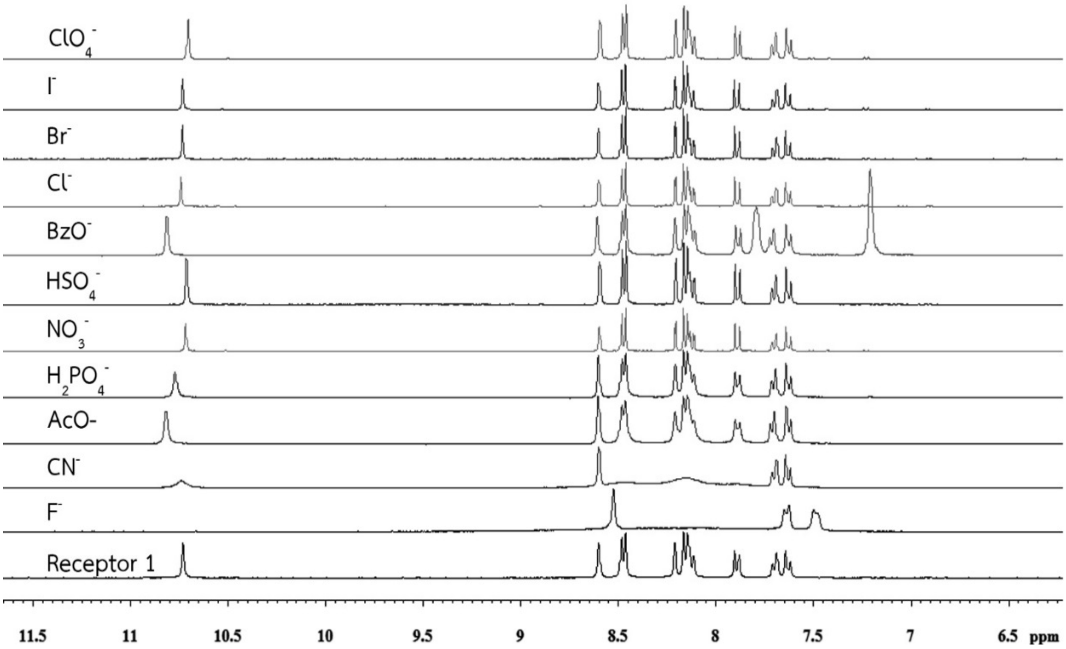


Figure 10 ^{1}H-NMR titration of **S1** (1x10^{-3} M) in DMSO-d_6 upon the addition of various anions (2 equiv).

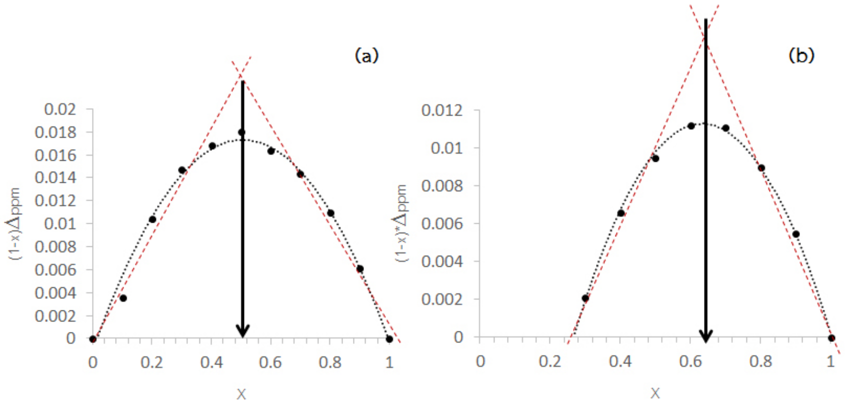


Figure 11 Job's plots from ^{1}H-NMR experiments for stoichiometry of (a) **S1**:AcO$^-$ and (b) **S1**:F$^-$.

CONCLUSIONS

A molecular sensor from carbazole and azobenzene showed its ability as a colorimetric sensor for cyanide and fluoride anions. The moderate yield of this compound was obtained and characterized. The selective detection for cyanide and fluoride in organic solvent involved initial 1:2 complex formation followed by deprotonation at higher concentration of basic anions. This deprotonation resulted in charge transfer transition through the nitro-azobenzene and eventually remarkable color change form pale yellow to red. The selectivity for fluoride was developed in a mixed solvent system which was acetonitrile : acetone (7:3). A limit of determination by the naked eye observation for cyanide and fluoride anions was studied and found that sensor **1** could detected fluoride ion at a lower concentration than cyanide ion.

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