Facile synthesis of an optical sensor for CO$_3^{2−}$ and HCO$_3^{−}$ detection
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A novel fluorogenic signalling probe (E)-3-(4-methoxyphenyl)-4-[(4-nitrobenzylidene) amino]-1H-1,2,4-triazole-5(4H)-thione (6) for the carbonate and bicarbonate ions has been developed through microwave assisted Schiff base formation reaction. The anion recognition occurs through hydrogen bonding assessed by $^1$H NMR titration experiments. The photo-physical results of probe 6 corroborates its applicability as an optical sensing platform for carbonate as well as bicarbonate ions in mixed aqueous organic media depending on pH of reaction solution. The fluorescence emission signal enhancement at 424 nm and considerable shift in the signal position as well as molar absorptivity to the probe absorption bands upon CO$_3^{2−}$ and HCO$_3^{−}$ addition suggest the affinity of probe 6 towards these ions in comparison to a variety of competitive ions in aqueous/ethanol (7:3, v/v) at neutral pH and ambient temperature. From the fluorescence titration experiment, the limit of detection was calculated to be 1.91 μM.

Keywords: microwave assisted synthesis; Schiff base formation; optical sensor; carbonate; bicarbonate; fluorescence increment

Introduction

In the recent years, considerable interest has been attracted in selective recognition and sensing of anionic species via artificial receptors in terms of their potential applications in the environmental, biological and clinical areas [1–9]. Metal carbonates and bicarbonates are ubiquitous in natural waters and soil environments as simple chemical compounds of industrial and environmental importance [10–12]. The major sources of carbonates are the hydrolysis of carbon dioxide into carbonic acid and consequential transformation into carbonates and bicarbonates which involved in rock weathering, mineral precipitation, ocean acidification and climate change [13]. Carbonates of calcium are important materials for marine and geological processes, often created as a result of biomineralisation that is used by nature to perform many diverse functions in marine organisms including skeleton and shell growth. Carbonate compounds are used extensively in the manufacturing of glass, rayon, rubber, plastic, paper, printing ink, cosmetics, toothpaste and food; meanwhile, carbonates are important candidates for electric vehicle and hybrid electric vehicle power sources where vinylene carbonate are used as additive electrolytes for rechargeable Li-ion batteries [14–18]. Effluents produced from these industries often contain substantial concentration of carbonates. In spite of essential roles in various processes, the carbonate ion is toxic in large doses. The strong caustic effect carbonate/bicarbonate to the gastro-intestinal tract may cause severe abdominal pain, vomiting, diarrhoea, collapse and even death [19]. In view of widespread occurrence of carbonate and bicarbonates in physiological, industrial and environmental samples, there exists a necessity of a durable,
accurate and rapid sensor for biomedical applications, clinical and environmental analysis with potential for real sample investigation without interference from endogenous substrates [20–26].

A number of analytical methods have been developed for carbonate detection including Fourier transform infrared (FT-IR) spectroscopy [27], passive acoustic emission [28,29], gas chromatography [30], pH-ion-sensitive field-effect transistor as a sensitive element [31,32], ion-selective electrodes [19,33–35] and chromoionophore based optodes [36]. Alongside, the development of luminescent signalling systems utilising organic chromophores is an active area of research in photochemistry to establish fluorescent chemosensors which can be applicable to industry, diagnostic and various kinds of environmental monitoring. The fluorescence-based techniques are important tools because of their appropriate acclamatory features of simple instrumentation, potentially rapid response time, non-invasive nature, high intrinsic sensitivity, appreciable detection selectivity, high temporal resolution and easy signal detection nature [6,37–39].

Herein, we report the synthesis of (E)-3-(4-methoxyphenyl)-4-[(4-nitrobenzylidene)amino]-1H-1,2,4-triazole-5(4H)-thione (6) as a fluorescent signalling probe for the selective detection of CO$_3^{2-}$ and HCO$_3^{-}$ in mixed aqueous–organic media. The absorption spectra of compound 6 (10 × 10$^{-6}$ molL$^{-1}$) in ethanol/water (3:7, v/v) reveal three absorption bands at 216, 253 and 278 nm with molar absorption coefficient values of 6.1 × 10$^4$, 3.6 × 10$^4$ and 3.3 × 10$^4$ M$^{-1}$ cm$^{-1}$, respectively. Interestingly, upon addition of carbonate as well as bicarbonate ions, there was considerable shift in the signal position as well as molar absorptivity on the probe absorption bands. Meanwhile, through fluorescence titration at 424 nm, it was confirmed that probe 6 exhibited ratiometric fluorescence response with remarkable enhancement in the emission intensity triggered by carbonate ions while no emission occurred in the case of the competitive ions (F$^-$, Cl$^-$, Br$^-$, NO$_2^-$, ClO$_2^-$, ClO$_3^-$, ClO$_4^-$, SO$_4^{2-}$, SiO$_3^{2-}$, NO$_3^{2-}$ and CH$_3$COO$^-$) in aqueous/ethanol (7:3, v/v) at neutral pH and ambient temperature. These characteristic changes in the absorption and emission spectra of probe 6 upon addition of carbonate as well as bicarbonate ions can be used as a tool for sensation of CO$_3^{2-}$ and HCO$_3^{-}$ in mixed aqueous organic media at the micro molar concentration level.

**Experimental**

**Substrates and reagents**

4-Methoxybenzoic acid, 4-nitrobenzaldehyde and phosphorous oxychloride were purchased from Aldrich. Hydrazine hydrate (80%), triethyl amine (TEA), CS$_2$, KOH and sodium hydrogen carbonate were obtained from Sigma-Aldrich. Sodium and potassium salts of F$^-$, Cl$^-$, Br$^-$, NO$_2^-$, ClO$_2^-$, ClO$_3^-$, ClO$_4^-$, SO$_4^{2-}$, SiO$_3^{2-}$, NO$_3^{2-}$, CH$_3$COO$^-$, HCO$_3^{-}$ and CO$_3^{2-}$ were obtained from Aldrich and Alfa Aesar. Ethanol, methanol, chloroform, water, acetonitrile, dimethyl sulfoxide, petroleum ether, ethyl acetate, n-hexane (Samchun Chemicals, Korea), H$_2$SO$_4$ and HCl (Jin Chemical & Pharmaceutical Co. Ltd., Korea) were used in these experiments.

**Instrumentations**

The reaction progress was monitored by thin layer chromatographic (TLC) analysis and the $R_f$ values were determined by employing pre-coated silica gel aluminium plates, Kieselgel 60 F$_{254}$ from Merck (Germany). TLC was visualised under a UV lamp (Vilber Lourmat-4 LC, France). The melting points were determined on a Digimelt MPA 160, USA, melting point apparatus and are uncorrected. The FT-IR spectra were recorded in KBr pellets on a Shimadzu FTIR-8400S
spectrometer (Kyoto, Japan). Proton and carbon nuclear magnetic resonance (1H NMR & 13C NMR) spectra were recorded on a Bruker Avance 400 MHz spectrometer with tetramethylsilane (TMS) as an internal standard. The chemical shifts are reported as δ values (ppm) downfield from the internal TMS of the indicated organic solution. Peak multiplicities are expressed as follows: s, singlet; d, doublet and m, multiplet. Abbreviations are used as follows: CD3OD, deuterated methanol; DMSO-d6, dimethyl sulfoxide-d6; D2O, deuterated water; FT-IR spectroscopy. The schematic representation of the synthetic route adopted to obtained (E)-3-(4-methoxyphenyl)-4-[(4-nitrobenzylidene)amino]-1H-1,2,4-triazole-5(4H)-thione (6) is shown in Scheme 1.

**General procedure for the synthesis of 4-methoxybenzohydrazide (3)**

4-Methoxybenzoyl chloride (2) was synthesised by the reaction of 4-methoxybenzoic acid (1) (1 mmol) in the presence of 1,2-dichloroethane (12 mL) solvent and the phosphorous oxychloride (0.4 mL) chlorinating agent under reflux for 3 h. Then, the resulting solution was cooled to room temperature, and the solvent was removed under reduced pressure to afford 2, which was directly used in the next step without further purification. Compound 2 was dissolved in acetonitrile (80 mL), added dropwise to a solution containing hydrazine hydrate (1 mmol), TEA (0.5 mL), acetonitrile (20 mL) and allowed to reflux for 3 h with monitoring by TLC. After consumption of the starting material, the reaction mixture was cooled to room temperature. Evaporation of the solvent under reduced pressure yielded crude 4-methoxybenzohydrazide (3) as a white solid upon cooling, which was purified by column chromatography if needed and crystallised in methanol.

White solid; yield: 84%; mp: 133–135°C; Rf: 0.38 (chloroform:methanol, 9:1); FT-IR (ν/cm−1): 3342, 3287 (NH2), 3158 (NH) 3030 (sp2 CH), 2937, 2874 (sp3 CH), 1628 (C=O), 1554, 1507, 1486 (C=C of phenyl ring); 1H NMR (400 MHz, DMSO-d6) δ 9.21 (s, 1H, NH), 7.36–7.24 (m, 2H, Ar-H), 6.97–6.84 (m, 2H, Ar-H), 4.27 (s, 2H, broad, NH2), 3.74 (s, 3H, OCH3); 13C NMR (100MHz, DMSO-d6) δ 168.7, 159.1, 136.5, 132.7, 129.4, 126.2, 114.5, 57.1.
**General procedure for the synthesis of 4-amino-3-(4-methoxyphenyl)-1H-1,2,4-triazole-5(4H)-thione (5)**

Potassium hydroxide (0.125 mol, 1 eq.) was dissolved in dry methanol (50 mL). To the solution, 3 (0.125 mol, 1 eq.) was added and the solution was cooled in ice. To this, carbon disulfide (0.125 mol, 1 eq.) was added with constant stirring for 1–2 h. The solid product of potassium 2-(4-methoxybenzoyl)hydrazine carbodithioate (4) formed was filtered, washed with chilled diethyl ether and dried. It was directly used for the next step without further purification. Compound 4 was taken in deionised water (50 mL) and hydrazine hydrate (0.250 mol) was added, followed by reflux overnight. The reaction mixture turned to yellowish green with the evolution of hydrogen sulphide and finally it became homogeneous. It was then poured in crushed ice and neutralised with dilute hydrochloric acid to afford white precipitates of 5 which was filtered, washed with cold water and crystallised in methanol.

White solid; yield: 78%; mp: 215–217°C; Rf: 0.23 (n-hexane:ethyl acetate, 7:3); IR (υ/cm−1) 3277, 3181 (NH2), 3057 (sp2 CH), 2932, 2874 (sp3 CH), 1597 (C=N), 1553, 1507, 1484 (C=C), 1320 (C=S); 1H NMR (400 MHz, CDCl3) δ 13.64 (s, 1H, NH), 7.41–7.34 (m, 2H, Ar-H), 6.98–6.85 (m, 2H, Ar-H), 5.61 (s, 2H, broad, NH2), 3.75 (s, 3H, OCH3); 13C NMR (100 MHz, CDCl3) δ 167.1, 159.5, 150.3, 135.2, 131.7, 126.1, 119.5, 114.5, 56.9.

**General procedure for the synthesis of target compound 6**

Compound 5 (0.3 g, 0.0018 mol, 1 eq.) was dissolved in methanol (20 mL) and 4-nitrobenzaldehyde (0.27 g, 0.0018 mol, 1 eq.) was separately dissolved in methanol (20 mL) and both solutions were mixed together, and two drops of concentrated H2SO4 was added, prior to exposure to microwave for 10 min with constant monitoring of the reaction progress by TLC after regular interval of 2 min. Upon complete consumption of the starting materials, the extra solvent was evaporated under reduced pressure and the resulting powder was crystallised on methanol. The purification of the product was done by column chromatography with the n-hexane:ethyl acetate solvent system. In order to synthesise the target compounds, the microwave assisted synthetic procedure was adopted due to its auspiciousness over the other methods. Upon using the microwave-assisted synthetic process, there is considerable advantage of less time consumption. The final product can be achieved in a good yield within 10 min. while without microwave the reaction time exceed a few hours. With these advantageous features of short reaction time and high product yield, the authors have selected the microwave-assisted synthetic procedure to synthesise the desired probe.

Yellow solid; yield: 85%; mp: 260–262°C; Rf: 0.42 (n-hexane:ethyl acetate, 7:3); 1H NMR (400 MHz, DMSO-d6) δ 14.22 (s, 1H, NH), 10.06 (s, 1H, imine proton), 8.39–8.14 (m, 2H, aromatic proton), 7.84–7.80 (m, 4H, aromatic proton), 7.11–7.07 (m, 2H, aromatic proton), 3.64 (s, 3H, OCH3); 13C NMR (100 MHz, DMSO-d6) δ 174.9, 161.5, 150.0, 130.4, 130.2, 124.7, 118.0, 114.7, 55.8; MS for C16H13N5O3S (ESI, negative mode, m/z), 354 [M − H].

**General procedure for spectroscopic measurement**

Probe 6 stock solution (500 μM) was prepared by dissolving 1.77 mg probe 6 in ethanol (total volume 10 mL). Similarly, to prepare 500 μM K2CO3 stock solutions, 0.691 mg of K2CO3 was dissolved in distilled water (total volume 10 mL) and the same procedure was followed in order to prepare other ion solutions. For spectroscopic measurements, a test solution of 3 mL was prepared with 2.77 mL of ethanol/water, 10 μL of ligand stock solution, 0.3 mL of buffer solution (EtOH/Tris–HCl buffer, 100 mM) and 20 μL of K2CO3 stock solution. The resulting solutions were mixed
before measurement and the final volume was fixed at 3 mL for UV-visible and fluorescence studies. All recognition studies were performed at 25°C while the samples were shaken to ensure solution uniformity prior to spectrum recording on a [SCINCO] UV-Vis Spectrophotometer ‘S-3100’ and FS-2 fluorescence spectrometer (SCINCO, Korea), respectively.

Results and discussions

Synthesis

The conversion of 1 to 2 was indicated by the FT-IR spectral data by the disappearance of a broad signal in the range of 3400 – 2500 cm⁻¹ due to an acid hydroxyl group. The formation of compound 3 was indicated by the FT-IR spectral data by the appearance of new signals at 3342, 3287 and 3158 due to primary and secondary amino groups of acid hydrazide. Meanwhile, there was a slight shift in the carbonyl stretching vibration from 1733 to 1628 cm⁻¹ indicating the successful conversion of compound 2 into 3. The appearance of a broad singlet due to primary and secondary amino groups at 9.21 ppm as well as 4.27 ppm, respectively, further confirms the formation of 3. The formation of compound 5 was characterised by FT-IR, ¹H NMR and ¹³C NMR spectral analysis. In the FT-IR spectrum, a relatively broad peak in the range of 3277 and 3181 cm⁻¹ with shoulder for NH₂ stretching vibration and a new signal at 1320 cm⁻¹ for C=S stretching vibration were observed, indicating the transformation of 3 into 5. In the ¹H NMR spectrum, characteristic highly downfield signals at 13.64 ppm due to triazole ring secondary amino proton resolution and at 5.61 ppm due to the primary amino proton were observed, confirming the formation of 5. The condensation of 5 with 4-nitrobenzaldehyde was confirmed by NMR (¹H NMR & ¹³C NMR) spectral analysis. The slight downfield shift in the NH signal from 13.64 to 14.22 ppm and complete disappearance of the NH₂ signal at 5.61 ppm confirms the Schiff base formation. Meanwhile, the appearance of a downfield singlet at 10.06 ppm due to imine protons further confirms the conversion of 5 into 6. Alongside the additional signal in both carbon and proton, the NMR spectrum confirms the successful synthesis of target compound 6. Additionally, probe 6 exhibited a strong molecular ion peak [M‒H]⁻ at m/z = 354 in the mass spectrum showing the correct mass of 6 (Figure S1, Supplemental data).

Proposed binding mechanism

In order to evaluate the possible binding mode of carbonates to the probe, the ¹H NMR titration experiment was conducted before addition of carbonate ions (solution of K₂CO₃ in D₂O) into the probe solution (probe 6 solution into DMSO-d₆) as well as after successive addition of carbonate ions into probe 6 solution. Theoretically, two isomeric conformations were possible for probe 6 due to the presence of a labile hydrogen atom, which may reside on the nitrogen atom of the triazole ring (thione tautomer) and it might be shifted to the sulphur atom (thiol tautomer). This labile hydrogen atom resonates at highly downfield area at 14.22 ppm as a singlet signal in the ¹H NMR spectrum while after addition of aqueous solution of carbonate ions into probe 6, this labile proton signal diminishes due to its replaceable characteristics with the solvent molecule. Meanwhile, the imine proton appears as a singlet at 10.06 ppm before addition of carbonate ions into the probe 6 solution. However, after addition of potassium carbonate solution into probe 6, there was momentous downfield shift in the imine proton and this downfield shift continuously increased upon massive addition of potassium carbonate solution into probe 6 as shown in Figure 1.

This downfield shift in the imine proton of probe 6 provides solid evidence for the involvement of imine protons in the binding with carbonate ions while the fate of the labile proton remains ambiguous due to its replaceable nature with the solvent molecule. On the basis of this ¹H NMR titration experiment, the proposed mechanism for carbonate binding is given in Scheme 2.
Figure 1. $^1$H NMR spectra of probe 6 alone and after successive addition of CO$_3^{2-}$ (1–3 eq., aqueous solution in deuterated water) into probe 6 solution (DMSO-$d_6$).

Scheme 2. The proposed binding mechanism of probe 6 with the carbonate ion on the basis of $^1$H NMR titration experiments.
Chromogenic response of the probe towards the anionic induction

The colorimetric response of probe 6 was consistent with that of the UV-visible/fluorescence and NMR results. The strong colorimetric change in the probe solution from dark yellow to maroon upon 4 eq. carbonate ion addition corroborates the successful interaction between the chromophore and the corresponding anions while there was no such response in the probe solution upon induction of varieties of the competing anionic species, indicating that probe 6 can serve as a potential candidate for ‘naked-eye’ detection of carbonate/bicarbonate anions (Figure 2). The exclusive selectivity of the sensor towards only carbonate/bicarbonate anions might be due to a particular high thermodynamic affinity of the probe to involve in the hydrogen bonding with that of the respective anions.

The probe behaves selectively towards the carbonate/bicarbonate ions in comparison to the number of competing anionic moieties i.e. F\(^-\), Cl\(^-\), Br\(^-\), NO\(_2\)\(^-\), ClO\(_2\)\(^-\), ClO\(_3\)\(^-\), ClO\(_4\)\(^-\), SO\(_4\)\(^{2-}\), SiO\(_3\)\(^{2-}\), NO\(_3\)\(^{2-}\) and CH\(_3\)COO\(^-\) as there are several reasons for this selectivity which can be summarised as below.

The fluoride ion is a very good base and due to its highest electronegativity among the discovered elements, its sensing mechanism towards Schiff bases followed the acid–base reaction as several reports can be found in the recent literature. In our designed probe, there is clear evidence for the involvement of the imine proton in the sensing process established by the NMR titration experiments (Figure 1). However, NMR titration experiments clearly represent that no acid–base reaction occurred in the molecule as a clear downfield shift was found in the imine proton but there is no evidence for this proton removal which ruled out the speculation of acid–base reaction in the probe. That is why no prominent signal shift was observed in the case of addition of the fluoride ions to the probe solution. Moreover, upon moving down group VII, the basicity of the element decreased and the nucleophilicity along with element size increased. Due to large size and high nucleophilic nature of the rest of the halogen elements, no pronounced changes were observed upon incubating them with the probe solution. Meanwhile, SO\(_4\)\(^{2-}\), SiO\(_3\)\(^{2-}\), NO\(_3\)\(^{2-}\) and CH\(_3\)COO\(^-\) are quite bigger size and have less basicity, which do not involve in the hydrogen bonding process with that of receptors due to their incompatibility towards the probe binding. Only the carbonate/bicarbonate ions were the basic elements, which selectively involved in the hydrogen bonding with that of the probe and shift the signal in the longer wavelength due to intermolecular charge transfer.

Photophysical properties

To examine the feasibility of probe 6 (10 μM) as an optical sensor for carbonate ions, the UV-visible and fluorescence properties were investigated in the absence and presence of carbonates by recording the absorption and emission spectra. Probe 6 was stable in a solvent mixture EtOH/
H$_2$O (v/v = 3/7, pH = 7, EtOH/Tris–HCl buffer). The absorption spectra of compound 6 (10 × 10$^{-6}$ molL$^{-1}$) in ethanol/water (3:7, v/v) reveal three absorption bands at 216, 253 and 278 nm with molar absorption coefficient values of 6.1 × 10$^4$, 3.6 × 10$^4$ and 3.3 × 10$^4$ M$^{-1}$ cm$^{-1}$, respectively. The absorption maxima obtained at longer wavelength were attributed to n→π* electronic transition of the conjugated system while high energy transition at shorter wavelength was considered due to π→π* electronic transition as energy gaps between π→π* energy levels are relatively high as compare to n→π*. Interestingly, after addition of 2 eq. K$_2$CO$_3$ into probe 6 solution, the intensity of the first absorption band was increased with the same absorption maxima bearing molar absorption coefficient values of 7.4 × 10$^4$ M$^{-1}$ cm$^{-1}$ while the second and third absorption bands were red shifted and new signals centred at 287 and 322 nm with the considerable molar absorption coefficient values of 2.8 × 10$^4$ and 2.7 × 10$^4$ M$^{-1}$ cm$^{-1}$, respectively, appeared. Meanwhile, upon bicarbonate addition, there was momentous rise in the first absorption band centred at 216 nm with the molar absorption coefficient values of 9.9 × 10$^4$ M$^{-1}$ cm$^{-1}$. At the same time, bicarbonate ions trigger 9 nm blue shift in the second absorption band with significant molar absorption coefficient values of 6.0 × 10$^4$ M$^{-1}$ cm$^{-1}$ while in the case of the third band, approximately the same molar absorption coefficient values (3.4 × 10$^4$ M$^{-1}$ cm$^{-1}$) at the same absorption maxima like that for the probe were observed. These characteristic changes in the absorption spectrum of probe 6 upon addition of carbonate and bicarbonate ions can be used as a tool for sensation of CO$_3^{2-}$ and HCO$_3^-$ in mixed aqueous organic media at the micro molar concentration level. The absorption spectrum of probe 6 in the presence and absence of carbonate as well as bicarbonate ions are shown in Figure 3.

The recognition ability of probe 6 towards carbonate ions was established upon titrating the probe solution with variable concentration of carbonate ions followed by subsequent measurement of absorption spectra. In the UV–visible titration experiment, the intensity of the newly red shifted signal triggered by carbonate ions continuously increases with successive increment of carbonate ions (2–10 eq.) into the probe solution as shown in Figure 4. This UV-visible titration

![Figure 3. UV–visible absorption spectra of probe 6 (10 μM) and its reaction solution with carbonate as well as bicarbonate ions (10 eq.) in ethanol/water (3:7, v/v) at pH 7.](image-url)
experiment highlights the ratiometric response of the probe towards carbonate ions as representation of the useful signalling system to assess the carbonate ions in real sample analysis.

To further assess the ratiometric response of probe 6, the fluorescence titration experiment was conducted with different concentrations of carbonate ions in EtOH/water (3:7, v/v, pH 7) at ambient temperature and the changes in the fluorescence spectra are indicated in Figure 5. In the absence of carbonate ions, the free probe solution did not show any fluorescent signal at 424 nm while upon successive addition of carbonate ions into probe 6 solution; there was a continuous increment in the fluorescent signal. A linear relationship was obtained between the fluorescence intensities at 424 nm upon increasing the concentration of carbonate ions. From this titration experiment, the minimal concentration of carbonate ions required to trigger the fluorescence signal from the probe solution (10 μM) was found to be about 10 μM under neutral pH conditions and the value of linearly dependent co-efficient ($R^2$) was found to be 0.9045 (inset of Figure 5). The comparably low value of linearly dependent coefficient and the poor linear relationship in the probe fluorescence spectrum upon incremental addition of carbonate ions partially ruled the 1:1 probe–anion binding stoichiometry. Moreover, from the fluorescence titration experiment (inset graph), the limit of detection was calculated to be 1.91 μM by using the relation $3 \sigma / \text{slope}$.

The selectivity behaviour over a wide range of background anions is obviously a matter of necessity for an excellent sensing material. To gain insight into the selective binding properties of probe 6 specifically with carbonate and bicarbonate ions, the recognition profiles of probe 6 (10 μM) towards various inorganic ions (20 μM) including F$^-$, Cl$^-$, Br$^-$, NO$_2^-$, ClO$_2^-$, ClO$_3^-$, ClO$_4^-$, SO$_4^{2-}$, SiO$_3^{2-}$, NO$_3^{2-}$ and CH$_3$COO$^-$ were primarily investigated by UV-visible spectroscopic analysis in EtOH/H$_2$O (3:7, v/v). The results showed that upon addition of carbonate as well as bicarbonate ions, there was considerable shift in the signal position as well as molar absorptivity on the probe absorption bands. At that time, the competitive ions did not exhibit such a kind of signal in the mentioned range (Figure 6). As a whole, there was no (blue or red) shift in the probe signal for the variety of competitive ions and the unique signal appearance only for carbonate and bicarbonate ions suggests the selective affinity of probe 6 with only CO$_3^{2-}$ and HCO$_3^-$ in comparison to a variety of competitive ions.
Figure 5. The fluorescence titration of probe 6 (10 μM) at emission maxima of 424 nm as a function of carbonate ion concentration (0–4 eq.) in ethanol/water (3:7, v/v) at pH 7; the inset described the linear relationship obtained between the fluorescence intensity at 424 nm upon increasing concentration of carbonate ions while $F/F_0$ was determined as a ratio between the maximum fluorescence intensity ($F$, after carbonate ion addition into probe solution) and the minimum fluorescence intensity ($F_0$, before addition of carbonate ions into probe 6 solution).

Figure 6. UV-visible absorption spectra of probe 6 (10 μM) in the presence of carbonate and bicarbonate ions (20 μM) and competing ions $F^-$, $Cl^-$, $Br^-$, $NO_2^-$, $ClO_2^-$, $ClO_3^-$, $ClO_4^-$, $SO_4^{2-}$, $SiO_3^{2-}$, $NO_3^{2-}$ and $CH_3COO^-$ in ethanol/water (3:7, v/v) at pH 7 and ambient temperature.
To further validate the fluorescence selectivity of probe 6 over carbonate and bicarbonate ions in practice, the sensing properties of probe 6 towards various inorganic ions were examined by treating probe 6 (10 μM) with a variety of environmentally and biologically relevant anions (20 μM) including F−, Cl−, Br−, NO2−, ClO2−, ClO3−, ClO4−, SO4^{2−}, SiO3^{2−}, NO3^{2−} and CH3COO− in EtOH/water (3:7, v/v, pH 7) at ambient temperature. No considerable fluorescent signal was observed for the probe alone, in the presence of bicarbonate ions as well as competitive anions in the range of 375–500 nm under tested conditions. In contrast, the fluorescence intensity was notably enhanced upon addition of 2 eq. of carbonate ions into probe 6 solutions which suggest the selective tendency of probe 6 towards carbonate ions in comparison to background ions under neutral conditions (Figure 7).

The quick response time for a sensing material is a prerequisite for practical applicability. To understand the carbonate binding reaction time with probe 6, the fluorescence emission spectra of probe 6 was recorded by variable time interval and the results revealed that the fluorescent signal appearance started immediately upon carbonate addition and completed within 1 min as shown in the fluorescence emission spectra at 424 nm at different time intervals (Figure 8).

The pH of the working solution is an important parameter for such a kind of sensing methodology. The effect of pH was evaluated in the pH range of 2–9 as shown in Figure 9. There was no emission signal at 424 nm for the probe (10 μM) alone in the entire pH range while after addition of carbonate ions into probe 6 solution, a drastic enhancement in the emission signal centred at 424 nm turns up in the broad pH span of 2–9. The lack of change in sensitivity of the probe towards pH gives an indication of the interference of bicarbonate ions towards the fluorescence signal enhancement because at pH values below 5–6, the carbonate ions may convert to bicarbonate ions and if the fluorescence signal emergence is considered due to only carbonate ions then there should be downfall in the fluorescence emission signal intensity under acidic conditions but the contradictory observation indicates the affinity of the probe towards both carbonate as well as bicarbonate ions. Finally, from these experiments, it was concluded that the probe behaves towards both carbonate and bicarbonate ions depending on the pH of the working solution.
The viability of the probe towards real sample analysis was investigated by employing distilled water as well as tap water under the same conditions. Almost the same fluorescence enhancement was observed in the case of distilled water as well as tap water, which suggests that the background ions present in the tap water do not interfere in the fluorescence emission spectra. However, it was believed that the tap water rich in carbonates may exert a prominent effect on the emission spectral characteristics (Figure 10).

Figure 8. Effects of reaction times on the fluorescence spectral intensity of probe 6 (10 μM) at emission maxima of 424 nm in the presence and absence of carbonate ions in ethanol/water (3:7, v/v) at pH 7.

Figure 9. The fluorescence emission spectral variation of probe 6 alone as well as after addition of carbonate ions with alteration of pH from 2 to 9.

The viability of the probe towards real sample analysis was investigated by employing distilled water as well as tap water under the same conditions. Almost the same fluorescence enhancement was observed in the case of distilled water as well as tap water, which suggests that the background ions present in the tap water do not interfere in the fluorescence emission spectra. However, it was believed that the tap water rich in carbonates may exert a prominent effect on the emission spectral characteristics (Figure 10).
Solvent effect

The solvent effect on the fluorescence emission spectra of the probe upon carbonate addition was observed by dissolving probe 6 in varieties of organic and aqueous-organic solvents and subsequent measurement of fluorescence emission spectra. The mixed aqueous organic solution because of the relevant features of pure aqueous or mixed aqueous media in biological samples analysis. The probe exhibited fair solubility in polar organic solvents including ethanol, methanol, acetonitrile and dimethyl sulphoxide. There was slight reduction in fluorescence intensity in polar aprotic solvent like DMSO while the maximum intensity fluorescence signal was observed in the case of ethanol, methanol and acetonitrile. These findings suggest that the most suitable solvent for carbonate binding to probe 6 is a polar protic solvent. The results of different solvents on the fluorescence emission intensities of probe 6 upon binding with carbonate ions are tabulated in Table 1.

Table 1. Solvent effect on the photophysical characteristics of the probe upon binding with carbonate ions.

| S. No | Solvent          | $\lambda_{em}$ (nm)$^a$ | $F/F_o$$^b$ | $\pi^*$$^c$ | $\beta$$^d$ |
|-------|------------------|--------------------------|-------------|-------------|-------------|
| 1     | EtOH:H$_2$O (7:3, v/v) | 424                      | 178         | 1.00        | 0.76        |
| 2     | MeOH:H$_2$O (7:3, v/v) | 424                      | 178         | 0.60        | 0.62        |
| 3     | MeCN:H$_2$O (7:3, v/v) | 424                      | 155         | 0.54        | 0.77        |
| 4     | DMSO:H$_2$O (7:3, v/v) | 424                      | 124         | 1.00        | 0.76        |

Notes: $^a$Fluorescence emission maxima.

$^b$F/F$_0$ was determined as a ratio between the maximum fluorescence intensity ($F$, after carbonate ion addition) and the minimum fluorescence intensity ($F_o$, free probe solution in the absence of carbonate ions).

$^c$Index of solvent polarity–polarisability for used organic solvent.

$^d$Parameters related to the organic solvent’s ability to accept a proton in a solute–solvent hydrogen bonding.
**Fluorescence quantum yield calculation**

The fluorescence quantum yield is perhaps an important characteristic of fluorophores. The fluorescence quantum yield is the number of emitted photons relative to the number of absorbed photons. The fluorescence quantum yield of the probe alone was almost equal to zero while a massive jump in the fluorescence quantum yield was observed upon addition of 2 eq. of carbonate ions into the probe solution. The probe exhibited a fluorescence quantum yield of 0.0097 upon addition of 2 eq. of carbonate ions calculated by using Equation (1) [41] utilising the emission spectra at 424 nm and excitation spectra at 322 nm. Quinine sulphate (0.5 M stock solution in 0.1 N sulphuric acid) was used as reference standard for relative fluorescence quantum yield determination [42–51].

\[
\Phi_{\text{unk}} = \Phi_{\text{std}} (I_{\text{unk}}/A_{\text{unk}})(A_{\text{std}}/I_{\text{std}})(n_{\text{unk}}/n_{\text{std}})^2, \tag{1}
\]

where \(\Phi_{\text{unk}}\) is the fluorescence quantum yield of the sample, \(\Phi_{\text{std}}\) is the quantum yield of the standard, \(I_{\text{unk}}\) and \(I_{\text{std}}\) are the integrated fluorescence intensities of the sample and the standard, respectively, \(A_{\text{unk}}\) and \(A_{\text{std}}\) are the absorbances of the sample and the standard at the absorption wavelength, respectively, \(n_{\text{unk}}\) and \(n_{\text{std}}\) are the refractive indices of the corresponding solvents.

**Computational study**

To get better sagaciousness in the distribution of an electronic cloud over probe upon binding with carbonate ions, the frontier molecular orbital i.e., highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO), was generated (Figure S2, Supplemental data). The extension of electron density was found over the acceptor unit of the molecule, triazole skeleton as well as carbonate moieties inside the molecule in the case of the HOMO. This electronic cloud extension might decrease the overall energy of the electronic transition and is responsible for the red shift of the absorption signals of the probe upon ionic binding. In the case of LUMO, the electron density was observed to spread on the triazole ring, electron withdrawing 4-nitrophenyl group, Schiff base skeleton of the molecule as well as further extension over the anionic territory. The minimum electron density was observed over the electron donor methoxy side of the molecule. Meanwhile, the energy minimised structure of probe with the total charge density after binding with carbonate ions was drawn to get insight into the rearrangement in probe upon binding with carbonate ions, which shows that the 4-nitrophenyl group and the 4-methoxyphenyl group in the core skeleton of probe arrange themselves in a trans conformation in order to minimise the repulsion to get better stability. However, the overall conformational structure adopted by the molecules upon binding with the carbonate ions is shown in Figure S2 (Supplemental data).

**Conclusion**

In summary, a simple, low cost and efficient Schiff base derivative was synthesised by reacting with 4-nitrobenzaldehyde in methanol upon exposure to microwave for 10 min. The formation of probe was characterised by FT-IR, \(^1\)H NMR, \(^13\)C NMR and mass spectrometric analysis. Probe (10 \(\times\) 10\(^{-6}\) mol L\(^{-1}\)) exhibited three absorption bands at 216, 253 and 278 nm with molar absorption coefficient values of 6.1 \(\times\) 10\(^4\), 3.6 \(\times\) 10\(^4\) and 3.3 \(\times\) 10\(^4\) M\(^{-1}\) cm\(^{-1}\), respectively. Interestingly, upon addition of carbonate as well as bicarbonate ions, there was considerable shift in the signal position as well as molar absorptivity on the probe absorption bands. Meanwhile, in the case of fluorescence emission spectral recording, the free
probe solution did not show any fluorescence signal at 424 nm in the absence of carbonate ions while upon successive addition of carbonate ions into probe 6 solution, there was a continuous increment in the fluorescence signal in aqueous/ethanol (7:3, v/v) at neutral pH and ambient temperature. From the fluorescence titration experiment, the limit of detection was calculated to be 1.91 μM (3σ slope$^{-1}$). The probe exhibited extraordinary selective tendency towards CO$_3^{2-}$ and HCO$_3^-$ over a wide range of back ground anions including F$^-$, Cl$^-$, Br$^-$, NO$_2^-$, ClO$_2^-$, ClO$_3^-$, ClO$_4^-$, SO$_4^{2-}$, SiO$_3^{2-}$, NO$_3^-$ and CH$_3$COO$^-$ investigated by UV-visible as well as fluorescence spectroscopic analysis. As a whole, the fluorescence emission signal enhancement at 424 nm and appearance of a unique shift in the absorption signal position as well as intensity upon CO$_3^{2-}$ and HCO$_3^-$ addition suggest the selective affinity of probe 6 towards these ions in comparison to a variety of competitive ions. It was believed that the proposed sensing methodology might be an efficient tool for analytical monitoring of trace CO$_3^{2-}$ and HCO$_3^-$ in real sample analysis.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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**Supplemental data**

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