Easy and rapid estimation of ammonia in cold storage potatoes: Precautions in environment

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1. **Table S1.** Performance comparison of existing methods and present method (fluorescence based chemosensor) for detection of NH$_3$.

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Method of detection</th>
<th>Sensor type</th>
<th>Detection limit</th>
<th>Detection state &amp; Selectivity</th>
<th>Sensitivity &amp; Selectivity</th>
<th>Response Time</th>
<th>Estimation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH$_3$</td>
<td>Fluorescence based chemosensor</td>
<td>Fluorescein-carbazole</td>
<td>4.3nM</td>
<td>Liquid</td>
<td>High</td>
<td>Instant</td>
<td>Yes</td>
<td>Current method</td>
</tr>
<tr>
<td>NH$_3$</td>
<td>colorimetric gas sensors</td>
<td>Porphyrin cobalt(I)-dansyl</td>
<td>40nM</td>
<td>Liquid</td>
<td>Moderate</td>
<td>Not mentioned</td>
<td>No</td>
<td>Tetrahedron Letters, 2011, 52, 2645–2648</td>
</tr>
<tr>
<td>NH$_3$</td>
<td>Optical sensor</td>
<td>Eosin ethylester and 2,7-dichlorofluorescein methylester</td>
<td>1µg/l</td>
<td>Liquid</td>
<td>Low</td>
<td>20-30min</td>
<td>No</td>
<td>Talanta, 2008, 77, 66–72</td>
</tr>
<tr>
<td>NH$_3$</td>
<td>Optical sensor</td>
<td>Coumarin 545T, Coumarin 30</td>
<td>~1 ppb</td>
<td>Liquid</td>
<td>Moderate</td>
<td>2 min</td>
<td>No</td>
<td>Chemistry Central Journal, 2012, 6, 124</td>
</tr>
<tr>
<td>NH$_3$</td>
<td>Optical sensor</td>
<td>NaYF$_4$: YbEr, phenol red</td>
<td>400 µM</td>
<td>Liquid</td>
<td>Low</td>
<td>5-7 min</td>
<td>No</td>
<td>Anal. Chem. 2010, 82, 5002–5004</td>
</tr>
<tr>
<td>NH$_3$, amines</td>
<td>Optical chemical sensor</td>
<td>Eosin dye</td>
<td>0.1-5ppm</td>
<td>Liquid</td>
<td>Low</td>
<td>20-200min</td>
<td>No</td>
<td>PHOTONIC SENSORS, 2016, 6, 2, 107–114</td>
</tr>
<tr>
<td>NH$_3$, amines</td>
<td>Optical Gas Sensor</td>
<td>MMPyP, TMPyP</td>
<td>0.05, 0.16ppm</td>
<td>Liquid</td>
<td>Moderate</td>
<td>4-5 minutes</td>
<td>No</td>
<td>Sensors, 2017, 17, 24</td>
</tr>
</tbody>
</table>
EXPERIMENTAL SECTION

Materials and Instruments:

All the reagents were purchased from Sigma-Aldrich Pvt. Ltd. (India). Unless otherwise mentioned, materials were obtained from commercial suppliers and were used without further purification. Solvents were dried according to standard procedures. Elix Millipore water was used in all respective experiments. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker 400 MHz instrument. For NMR spectra, DMSO-d$_6$ and for NMR titration DMSO-d$_6$ and D$_2$O were used as solvent using TMS as an internal standard. Chemical shifts are expressed in δ ppm units and $^1$H–$^1$H and $^1$H–C coupling constants in Hz. The mass spectrum (HRMS) was carried out using a micromass Q-TOF Micro$^\text{TM}$ instrument by using methanol as a solvent. Fluorescence spectra were recorded on a PerkinElmer Model LS55 spectrophotometer. UV spectra were recorded on a SHIMADZU UV-3101PC spectrophotometer. The following abbreviations are used to describe spin multiplicities in $^1$H NMR spectra: s = singlet; d = doublet; t = triplet; m = multiplet.

Scheme S1. Step-wise Synthesis of the chemosensor CFI.
**Compound 1** was synthesized from a known literature.\(^1\) Fluorescein (2.5 g, 7.5 mmol) and CH\(_3\)OH (6 mL) were taken in a 100 mL three-neck round-bottom flask at room temperature. The whole system was then cooled to 0ºC, followed by the addition of a mixture of NaOH aqueous solution (50%) and 15-crown-5 (30 \(\mu\)L) within 5 min. The resulting mixture was stirred for 10 min, and then allowed to warm gradually in an oil bath. CHCl\(_3\) (10 mL) was added dropwise while the reaction temperature was maintained at 55ºC. The reaction mixture was further stirred for 10 h at this temperature, and then cooled to room temperature. The mixture is acidified with H\(_2\)SO\(_4\) (15 mL, 10 M), and the purple-black precipitate appeared. This solid was filtered and dried and purified by column chromatography (CHCl\(_3\): EtOAc =3:1) on silica gel to get the crude monoaldehyde-functionalized fluorescein. \(^1\)H NMR (400 MHz, DMSO-d\(_6\)): \(d\) (ppm) =11.88 (s, 1H), 10.63 (s, 1H), 10.26 (s, 1H), 8.00-8.02 (d, 1H, \(J=8\) Hz), 7.71-7.82 (m, 2H, \(J=44\) Hz), 7.30-7.31 (d, 1H, \(J=4\) Hz), 6.93-6.96 (d, 1H, \(J=12\) Hz), 6.84-6.85 (d, 1H, \(J=4\) Hz), 6.69-6.72 (d, 1H, \(J=12\) Hz), 6.60-6.61 (d, 2H, \(J=4\) Hz). \(^{13}\)C NMR (400 MHz, DMSO-D\(_6\)): \(d\) (ppm)= 192.84, 168.54, 162.92, 159.62, 152.37, 152.16, 150.85, 136.52, 135.80, 130.32, 129.00, 125.90, 124.79, 123.99, 113.52, 113.38, 109.69, 109.21, 109.14, 102.64, 81.80.

**CFI** was synthesized from compound 1 by one-step imine formation reaction. compound 1 (2.0 g, 5.5 mmol), 3-amino-9-ethyl carbazole (1.40 g, 6.67 mmol), EtOH (40 ml) were taken in a 100 ml RB flask and reflux the mixture at 83ºC for 24 hrs. The product was extracted with CHCl\(_3\) after evaporation of EtOH. After drying it over anhydrous Na\(_2\)SO\(_4\), the organic layer was evaporated completely. The residue was purified by column chromatography with the eluent CHCl\(_3\): EtOAc (6:1,v/v) to get the product **CFI** with 75% yield (Fig.). \(^1\)H NMR (400 MHz, DMSO-d\(_6\)): \(d\) (ppm) =10.18 (s, 1H), 9.69 (s, 1H), 8.51 (s, 1H), 8.30-8.32 (d, 1H, \(J=8\)Hz), 8.01-8.03 (d, 1H, \(J=8\)Hz), 7.80-7.82 (d, 2H, \(J=8\)Hz), 7.73-7.77 (m, 3H, \(J=16\)Hz), 7.65-7.67 (d, 1H, \(J=8\)Hz), 7.51-7.53 (t, 1H, \(J=8\)Hz), 7.32-7.34 (d, 1H, \(J=8\)Hz), 7.24-7.28 (t, 1H, \(J=16\)Hz), 7.06-7.07 (d, 1H, \(J=4\)Hz), 6.69-6.76 (m, 2H, \(J=28\)Hz), 6.61-6.62 (t, 2H, \(J=4\)Hz), 4.50-4.52 (d, 2H, \(J=8\)Hz), 1.34-1.37 (t, 3H, \(J=12\)Hz). \(^{13}\)C NMR (400 MHz, DMSO-D\(_6\)): \(d\) (ppm)= 168.62, 164.07, 159.52, 154.31, 152.28, 151.29, 150.59, 140.28, 139.00, 135.70, 132.27, 130.21, 128.88, 126.08, 124.70, 124.01, 122.94, 122.29, 120.93, 120.16, 119.07, 113.84, 113.22, 109.88, 109.47, 108.20, 106.83, 102.97, 82.61, 37.18, 13.73. HRMS (TOF MS): (m/z, %): Calcd. for C\(_{35}\)H\(_{24}\)N\(_2\)O\(_5\): 552.1685. Found: m/z = 553.1755 (M+H\(^+\)).
2. NMR Studies:

$^1$H NMR of Compound-1 in DMSO-d$_6$:

![Fig. S1 $^1$H NMR of Compound 1 in DMSO-d$_6$ (400 MHz).]

$^{13}$C NMR of Compound-1 in DMSO-d$_6$:

![Fig. S2 $^{13}$C NMR of Compound 1 in DMSO-d$_6$ (400 MHz).]

"Fig. S1 $^1$H NMR of Compound 1 in DMSO-d$_6$ (400 MHz).

"Fig. S2 $^{13}$C NMR of Compound 1 in DMSO-d$_6$ (400 MHz)."
$^1$H NMR of CFI in DMSO-d$_6$:

![1H NMR of CFI in DMSO-d$_6$](image)

*Fig. S3* $^1$H NMR of CFI in DMSO-d$_6$ (400 MHz).

$^{13}$C NMR of CFI in DMSO-d$_6$:

![13C NMR of CFI in DMSO-d$_6$](image)

*Fig. S4* $^{13}$C NMR of CFI in DMSO-d$_6$ (400 MHz).
3. Mass spectrum of CFI:

![Mass spectrum of CFI](image)

**Fig. S5** HRMS of CFI

4. Probable mechanistic pathway of the interaction between CFI and NH₃:

![Probable mechanistic pathway](image)

**Fig. S6** Probable mechanistic pathway of the reaction between CFI and NH₃
5. Job’s plot for determining the stoichiometry of interaction by fluorescence method:

![Job's plot](image)

**Fig. S7** Job’s plot of CFI (c =1µM) with NH$_3$ (1µM) in acetonitrile:water (1:10, v/v) at pH 7.0, (10 mM phosphate buffer) by fluorescence method, which indicate 1:1 stoichiometry for CFI with NH$_3$. Standard deviations are represented by error bar (n=3).

6. Calculation of limit of detection (LOD) of CFI with NH$_3$:

The detection limit of the chemosensor CFI for NH$_3$ was calculated on the basis of fluorescence titration. To determine the standard deviation for the fluorescence intensity, the emission intensity of four individual receptors without NH$_3$ was measured by 10 times and the standard deviation of blank measurements was calculated.

The limit of detection (LOD) of CFI for sensing NH$_3$ was determined from the following equation$^2$.

\[
\text{LOD} = K \times \frac{SD}{S}
\]

Where K = 2 or 3 (we take 3 in this case); SD is the standard deviation of the blank receptor solution; S is the slope of the calibration curve.
Fig. S8 Linear fit curve of CFI at 516 nm with respect to NH$_3$ concentration. Standard deviations are represented by error bar (n=3).

From the linear fit graph we get slope = 1.197 × 10$^8$, and SD value is 0.17305
Thus using the above formula we get the Limit of Detection = 4.33 × 10$^{-9}$ M, i.e 4.33 nM. Therefore CFI can detect NH$_3$ up to this very lower concentration by fluorescence technique.

7. pH titration curve of CFI upon gradual addition of NH$_3$:

Fig. S9 Effect of pH on the fluorescence intensity of CFI (1 μM) in the absence of NH$_3$ (black line) and in the presence of NH$_3$ (10 μM, red line).
8. Competitive fluorescence studies of CFI with various analytes:

**Fig. S10** Fluorescence emission spectra of CFI (1 μM) upon addition of different analytes at 516 nm (λ<sub>ex</sub> = 450 nm) in CH<sub>3</sub>CN:H<sub>2</sub>O (1:10, v/v) at pH 7.0 (10 mM phosphate buffer) (Guests conc. = 10 μM).

**Fig. S11** Histogram representing competitive fluorescence spectra of CFI with other interfering analytes at 516 nm (λ<sub>ex</sub> = 450 nm) in CH<sub>3</sub>CN: H<sub>2</sub>O (1:10, v/v) at pH 7.0 (10 mM phosphate buffer).
Fig. S12  Histogram representing competitive fluorescence spectra of CFI with ROS, RNS and various anions at 516 nm (λ<sub>ex</sub> = 450 nm) in CH<sub>3</sub>CN: H<sub>2</sub>O (1:10, v/v) at pH 7.0 (10 mM phosphate buffer).

9. <sup>1</sup>H NMR titration spectrum of CFI with NH<sub>3</sub>:

Fig. S13  <sup>1</sup>H NMR titration [400 MHz] of CFI in DMSO-d<sub>6</sub> at 25<sup>0</sup>C and the corresponding changes after the gradual addition of 1 equiv. of NH<sub>3</sub> in D<sub>2</sub>O.
10. $^{13}$C NMR titration spectrum of CFI with NH$_3$:

![C NMR titration spectrum of CFI with NH$_3$](image)

**Fig. S14** $^{13}$C NMR titration [400 MHz] of CFI in DMSO-d$_6$ at 25°C and the corresponding changes after addition of one equiv. of NH$_3$ in D$_2$O. The red spot indicates the formation of aromatic keto group at 182 ppm.

11. Partial HRMS of the mixed assay system:

![Partial HRMS spectra of CFI-NH$_3$ mixture in acetonitrile](image)

**Fig. S15** Partial HRMS spectra of CFI-NH$_3$ mixture in acetonitrile, taken after two hours of mixing.
12. Details of energy calculations using Density Functional Theory (DFT):

**Table S2.** Details of the geometry optimization in Gaussian 09 program

<table>
<thead>
<tr>
<th>Details</th>
<th>CFI</th>
<th>CFI-open</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calculation method</td>
<td>B3LYP</td>
<td>B3LYP</td>
</tr>
<tr>
<td>Basis set</td>
<td>6-311G**</td>
<td>6-311G**</td>
</tr>
<tr>
<td>E(CAM-B3LYP) (a.u.)</td>
<td>-1834.296</td>
<td>-1834.302</td>
</tr>
<tr>
<td>Charge, Multiplicity</td>
<td>0, 1</td>
<td>0, 1</td>
</tr>
<tr>
<td>Solvent (CPCM)</td>
<td>Water</td>
<td>Water</td>
</tr>
</tbody>
</table>

**Table S3.** Selected electronic excitation energies (eV), oscillator strengths (f), main configurations of the low-lying excited states of all the molecules and complexes. The data were calculated by TDDFT/B3LYP/6-311G(d,p) based on the optimized ground state geometries.

<table>
<thead>
<tr>
<th>Molecules</th>
<th>Electronic Transition</th>
<th>Excitation Energy(^a)</th>
<th>(f)(^b)</th>
<th>Composition(^c) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFI</td>
<td>(S_0 \rightarrow S_1)</td>
<td>3.01 eV 399.93 nm</td>
<td>0.6751</td>
<td>H (\rightarrow) L (69%)</td>
</tr>
<tr>
<td></td>
<td>(S_0 \rightarrow S_{13})</td>
<td>4.35 eV 284.76 nm</td>
<td>0.5768</td>
<td>H (-1 \rightarrow) L+2 (42%)</td>
</tr>
<tr>
<td>CFI-Open</td>
<td>(S_0 \rightarrow S_3)</td>
<td>2.9731 eV 450.02 nm</td>
<td>0.3837</td>
<td>H (-1 \rightarrow) L (68.6%)</td>
</tr>
<tr>
<td></td>
<td>(S_0 \rightarrow S_4)</td>
<td>3.1155 eV 397.96 nm</td>
<td>0.1727</td>
<td>H (-2 \rightarrow) L (65.7%)</td>
</tr>
</tbody>
</table>

\(^a\)Only selected excited states were considered. The numbers in parentheses are the excitation energy in wavelength. \(^b\)Oscillator strength. \(^c\)H stands for HOMO and L stands for LUMO.
Table S4. Energies of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO)

<table>
<thead>
<tr>
<th>Species</th>
<th>$E_{\text{HOMO}}$ (a.u)</th>
<th>$E_{\text{LUMO}}$ (a.u)</th>
<th>$\Delta E$ (a.u)</th>
<th>$\Delta E$ (eV)</th>
<th>$\Delta E$ (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFI</td>
<td>-0.20148</td>
<td>-0.06945</td>
<td>0.13203</td>
<td>3.59</td>
<td>82.79</td>
</tr>
<tr>
<td>CFI-Open</td>
<td>-0.22212</td>
<td>-0.10152</td>
<td>0.1206</td>
<td>3.28</td>
<td>75.63</td>
</tr>
</tbody>
</table>

13. Paper strip detection:

Fig. S16 Display of paper strip sensing of NH$_3$ using a CFI coated paper strip in visual (top) and under a UV lamp (bottom): (a) only an CFI coated filter paper (b) after 15 seconds of incubation of NH$_3$ (c) after 15 seconds of dipping in NH$_3$ solution.

NH$_3$ preparation in liquid and gas phase. An aqueous solution of liq. NH$_3$ (25%) was prepared by dissolving liquor ammonia in distilled water. Gaseous NH$_3$ was generated from concentrated liquor ammonia in a closed container.
**Potato extraction:**

One fresh potato (P1) sample was collected from our own garden (harvested without any pesticide) and the other three samples (P2, P3, P4) were collected from nearby cold storage. Then the potato samples were cut into thin slices and the slices were ground thoroughly in a pestle mortar to get potato juice. This potato juice was then filtered and the filtrate treated as potato extract. The potato extract from different potato samples were subjected to fluorimetric analysis at pH 7.0 (10 mM phosphate buffer) to quantify the amount of $\text{NH}_3$ present therein.

**14. Reference:**
