

Proceedings

Carbon Dots and Fluorescein: The Ideal FRET Pair for the Fabrication of a Precise and Fully Reversible Ammonia Sensor [†]

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Abstract: Monitoring of ammonia in the human breath is of paramount importance to monitor diseases link to liver and kidney malfunctioning. The present paper describes a solid-state optical ammonia sensor based on Förster resonance energy transfer (FRET) between narrowly dispersed blue-emitting carbon nanodots (CNDs) as FRET donor and fluorescein as FRET acceptor. The fluorophores were physically entrapped in a close to superhydrophobic sol-gel matrix, in turn deposited on a PVDF-HFP electrospun fiber membrane. The sensor shows a linear calibration with a remarkably low limit of detection, i.e., 110 ppb, and adequate reproducibility up to six N₂/NH₃ cycles.

Keywords: carbon dots; ammonia sensor; Förster resonance energy transfer

1. Introduction

Ammonia is a key bio-marker to identify liver and kidney malfunctioning [1], *H. pylori* infection [2] and halitosis [3].

Hence, there is an impelling demand for a reversible, robust and accurate sensor able to monitor ammonia in human breath within the targeted concentration window (i.e., 0.2–1.0 ppm).

Although electrochemical sensors have shown remarkable limits of detection and an adequate operational window, they present the usual drawbacks associated with an electrochemical transduction, namely strong read-out interferences by other gaseous analytes.

Optical sensors overcome the previously mentioned drawbacks albeit the simple readout by fluorescence leads to a considerable drift in signal according to the dye loading in the solid matrix, which jeopardizes the robustness and reproducibility of the sensor.

Optical sensors based on Förster resonance energy transfer (FRET) between two dyes with overlapping excitation/emission spectra tackle such issue by calibrating the sensor via the ratio between FRET donor and acceptor emission peaks, which eliminates any signal variation due to dye loading in the matrix [4].

In the last decade, carbon nanodots (CNDs) have emerged as a photostable, non-toxic and cost-efficient class of optical nanostructures [5]. Their tunable excitation-dependent emission characteristics render them particularly appealing for their use in FRET detection systems. Indeed, a FRET pair composed of carbon dots and sodium rhodizonate showed a remarkable limit of detection of 3 ppm and a response time upon NH_3 exposure of 5 s [6]. However, reversibility of the sensor was only tested by adding hydrochloric acid, which is not viable for continuous monitoring of ammonia in occupational environments.

Here, we present an alternative route for the synthesis of CNDs by pyrolysis of ethylenediaminetetracetic acid (EDTA) in the presence of a hydrogen peroxide solution. These CNDs, used as FRET donor, were embedded in a flexible sol-gel matrix in the presence of fluorescein as FRET acceptor to ensure the physical entrapment of the fluorophores. The sol-gel mixture was then drop cast on electrospun PVDF-HFP membranes to impart a highly porous morphology to the sol-gel layer, with the target of decreasing the response time for the resulting solid-state ammonia sensor.

2. Materials and Methods

2.1. Materials

Fluorescein (for fluorescence, free acid), ammonium hydroxide (ACS reagent, 28–30% NH_3 basis), methyltriethoxysilane (MTES) (99%), diethoxydimethylsilane (DMDES) ($\geq 97\%$), ethylenediaminetetracetic acid (EDTA) (purified grade 99%), acetic acid (reagent plus $\geq 99\%$) and hydrogen peroxide 30 wt % solution in water (Trace Select) were purchased from Sigma-Aldrich and used as received without any further purification.

Compressed gas cylinders with selected NH_3 - N_2 standard mixtures containing 100 ppb, 500 ppb, 10 ppm and 20 ppm of NH_3 were obtained from Carbagas and employed for testing the sensing performances.

2.2. Synthesis of CNDs, Sol-Gel, PVDF-HFP Electrospun Fibers and Sol-Deposition

Details on the synthetic methods to prepare the single components for the solid-state optical ammonia sensors are described elsewhere in a recently submitted publication [7].

3. Results

The performance of our solid state ammonia sensor was tested in terms of sensitivity, reproducibility, reversibility and response time.

By calibrating the signal with respect to the fluorescein emission peak, a sharp response to extremely low concentration of ammonia (i.e., 110 ppb) is observed. The sensor shows a linear fitting within the tested dynamic range from 100 ppb till 20 ppm of ammonia, which is the relevant range for detecting ammonia exposure in occupational environments (Figure 1a).

Although fluorescein is well-known to increase its emission peak at higher pH values [8–10], such remarkable sharp response to ammonia cannot be exclusively ascribed to the fluorescein pH behavior. Indeed, as it has been observed by studying the FRET behavior in solution, a prominent increase in FRET efficiency from 85 to 88% is observed when 10 v/v % of 0.1 M NH_4OH solution in water is added to the mixture. Although such measurements give us a qualitative contribution of the energy transfer to the variation in signal of the fluorescein peak upon ammonia exposure, the translation of such efficiencies to the solid state sensor is not possible, as the FRET efficiency might diverge due to a different contribution of non-covalent interactions in the solid state.

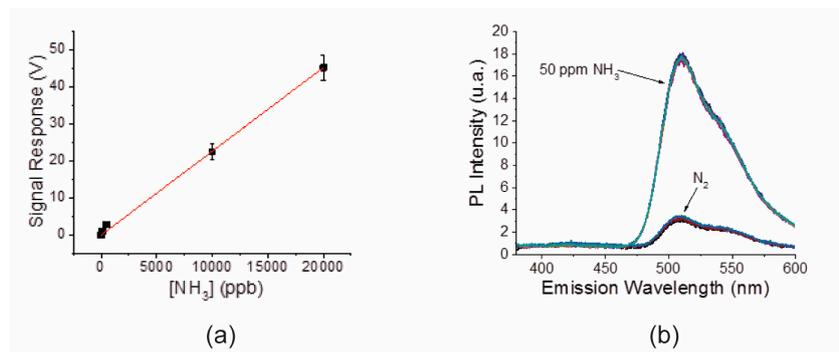


Figure 1. (a) Optical response of the fluorescein emission peak to five different ammonia concentrations. The results were fitted to the equation: $y = 0.0026 \cdot x$ ($R^2 = 0.999$); (b) Ammonia sensor reversibility through six different N₂/NH₃ cycles [NH₃] = 50 ppm.

Nevertheless, it can be stated that the fluorescein response to ammonia within the FRET pair is a combination of both factors: increased FRET efficiency due to reduced FRET inter-distance between the fluorophores and the pH-dependent behavior of fluorescein.

The reduced FRET inter-distance can be tentatively explained as a reversible formation of imine derivatives from the ketone groups observed on the CNs surface, which render the CNs surface prone to interact more strongly with the fluorescein carboxyl group, thereby establishing a stronger electrostatic interaction.

This translates into a lower limit of detection for the optical ammonia sensor (LOD_{NH_3}). Indeed, the calculated LOD_{NH_3} (as the extrapolated concentration at which the signal is three times the averaged standard deviation of the readouts) resulted to be 110 ppb. Hence, the proposed sensor reduces by roughly three orders of magnitude the limit of detection obtained by the previous FRET pair evaluated by our group (i.e., 115 ppm) [8]. This result so far represents the lowest limit of detection of any optical ammonia sensor published in literature. Indeed, previous studies have shown limits of detection of 1.2 ppm by utilizing CdSe/SiO₂ semi-conductor quantum dots [11] and 3 ppm by using a FRET between carbon nanodots and sodium rhodizonate [6].

More importantly, the solid-state sensor presented here undergoes a completely reversible switching of the fluorescein optical signal up to six cycles upon exposure to 50 ppm of ammonia (Figure 1b). Indeed, the relative standard deviation (RSD) at 50 ppm obtained during the sensor calibration was 3.8%. However, the variation in fluorescence intensity between first and sixth cycle was only a mere 0.5%. The high reversibility represents an obvious advantage if compared to the previous FRET sensor based on carbon dots and sodium rhodizonate as in that case the reversibility was tested only upon the addition of gaseous HCl [6]. In our case, the strong hydrophobicity of the coating and the support membrane (water contact angle of 97.15° and 142.5°, respectively) drives the desorption of ammonia. The same effect cannot be achieved with more hydrophilic structures such as cotton fibers used as support in the previous study [6].

The response time of the sensors calibrated on the fluorescein emission peak (defined as the time required to achieve 95% of the stable fluorescence intensity) shows a relatively sharp response time of 8 ± 2 min in adsorption and 15 ± 2 min in desorption upon exposure to 20 ppm of ammonia, which represent a noticeable improvement if compared to our previous work [8], where even the thinnest sol-gel coating showed a relatively longer response time (i.e., 18 ± 2 min). Thus, the deposition of a sol-gel layer onto the electrospun fiber (localized either directly on the fiber or in the surroundings of several entangled fibers) increases the overall accessible surface area. This translates into a higher diffusion rate of the analyte and lower response time.

4. Conclusions

In this work, we demonstrated highly performing and fully reversible optical ammonia sensor based on FRET between fluorescein and carbon nanodots (CNDs). The integration of the FRET system into a flexible sol-gel coated PVDF/HFP electrospun fiber matrix allows an easy encapsulation within lab on chips for the detection of ammonia in human breath. The robustness, in terms of reproducibility and photostability, as well as the full reversibility of this ammonia sensor is appealing not only for the present system, but also for the development of alternative optical chemosensors.

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Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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