Reflectometric Optosensor for Visual Detection of Ammonia Based on Silica Pellet Sensing Material

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Abstract Ammonia (NH_3) has been widely used in the manufacture of fertilizers that applied to soil, but the high consumption of fertilizers will end up with water pollution. Owing to the deleterious effects of NH_3 to human and environment, a new optosensor for NH_3 has been fabricated based on silica pellet sensing material. Microsilica was synthesized by sol-gel method in the presence of cobalt(II) chloride hexahydrate ($CoCl_2 \cdot 6H_2O$), followed by manual grinding process to obtain micro-sized silica particles. Due to the non-transparent pellet material used for NH_3 sensing, a fiber optic reflectance spectrophotometer was employed for monitoring of reflectance signal transduction event as the pellet colour changed from pink to blue hue upon reaction with NH_3 at optimum pH 13. Due to the high porosity and surface area of silica microparticles were used as immobilization matrix, the immobilized Co^{2+} ion demonstrated broad dynamic linear range from 18 to 100 ppm NH_3 with a fast response time of 3 min. The reflectometric sensing protocol involves a single-step NH_3 assay which merely requires dispensing small aliquots of NH_3 onto the reaction surface of the pellet sensor. This makes on-site

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NH$_3$ detection more user-friendly and convenient when compared to traditional electrochemical-, infrared- and gas chromatography-based methods.

Keywords Optosensor · Ammonia detection · Reflectance spectrophotometer · Sol-gel

Introduction

NH$_3$ is produced by nitrogen fixation in nitrogen cycle and occurred naturally in soil from bacterial processes. NH$_3$ is the precursor for fertilizer productions. NH$_3$ can be either converted into solid fertilizers e.g. urea, ammonium nitrate, ammonium phosphate or directly applied to arable soil [1]. The excess of NH$_3$ in surrounding may create potential hazards to human and ecosystems. This indicates that determination of NH$_3$ is of paramount importance in ecological monitoring.

Ministry of Health Malaysia has regulated the standard limit for NH$_3$ in Ammonia detection both raw and drinking waters is less than 1.5 ppm [2, 3], meanwhile, according to WHO [4], NH$_3$ threshold odour concentration at alkaline pH is approximately 1.5 ppm and a taste threshold of 35 mg/L is advocated for ammonium (NH$_4^+$) ion.

NH$_3$ detection based on wet chemical methods for example titrimetry and gravimetry require long analysis time, high chemical consumption and non-continuous. Several conventional methods for quantitation of NH$_3$ such as fourier transform infrared spectroscopy (FTIR), non-dispersive infrared gas analyzer, electrochemical method [5], gas chromatography (GC) [6] and GC coupled with fluorescence detection [7, 8]. Electrochemical method based on potentiometric electrodes are sensitivity, yet still they sustain strong interference from alkali metal ions such as Na$^+$ and K$^+$ ions. Other traditional methods for NH$_3$ assay are generally cumbersome, time-consuming, consumptive of the analyte, expensive and rather inaccurate. Therefore, alternative means for rapid detection of NH$_3$ based on low cost and handy device is highly required.

Over the past two decades, the development and applications of optical chemical sensors (optodes) have flourished rapidly which applied for determination of various analytes of interest, including cations, anions, neutral and gaseous species [9]. In terms of portability, low cost and fast assay, optosensor is beneficial for swift detection of NH$_3$ pollutants in the environment.

Experimental

Reagents
Sol-gel was prepared by mixing tetraethyl orthosilicate (Si(OC$_2$H$_5$)$_4$, 98 %, Acros Organics), ethanol (C$_2$H$_5$OH, 99.8 %, R&M Chemicals) and deionized water at a

molar ratio of 1:4:16 followed by addition of 3–4 drops of 12 M hydrochloric acid (HCl, 37 %, Friendemann Schmidt). The mixture was then stirred until a homogenous sol solution obtained at room temperature (25 °C). Phosphate buffer solution was prepared by mixing monosodium phosphate (NaH_2PO_4, 99 %, Bio Basic Inc.) and disodium phosphate (Na_2HPO_4, 99.8 %, Sigma Aldrich) in deionized water. The buffer pH was later adjusted with 2 M sodium hydroxide (NaOH, 99 %, Friendemann Schmidt). NH_3 stock solution was prepared by dissolving ammonium chloride (NH_4Cl, 99.8 %, Sigma Aldrich) salt in deionized water.

Instrumentation

Reflectance intensity measurements of pellet sensor were accomplished by using fiber optic reflectance spectrophotometer (Ocean Optics, USB4000-UV-Vis). Mettler Toledo pH meter was used to measure buffer pH. Silica pellet was made using Parr 2811 Pellet Press.

Preparation of Silica-based Pellet Sensor

About 0.049 g of $CoCl_2 \cdot 6H_2O$ (99 %, R&M Chemicals) salt was dissolved in silica sol-gel. The sol solution was stirred for 1 h and kept at room temperature for 24 h. The solution was then oven-dried at 80 °C for 1 h in a petri dish in order to obtain the gel monolith. Then, the sol-gel disc immobilized with Co^{2+} ion was manually ground by using mortar and pestle and sieved to obtain microsilica at <75 μm. The CO^{2+} ion immobilized microsilica was then mixed with methylcellulose (Sigma Aldrich) at a volume ratio of 1:1 and transferred into the die set of pellet press tool to be manually compressed to obtain a 12.7 mm diameter × 2 mm height sensor in circular pellet format.

Optimization on NH_3 Pellet Sensor

pH effect on the optosensor was studied by varying the pH of the reaction medium from pH 1 to pH 13. Dynamic linear range of the NH_3 optosensor was determined at 637 nm using various NH_3 concentrations at pH 13. Detection limit of the optosensor was determined based on the summation of average blank response and three times the standard deviation of the blank. Response time study of the optosensor was accomplished by dispersing constant NH_3 concentration solution on the pellet sensor and the reflectance response was measured every 30 s for 10 min. Interference study was conducted by adding elements (e.g. Na^+, K^+, Ca^{2+}, Mg^{2+} and SO_4^{2-} ions) that are commonly co-exist with NH_3 in the water system onto the pellet sensor at various concentrations, and NH_3 concentration was maintained at 50 ppm. *t*-test was used to determine significant difference in the sensor responses.

Results and Discussion

Characterization of NH3 Pellet Sensor

The immobilized Co^{2+} ion is pink in colour as it is a transition metal ion with partially filled *d*-orbital, and gave maximum reflectance intensity at the wavelength

Fig. 1 Reflectance spectra of the immobilized Co^{2+} ion (*a*) and its complex with 50 ppm NH_3 (*b*) at pH 13
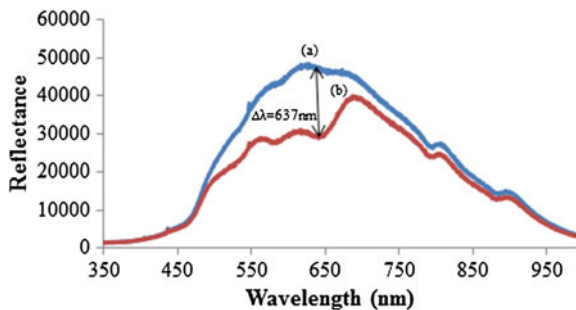
of 630 nm. When Co^{2+} ion-immobilized silica pellet reacted with NH_3, it changed to blue colour and gave maximum reflectance at 683 nm. However, the reflectance intensity was diminished when the bright pink-coloured Co^{2+} ion silica pellet was turned to dark blue. Figure 1 depicts the reflectance spectra of immobilized Co^{2+} ion and its NH_3 complex at pH 13.

The chemical reaction taken place between Co^{2+} ion and NH_3 can be described with Eq. 1:

$$CO^{2+}_{(aq)} + 6NH_{3(aq)} \rightleftharpoons \left[CO(NH_3)_6\right]^{2+}_{(aq)} \tag{1}$$
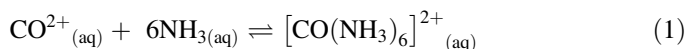
In general, NH_3 acts as both a base and a ligand. When an aliquot of NH_3 is introduced towards immobilized Co^{2+} ion, NH_3 molecules replace water ligands attached to the pink hexaaquacobalt(II) $[Co(H_2O)_6]^{2+}$ ion and that dark blue colours of immobilized hexamminecobaltate(II) $([Co(NH_3)_6]^{2+})$ complex are formed. The formation of dark blue immobilized $[Co(NH_3)]_6^{2+}$ complex absorbed incident light transmitted by the bifurcated optical fiber and attenuated the reflectance signal over the visible wavelength. The immobilized Co^{2+} ion, on the other hand, demonstrated higher reflectance intensity as bright colours reflect better than dark. Because maximum reflectance difference between immobilized reagent and complex was observed at 637 nm, therefore, this wavelength was used as working wavelength for subsequent experiments.

pH Effect

Basically, NH_3 in water exist in equilibrium with NH_4^+ ion as shown in Eq. 2:

$$NH_4^+ + H_2O \rightleftharpoons NH_3 + H_3O^+ \tag{2}$$
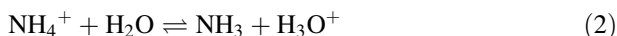
The equilibrium is extremely dependant on the pH where high pH favours the formation of NH_3 and vice versa [10]. Figure 2 shows that the optimum response for NH_3 pellet sensor was obtained at pH 13 and 637 nm, whereby the largest relative reflectance acquired between immobilized reagent and complex.
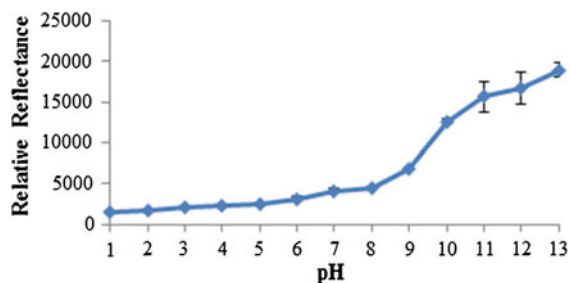
Fig. 2 The effect of pH on the immobilized $[Co(NH_3)_6]^{2+}$ complex at 637 nm using 50 ppm NH_3 at pH 13

From pH 1 to pH 6, the acidic conditions favouring the protonation of NH_3, thereby the formation of blue-coloured $[Co(NH_3)_6]^{2+}$ complex was not discernible, and rendered higher reflectance signal which was relatively similar to that of the immobilized Co^{2+} ion with no NH_3 was added. As the pH increased from pH 8 and onwards, the blue colouration of silica pellet was getting obviously developed. The deprotonation of analyte was high in basic conditions especially from pH 11.6 to pH 12.5, and the blue colour became darkest at pH 13 due to the presence of excess NH_3 in basic conditions, which promoted the formation of immobilized $[Co(NH_3)_6]^{2+}$ complex [11]. Thus, optimum pH 13 was applied for further optimization analyses.

NH_3 Detection

The silica pellet sensor was tested with a range of NH_3 concentrations. The reflectance signal increased linearly with increasing NH_3 concentration and gradually levelling off at a steady-state relative reflectance from 500 ppm NH_3 onwards (Fig. 3).

The calibration curve of relative reflectance versus NH_3 concentration was found linear over the range of 18–100 ppm NH_3 with a correlation coefficient (R^2) of 0.9866. The limit of detection (LOD) of the NH_3 pellet sensor was calculated to be 15 ppm NH_3. The pellet sensor was found taking 3 min for full colour development upon addition of NH_3 solution. Therefore, 3 min reaction time was fixed for every optical NH_3 detection. The measurement for identical addition of analyte was reproducible at 0.5 % RSD ($n = 3$), and the sensor repeatability was obtained at <5.0 % RSD ($n = 5$) indicated that the NH_3 pellet sensor can be satisfactorily reused for *in situ* environmental monitoring of NH_3 pollutant. The NH_3 optode retained almost 100 % of its initial reflectance response over 6 months' storage period at ambient temperature indicative of a highly stable immobilized reagent. Based on the linear range obtained, it can be deduced that this silica-based pellet sensor can be used for continuous monitoring of NH_3 in sewage, domestic waste water, landfill leachate, etc. whereby the typical NH_3 levels in those waters are normally between
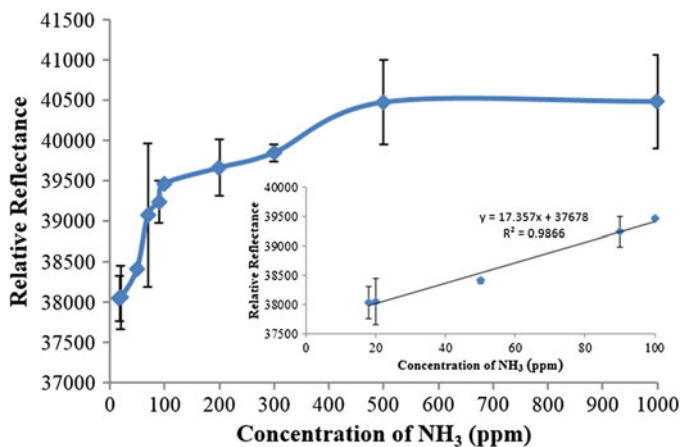
Fig. 3 The response curve of the pellet sensor generated using NH_3 concentrations from 18 to 100 ppm at pH 13. The inset shows the dynamic linear NH_3 concentration range for the silica-based pellet sensing material

10 and 40 ppm. However, it can be excessive up to 100 ppm or more in the highly polluted waters e.g. river waters.

Optical sensor for NH_3 detection in aqueous environment has recently been developed by Varghese et al. [12] based on evanescent wave fiber optic sensor with dual wavelength probing for NH_3 detection in water at ppb levels. Pre-treatment of the water sample (i.e. dilution) becomes necessary when the sensor is used for on-site analysis of sewage for sample concentration >17 ppm, and that direct determination of NH_3 in envronmental samples become impposible. Optical NH_3 sensor for higly polluted water has also been fabricated by Tan et al. [13] with broader dynamic linear range ($\sim$1000–5000 ppm NH_3) using reflectance approach. However, it took some 6 min long for assay of NH_3 in water. This suggests the potential of the proposed pellet-based sensor for in situ analysis of NH_3 in aqueous media, which may be performed in a quantitative or semi-quantitative manner based on pellet colour change.

Interference

Interference study is crucial to determine the pellet sensor performance for optical sensing of NH_3 in solution. Based on the results shown in Table 1, alkali, alkaline earth and SO_4^{2-} ions do not pose significant interference to the determination of 50 ppm NH_3 using the developed silica pellet sensor. The presence of those elements in the concentration higher than NH_3 is usually not possible in water samples [14].

Table 1 The pellet sensor response to various interfering agents at different concentration ratios

Interfering ion	Pellet sensor response at different interfering ion concentration to NH_s concentration ratios				
	1:0	1:1	1:10	1:100	1:1000
Na^+	38412.61 ± 41.69	38313.90 ± 353.85	31157.45 ± 354.09^a		33288.46 ± 5.53^a
K^+	38412.61 ± 41.69	38301.46 ± 268.26	38321.12 ± 346.82	38402.04 ± 43.19	
Mg^{2+}	38412.61 ± 41.69	38333.95 ± 340.64	38317.78 ± 83.88	33063.76 ± 118.79^a	
Ca^{2+}	38412.61 ± 41.69	38426.01 ± 26.12	38417.17 ± 163.53	31245.89 ± 329.83^a	
SO_4^{2-}	38412.61 ± 41.69	38423.42 ± 48.62	38404.83 ± 122.15	38333.04 ± 48.15	30133.04 ± 32.89^a

[a]t–statistic exceeded t–critical value

Conclusion

The proposed pellet-based optosensor has the potentiality for visual determination of NH_3 in environmental water samples. The small size of the silica microparticles presented no significant barrier for diffusion of NH_3. The large interfacial contact area of the immobilized Co^{2+} ion on the microsilica with NH_3 allows mass transfer of NH_3 molecule to the microparticles' surfaces to effect the immobilized charge-transfer complex formation, thus faster response time and broader dynamic range were achieved. The proposed NH_3 pellet sensor involved fast detection procedure, which merely requires dispensing a small aliquot of sample over the sensor surface. It is also an economical sensing device since no substrate is required to contain the sensing matrix, whereby the stand-alone reagent phase in its finished form is free from physical support. The simplicty-based sensing method allows NH_3 determination for a large number of environmental samples to be carried out in a short period of time.

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