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Electrochemical Detection of Ammonia in Aqueous Solution Using Fluorescamine: A Comparison of Fluorometric Versus Voltammetric Analysis

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Abstract: Fluorescamine is a non-fluorescent reagent widely used for the quantitative determination of primary amines by fluorescence spectroscopy as it reacts readily with primary amines to form a fluorescent product. In this work, a new sensitive voltammetric method for the detection of ammonia in aqueous solution by the reaction with fluorescamine has been developed. First, the electrochemical behaviour of fluorescamine in the absence and presence of ammonia was investigated in 0.1 mol·L^{-1} borate buffer solution (pH 9.0) by cyclic voltammetry using a glassy carbon (GC) electrode. As for fluorescamine itself, a well-defined irreversible oxidation peak could be observed at ca. 0.70 V vs. SCE. When ammonia was added to the fluorescamine solution, another irreversible oxditaion peak corresponding to the oxidation of the reaction product formed between fluorescamine and ammonia could be observed at ca. 0.46 V vs. SCE. Upon the addition of ammonia, the oxidation peak of fluorescamine became smaller while the oxidation peak of the reaction product formed increased in height, due to the stoichiometric chemical consumption of fluorescamine by ammonia and the formation of the product during the reaction, respectively. These two anodic peaks corresponding to the oxidation of fluorescamine and its fluorescent product formed were then used for the quantitative detection of ammonia, explored by square wave voltammetry and by fluorescence spectroscopy. The square wave voltammetric response of the reaction product formed showed a linear response over ammonia concentration range of 0 to 60 μmol·L^{-1}. The limits of detection (LOD) was found to be 0.71 μmol·L^{-1} and 3.17 μmol·L^{-1} determined based upon Signal/Noise (S/N) = 3 and 3σ, respectively. These limits of detection are similar to those obtained with the fluorometric method.

Key words: electrochemical detection; cyclic voltammetry; square wave voltammetry; fluorescence spectroscopy; ammonia; fluorescamine

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1 Introduction

Ammonia is a natural basic gas and the third most abundant nitrogen compound present throughout the atmosphere[1-2]. The three major sources of ammonia entering into the environment include (1) the conversion of atmospheric nitrogen to ammonium salts and the addition of these particulates to the soil in the form of dissolved dust or particulates in rain water, (2) the metabolic activities of manure decomposition in agriculture and wildlife, and (3) the combustion from either chemical plants or motor vehicles[1-4]. When an excess of ammonia is released into the environment, it can disturb the nutrition cycle and the ecological nitrogen balance[1-3]. High concentrations of ammonia are potentially harmful to human health that can cause ulceration to the eyes, coughing chest pain and severe irritation to the respiratory tract[1-6]. According to the recommendations from the U.S. Occupational Safety and Health Administration (OSHA)[6-7] and the National Institute

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for Occupational Safety and Health (NIOSH)[8] for the allowable exposure concentration limit of ammonia, using a time weighted average, should be no more than 35 ppm (ca. 2.1×10^{-3} mol·L^{-1}) in air over an eight-hour work shift. The maximum limit of ammonia set by the European Association for drinking water is approximately 0.5 ppm (ca. 2.9×10^{-5} mol·L^{-1})[9-11]. Hence, the determination of ammonia is typically necessary for the health and well-being of both humans and animals[1-3, 5-6, 12]. One of the most commonly used methods for the detection of ammonia is based on fluorescence spectrophotometry of the fluorescent species formed via the reaction with either o-phthalaldehyde (OPA) and mercaptoethanol[13-17] or fluorescamine[5, 16-18].

As comprehensively reported in the literature[5, 17, 19-26], fluorescamine is intrinsically non-fluorescent; however, it reacts readily with primary amines in aqueous solution (at pH 8.0 ~ 9.5) to form a highly fluorescent product, pyrrolinone. Fluorescamine is therefore a well-known reagent for the fluorometric determination of primary amines[16-17, 19-26]. The use of fluorescamine for the determination of amine group-containing organic molecules, for example amino acids, peptides and proteins, is also well-documented[16-17, 19-26]. In addition, the use of fluorescamine for the fluorometric detection of ammonia in vapour phase has previously been investigated on a sodium lauryl sulfate (NaLS) -treated paper substrate by Pal and co-workers[5]. Once fluorescamine reacted with gaseous ammonia on the paper substrate, an intense fluorescent product was produced (illustrated in Fig. 1) and determined by fluorescence spectroscopy with excitation and emission wavelengths of 380 nm and 460 nm, respectively[5]. This fluorescence method was subsequently employed as a sensitive measurement of the air vapour concentration (AVC) of ammonia[5].

Although fluorescence spectroscopy is an extremely sensitive analytical technique which has previously been proposed for ammonia detection[5], it has some disadvantages that cannot be overlooked[27].

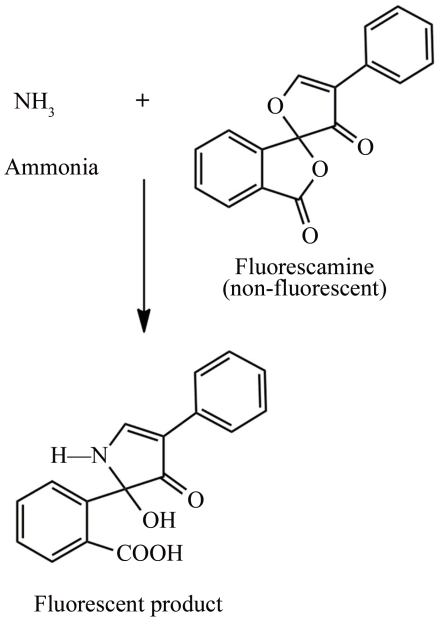

Fig. 1 Reaction of fluorescamine and ammonia, yielding a highly fluorescent product[5].

For instance, many fluorescent compounds can interact with the excitation UV light resulting in the photochemical change or the destruction of these fluorescent compounds, which can then cause a decrease in fluorescence intensity. Moreover, the fluorescence intensity is significantly affected by many experimental parameters such as pH, ionic strength and temperature which therefore need to be carefully controlled during the measurements in order to obtain the reproducible results. The quenching of fluorescence intensity resulting from the interaction between a fluorophore and other non-fluorescent substances present in the system should also be taken into account as it can produce a false positive response in the fluorometric measurement. As highlighted in the literature[28-31], for example, molecular oxygen is a common fluorescence quencher for various fluorophores due to its high solubility in aqueous solutions and organic solvents. Since oxygen is a particularly good quencher which can quench almost all known fluorophores, an additional time-consuming and laborious process is often required in order to get rid of dissolved oxygen from the solution before

measuring fluorescence spectra[28-30]. Considering the fluorescence spectrometer, the instrument itself is somewhat sophisticated and relatively expensive; the instrument operation requires appropriate background knowledge and proper training, and the periodic checks of instrumental sensitivity and replacement of the lamps are normally required for the instrument[27]. According to these drawbacks of fluorescence spectroscopy mentioned above, it is valuable to develop a new reliable, cheap and sensitive analytical method which can be used as an alternative for ammonia detection.

Electrochemical methods are sensitive, fast, versatile, inexpensive, environmentally friendly, and effective techniques for the quantitative determination of a variety of organic and inorganic compounds in aqueous and nonaqueous solutions[27]. Typically, the major advantages of the voltammetric techniques include good accuracy and precision, excellent sensitivity with a very large linear concentration range, a large number of useful solvents and electrolytes, a wide range of temperatures, rapid analysis times and simultaneous determination of several analytes[27].

In this work, we aim to develop a new sensitive voltammetric method for the determination of ammonia in aqueous solution based on the reaction between fluorescamine and ammonia. In essence, the electrochemical behaviour of fluorescamine in the absence and presence of ammonia was first investigated by cyclic voltammetry using a glassy carbon (GC) electrode. Then the quantitative detection of ammonia via the treatment with fluorescamine was thoroughly examined by square wave voltammetry. The limits of detection were found to be comparable to those obtained by fluorescence spectroscopy, highlighting that this new electroanalytical method is a sensitive, simple, cheap and rapid technique for ammonia detection.

2 Experimental

2.1 Reagents and Equipments

Fluorescamine ($C_{17}H_{10}O_4$, 98%), ammonium chloride (NH_4Cl, ⩾ 99.5%) and all other chemicals were purchased from Sigma-Aldrich (Gillingham, UK). All solutions were prepared using deionised water of resistivity not less than 18.2 MΩ·cm at 298 ± 2 K (Millipore UHQ, Vivendi, UK).

Sonication was carried out using a D-78224 Singen/Htw sonicator (50/60 Hz, 80 W, UK). Centrifugation was carried out using a Centrifuge 5702 (Eppendorf, UK). pH measurement was made using a pH213 pH meter (Hanna instrument, UK).

Electrochemical measurements were recorded using an Autolab PGSTAT 20 computer-controlled potentiostat (EcoChemie, Utrecht, The Netherlands) with a standard three-electrode configuration. A glassy carbon electrode (GC, 3 mm diameter, BAS Technical, UK) was used as the working electrode. A saturated calomel electrode (SCE) and a carbon rod acted as the reference and counter electrodes respectively. The GC was polished using diamond pastes of decreasing sizes (Kemet, UK). Cyclic voltammetry (CV) was recorded at a scan rate of 50 $mV \cdot s^{-1}$, otherwise stated. Square wave voltammetry (SWV) was recorded with a frequency of 12.5 Hz, a step potential of 4 mV and a pulse amplitude of 10 mV. All solutions were thoroughly degassed with pure N_2 for 10 min prior to performing any voltammetric measurements. Electrochemical experiments were carried out at room temperature in 0.1 $mol \cdot L^{-1}$ borate buffer solution (pH 9.0).

UV-Visible spectroscopy was performed using a Varian Cary-100 Bio UV-Vis Spectrophotometer (Varian, Oxford, UK) with Cary WinUV software. Fluorescence spectroscopy was performed on a Varian Cary Eclipse Fluorescence Spectrophotometer (Varian, Oxford, UK) with Cary Eclipse software. The slit width for both excitation and emission monochromators was set to be 5 nm.

2.2 Preparation of Ammonia and Fluorescamine Stock Solutions

Ammonia stock solution was prepared by dissolving ca. 14.5 mg of ammonium chloride in 50 mL of 0.1 mol·L^{-1} borate buffer solution (pH 9.0). Free ammonia was subsequently generated in the solution according to the ammonium/ammonia equilibrium reaction (Equation 1).

$$NH_4^+ + H_2O \rightleftharpoons NH_3 + H_3O^+ \quad (1)$$

The exact concentration of free ammonia can then be calculated at known pH and temperature by using the dissociation constant of ammonium ion (K_a), as shown in Equation 2.

$$[NH_3] = \frac{TAN}{1 + \frac{[H^+]}{K_a}} \quad (2)$$

where $[NH_3]$ is the concentration of free ammonia present at equilibrium. TAN (total ammonia nitrogen) is the concentration of ammonium chloride prepared, giving the total number of ammonia and ammonium ion present in the solution. $[H^+]$ is the concentration of hydronium ion, determined from pH of the solution, and K_a is the dissociation constant of ammonium ion ($K_a = 5.85 \times 10^{-10}$ mol·L^{-1} at 20 ℃)[32-33].

Since the total ammonia concentration (TAN) in the pH 9.0 NH_4Cl/NH_3 stock solution prepared here was 5.42 mmol·L^{-1}, the exact concentration of free ammonia and the ammonium ion present in the pH 9.0 NH_4Cl/NH_3 stock solution could then found to be 2 mmol·L^{-1} and 3.42 mmol·L^{-1}, respectively. Note that when free ammonia was consumed by fluorescamine in the chemical reaction (Fig. 1), the ammonia/ammonium equilibrium was disturbed and subsequently driven to the right (Equation 1) in order to produce more free ammonia into the system via further dissociation of ammonium ion. Consequently, the total concentration of ammonia employed in this study is the sum of free ammonia either (1) originally present in the equilibrium system before the chemical reaction of fluorescamine and ammonia taking place or (2) later produced by further dissociation of ammonium ion due to the disturbance of the ammonia/ammonium equilibrium during the reaction between fluorescamine and ammonia. This total concentration of ammonia is the quantity which is relevant analytically as the reaction of ammonia with fluorescamine is chemically irreversible.

Fluorescamine can be dissolved in water-miscible solvents such as acetone, acetonitrile and dioxane[20, 26]. However, acetone has been found to be particularly suitable due to the commercial availability of grades with low fluorogenic impurities and the high stability of fluorescamine which is stable for at least 12 weeks in acetone[20]. In this study, fluorescamine stock solution was prepared in acetone, protected from light and stored refrigerated when not in use.

2.3 Electrochemical Determination of Ammonia

The electrochemical behaviour of fluorescamine in the absence and presence of ammonia was first investigated by cyclic voltammetry (CV) in 0.1 mol·L^{-1} borate buffer solution (pH 9.0) using a glassy carbon (GC) electrode. The quantitative electrochemical determination of ammonia using fluorescamine was then performed by square wave voltammetry (SWV) via a standard addition protocol.

3 Results and Discussion

3.1 Fluorescence Excitation and Emission Profiles of the Product Formed Between Fluorescamine and Ammonia

The absorption and fluorescence emission of the fluorescent product formed between fluorescamine and aqueous ammonia was initially explored by UV-Vis spectroscopy and fluorescence spectroscopy in order to measure the excitation (λ_{EX}) and emission (λ_{EM}) wavelengths for further studies.

Fig. 2 shows the fluorescence excitation and emission profiles of the fluorescent product resulting from the reaction between 200 μmol·L^{-1} fluorescamine and 200 μmol·L^{-1} total NH_3 in pH 9.0 borate buffer solution. The wavelength of maximum

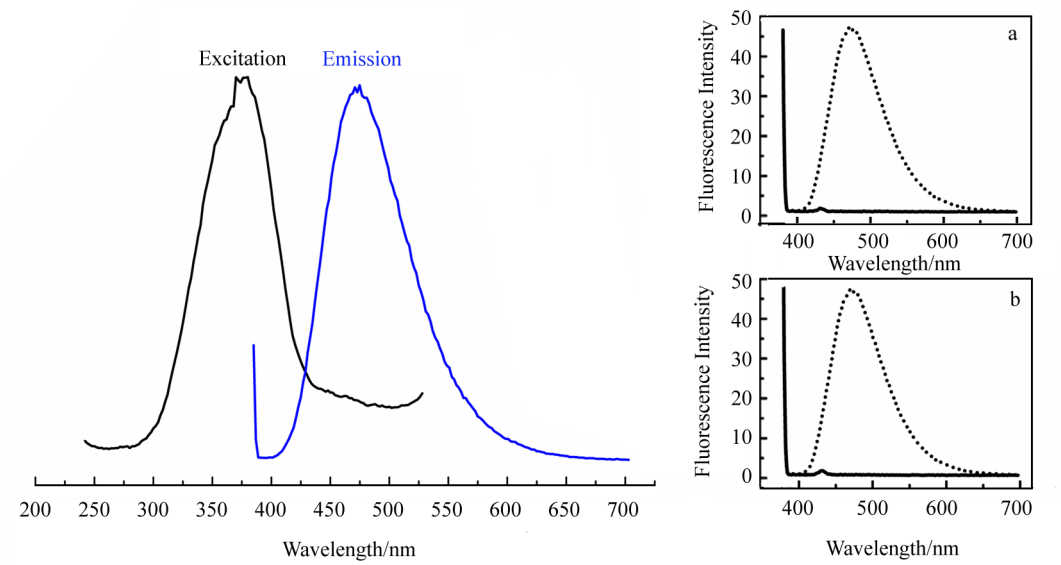

Fig. 2 Fluorescence excitation and emission profiles of the fluorescent product resulting from the reaction between 200 μmol·L^{-1} fluorescamine and 200 μmol·L^{-1} total NH_3 in pH 9.0 borate buffer solution, with excitation and emission wavelengths of 378 and 472.87 nm, respectively. Inset: Fluorescence emission spectra of either (a) 200 μmol·L^{-1} fluorescamine or (b) 200 μmol·L^{-1} total NH_3, in pH 9.0 borate buffer solution, before (solid line) and after (dotted line) mixing them together.

absorbance (λ_{max}) was observed at ca. 378 nm. This wavelength was then employed as an excitation wavelength (λ_{EX}) for the fluorescence emission of 200 μmol·L^{-1} fluorescamine in the presence of 200 μmol·L^{-1} total NH_3, and its fluorescence emission spectrum was observed at a longer wavelength with the maximum fluorescence intensity (λ_{EM}) at 472.87 nm.

Next, the fluorescence properties of fluorescamine and ammonia were checked separately for each species before mixing them together, and the resulting fluorescence emission spectra are displayed as an inset in Fig. 2. No fluorescence emission peak could be observed for either fluorescamine (a) or ammonia (b), confirming the non-fluorescent properties of these starting materials (solid line). In contrast, a large fluorescence emission peak could be seen after mixing these two components together (dotted line) ascribed to the fluorescent product formed from the fluorescamine and ammonia reaction[5].

In terms of the stability of the fluorescent product formed, the fluorescence intensity of 100 μmol·L^{-1} fluorescamine in the presence of 60 μmol·L^{-1} total NH_3 in pH 9.0 borate buffer solution was monitored as a function of time for up to 24 h after the treatment. The resulting fluorescence emission spectra showed that the fluorescence intensity of the fluorescent product was comparatively stable with very little change in fluorescence signal (ca. 0.33% increase in fluorescence intensity after 12 h or so).

3.2 Detection of Ammonia by Fluorescence Method

Fig. 3 displays the fluorescence emission spectra of 100 μmol·L^{-1} fluorescamine in pH 9.0 borate buffer solution with varying total concentration of ammonia from 0 to 400 μmol·L^{-1}. It can be seen from Fig. 3 that the fluorescence intensity of fluorescamine increased with the increase of ammonia concentration. In addition, there was no change in emission maxima and shape of peaks in these fluorescence spectra, demonstrating no observable photochemical side reaction between fluorescamine and ammonia[28, 34].

Fig. 4 shows the plot of fluorescence intensity of the fluorescent product formed between 100 μmol·L^{-1} fluorescamine and ammonia in pH 9.0

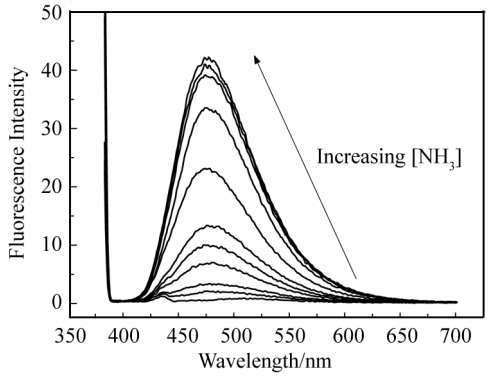

Fig. 3 Fluorescence emission spectra of 100 μmol·L^{-1} fluorescamine in pH 9.0 borate buffer solution, in the presence of ammonia in the total concentration range of 0, 2, 4, 10, 14, 20, 40, 60, 100, 200 and 400 μmol·L^{-1}.

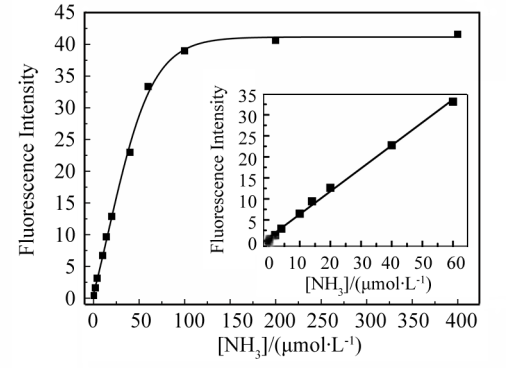

Fig. 4 Plot of fluorescence intensity of the fluorescent product obtained from the reaction between 100 μmol·L^{-1} fluorescamine and ammonia in the total concentration range of 0 to 400 μmol·L^{-1} versus ammonia concentration. Inset: Linear plot of fluorescence intensity of the fluorescent product obtained from the reaction between 100 μmol·L^{-1} fluorescamine and ammonia in the total concentration range of 0 to 60 μmol·L^{-1}, with correlation coefficient (R^2) of 0.997.

borate buffer solution versus total ammonia concentration in the range of 0 to 400 μmol·L^{-1}. As the concentration of ammonia increased, the fluorescence intensity increased consistently and then completely levelled off at the total concentration of ammonia of 100 μmol·L^{-1}, confirming a 1:1 stoichiometric ratio of fluorescamine to ammonia, previously reported in the literature and consistent with the data in Fig. 1[5]. A plot of fluorescence intensity of the fluorescent product formed versus ammonia concentration was found to be linear in the total ammonia concentration range of 0 to 60 μmol·L^{-1} (shown as an inset in Fig. 4), and the limit of detection (LOD) determined based on 3σ, where σ is the standard deviation (root mean square value), was found to be 3.67 μmol·L^{-1} (shown in Tab. 1). Besides, the LOD derived from the method of $S/N = 3$ (three times the standard deviation of the blank for $n = 10$)[35] was also examined here and found to be 0.24 μmol·L^{-1} (shown in Tab. 1). It should be noted that the method of $S/N = 3$ focuses on background signals (from blank measurements) rather than analyte signals [35], therefore its LOD value is typically lower than that calculated from the method of 3σ[36].

3.3 Voltammetric Behaviour of Fluorescamine

According to the literature[5], the reaction between fluorescamine and ammonia in vapour phase has previously been examined on a sodium lauryl sulphate (NaLS)-treated paper substrate, and the fluorescent compound formed by this reaction has then been determined by fluorescence spectroscopy. However, no research concerning the electrochemistry of fluorescamine nor the electrochemical detection of ammonia by fluorescamine has been carried out so far to the authors' knowledge.

In this study, the voltammetric behaviour of fluorescamine in the absence and presence of ammonia was preliminary investigated by cyclic voltammetry. Fig. 5 displays the overlaid cyclic voltammograms for a glassy carbon (GC) electrode in 0.1 mol·L^{-1} borate buffer solution (pH 9.0) in the absence (dotted line) and presence (solid line) of 100 μmol·L^{-1} fluorescamine. As shown in Fig. 5, an irreversible oxidation peak of fluorescamine was clearly observed at ca. 0.70 V vs. SCE, while no oxidation peak could be observed in the blank solution (dotted line). On the reverse scan, no reverse reduction peak for fluorescamine was observed, probably suggesting an oxidation process followed by a fast irreversible chemical reaction, which rapidly removed the generated product[37-38]. In

addition, it was found that the oxidation peak current for fluorescamine decreased moderately in the consecutive scans (solid line), possibly due to the depletion of fluorescamine near the electrode surface during the successive scans. After waiting ca. 1 ~ 2 min (after the 5th cycle) in order to allow the concentration gradient near the electrode to relax, the subsequence oxidation peak current slightly increased compared to the previous scan (the 5th scan), but still lower than that observed in the first two scans. This suggested that the oxidation of fluorescamine is affected not only by the concentration gradient of fluorescamine across the diffusion layer, but also by the passivation of the electrode surface.

As seen in an inset in Fig. 5, the chemically irreversible oxidation peak of fluorescamine got noticeably smaller with the cycle numbers (≥ 10 cycles), and it then disappeared completely after the 30th scan, suggesting the electrode passivation, developed only very slowly owning to low concentration of fluorescamine (100 μmol·L^{-1}) and probably caused by the gradual formation of polymeric films on the electrode surface. In order to confirm this slow electrode passivation observed here, first the GC electrode was deliberately passivated by 50 successive scans in fluorescamine solution. Next, it was removed from the solution, rinsed roughly, and then immersed back again in the same fluorescamine solution. As expected, no oxidation of fluorescamine could be observed in this case as the electrode surface was entirely blocked by the electrochemical reaction product formed. The GC electrode was then removed, polished to get rid of the passivation layer and placed back in the fluorescamine solution. The resulting oxidation peak of fluorescamine at the clean GC electrode had an identical response to that observed earlier in the first scan with no shift in peak potential. No electrode passivation could be observed in pure pH 9.0 borate buffer solution with absence of fluorescamine.

As for the reproducibility of the oxidation of fluorescamine, triplicate experiments were performed

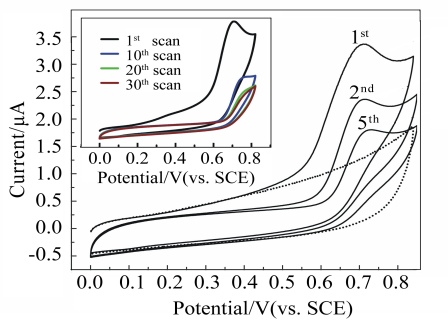

Fig. 5 Overlaid cyclic voltammograms for a bare glassy carbon (GC) electrode in 0.1 mol·L^{-1} borate buffer solution (pH 9.0), in the absence (dotted line) and the presence (solid line) of 100 μmol·L^{-1} fluorescamine. Inset: Overlaid successive cyclic voltammograms (1st, 10th, 20th and 30th scans) of 100 μmol·L^{-1} fluorescamine in 0.1 mol·L^{-1} borate buffer solution (pH 9.0), demonstrating electrode passivation from electrochemical oxidation of fluorescamine. All scan at 50 mV·s^{-1}.

in 500 μmol·L^{-1} fluorescamine in 0.1 mol·L^{-1} borate buffer solution (pH 9.0), with the electrode freshly-prepared for each measurement. From the cyclic voltammograms, it was determined that the relative standard deviation (%RSD) of oxidative peak heights was 1.4 %, indicating good reproducibility of fluorescamine oxidation.

The effect of scan rate on the voltammetric behaviour of fluorescamine was next investigated and the resulting voltammograms are shown in Fig. 6. The plot of log of anodic peak current versus log of scan rate (plotted as an inset in Fig. 6) shows a linear response with a slope of 0.50, indicating that the electrode process is diffusion-controlled, as opposed to a surface adsorption-controlled process[37-38]. Typically, the theoretical slope values of 0.5 and 1.0 are expressed for ideal reactions of diffusion-controlled and adsorption-controlled electrode processes, respectively[37-38]. In addition, the peak potential of fluorescamine did not significantly change with the increase of scan rate, consistent with that the electrode process being an EC process, as discussed earlier.

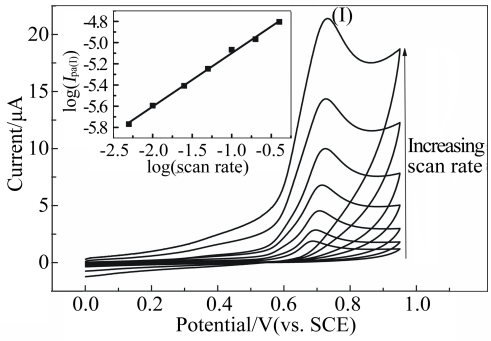

Fig. 6 Overlaid cyclic voltammograms of scan rate study (5 ~ 400 mV·s^{-1}) for 500 μmol·L^{-1} fluorescamine in 0.1 mol·L^{-1} borate buffer solution (pH 9.0), recorded at a glassy carbon (GC) electrode. Inset: Plot of log of anodic peak current (I_{pa}) of 500 μmol·L^{-1} fluorescamine in 0.1 mol·L^{-1} borate buffer solution pH 9.0 (Peak I) versus log of scan rate (R^2 = 0.997 with a slope of 0.50).

3.4 Voltamm etric Behaviour of Fluorescamine in the Presence of Ammonia

Fig. 7 displays typical cyclic voltammograms of 1 mmol·L^{-1} free ammonia (a) and 100 μmol·L^{-1} fluorescamine in the absence (b) and presence of (c) 10 μmol·L^{-1} and (d) 100 μmol·L^{-1} total ammonia, in 0.1 mol·L^{-1} borate buffer solution (pH 9.0). Fig. 7a shows the voltammetric response of ammonia, whereas Fig.7b represents the chemically irreversible oxidation of fluorescamine, labelled peak (I). Note that for the former process, no anodic feature could be observed prior to the oxidation of the electrolyte.

When ammonia (in 100 μmol·L^{-1} fluorescamine) was added to a 100 μmol·L^{-1} fluorescamine solution, the colour of the solution changed from colourless to pale greenish-yellow as a result of the formation of the fluorescent product formed between fluorescamine and ammonia. The cyclic voltammetric behaviour of the reaction product was investigated after 2 min since ammonia was added and mixed in the fluorescamine solution. As seen in Fig. 7c and 7d, a new chemically irreversible oxidation peak, labelled peak (II), corresponding to the oxidation of the product formed could be observed at ca. 0.46 V vs. SCE. Upon the addition of ammonia, the anodic peak current of the reaction product (peak (II)) increased in height while the other anodic peak attributed to the oxidation of fluorescamine itself (peak (I)) became noticeably smaller with a slight shift of the peak potential towards more positive values, simply due to the chemical consumption of fluorescamine by ammonia.

The effect of scan rate on the voltammetric behaviour of fluorescamine in the presence of ammonia was also examined and is shown in Fig. 8. The plot of log of anodic peak current (peak II) correspond-

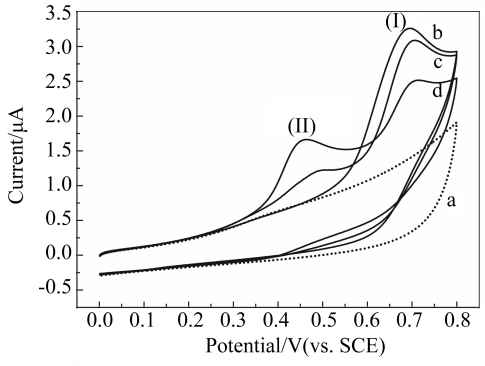

Fig. 7 Overlaid cyclic voltammograms of 1 mmol·L^{-1} ammonia (a) and 100 μmol·L^{-1} fluorescamine in the absence (b) and presence of (c) 10 μmol·L^{-1} and (d) 100 μmol·L^{-1} total ammonia, in 0.1 mol·L^{-1} borate buffer solution (pH 9.0), recorded at a glassy carbon (GC) electrode after 2 min since ammonia was added to the fluorescamine solution. All scan at 50 mV·s^{-1}.

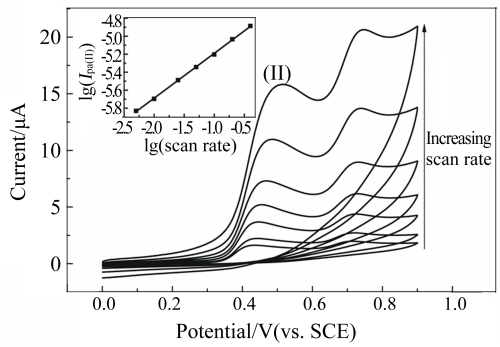

Fig. 8 Overlaid cyclic voltammograms of scan rate study (5 ~ 400 mV·s^{-1}) for 500 μmol·L^{-1} fluorescamine with 500 μmol·L^{-1} total ammonia, in 0.1 mol·L^{-1} borate buffer solution (pH 9.0), recorded at a glassy carbon (GC) electrode. Inset: Plot of log of anodic peak current (I_{pa}) corresponding to the oxidation of the product (peak II) versus log of scan rate (R^2 = 0.999 with a slope of 0.50).

ing to the oxidation of the reaction product versus log of scan rate (plotted as an inset in Fig. 8) shows a linear response with a slope of 0.50, demonstrating the diffusion-controlled nature of this oxidation process. Moreover, we can see from Fig. 8 that as the scan rate increased, a slight shift in the anodic peak potential of the product formed (peak II) towards more positive values could be observed, confirming that the electrode process of the product is electrochemically irreversible (as well as chemically irreversible).

3.5 Electrochemical Detection of Ammonia by Square Wave Voltammetry

Quantitative electrochemical detection of ammonia was next carried out using square wave voltammetry (SWV), a highly sensitive technique widely used for trace analysis[27, 39]. In comparison to cyclic voltammetry, square wave voltammetry has many promising features, including the background suppression, the wider range of time scale, the shorter analysis time and the lower limit of detection because of its efficient discrimination of capacitance current[27, 37, 39-40]. As for the reaction of fluorescamine and ammonia studied here, the well-defined square wave voltammetric response could be obtained with the following SWV parameters: amplitude 10 mV; frequency 12.5 Hz and step potential 4 mV.

Fig. 9 displays the overlaid square wave voltammograms of 0.1 mol·L^{-1} borate buffer solution pH 9.0 (dotted line) and 100 μmol·L^{-1} fluorescamine with ammonia in the total concentration range of 0 to 400 μmol·L^{-1} (solid line). It can be seen from Fig. 9 that no obvious oxidation feature could be observed for the blank solution (dotted line), whereas for 100 μmol·L^{-1} fluorescamine as the starting reagent (blue solid line), a single oxidation peak corresponding to the oxidation of fluorescamine, labelled peak I, was observed at ca. 0.67 V vs. SCE. When ammonia (in 100 μmol·L^{-1} fluorescamine) was added to 100 μmol·L^{-1} fluorescamine solution, a new oxidation peak attributed to the oxidation of the product

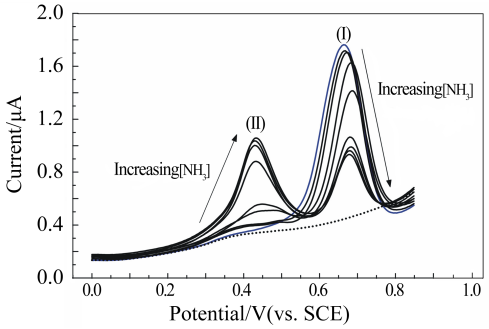

Fig. 9 Overlaid square wave voltammograms of either 0.1 mol·L^{-1} borate buffer solution pH 9.0 (dotted line) or 100 μmol·L^{-1} fluorescamine in the absence (blue solid line) and presence (black solid line) of ammonia in the total concentration range of 2, 4, 10, 20, 60, 100, 200 and 400 μmol·L^{-1}, in 0.1 mol·L^{-1} borate buffer solution (pH 9.0), recorded at a glassy carbon (GC) electrode after 2 min since ammonia was added to the fluorescamine solution with a frequency of 12.5 Hz, a step potential of 4 mV and an amplitude of 10 mV.

formed between fluorescamine and ammonia, labelled peak II, could be observed at ca 0.43 V vs. SCE with an increase in its peak height upon the addition of ammonia. Above 20 μmol·L^{-1} total ammonia added, the oxidation peak of the product (peak II) became increasingly well defined as the amount of product being formed increased. Regarding the oxidation peak current of fluorescamine itself (peak I), it decreased with the addition of ammonia and its oxidation peak potential slightly shifted towards more positive potentials, consistent with that observed earlier by cyclic voltammetry. Note that either peak I or peak II which decreased and increased, respectively upon the concentration of ammonia present in fluorescamine solution could basically be used for the determination of ammonia by electrochemical method studied here.

Fig. 10 shows the plots of square wave voltammetric response for peak I (A) and peak II (B) of fluorescamine in the presence of ammonia in the total concentration range of 0 to 400 μmol·L^{-1} versus total ammonia concentration. As seen in Fig.

10A, the oxidation peak current of the starting material (fluorescamine, peak (I)) decreased consistently with the increasing concentration of ammonia before levelling off after 100 μmol·L^{-1} total ammonia was added to 100 μmol·L^{-1} fluorescamine. On the other hand, the oxidation peak current of the product formed (peak II) shown in Fig. 10B increased instantly with the addition of ammonia up to 100 μmol·L^{-1} before reaching a plateau. In general, these plots demonstrated that the chemical reaction between fluorescamine and ammonia reached its completion when the molar stoichiometric ratio of fluorescamine to ammonia was 1:1 as previously observed and confirmed by fluorescence method since the equimolar quantities of ammonia and fluorescamine were needed in order to reach the limiting signal value.

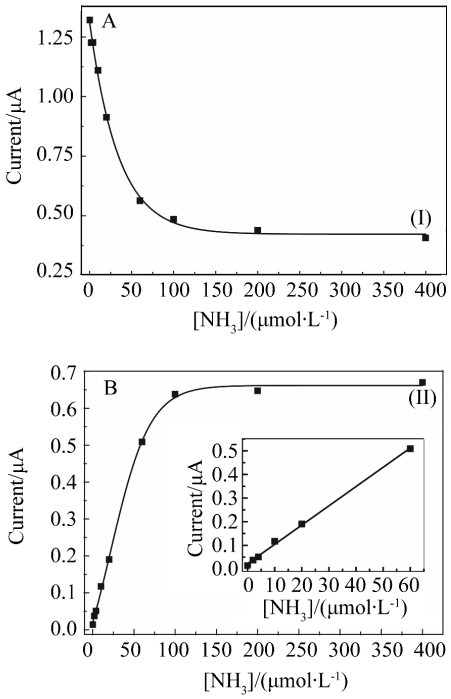

Fig. 10 Plots of square wave voltammetric peak current versus ammonia concentration of the oxidation peaks I (A) and II (B) of 100 μmol·L^{-1} fluorescamine in the presence of ammonia in the total concentration range of 0 to 400 μmol·L^{-1}. Inset in (B): Linear plot of peak current II of 100 μmol·L^{-1} fluorescamine and ammonia in the total concentration range of 0 to 60 μmol·L^{-1}, with correlation coefficient (R^2) of 0.998.

A plot of peak current of peak II corresponding to the oxidation of the product formed against total ammonia concentration (plotted as an inset in Fig. 10B) shows a linear response over a wide ammonia concentration range of 0 to 60 μmol·L^{-1} with a limit of detection (LOD) of 3.17 μmol·L^{-1} (shown in Tab. 1), determined based on 3σ, where σ is the standard deviation (root mean square value). The method of S/N = 3, involving an analysis of the background noise, was also used here to calculated the limit of detection, and it was found to be 0.71 μmol·L^{-1} (shown in Tab. 1).

Tab.1 Limits of detection (LOD), derived from the methods of 3σ and S/N = 3 for n = 10, for the fluorescence and elelctrochemical detection of ammonia in aqueous solution by fluorescamine.

Method	LOD/(μmol·L^{-1})	
	Fluorescence spectroscopy	Square wave voltammetry
3σ	3.67	3.17
S/N = 3	0.24	0.71

3.6 Comparison of Electrochemical and Fluorescence Methods for Ammonia Detection

Although fluorescence spectroscopy is recognised as a very sensitive technique widely used for trace analysis and ideally suitable for the measurement of fluorescent product obtained by the reaction of fluorescamine and ammonia investigated here, it is generally much more expensive, labour-intensive and complicated to use compared to voltammetric methods discussed earlier[27]. In this work, the new voltammetric method which possesses many desirable characteristics including high sensitivity, comparative simplicity, rapid response and low cost has been developed for the detection of ammonia.

Basically, the detection of ammonia in aqueous solution using fluorescamine has been investigated by both fluorometric and voltammetric methods.

The limits of detection (shown in Tab. 1), derived from the methods of 3σ and $S/N = 3$, for the determination of ammonia by both methods have also been reported and compared. As seen in Tab. 1 when the more reliable method of 3σ[36] has been employed, the voltammetric method for the detection of ammonia by fluorescamine could obtain the limit of detection of 3.17 $\mu mol \cdot L^{-1}$ comparable to that of 3.67 $\mu mol \cdot L^{-1}$ for fluorescence spectroscopic method, indicating that these two detection techniques are completely in agreement and either can be used for the detection of ammonia in aqueous solution by the reaction with fluorescamine. It is important to note that these detection limits of 3.17 $\mu mol \cdot L^{-1}$ and 3.67 $\mu mol \cdot L^{-1}$ (derived base upon 3σ) reported here are approximately one order of magnitude below the limit of ammonia for drinking water suggested by the European Association[9-11].

Despite the fact that much lower limits of detection for ammonia (0.71 $\mu mol \cdot L^{-1}$ and 0.24 $\mu mol \cdot L^{-1}$ for square wave voltammetry and fluorescence spectroscopy, respectively) could be achieved when the method of $S/N = 3$ which basically focuses on the blank signals has been applied, the voltammetric method is still a highly sensitive technique capable of extremely low detection limit for ammonia, favourably compared with the fluorometric method.

4 Conclusions

The electrochemical behaviour of fluorescamine in the presence of ammonia in 0.1 $mol \cdot L^{-1}$ borate buffer solution (pH 9.0) has been investigated by cyclic voltammetry and square wave voltammetry. As for fluorescamine itself, a chemically irreversible oxidation peak could be observed at ca. 0.70 V vs. SCE corresponding to the oxidation of fluorescamine. When ammonia was added to the fluorescamine solution, a chemical reaction between these two components generally occurred, producing a highly fluorescent product in the system. The resulting reaction product subsequently introduced a new chemically and electrochemically irreversible oxidation peak observed at ca. 0.46 V vs. SCE, attributed to the oxidation of the product formed. In contrast, the oxidation peak height of fluorescamine observed earlier decreased accordingly due to the fact that fluorescamine was readily consumed by ammonia during the reaction. Upon the addition of ammonia, the oxidation peak of fluorescamine decreased constantly while the oxidation peak of the product formed increased regularly until the reaction completion was achieved. These two anodic peaks corresponding to the oxidation of fluorescamine and its product formed by the reaction with ammonia could then be used for the electroanalytical determination of ammonia in aqueous solution. The limit of detection for this new voltammetric method was found to be comparable with that for fluorescence spectroscopic method. Considering its high sensitivity, comparative simplicity, rapid response and low cost, the electrochemical method developed here thus appears to be a simple, fast, cheap and effective means of ammonia detection although interference by other primary amines would be expected.

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荧光胺电化学检测水溶液中的氨:荧光检测与伏安分析比较

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摘要: 荧光胺是一种非荧光剂,易与伯胺反应形成荧光产物,被普遍用于伯胺的荧光光谱定量分析. 本文利用荧光胺与伯胺反应发展了一种新型灵敏的伏安法用于检测水溶液中的伯胺. 首先,在有、无伯胺的 0.1 mol·L^{-1} PBS (pH 9.0) 缓冲液中, 研究了玻碳电极表面荧光胺的循环伏安电化学行为. 荧光胺的不可逆氧化峰出现在 0.70 V (vs. SCE),当加入伯胺时,在 0.46 V (vs. SCE)出现另一不可逆的氧化峰,为荧光胺与伯胺反应的产物. 继续加入氨水,荧光胺的氧化峰变弱,反应产物的氧化峰则由于荧光胺按反应化学计量比随氨消耗增多而随之增大. 上述两个阳极峰分别对应于荧光胺及其反应产物, 采用方波伏安和荧光光谱技术可实现水溶液中伯胺的定量检测. 在 0 ~ 60 μmol·L^{-1} 氨浓度范围内,该反应产物方波伏安检测成线性响应. S/N = 3 或 3σ 时检测下限分别为 0.71 μmol·L^{-1} 和 3.17 μmol·L^{-1},与荧光法检测的结果相近.

关键词: 电化学检测; 循环伏安; 方波伏安; 荧光光谱; 氨; 荧光胺