

Quantitative Determination of Ammonium Ion in Aqueous Environment Using Riegler's Solution and Artificial Neural Network

(Penentuan Kuantitatif Ion Ammonium dalam Persekitaran Aqueus
Menggunakan Larutan Riegler dan Jaringan Neural Tiruan)

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ABSTRACT

A quantitative analysis has been conducted to determine the concentration of ammonium (NH_4^+) ion in solution by using Ultraviolet-visible spectrophotometry method and artificial neural network (ANN). Riegler's reagent was used to form Riegler- NH_4^+ complex. The characterisations of Riegler's reagent in solution such as photostability, pH effect, reagent concentration, dynamic range and reproducibility were conducted. The colour change of the Riegler's reagent after reaction with NH_4^+ was yellow to red. The Riegler's reagent responds linearly to NH_4^+ ion concentration in the range of 1-7 ppm with optimum response at pH7. Satisfactory reproducibility (2.0-2.8%) were obtained with this reagent. The effect of interfering ions that may contain in the leachate on the determination of NH_4^+ ion was also studied. The application of ANN enabled the extension of the useful dynamic concentration range of NH_4^+ ion to 1–24 ppm. The best ANN architecture for Riegler- NH_4^+ complex was built from 29 hidden neurons, 21,389 epochs number and 0.001% learning rate which produced sum square error (SSE) value of 0.0483 with an average calibration error of 1.4136.

Keywords: Ammonium ion; artificial neural network; Riegler's reagent; ultraviolet-visible spectrophotometry

ABSTRAK

Analisis kuantitatif telah dilakukan untuk menentukan kepekatan ion ammonium (NH_4^+) dalam larutan dengan menggunakan kaedah spektrofotometri utralembahyung-nampak dan jaringan neural tiruan (ANN). Reagen Riegler telah digunakan untuk membentuk kompleks Riegler- NH_4^+ . Pencirian terhadap reagen Riegler dalam larutan termasuk analisis kestabilan foto reagen, kesan pH, kesan kepekatan reagen, julat kepekatan dinamik dan kebolehlungan telah dilakukan. Perubahan warna reagen Riegler selepas bertindak balas dengan NH_4^+ adalah kuning ke merah. Reagen Riegler memberi rangsangan linear kepada ion NH_4^+ dalam julat 1-7 ppm dengan rangsangan optimum pada pH7. Kebolehlungan yang memuaskan (2.0-2.8%) telah diperolehi dengan reagen ini. Kesan ion pengganggu yang boleh didapati dalam air larut lesap dalam penentuan ion NH_4^+ juga dikaji. Penggunaan ANN telah berupaya memanjangkan julat kepekatan dinamik ion NH_4^+ sehingga julat kepekatan 1 – 24 ppm. Arkitektur jaringan ANN yang terbaik untuk kompleks Riegler- NH_4^+ dibina daripada 29 neuron terlindung, 21,389 unit bilangan kitaran dan kadar pembelajaran 0.001% yang menghasilkan nilai ralat jumlah kuasa dua (SSE) sebanyak 0.0483 dengan purata ralat sebanyak 1.4136.

Kata kunci: Ion ammonium; jaringan neural tiruan; reagen Riegler's; spektrofotometri utralembahyung-nampak

INTRODUCTION

Ammonium (NH_4^+) ion is often found at low levels (at ppm) or higher levels in natural waters as a result from the pollution by sewage (Abass et al. 1998; Andrew et al. 1995). The high levels of NH_3 detected prompted the water distribution company Syabas to cut off water supply to two million people in Semenyih, Bangi, Putrajaya, Cyberjaya, Salak Tinggi, USJ, Pulau Meranti, Seri Kembangan, Sepang and KLIA of Malaysia in year 2006 (Krishnan 2006). For human health, ingestion of NH_4^+ contaminated food may result in corrosion of mouth lining, esophagus and stomach (Kwan et al. 2005). An elevated NH_4^+ blood level is considered a strong indicator of an abnormality in nitrogen homeostasis that related is to liver dysfunction (Pranaityte et al. 2007).

Leachate is the liquid that is found in association with landfill. Basically, landfill leachate contained high concentration of sulfate, iron and NH_4^+ ions which can pose environmental or health hazard. Typically, high concentrations of NH_4^+ in water can lead to nutrient enrichment of water systems and potential for turbidity, taste, odour and toxicity problems (Pranaityte et al. 2007). In Malaysia, the typical NH_4^+ concentration is present in the leachate are in the range of 1200-3000 ppm (Renault et al. 1999).

Artificial neural network (ANN) are used for modeling non-linear problems and to predict the output values for a given input parameters from their training values (Senthilkumar & Selvakumar 2005). ANN is defined as a computing system made up of a number of simple,

highly interconnected processing elements, which process information by its dynamic state response to the external inputs. ANN is also described as a data processing system that stimulates the human brain by building on information that it 'learn' (Faiz Bukhari Mohd Suah et al. 2003b).

The commonest type of ANN consists of three groups or layers of units. A layer of input units connected to a layer of hidden units, which in turn is connected to a layer of output units. The activity of the input units represents the raw information that is fed into the network. The activity of each hidden units is determined by the activities of the input units and the weights on the connections between the input and the hidden units. Similarly, the behaviour of the output units depends on the activity of the hidden units and the weights between the hidden and the output units (Senthilkumar & Selvakumar 2005). The range of chemical applications of ANN is very large and it includes fields as diverse as modelling structure of protein, molecular dynamics, process control, interpretation of spectra, calibration, pattern recognition, optimisation of the linear signal range and signal processing (Faiz Bukhari Mohd Suah et al. 2003a).

A variety of methods have been reported for monitoring NH_4^+ ion in water. These include flow spectrophotometric (Icardo et al. 1999), solvent extraction- spectrophotometric (Parham & Mobarakzadeh 2002), potentiometric (Deyhimi & Choobar 2005; Hassan et al. 2001), flow injection analysis with fluorometric (Aminot et al. 2001), enzymatic-amperometric methods (Abass et al. 1998; Kwan et al. 2005), molecular method with ammonia-oxidizing bacteria (Boer et al. 1995; Strotmann & Windecker 1997; Taylor & Bottomley 2006), continuous colorimetric assays (Li et al. 2005) and fluorometric (Kuo et al. 2005). However, these methods are either time-consuming or require elaborate preparation procedures. Optical chemical sensor for the determination of NH_4^+ ion has found wide application. All are based on the colour change occurring as a result of a chemical reaction. These methods are suitable for determination of total NH_4^+ concentration (Trinkel et al. 1996). In this study, a chemical reagent known as Riegler's solution which yields a red complex when reacted with NH_4^+ ion in neutral medium was used for the quantitative determination of this ion in solution.

MATERIALS AND METHODS

PREPARATION OF RIEGLER'S REAGENT AND AMMONIA SOLUTION

All chemicals used were of analytical grade and deionised water was used throughout for solution preparation. Riegler's reagent was prepared by dissolving 1 g of p-nitroaniline (MERCK) in 25 mL of 2 M hydrochloric acid (HCl) (Ashland Chemical) with warming and diluted with 160 mL of deionised water. After cooling, 20 mL of 5% sodium nitrite (MERCK) solution was added to the solution with vigorous shaking (Vogel 1979). The solution was shaken thoroughly for two minutes to allow the

diazotization reaction to go to completion (Cherian et al. 2006). The first equation in Figure 1 shows the diazotization reaction occurred between the p-nitroaniline and nitrite ion (NO_2^-) to produce the Riegler's reagent. A standard stock ammonia solution (10,000 ppm) was prepared by dissolving the required amount of concentrated ammonia solution (MERCK) in deionised water. The ammonia solution used has been standardised with Nessler method using ammonium chloride. Solutions containing the working concentrations range of NH_4^+ ion were prepared daily by diluting the stock solution with deionised water. Buffer solutions were prepared according to methods from Robert (1972).

INSTRUMENTATION

A single beam ultraviolet-visible spectrophotometer (model Varian-Cary Win UV 50) was employed for all direct absorbance measurements. The instrument parameters were controlled by the Cary-Win software (Varian). Data were collected and processed by this software as well. Measurements of pH were made with a pH-meter (MeterLab PHM 210) using a combined glass electrode. A magnetic stirrer was used to stir the solution. The ANN training and data treatment were realised by using Matlab program version 7.0 under a Intel (R) Pentium (R) M 1500 MHz processor having 240 MB RAM.

CHARACTERISATION OF REACTION BETWEEN RIEGLER'S REAGENT AND NH_4^+ ION

The characterisation of the reactions between Riegler's reagent and NH_4^+ ion involved the study on the photostability of the reagent, pH effect, the effect of reagent concentration, dynamic range, reproducibility and interference. The photostability of the Riegler's reagent was studied by exposing the Riegler's reagent to a light source continuously for 8 h. The absorbance measurements were collected at 1 h intervals. The shelf-life of the Riegler's reagent was also studied where the absorbance measurements were collected at a fixed time interval of 2 days over a period of a month.

The effect of pH was studied by varying the pH of the medium solution. The study on the effect of reagent concentration was carried out by varying the concentration of Riegler's reagent i.e. $0-9.70 \times 10^{-5}$ M, whereas the dynamic range of NH_4^+ ion was determined by varying the concentration of NH_4^+ ion solution in the range of 0-26 ppm. The absorbance measurements were performed in triplicates. The reproducibility of the Riegler's reagent was studied at NH_4^+ ion concentrations of 6 ppm and 12 ppm. Interferences from foreign ions were investigated by introducing the Riegler's reagent into a known amount of interfering ions during the NH_4^+ ion determination. The degree of interference of these ions was evaluated with absorbance measurements recorded in the presence and absence of the interfering ion in the determination of 5 ppm NH_4^+ ion. The tolerance limit was set as the amount of interfering ion causing $\pm 5\%$ error in the determination

of NH_4^+ ion.

ANN training, which include optimisation and validation of the absorbance intensities from each spectrum of the selected wavelength points were established by using Matlab version 7.0. A total of 14 absorbance spectra were obtained and used for the ANN training. Five of these spectra (2, 6, 12, 17 and 24 ppm) were used for testing the trained networks whilst the remaining spectra (1, 4, 5, 7, 10, 15, 20, 22 and 26 ppm) were used for networks training. These spectra were selected to represent the data from the linear region (2, 6, 12, 17, 24 ppm) and non-linear region (1, 4, 5, 7, 10, 15, 20, 22 and 26 ppm) of the response curve. The ANN was optimised with respect to the number of hidden neurons, epochs and learning rate constants. A feed-forward neural network having a single hidden neuron layer with back-propagation training algorithm was adapted for data treatment. The input layer consists of 7 neurons that corresponds to the absorbance intensities measured at 7 different wavelengths from each spectrum. The output layer involves a single neuron representing the variable NH_4^+ concentration values. The network having up to 32 neurons in the hidden layer have been considered in this study. The networks were trained up to 21,389 epochs and the progress of sum square error (SSE) between the calculated and the measured output was recorded. Lastly, a new set of input data was introduced to the network to check for its forecast capability and precision.

RESULTS AND DISCUSSION

Riegler's reagent is prepared from the mixture of p-nitroaniline, HCl and NaNO_2 . This reagent is commonly used for qualitative determination of NH_4^+ ion in solution. The chemical reaction between Riegler's reagent and NH_4^+ ion is depicted in Figure 1. The reagent (I) yields a

red colouration due to the formation of complex (II) with an NH_4^+ salt in the presence of sodium hydroxide solution (Vogel 1979). Figure 2 shows the absorbance spectra of the Riegler's reagent alone and its NH_4^+ complex. The decrease in absorbance at the wavelength of 261 nm is due to the consumed Riegler's reagent during reaction with NH_4^+ ion. The working wavelength was chosen due to the greatest divergence between the two absorbance spectra before and after reaction with NH_4^+ ion. Therefore, 380 nm was used for further analysis. The colour change of the Riegler's reagent after reaction with NH_4^+ was from yellow to red.

To optimize the chemical reaction between Riegler's reagent and NH_4^+ ion, an experiment at different pH ammonia solution was conducted. The optimum pH value for complex formation was found to be pH7 (Figure 3). The greatest absorbance difference occurred at pH7. Therefore, this pH was employed in subsequent experiments. In more acidic environments (lower pH), higher concentration of hydrogen (H^+) ions are present in the solution, resulting in higher concentrations of NH_4^+ ion. In more basic environments (higher pH), the concentration of H^+ ions will be lower and a higher concentration of NH_3 are produced. In fact, the degree to which NH_3 forms the NH_4^+ ion depends on the pH of the solution. At pH ~ 7 , about 99% of the NH_3 molecules are protonated to become NH_4^+ ion (Kwan et al. 2005; Staden & Taljaard et al. 1997). This would allow more Riegler's reagent molecules to form the Riegler- NH_4^+ complexes and thus increased the absorbance signal. In the qualitative analysis of NH_4^+ ion, the Riegler's solution was used to detect the presence of NH_4^+ ion in neutral or slightly acid test solution (Vogel 1979).

A study on the photostability of the Riegler's reagent was conducted to monitor the possibility of photodecomposition of the reagent when it was

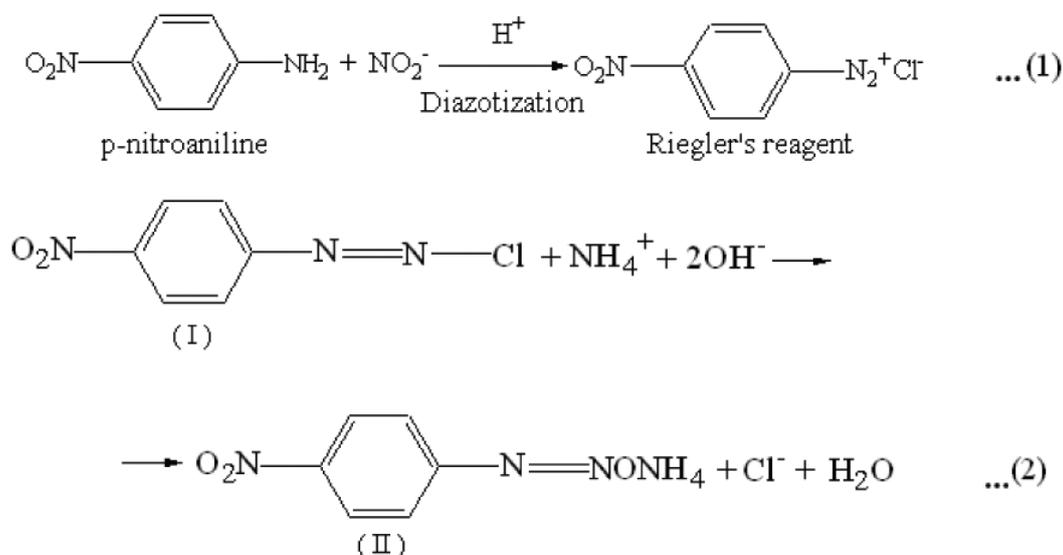


FIGURE 1. Diazotization reaction between p-nitroaniline and NO_2^- ion and the chemical reaction between Riegler's reagent and NH_4^+ ion

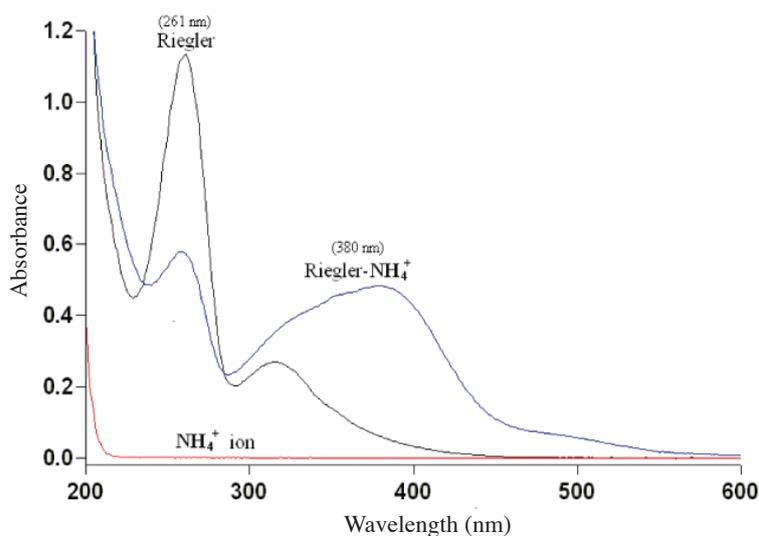


FIGURE 2. Absorbance spectra of the Riegler's reagent alone and its complex with NH_4^+

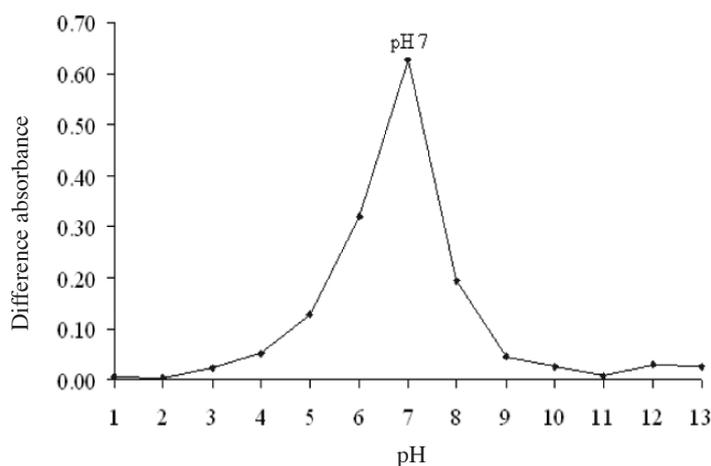


FIGURE 3. The effect of pH for the complex formation of NH_4^+ with Riegler's reagent $[\text{NH}_4^+] = 100 \text{ ppm}$

continuously exposed to a light source for a long period of time (Azizul Isha et al. 2006). For continuous monitoring period of 8 hours, the relative standard deviation (RSD) value for the study was found to be 4.5%. Whereas, the RSD values for the photostability of the Riegler's reagent study for a period a month at 4°C and 25°C were found to be 36.9% and 60.0%, respectively. Furthermore, it was noticed that the reagent was reasonably stable when it was continuously exposed to the light source for about 9 days at 4°C (RSD, 1.3%). Cherian and Narayana (2006) have reported that the diazotization between the p-nitroaniline and NO_2^- ion occurred at $0\text{--}5^\circ\text{C}$.

Figure 4 shows the plot of Riegler- NH_4^+ complex absorbance versus concentration of Riegler's reagent in concentration range of $0\text{--}9.70 \times 10^{-5} \text{ M}$. The optimum concentration of the reagent was obtained at $7.54 \times 10^{-5} \text{ M}$. Increasing the reagent concentration gives increasing

absorbance signal at the same NH_4^+ ion concentration until a plateau region was achieved where the entire NH_4^+ ion had been consumed during the reaction with Riegler's reagent. Nor Azah Yusof and Musa Ahmad (2002) have reported the same trend of result for the response of lead based on the use of immobilised gallocynin in chitosan membrane.

Typical calibration plots over the range of $0\text{--}26 \text{ ppm}$ is shown in Figure 5. A plateau region is achieved when the concentration of NH_4^+ ion is higher than 20 ppm . This point, represent the state where all the Riegler's reagent had been consumed during reaction with NH_4^+ ion. A linear relationship was obtained between absorbance and the NH_4^+ ion over the concentration range of $1\text{--}7 \text{ ppm}$.

The reproducibility of the Riegler's reagent was evaluated for two analyte concentrations, i.e. 6 ppm and 12 ppm . The absorbance measurements were carried out

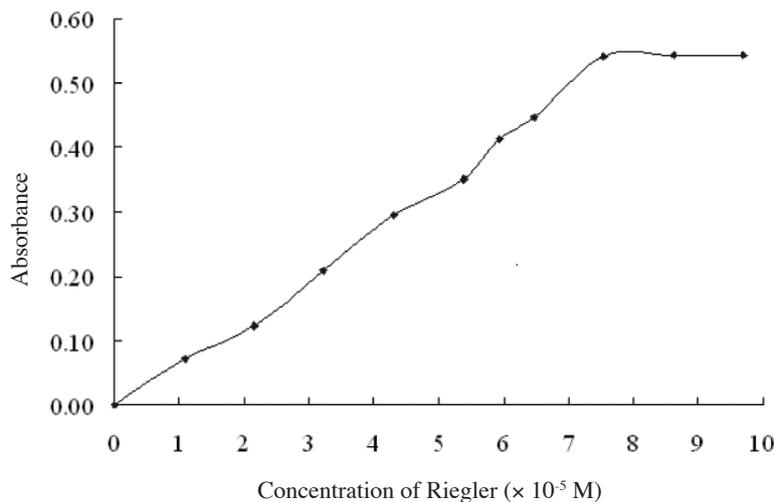


FIGURE 4. The effect of Riegler's reagent concentration for the complex formation

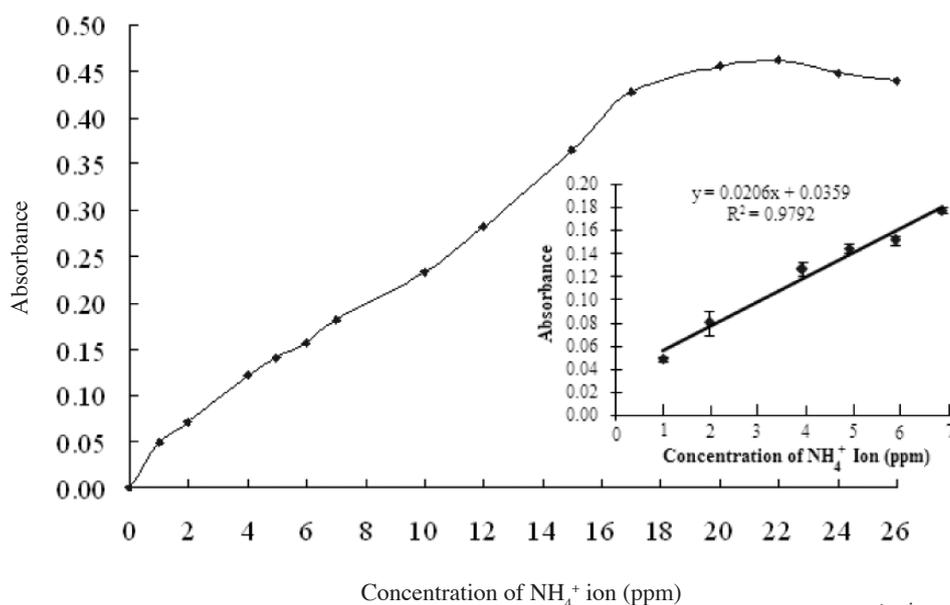


FIGURE 5. The response curve of the Riegler's reagent towards different NH_4^+ ion concentration. Inset shows the linear response produced by the Riegler's reagent in the range of 1-7 ppm NH_4^+ ion concentration

ten times for each analyte concentration which yielded satisfactory RSD values of 2.0% and 2.8% at NH_4^+ ion concentrations of 6 ppm and 12 ppm, respectively (Figure 6).

The effect of foreign ions on the absorbance signal of the Riegler- NH_4^+ complex is summarised in Table 1. Under the optimum conditions, the interfering ion in various concentrations was added to the solution containing 5 ppm NH_4^+ ion and examined separately. The tolerable ratios according to the criterion with $\pm 5\%$ in the absorbance at the wavelength of 380 nm. The only serious interference was Fe^{3+} ion. Fe^{3+} ion interferes significantly because Fe^{3+} ion exist as a yellow transition metal ion and the colour turned brownish at high concentration (Chang 2002), thus increased the absorbance signal. K^+ , Na^+ , Ca^{2+} , Mg^{2+} and

SO_4^{2-} ions could interfere in the determination of NH_4^+ ion when present in high concentrations. Ca^{2+} ion interferes at molar ratio of 0.1 due to the formation of the insoluble $\text{Ca}(\text{OH})_2$ (Chang 2002).

Figure 7 shows the three dimensional absorbance spectrum of Riegler- NH_4^+ complex at NH_4^+ ion concentration range of 0-26 ppm and wavelength range of 200-600 nm. As shown, the beneficial linear range of Riegler's reagent response is limited in the NH_4^+ ion concentration range of 1-7 ppm. Subsequently, ANN was operated to process the signal of the Riegler's reagent response. A total of 14 spectra (1, 2, 4, 5, 6, 7, 10, 12, 15, 17, 20, 22, 24 and 26 ppm) were employed for the ANN training. Seven wavelengths points (207, 229, 261, 385, 380, 456 and 568 nm) from each spectrum were chosen to represent

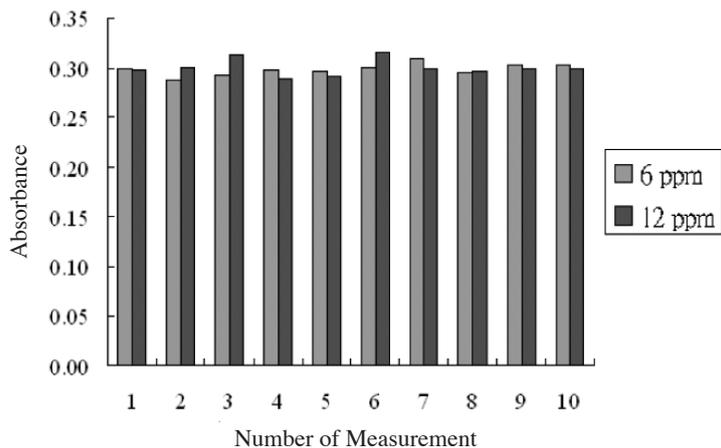


FIGURE 6. The repeatability of the Riegler's reagent for the measurement of NH_4^+ ion concentration of 6 and 12 ppm

TABLE 1. Tolerance of foreign ions on the determination of 5 ppm NH_4^+ ion (n=3)

| Interfering ion | Tolerable ratio of molar concentrations |
|------------------------------|---|
| Na^+ | 500 |
| $\text{K}^+, \text{Mg}^{2+}$ | 250 |
| SO_4^{2-} | 0.5 |
| Ca^{2+} | 0.1 |
| Fe^{3+} | 0.0001 |

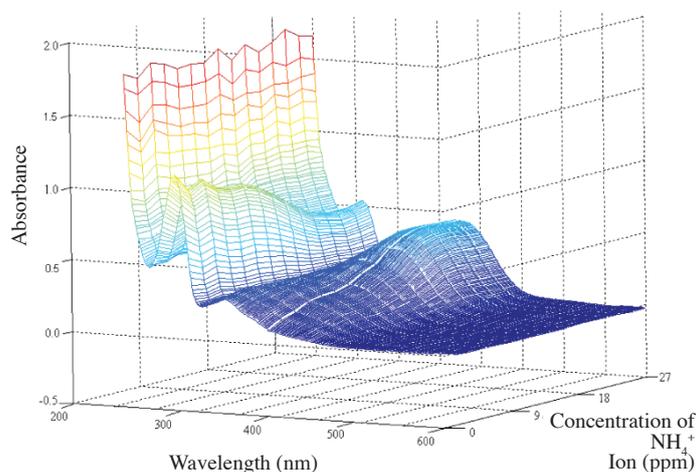


FIGURE 7. The three dimensional absorbance spectrum of Riegler- NH_4^+ complex at NH_4^+ ion concentration range of 0-26 ppm and wavelength range of 200-600 nm

the input data for the ANN. The network optimisation was performed by changing the number of hidden neuron. The SSE was measured at the end of each training. The SSE was used to check the convergence of the trained networks (Faiz Bukhari Mohd Suah et al. 2003b). The fastest convergence was achieved for the network using 29 hidden neurons (Figure 8). To evaluate the effect of increasing the training epoch on the fitting capability of

the network, the training cycles was changed from 5000 epochs to 21389 epochs. The results indicate that training over 21389 epochs reduced the SSE of these networks. In order to improve the process in choosing the best network's architecture, all of these networks were presented with 5 calibration spectra (2, 6, 12, 17 and 24 ppm) to establish their prediction capability. Table 2 displays and compares the predicted NH_4^+ ion concentration values given by these

networks with the expected NH_4^+ ion concentration. From Table 2, the network with 29 hidden neurons created the best prediction with an average calibration error of 1.4136. This network also extends the limited linear response range

from 1-7 ppm of the NH_4^+ ion to full calibration range of 1-24 ppm as indicated in Figure 9.

CONCLUSION

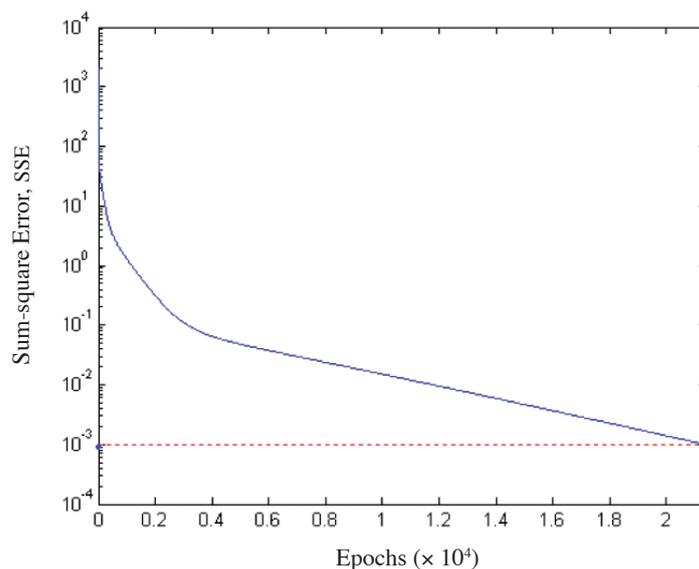


FIGURE 8. Training SSE plot over 21389 epochs for network with 29 hidden neurons

TABLE 2. The networks NH_4^+ ion concentration prediction using calibration data

| Number of hidden neuron | Expected NH_4^+ ion concentration (ppm) | | | | | | | | | | Average calibration error |
|-------------------------|--|--------|------------|--------|------------|--------|------------|--------|------------|--------|---------------------------|
| | 2 | | 6 | | 12 | | 17 | | 24 | | |
| | Prediction | Error | Prediction | Error | Prediction | Error | Prediction | Error | Prediction | Error | |
| 9 | 1.3777 | 0.6223 | 3.0204 | 2.9796 | 12.9431 | 0.9431 | 18.7728 | 1.7728 | 26.1583 | 2.1583 | 1.4271 |
| 13 | 3.7926 | 1.7926 | 5.0303 | 0.9697 | 11.5845 | 0.4155 | 18.1464 | 1.1464 | 28.6031 | 4.6031 | 1.5091 |
| 19 | -0.0157 | 1.9843 | 4.4508 | 1.5492 | 15.6679 | 3.6679 | 19.4977 | 2.4977 | 27.4735 | 3.4735 | 2.1963 |
| 25 | 1.4833 | 0.5167 | 4.6025 | 1.3975 | 15.2010 | 3.2010 | 18.9058 | 1.9058 | 26.5557 | 2.5557 | 1.5975 |
| 28 | 1.8117 | 0.1883 | 4.9318 | 1.0682 | 18.6946 | 6.6946 | 18.3539 | 1.3539 | 25.2176 | 1.2176 | 1.7621 |
| 29 | 3.2279 | 1.2279 | 5.1077 | 0.8923 | 13.1527 | 1.1527 | 19.0947 | 2.0947 | 27.0877 | 3.0877 | 1.4136 |
| 30 | 4.7723 | 2.7723 | 4.2380 | 1.7620 | 13.8279 | 1.8279 | 19.1092 | 2.1092 | 27.4118 | 3.4118 | 1.9894 |

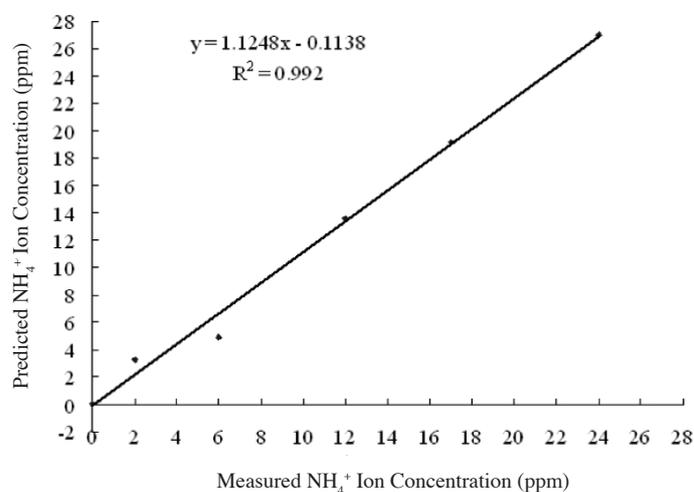


FIGURE 9. Calibration data prediction by the network with 29 hidden neurons

Riegler's reagent has been characterised in this study for its use as the chemical reagent for the quantitative determination of NH_4^+ based on absorbance measurements. The response of the Riegler's reagent were sensitive and repeatable with optimum response at pH7 as well as a linear response at NH_4^+ ion concentration range of 1-7 ppm. A network structure composed of 7 input neurons, 29 hidden neurons and 1 output neuron was found suitable for the signal processing purpose. ANN also useful in extending the response range of the Riegler's reagent from 1-7 ppm to 1-24 ppm.

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