

Research Article

Determination of NH_4^+ in Environmental Water with Interfering Substances Using the Modified Nessler Method

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Received 30 August 2012; Revised 19 December 2012; Accepted 8 January 2013

Academic Editor: Eva Pocurull

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Nitrogen is an essential element in the environment. If excess nitrogen including NH_4^+ is present in water, however, it can result in algae blooming and eventually the destruction of the aquatic ecosystem. Therefore, the determination of NH_4^+ in streams, lakes, and effluents of the treatment facilities has long been carried out. The Nessler method is the most common spectrophotometric method to measure NH_4^+ in water. However, the result of the method becomes inaccurate if there are interfering substances such as Cl_2 , Cl^- , hardness-causing compounds (e.g., Mg^{2+}), and Fe^{2+} in target water samples. In this study, therefore, the traditional Nessler method has been modified to eliminate the effects of interfering substances; the so-called MS was added to water samples. In addition, the polyvinyl alcohol reagent as a dispersing agent was added to water samples to increase the sensitivity and reproducibility of the method. The modified method could successfully analyze NH_4^+ of water samples even with the interfering substance at high concentration.

1. Introduction

The nitrogen cycling in the environment is an important process because it is an essential element for the growth of all the lives; from a simple bacterium to a complex organism like a human [1, 2]. In general, nitrogen is present in natural water in the forms of organic nitrogen, ammonia, nitrate, and nitrite. In the case of raw wastewater, nitrogen is normally present in the organic nitrogen and NH_4^+ forms. Ammonia also can be naturally generated in the environment [3].

The presence of NH_4^+ at high concentrations in a stream or a lake can contribute to eutrophication, that is, massive algae blooming resulting in the depletion of dissolved oxygen in water and the subsequent devastation of aquatic life. Therefore, many countries regulate NH_4^+ concentration of surface water and wastewater. Some examples of the regulation on the NH_4^+ levels of different types of water are listed in Table 1 [4].

Since aqueous NH_4^+ is controlled by regulation, many authorities associated with water regularly collect water samples and analyze their NH_4^+ concentration. In general, the NH_4^+ concentration of a water sample can be determined

by a titrimetric method [5], the method using an ammonia-selective electrode [6], or a colorimetric method [7–10].

In the titrimetric method, a color indicator is added to a water sample, which is subsequently titrated with 0.02 N H_2SO_4 . At the end point of the titration, the color of the sample turns to a pale lavender color. The amount of sulfuric acid used for the color change is proportional to the amount of NH_4^+ in water [5].

The method using an NH_3 -selective electrode is probably the easiest one; at pH 11, ammonia exists in the form of NH_3 , which diffuses through a specially designed membrane at the tip of the electrode, resulting in the change of electrical potential which is measured to determine the NH_4^+ concentration [6].

There are two colorimetric methods available for the determination of NH_4^+ concentration. The first one is the Nessler method in which potassium, mercury, and iodine react with NH_4^+ to create a yellow-brownish colored compound; it is called the Nesslerization [8]. The second one is the phenate method in which phenol and HOCl react with NH_4^+ to create a blue-colored compound [9, 10]. In both methods,

TABLE 1: Examples of regulation on maximum allowable NH_4^+ concentrations for different waters.

Type of sample	WHO	Korean legislation	EPA	European legislation
Drinking water	—	0.5 mg L ⁻¹	—	0.5 mg L ⁻¹
Surface water	<0.2 mg L ⁻¹ (up to 0.3 mg L ⁻¹ in anaerobic waters)	—	0.02 mg L ⁻¹	1.5 mg L ⁻¹
Wastewater	—	—	—	20 mg L ⁻¹ (expressed as N total)

the color intensity of the final compound is proportional to the NH_4^+ concentration of target samples.

In fact, the colorimetric methods are the most commonly used in practice [11]. Particularly, the Nessler method, since it was developed in 1856, has been the most widely applied for the analysis of NH_4^+ in water, especially at low concentrations (i.e., 0.4 mg L⁻¹–1.0 mg L⁻¹ NH_4^+ -N) [12].

When NH_4^+ in a water sample is analyzed, the Nesslerization is carried out. The product from the reaction between the Nessler reagent and aqueous NH_4^+ can absorb light over a wide wavelength range, that is, 400 nm–425 nm (= yellow brownish-colored light).

If the water sample is clean enough, the Nesslerization can be applied to it without any pretreatment. However, if a water sample contains other color or turbidity-causing substances (e.g., alkali ions such as Ca^{2+} , Fe^{2+} , Mg^{2+} , and HS^-), the Nesslerization cannot be applied directly. The alkali ions and sulfide can form turbidity-causing substances or flocs when the water sample is treated with the Nessler reagent. In this case, the water sample should be pretreated with ZnSO_4 , ethylenediaminetetraacetic acid (EDTA), or Rochelle solution to remove the turbidity-causing substances. One of the disadvantages of using EDTA is that excess amount of the Nessler reagent should be provided to insure the reaction between the Nessler reagent and NH_4^+ .

In this study, an MS (MS) was proposed to suppress the interference from the residual Cl_2 , Cl^- , Mg^{2+} (as a hardness-causing compound), and Fe^{2+} in the determination of water NH_4^+ using the Nessler method. In fact, Boopathy [13] applied the stabilizer to remove hardness from the samples, when he analyzed NH_4^+ in the samples collected from an anaerobic soil slurry system. In addition, the polyvinyl alcohol (PVA) reagent was added to increase the light absorbance of a water sample and the sensitivity of the Nessler method [14, 15]. We believe that the addition of the MS and the PVA reagents will increase the usability of the Nessler method in water chemistry researches involving NH_4^+ measurement.

2. Materials and Methods

2.1. Preparation of MS and PVA Dispersing Agent. Unless stated otherwise, all the chemicals used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA). In addition, all the reagents used in experiments were prepared with ammonia-free water.

First, the MS was formulated to suppress the interference from various ions and hardness of water samples to the NH_4^+ determination via the Nessler method. It was synthesized by carefully dissolving 250 g of sodium citrate and 300 g of $\text{NaKC}_4\text{H}_4\text{O}_6$ in 1 L deionized water.

Second, the PVA as a dispersing agent, which was used to help the yellow color to well develop in the reaction with the Nessler reagent and NH_4^+ in a water sample, was formulated by dissolving PVA of 1.35 g in 1 L deionized water.

Sodium citrate (purity = 99.8%) and PVA (purity = 75% hydrolyzed, approx. M.W.: 2000) was obtained from Acros Organics (Geel, Belgium), and $\text{NaKC}_4\text{H}_4\text{O}_6$ (purity = 99.0%) were obtained from Samchun Pure Chemical Inc. (Pyeongtaek, Korea).

The Nessler reagent for color development was prepared following the Standard Method [16].

2.2. Preparation of Standard NH_4^+ and Interfering Chemical Solutions. In order to evaluate the feasibility of the developed interference suppressor and the color development aid in the Nessler method, they were applied to the quantitation of NH_4^+ in synthesized solutions with different interfering chemicals, that is, residual Cl_2 , Cl^- , hardness (or Mg^{2+}), or Fe^{2+} .

Standard solutions of NH_4^+ and the interfering chemicals were prepared by diluting premade stock solution for each of the chemicals with deionized water. A NH_4^+ stock solution for the experiment was made by dissolving 3.819 g anhydrous NH_4Cl which was previously dried at 103 °C for 3 h in 1 L water. If 1 mL stock solution is added to 1 L deionized water, the NH_4^+ concentration of the final solution is 1 mg N L⁻¹.

In the same or similar ways, the standard solutions for each of four interfering chemicals were prepared. First, standard chlorine solutions were prepared by diluting appropriate amount of 50–75 mg Cl_2 L⁻¹ certified standard solution (2 mL PourRite Ampules; HACH, USA) in deionized water.

Standard Cl^- solution was prepared by appropriately diluting the premade 2000 mg L⁻¹ Cl^- stock solution (1.75 g anhydrous NaCl in 50 mL water) with deionized water. Standard hardness (or Mg^{2+}) solution was prepared by diluting premade 500 mg L⁻¹ Mg^{2+} stock solution (0.123 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in 100 mL water) with deionized water. Lastly, to prepare standard Fe^{2+} solutions, a 100 mg L⁻¹ Fe^{2+} stock solution was made by dissolving 0.0702 g NH_4FeSO_4 -hexahydrate in 100 mL.

2.3. Experimental Procedure. Two sets of experiments were carried out. In the first set of the experiments, the effects of the developed MS and the PVA on the light absorbance of NH_4^+ standard solutions of 0.5, 1.0, and 1.5 mg N L⁻¹ were evaluated. In the second set, the effects of the presence of Cl_2 , Cl^- , Mg^{2+} (as a hardness-causing chemical), or Fe^{2+} on the determination of NH_4^+ using the Nessler method were evaluated.

The recovery efficiency of the proposed method was also evaluated with stream water and domestic sewage. On collection, the stream and sewage samples were filtered with $0.4\ \mu\text{m}$ glass fiber filters. $\text{NH}_4^+\text{-N}$ of $1\ \text{mg L}^{-1}$ was then spiked into water samples. For each water matrix, a total of seven replicates were prepared.

All the experiments were carried out as follows. First, a 25 mL water sample was filled into a 100 mL graduated cylinder. Then, three drops of the MS were added to the cylinder, which was subsequently put a cover on and shaken to mix its solution inside. After the solution was mixed, three drops of the PVA reagent were also added to the solution. Then, the solution was again covered and mixed.

Lastly, 1.0 mL of the Nessler reagent was added into the cylinder with mixture. Once the final solution was prepared, its portion was transferred into a spectrophotometer (C-MAC QVIS 3000 Spectrophotometer, Daejeon, Korea) cell and the UV light absorbance was measured. The wavelength for the light used in the measurement was set at 425 nm.

Prior to the measurement of each water sample, a blank solution was prepared for reference. When only the MS was added to the blank solution with the Nessler reagent and without NH_4^+ , the absorbance did not increase (Figure 1). However, when the PVA was alone added to the NH_4^+ blank solution, a slight increase in the absorbance could be observed, since the PVA is a color-dispersing agent. The same level of absorbance increase could be observed when both the PVA and the MS were added to the NH_4^+ blank solution. Therefore, when NH_4^+ in water samples is quantitated using the proposed method, zero point of the spectrometer should be adjusted accordingly.

3. Results and Discussion

3.1. Effects of PVA and/or MS on NH_4^+ Analysis Using Nessler Method. First, the effects of the MS or the PVA reagent on the determination of NH_4^+ in water by the Nessler method were evaluated as follows. For each test, samples with three different NH_4^+ concentrations, that is, 0.5, 1.0, and $1.5\ \text{mg N L}^{-1}$ were prepared. Before the NH_4^+ concentration of each sample was quantitated by the Nessler method, the sample was pretreated as follows. The first one was added with three drops of the MS. The second one was added with three drops of the PVA reagent. In the case of the third sample, both MS and PVA reagent were added. Lastly, the fourth one was not pretreated as a control. Therefore, a total of four test samples for each NH_4^+ concentration level were prepared.

Once the samples were prepared, the light absorbance of each sample was monitored over 20 min using the spectrophotometer. The result from the measurement of the four samples is provided in Figure 2.

Apparently, it was evident that the addition of PVA increased the light absorbance of water samples with NH_4^+ ; the UV absorbance increase of 33–53% could be obtained for all the NH_4^+ concentration levels (Figure 2). The result indicates that the Nessler method with the PVA addition would be more sensitive than the one without; lower NH_4^+ concentration can be determined. In addition, the absorbance of the sample with the PVA added did not change over 20 min

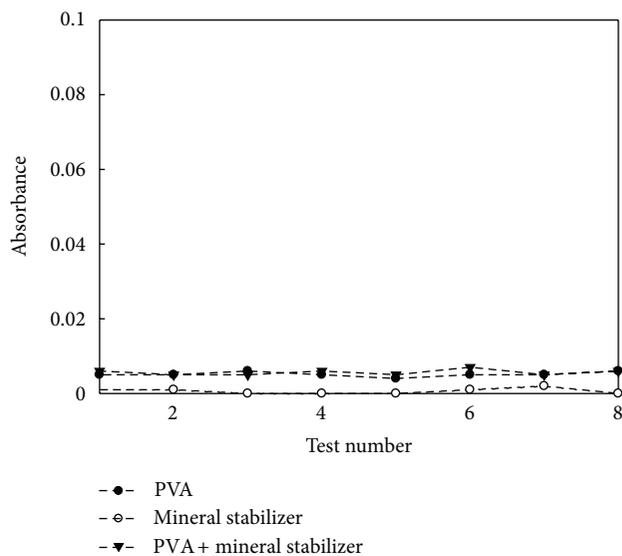


FIGURE 1: Effect of PVA and/or MS on UV absorbance of blank solution.

(c.v. = 0.38–3.90%); the absorbance of the sample with the PVA reached its maximum instantaneously. Therefore, it was confirmed that the PVA would help the color development of the samples analyzed with the Nessler method. In fact, only a slight change in the light absorbance of the sample without any treatment (i.e., control) also could be observed.

In the case of the MS, it did not have an effect on the result of the NH_4^+ measurement using the Nessler method; the sample with the MS only showed the same absorbance with the one without any pretreatment for all the NH_4^+ concentration levels. However, the water sample with both the MS and the PVA reagents added showed lower or higher light absorbance than the one with the PVA only (Figure 2). Probably an interaction between the MS and the PVA occurred and affected the sample absorbance.

Nonetheless, it was concluded that the addition of the MS would not have any effect on the analysis of NH_4^+ in water samples using the Nessler method. Particularly, it was concluded that the PVA addition would increase the sensitivity of the Nessler method for the NH_4^+ analysis.

3.2. Effects of PVA and/or MS on NH_4^+ Analysis Using Nessler Method for Water with Interfering Substances. As stated in Section 1, residual Cl_2 , Cl^- , hardness, and iron salts in water are known common interfering substances in the NH_4^+ analysis using the Nessler method. In this section, therefore, the effects of the addition of the PVA and the MS on the result of the Nessler method application for the quantitation of NH_4^+ in water samples with residual Cl_2 , Cl^- , Mg^{2+} for hardness, or Fe^{2+} as an interfering substance were evaluated.

3.2.1. Effect of PVA and MS Addition on NH_4^+ Analysis of Water with Residual Chlorine. Water samples with the residual Cl_2 of 0.0, 0.1, or $0.5\ \text{mg L}^{-1}$ were analyzed with the modified Nessler method in which the MS and the PVA reagents had been added into the samples before the

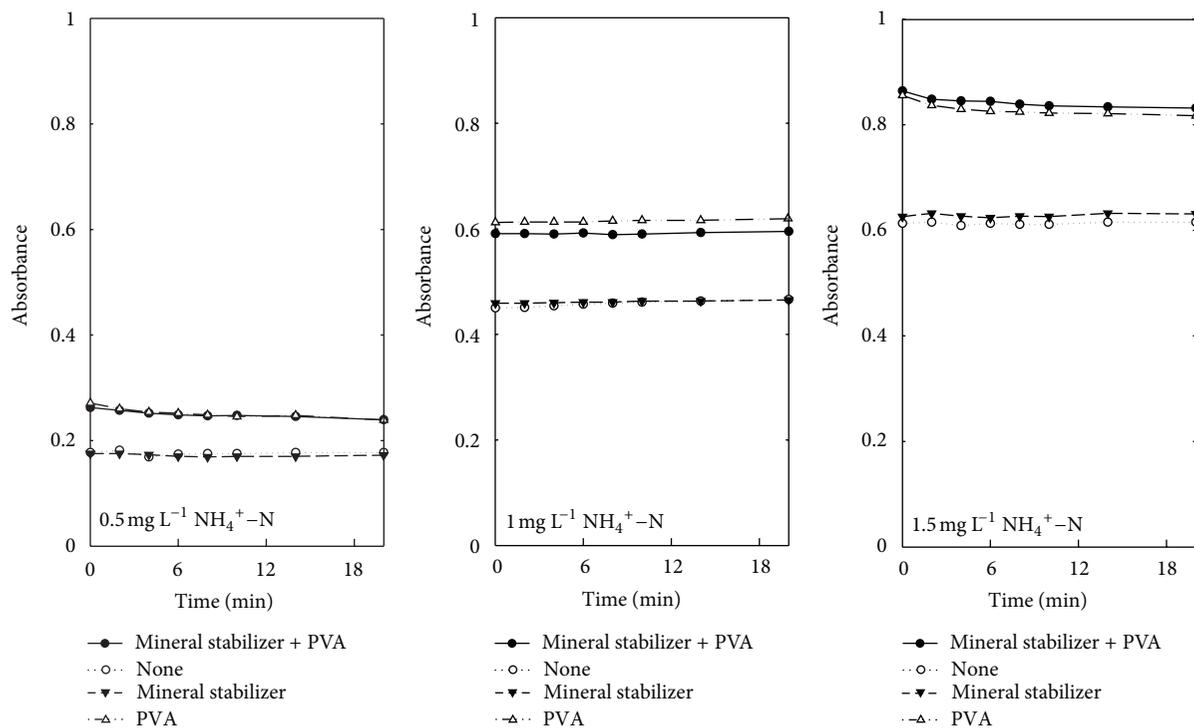


FIGURE 2: Light absorbance of water samples with different pretreatments.

Nessler reagent was added. The result was compared with the one from the measurement with the original Nessler method (Figure 3). The effect of residual chlorine on both the modified and the original Nessler methods was evaluated with the samples of three different NH_4^+ concentrations, that is, 0.5, 1.0, and 1.5 mg N L^{-1} .

Again, the modified Nessler method showed higher UV absorbance than the original one for all the NH_4^+ concentration levels. However, the light absorbance was slightly affected by the presence of residual chlorine when NH_4^+ concentration was 1.5 mg N L^{-1} ; absorbance difference of 12% was observed. In fact, the original method also showed similar difference in the UV absorbance for the water sample with NH_4^+ concentration of 1.5 mg N L^{-1} , when the samples contained residual Cl_2 . Unlike the modified Nessler method, the original method's UV absorbance was also unstable when NH_4^+ concentration was low, that is, 0.5 mg N L^{-1} .

However, it should be stated that in practice it would take a while until a water sample would be analyzed after its collection. If residual Cl_2 is present in water samples, it can react with NH_4^+ in water to form chloramines [17], resulting in the underestimation of NH_4^+ concentration. Therefore, it would be a good practice to remove residual Cl_2 by adding NaHSO_3 right after sample collection, if the presence of chlorine in water is suspected.

3.2.2. Effect of PVA and MS Addition on NH_4^+ Analysis of Water with Cl^- . Water samples with Cl^- of 0.0, 2000, 3000, or 10000 mg L^{-1} were analyzed with the modified Nessler method in which the MS and PVA reagents had been added into the samples before the Nessler reagent was added. The

result was compared with the one from the measurement by the original Nessler method (Figure 4). The effect of Cl^- on both the modified Nessler method and the original Nessler one was evaluated with samples of three different NH_4^+ concentrations, that is, 0.5, 1.0, and 1.5 mg N L^{-1} .

For water samples with 1.5 mg NH_4^+ -N and 10000 mg L^{-1} Cl^- , the UV absorbance values measured by both the modified and the original Nessler methods were higher than the one for water samples with the same NH_4^+ concentration but with lower Cl^- (Figure 4); 12% higher UV absorbance was observed for the sample with 10000 mg L^{-1} Cl^- , compared to the one with those lower Cl^- concentrations.

However, the modified Nessler method showed almost the same absorbance for the samples with lower NH_4^+ concentrations regardless of Cl^- concentration levels. On the other hand, the original method still shows significantly different absorbance for the water samples even of lower NH_4^+ concentrations, if Cl^- concentration was high (Figure 4).

In fact, 1000–3000 mg L^{-1} Cl^- is not uncommon to water samples collected from industrial wastewater plants. Therefore, the modified Nessler method can be beneficial in the analysis of NH_4^+ in industrial wastewater.

3.2.3. Effect of PVA and MS Addition on NH_4^+ Analysis of Water with Hardness. Water samples with Mg^{2+} (as a hardness-causing substance) of 0.0, 500, 750, or 1000 mg L^{-1} as CaCO_3 were analyzed with the modified Nessler method in which the MS and PVA reagents had been added into the samples before the Nessler reagent was added. The result was compared with the one from the measurement with

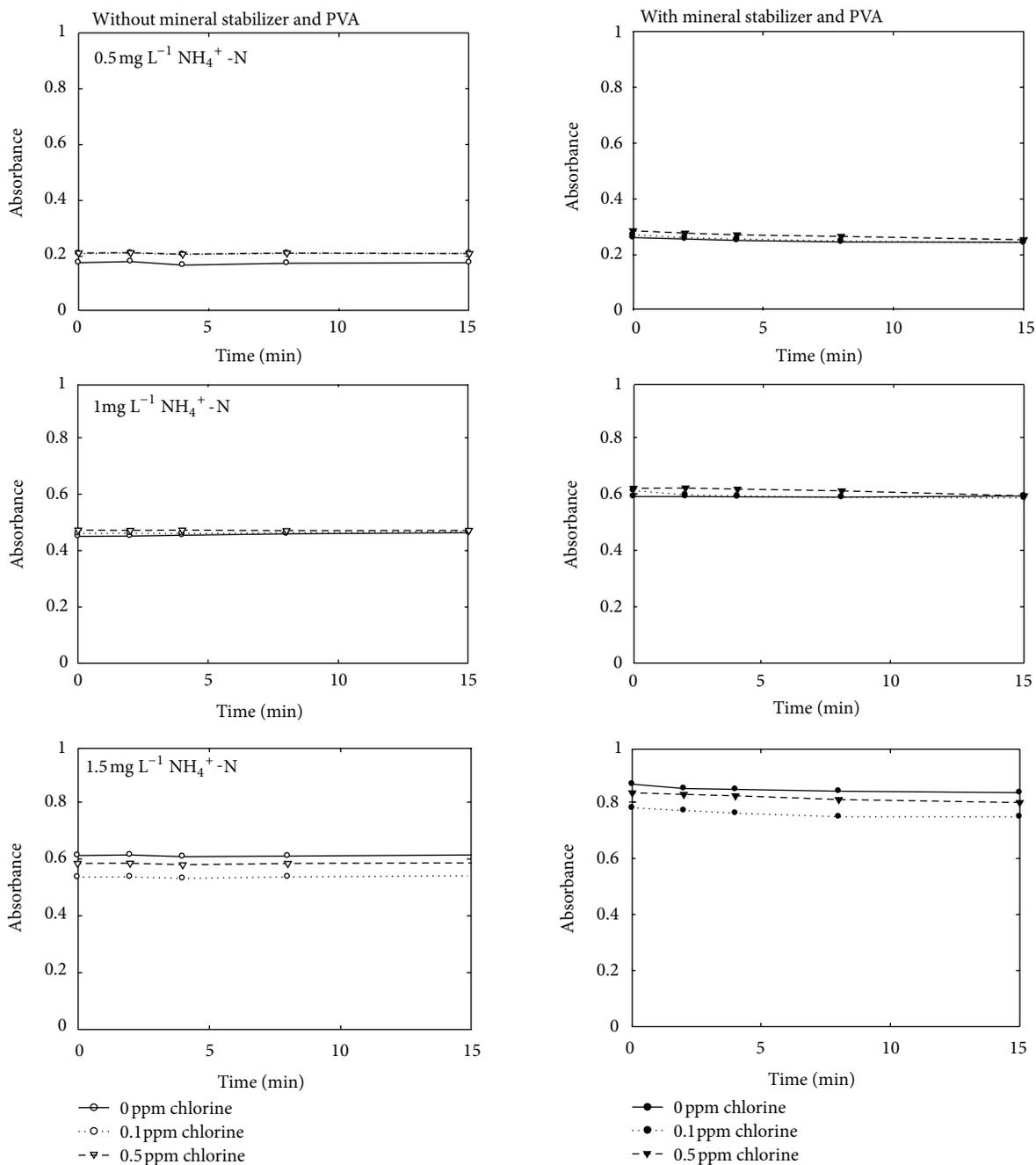


FIGURE 3: UV absorbance at water samples of different NH_4^+ concentrations with different reagents, and with chlorine as interfering substance.

the original Nessler method (Figure 5). The effect of Mg^{2+} on both the modified Nessler method and the original Nessler one was evaluated with samples of three different NH_4^+ concentrations, that is, 0.5, 1.0, and 1.5 mg N L^{-1} .

In the case of the original Nessler method, an accurate analysis of NH_4^+ content could not be possible when Mg^{2+} was present in water samples. Even at the lowest concentration (i.e., 500 mg L^{-1}), Mg^{2+} increased the absorbance more than double, resulting in large overestimation for all the NH_4^+ concentrations levels. In fact, the absorbance of water samples was very proportional to the Mg^{2+} concentration;

higher absorbance could be observed at higher Mg^{2+} concentration. Interestingly, the exaggerated UV absorbance rapidly dropped below that of the control (Figure 5). Probably, the Mg^{2+} precipitates formed from the Nessler reaction settled down over time and turned the turbid water samples to clearer ones [18].

On the other hand, the water samples analyzed with the modified Nessler method showed relatively smaller difference in the UV absorbance if the Mg^{2+} concentration of the water samples was less than 750 mg L^{-1} as CaCO_3 (Figure 5). Nonetheless, in the presence of a hardness-causing chemical

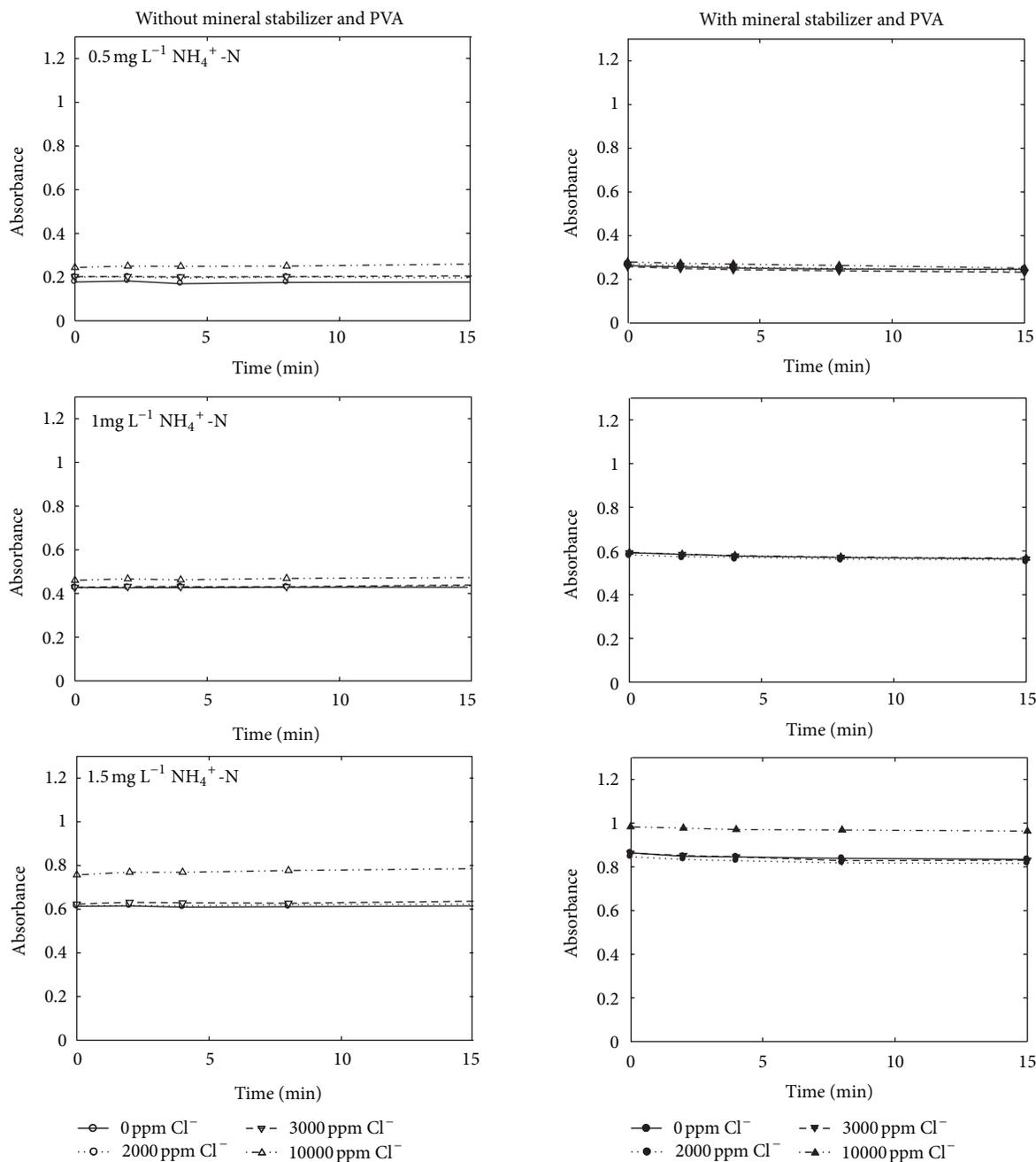


FIGURE 4: UV absorbance at water samples of different NH_4^+ concentrations with different reagents, and with Cl^- as interfering substance.

like Mg^{2+} , the accurate measurement might be to some degree difficult. Therefore, it is necessary to remove it from a sample before NH_4^+ analysis is carried out, if higher hardness is suspected for the sample.

3.2.4. Effect of PVA and MS Addition on NH_4^+ Analysis of Water with Fe^{2+} . Water samples with Fe^{2+} of 0.0, 0.5, 1.0, or 2.0 mg L^{-1} were analyzed with the modified Nessler method in which the MS and PVA reagents had been added into the samples before the Nessler reagent was added. The result was compared with the one from the measurement by the original Nessler method (Figure 6). The effect of Fe^{2+} on

both the modified Nessler method and the original Nessler one was evaluated with samples of three different NH_4^+ concentrations, that is, 0.5, 1.0, and 1.5 mg L^{-1} .

In the case of the original Nessler method, an accurate analysis of NH_4^+ content could not be possible when Fe^{2+} content of water samples was more than 1.0 mg L^{-1} . In fact, Fe^{2+} in water is known to form a complex with salts in the Nessler reagent, and increases turbidity and the absorbance.

The modified Nessler method also could not accurately quantitate NH_4^+ in water samples with Fe^{2+} . For any NH_4^+ concentration, the water sample even with 0.5 mg L^{-1} Fe^{2+} showed significantly higher UV absorbance than that of the

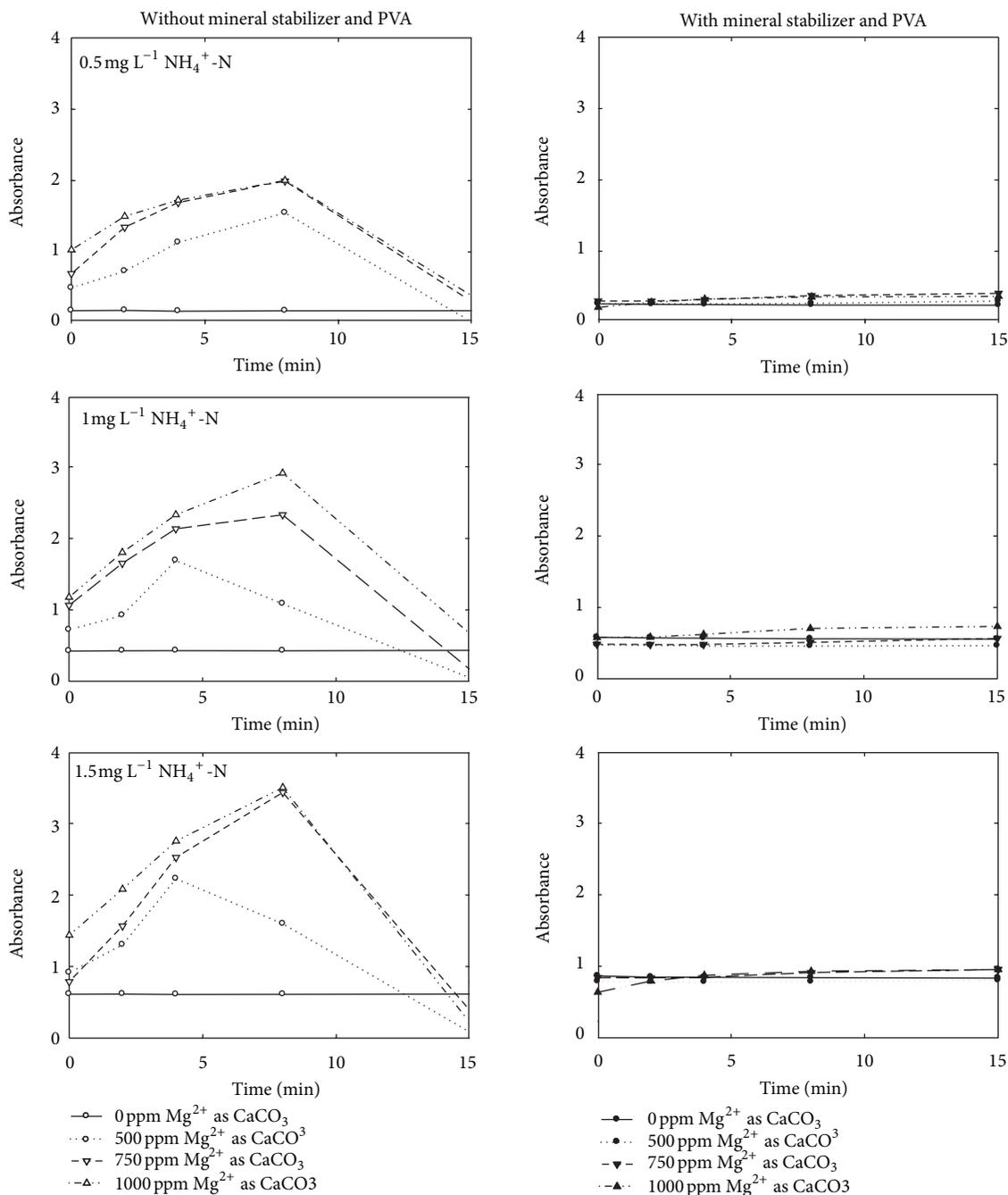


FIGURE 5: UV absorbance at water samples of different NH_4^+ concentrations with different reagents, and with Mg^{2+} (as hardness-causing chemical) as interfering substance.

sample without Fe^{2+} (Figure 6). Therefore, it was concluded that both the original and the modified Nessler methods for NH_4^+ quantitation cannot be applied to water samples with Fe^{2+} . Like the case of Mg^{2+} as an interfering substance, it is necessary to remove Fe^{2+} from a sample before NH_4^+ analysis is carried out, if the sample is suspected to have Fe^{2+} .

3.3. Recovery Efficiency of the Proposed NH_4^+ Analysis Method. Recovery efficiency of the modified Nessler method was performed with sewage and fresh water samples which

were collected from a local sewage treatment plant and a nearby river. Upon delivery to the laboratory, the water samples were filtered and analyzed for their NH_4^+ contents. The sewage contained $\text{NH}_4^+\text{-N}$ of 12.4 mg L^{-1} , while the surface water did contain 0.8 mg L^{-1} $\text{NH}_4^+\text{-N}$. In fact, the sewage sample was ten times diluted before being used for the recovery test. Then, the sewage and stream water samples of 100 mL each were spiked with NH_4^+ stock solution to increase their NH_4^+ concentration as much as 1 mg N L^{-1} . Then, the samples analyzed for NH_4^+ concentration using the modified

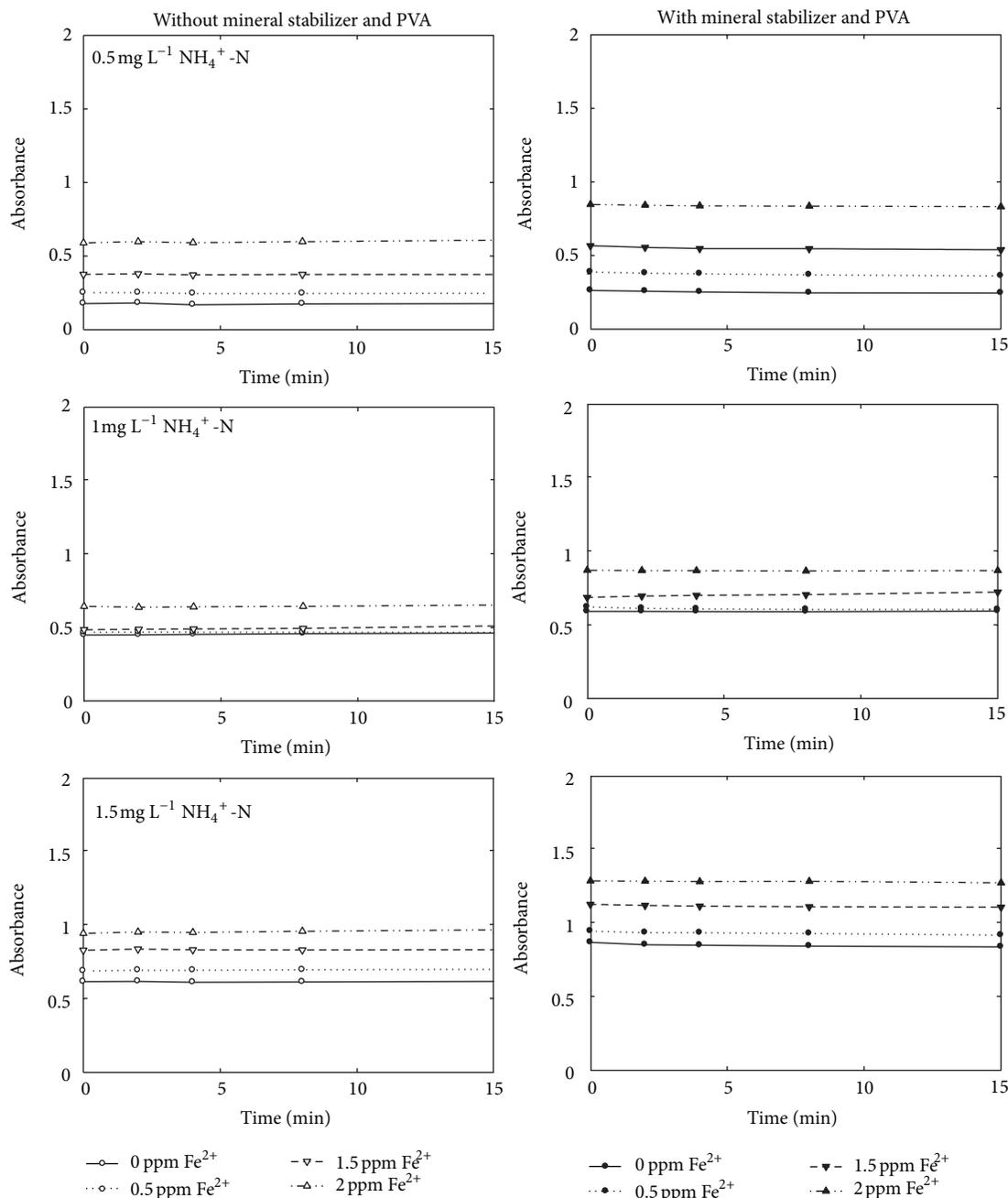


FIGURE 6: UV absorbance at water samples of different NH_4^+ concentrations with different reagents and with Fe^{2+} (as hardness-causing chemical) as interfering substance.

Nessler method. In short, the recovery efficiency of 96.5–104.5% could be obtained using the proposed method.

4. Conclusion

The traditional Nessler method for the analysis of water NH_4^+ was modified by introducing the additional reagents, that is, the MS and the PAV. With the help of the PVA, the light absorbance of the water samples under analysis could be increased significantly; so the sensitivity of the Nessler method increased. In addition, the maximum absorbance could be rapidly achieved and did not change over time.

Therefore, the sensitivity and stability of the original Nessler method could be improved decently.

Due to the addition of the MS, the modified method could accurately analyze NH_4^+ in water samples with residual Cl_2 , Cl^- , or Mg^{2+} , each of which is a well-known substance causing interference to the accurate determination of the NH_4^+ concentration. However, both the traditional and modified Nessler methods could not accurately determine NH_4^+ of a water sample with Fe^{2+} . Nonetheless, we believe that the proposed method will be beneficial to researchers who regularly analyze NH_4^+ in water samples.

Acknowledgment

This work was supported by the R&D Program of MKE/KEIT (R&D Program no. 10037331, Development of Core Water Treatment Technologies based on Intelligent BT-NT-IT Fusion Platform).

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