

# Possible Influences on Ammonia Nitrogen Determination by Nessler's Reagent Spectrophotometry

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## Abstract

Nessler's reagent spectrophotometry is the traditional method for ammonia nitrogen (NH<sub>3</sub>-N) determination, which is adopted in the national standard of the People's Republic of China (HJ 535-2009). In order to improve the accuracy of such method, possible influences, Nessler's Reagent preparation, pH value of the solution, metal ions, organic solvents and natural organic matters, on NH<sub>3</sub>-N determination were discussed. The results suggested that the method B, employing KI, HgI<sub>2</sub> and NaOH, is preferred in Nessler's Reagent preparation. And the Nessler's Reagent Spectrophotometry may be executed over a wide pH range (4-11). The metal ions, organic solvents and natural organic matters have significant influences in NH<sub>3</sub>-N detection.

**Keywords** Nessler's Reagent, Ammonia Nitrogen, pH, Metal Ion, Organic Solvent, Natural Organic Matter.

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## 1. Introduction

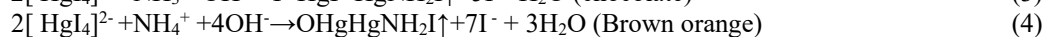
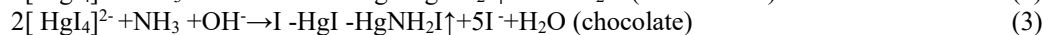
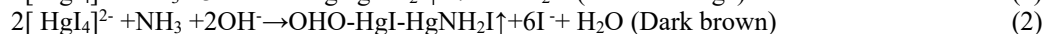
The concept, ammonia-nitrogen (NH<sub>3</sub>-N) is aquatic nitrogen in form of NH<sub>3</sub> or NH<sub>4</sub><sup>+</sup>. The main resources of NH<sub>3</sub>-N are nitrogen-containing compounds from domestic wastewater, industrial wastewater and agricultural effluent. Thus, NH<sub>3</sub>-N becomes a typical indicator in (waste)water quality determination. Presently, the classical method for NH<sub>3</sub>-N determination is Nessler's reagent spectrophotometry. With the advantages of high sensitivity and easy operation, Nessler's reagent spectrophotometry is adopted in the national standard of the People's Republic of China (HJ 535-2009)<sup>[1]</sup>.

Generally, the detection limit of Nessler's reagent spectrophotometry was 0.025 mg/L. Its lower limits and the upper limits are 0.10 and 2.0mg/L, respectively<sup>[2]</sup>. This method is popular in routine NH<sub>3</sub>-N monitoring and in experiment course in universities. According to the quality assurance and quality control required by the national standard (HJ 535-2009), critical controls are settled on the (waste)water pre-treatment, reagent preparations and the operation procedure. However, there are still some factors may influence the NH<sub>3</sub>-N determination. Therefore, substantial investigations were given on the Nessler's reagent preparation, pH value of solution and coexisting interferants. The present work may provide valuable reference for national standard optimization.

## 2. Principles and methods

### 2.1 Principle of ammonia nitrogen reaction

NH<sub>3</sub>-N reacts with Nessler's reagent forming reddish-brown complexes. And the complicated reactions are outlined in equation (1)-(5). Furthermore, the NH<sub>3</sub>-N concentration is proportional to the spectrophotometric absorbance of the complex.



### 2.2 Methods

Standard solution of NH<sub>3</sub>-N: standard solutions of NH<sub>3</sub>-N were prepared employing NH<sub>4</sub>Cl (high-grade purity), which was dried at 105 °C more than 2 hours before use.

Unless stated, all solutions were freshly prepared using ultrapure water (18.2 mΩ·cm<sup>-1</sup>, Millipore Corp., USA). The soluble interferants were added into the standard solution of NH<sub>3</sub>-N, directly. In addition, the insoluble interferants were soaked in 5 mol·L<sup>-1</sup> NaOH solution for 24 hour, and the filtrate was added into the standard solution of NH<sub>3</sub>-N.

After the water sample was treated, 20 mL was put into the 50 mL colorimetric tube, 1.0 mL potassium and sodium tartrate solution, and 1.5 mL Nessler's reagent were added, sequentially. Then ultrapure water was added

into the colorimetric tube to dilute the mixture to the scale mark and the colorimetric determination was conducted at the wavelength of 420 nm.

### 3 Results and discussion

#### 3.1 The influence from the methods of Nessler's reagent preparation

Given by the national standard, KI, HgCl<sub>2</sub> and KOH (Method A), and KI, HgI<sub>2</sub> and NaOH (Method B), are employed in Nessler's reagent preparation. [HgI<sub>4</sub>]<sub>2</sub>, the critical chromophore group in the spectrophotometry can be derived in both methods. According to experimental results, the blank value obtained in Method A is 0.025, while that is 0.029 in Method B. Both of them are within the scope acquired by the national standard. Additionally, during the calibration employing two type of Nessler's reagents, the correlation coefficients are 0.9986 (Method A) and 0.9990 (Method B), respectively.

Considering the preparation of Nessler's reagent, Method B is preferred based on the results above. Firstly, in Method A, it takes a lot of time to prepare saturated HgCl<sub>2</sub> solution, because of the slow dissolution of HgCl<sub>2</sub> in KI solution. Secondly, the impurities of KOH in Method A produce some sediment, which cause long-time precipitation process and complicated preparation. Thirdly, Nessler's reagent prepared in Method B has longer shelf life about 1 year, which minimizes the Hg-containing reagent waste.

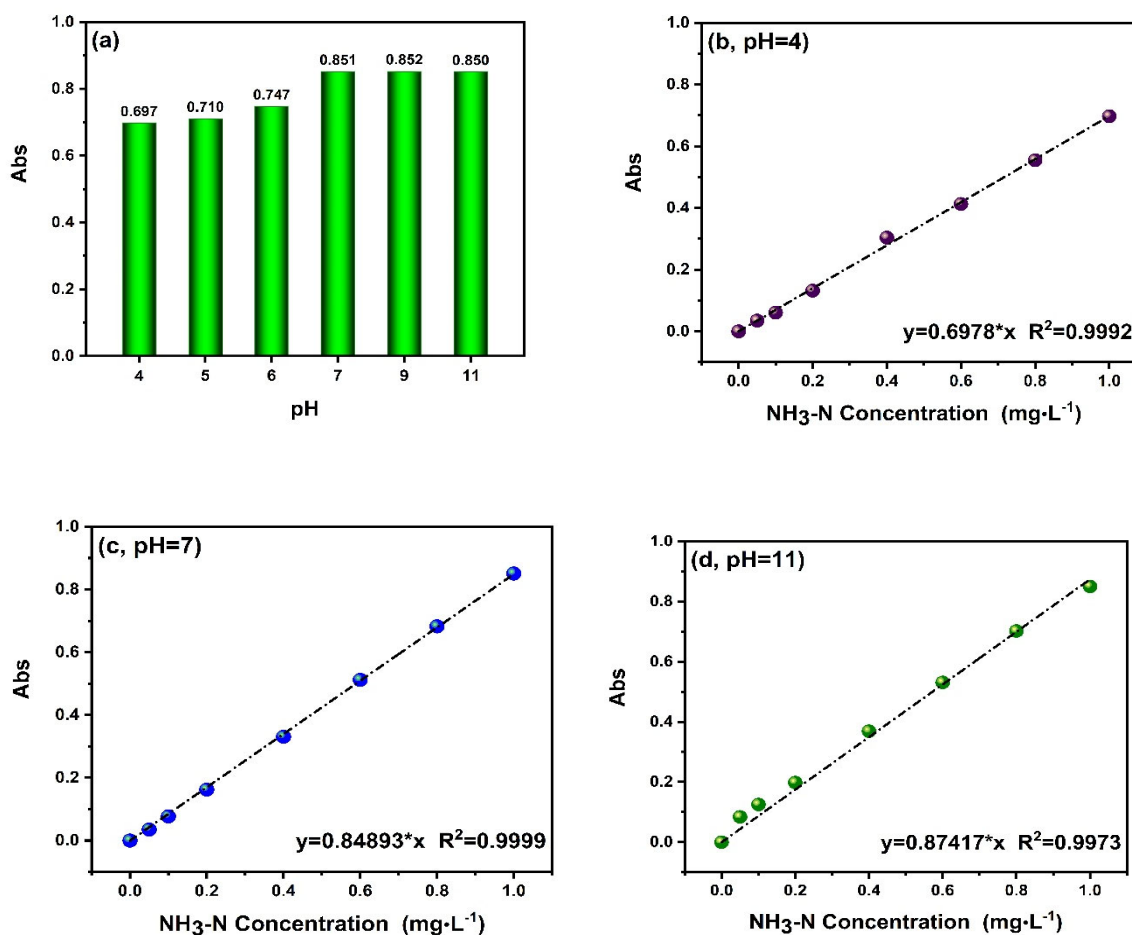


Figure 1. The influence from pH on NH<sub>3</sub>-N detection.

#### 3.2 The influence from pH on NH<sub>3</sub>-N detection

Solution pH is investigated for its possible impacts on NH<sub>3</sub>-N detection<sup>[3]</sup>. The NH<sub>3</sub>-N solutions (1 mg·L<sup>-1</sup>) with desired pH were adjusted by H<sub>2</sub>SO<sub>4</sub> (0.5 mol·L<sup>-1</sup>) or NaOH (1 mol·L<sup>-1</sup>) solution. As shown in Figure 1a, the absorbance in the spectrophotometry (Abs) increases with raised pH value (pH<7), while the Abs is insensitive to pH value in the range of 7-11. As depicted in Figure 1b-d, the Abs is in good correlation with NH<sub>3</sub>-N concentration over acidic, neutral and alkaline conditions.

#### 3.3 The influence from metal ions

The effect of certain metal ions, Mn<sup>2+</sup>, Zn<sup>2+</sup>, Cd<sup>2+</sup>, Co<sup>2+</sup>, Cu<sup>2+</sup>, Ni<sup>2+</sup> and Pb<sup>2+</sup>, on the NH<sub>3</sub>-N detection was

investigated. The  $\text{NH}_3\text{-N}$  solutions ( $1 \text{ mg}\cdot\text{L}^{-1}$ ) with certain metal ion ( $5 \text{ mmol}\cdot\text{L}^{-1}$ ) component were prepared at pH 4. To exclude the interference stems from the color of ions, special reference was prepared with corresponding metal ion, but without  $\text{NH}_3\text{-N}$  and Nessler's reagent. The results presented in Figure 2 shows the influence varies with metal ions. Specifically,  $\text{Mn}^{2+}$  has imperceptible interference,  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$  and  $\text{Co}^{2+}$  have weak interference, however  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Pb}^{2+}$  exerts obvious interference. For example,  $\text{Pb}^{2+}$  caused Abs increase about 13%. Considering the  $\lg\beta_n$  between corresponding metal ions and ligand (Table 1),  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Pb}^{2+}$ , especially for  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Pb}^{2+}$ , have intensive complexation tendency towards  $\text{NH}_3$ . Thus, it is hard for potassium sodium tartrate with a final concentration about  $40 \text{ mmol}\cdot\text{L}^{-1}$  to mask the interference from such metal ions totally.

### 3.4 The influence from organic solvents

Organic solvents, such as methanol (MT), dimethyl formamide (DMF), dimethyl sulfoxide (DMSO), ethanol (ET), acetonitrile (ACN), acetone (CP) and trimethylamine (TEA), are extensively used in manufacturing process. These organic solvents coexist with  $\text{NH}_3\text{-N}$  in the (waste)water samples<sup>[4]</sup>. Therefore, it was of great significance to evaluate the influences from such organic solvents on  $\text{NH}_3\text{-N}$  detection employing the Nessler's reagent spectrophotometry. Accordingly, the  $\text{NH}_3\text{-N}$  solutions ( $1 \text{ mg}\cdot\text{L}^{-1}$ ) with certain organic solvent ( $5 \text{ mmol}\cdot\text{L}^{-1}$ ) were prepared at pH 7. As shown in Figure 3, the organic solvents cause non-determinacy in  $\text{NH}_3\text{-N}$  detection. Furthermore, the interference depends on the type of the organic compounds. The impact from methanol (MT), dimethyl formamide (DMF), acetone (CP) and ethanol (ET) is negligible. However, acetonitrile (ACN), dimethyl sulfoxide (DMSO) and trimethylamine (TEA) raise the Abs of  $\text{NH}_3\text{-N}$  detection significantly. As for trimethylamine (TEA), these is an increase more than 16% observed.

Table 1. The parameters of  $\lg\beta_n$  between corresponding metal ions and ligands<sup>[4]</sup>.

Ligand	Metal ion	Number of ligand	$\lg\beta_n$	
potassium sodium tartrate	$\text{Hg}^{2+}$	1	7.0	
	$\text{Cd}^{2+}$	1	2.8	
	$\text{Co}^{2+}$	1	2.1	
	$\text{Cu}^{2+}$	1,2,3,4	3.2, 5.11, 4.78, 6.51	
	$\text{Mn}^{2+}$	1	2.49	
	$\text{Ni}^{2+}$	1	2.06	
	$\text{Pb}^{2+}$	1,3	3.78, 4.7	
	$\text{Zn}^{2+}$	1,2	2.68, 8.32	
	$\text{NH}_3$	$\text{Hg}^{2+}$	1,2,3,4	8.8, 17.5, 18.5, 19.28
		$\text{Cd}^{2+}$	1,2,3,4,5,6	2.65, 4.75, 6.19, 7.12, 6.80, 5.14
$\text{Co}^{2+}$		1,2,3,4,5,6	2.11, 3.74, 4.79, 5.55, 5.73, 5.11	
$\text{Cu}^{2+}$		1,2,3,4,5	4.31, 7.98, 11.02, 13.32, 12.86	
$\text{Mn}^{2+}$		1,2	0.8, 1.3	
$\text{Ni}^{2+}$		1,2,3,4,5,6	2.80, 5.04, 6.77, 7.96, 8.71, 8.74	
$\text{Pb}^{2+}$		1,2,3,4	9.6, 18.5, 26.0, 32.8	
$\text{Zn}^{2+}$		1,2,3,4	2.37, 4.81, 7.31, 9.46	

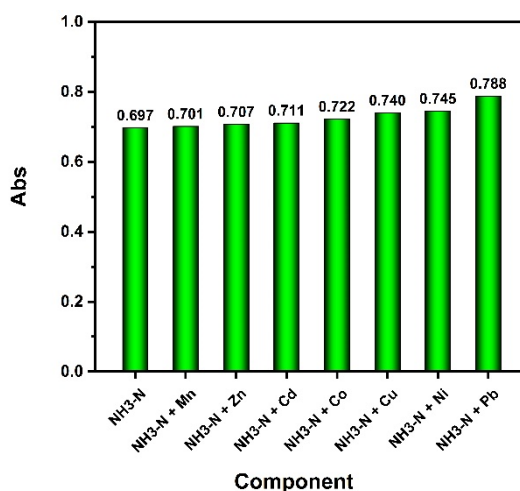


Figure 2. The influence from metal ions on  $\text{NH}_3\text{-N}$  detection.

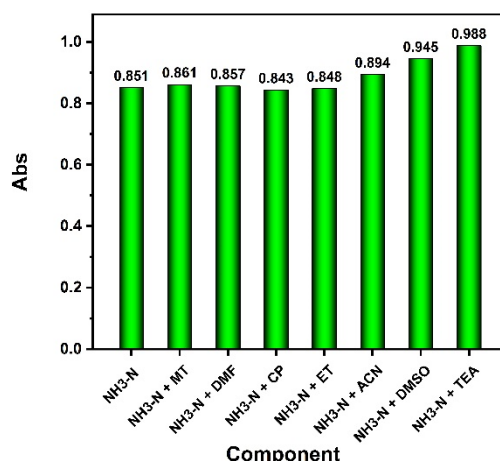


Figure 3. The influence from organic solvents on NH<sub>3</sub>-N detection.

### 3.5 The influence from natural organic matters

Natural organic matter (NOM) is a series of complicated organic materials and a ubiquitous component in (waste)water samples<sup>[3]</sup>. Interactions between the hydrologic cycle and the biosphere and geosphere shape the amount, character and properties of NOM in the environments. In this study, to evaluate the influence from NOM, the sediments (20 g) from marine (MS), river (RS), lake (LS) and swamp (SS) were soaked in 5 mol·L<sup>-1</sup> NaOH solution (50 mL) for 24 hour, and the filtrates (20 mL) were added into the standard solutions of NH<sub>3</sub>-N, using humic acid (HA) and fulvic acid (FA) as the references. Additionally, the NH<sub>3</sub>-N solutions (1 mg·L<sup>-1</sup>) with certain NOM were prepared at pH 11. As presented in Figure 4, the NOMs have significant influence on NH<sub>3</sub>-N detection. The leaching solutions from the various sediments reinforce the Abs in the spectrophotometry, resembling the influences of humic acid (HA) and fulvic acid (FA). The results indicate that the sediments may reinforce the Abs in the Nessler's Reagent Spectrophotometry, by releasing the NOMs, such as humic acid and fulvic acid.

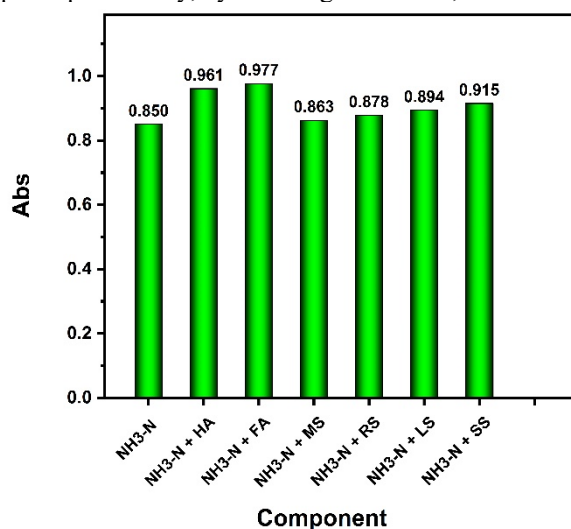


Figure 4. The influence from natural organic matters on NH<sub>3</sub>-N detection.

### 4. Conclusion

In summary, the influences from the Nessler's Reagent preparation, pH value of the solution, metal ions, organic solvents and natural organic matters were investigated. The results indicated that the method B (KI, HgI<sub>2</sub> and NaOH) is preferred in Nessler's Reagent preparation. The Nessler's Reagent Spectrophotometry may be executed over a wide pH range (4-11). The metal ions, organic solvents and natural organic matters have significant influences in NH<sub>3</sub>-N detection, which need to be eliminated.

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