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**SPECTROPHOTOMETRIC DETERMINATION OF TRACE  
NITRITE WITH A NOVEL SELF-COUPLING DIAZOTIZING  
REAGENT: J-ACID**

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*A simple and sensitive method for the spectrophotometric determination of nitrite was described and optimum reaction conditions along with other important analytical parameters were established. In the presence of potassium bromide at 25°C, nitrite reacted with J-acid in hydrochloric acid producing diazonium salt and then coupled with excess J-acid in the sodium carbonate solution yielding red colored azo compounds. At wavelength of 500 nm, Beer's law was obeyed over the concentration range of 0,02 – 0,60 mg · L<sup>-1</sup>. The molar absorptivity was 3,92 · 10<sup>4</sup> L · mol<sup>-1</sup> · cm<sup>-1</sup>. This method was easily applied to the determination of trace nitrite in environmental water with recoveries of 98,7 – 101,2%.*

**Keywords:** nitrite, J-acid, self-coupling, spectrophotometric.

### **Introduction**

Nitrite is a kind of harmful substances present extensively around us. When it in human body greatly, it will react with iron (III) in the hemoglobin of the red blood cells, forming methemoglobin which is unable to carry oxygen thus causing hypoxia and acute poisoning. Moreover, nitrite has direct impact on the health because of its reaction with secondary and tertiary amines and amides in human body, which produce toxic and carcinogenic nitrosamines compounds [1 – 4]. Therefore, the concentration of nitrite in foods and environmental water is limited strictly by the U.S. Public Health Association [5]. Researchers make great amounts of efforts to establish accurate and simple analytical methods.

Under acidic condition, the diazotization with nitrite and primary aromatic amine is always simple and very general. The reaction has the advantages of free from interference, high sensitivity and excellent selectivity. Consequently, scientists all over the world study spectrophotometric determination of nitrite

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by diazo-coupling reaction. Szczepaniak [6] modified Griess method (-Griess method is the diazo-coupling reaction with sulfanilamide and N-(1-naphthyl) ethylenediamine hydrochloride (NEDA)). He used sulfanilamide for diazo reagent, 1-naphthol-4-sulphonic acid or 8-amino-2-naphthalene sulfonic acid for coupling reagent. The sensitivity and accuracy of this method was pretty high. Reddy [7] used DSD acid (4,4'-diaminodiphenylethylene-2,2'-disulfonic acid) for diazo reagent and 2-naphthol for coupling reagent. The molar absorptivity was  $3,0 \cdot 10^4 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ .

Nagarja [8 – 9] had found dapsone-phloroglucinol for coupling reagent, the molar absorptivity was  $4,28 \cdot 10^4 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ . Later he found 4-amino-5-hydroxynaphthalene-2,7-disulphonic acid monosodium salt for coupling, the molar absorptivity was  $2,60 \cdot 10^4 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ . The linear range was within  $0,1 - 1,6 \mu\text{g} \cdot \text{mL}^{-1}$ . Sreekumar [10] used *p*-nitroaniline for diazo reagent and sulfonamide or ethyl acetoacetate for coupling reagent. The maximum absorption wavelength for azo compound was at 439 and 465 nm. The molar absorptivity was  $1,59 \cdot 10^4$  and  $1,22 \cdot 10^4 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ . Linearity was within  $0,05 - 6,0$  and  $0,2 - 3,9 \mu\text{g} \cdot \text{mL}^{-1}$ . Zhong-Xiaoyong [11] used sulfanilamide for diazo reagent and aminonaphthol sulfonic acid for coupling reagent to determine the nitrite in foods. The Beer's law was found to obey for nitrite in the concentration range of  $0,02 - 1,4 \mu\text{g} \cdot \text{mL}^{-1}$ . The molar absorptivity was  $3,90 \cdot 10^4 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ . Yu Yun [12] found sulfanilamide and N-phenyl J-acid. She replaced NEDA with N-phenyl J-acid. The molar absorptivity was  $4,63 \cdot 10^4 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ . The linear range is within  $0,003 - 0,7 \mu\text{g} \cdot \text{mL}^{-1}$ , the recoveries were in the range of 98,7 – 101,2%, Relative Standard Deviation (R.S.D.) was 2,10%.

From the above literatures, it can be found that Griess reaction still is the most used methodology for the determination. The sensitivity is greater with NEDA. However, NEDA is strong carcinogens and it will cause secondary pollution. In this paper, a very simple and less toxic method for nitrite determination based on self-coupling reaction with J-acid (2-amino-5-naphthol-7-sulfonic acid) has been developed. The proposed method is sensitive, requires no control of temperature and do not suffer from most of the potential interferents.

## Experimental

*Reagents and equipment.* All chemicals were analytical grade without further purification. Double-distilled water was used throughout the experiments for dilution of the reagents and samples.

Stock sodium nitrite solution –  $0,25 \text{ mol} \cdot \text{L}^{-1}$ ; working sodium nitrite solution  $2,5 \cdot 10^{-4} \text{ mol} \cdot \text{L}^{-1}$ ; hydrochloric acid solution –  $1,5 \text{ mol} \cdot \text{L}^{-1}$ ; sodium carbonate solution –  $1,0 \text{ mol} \cdot \text{L}^{-1}$ ; 1% (mass fraction) potassium bromide aqueous solution; J-acid (2-amino-5-naphthol-7-sulfonic acid) –  $1,0 \cdot 10^{-2} \text{ mol} \cdot \text{L}^{-1}$ ; H-acid (4-amino-5-hydroxynaphthalene-2,7-disulphonic acid) –  $1,0 \cdot 10^{-2} \text{ mol} \cdot \text{L}^{-1}$ . UV757CRT spectrophotometer, Shanghai Precision and Scientific Instrument Corporation. PHS – 2C precision acidity meter, Shanghai ray magnetic instrument factory.

*General procedures.* 1,0 ml of sodium nitrite standard solution, 9,0 ml of distilled water, 1,0 ml of potassium bromide solution, 1,0 ml of J-acid solution and 1,0 ml of hydrochloric acid solution were added into a 25 ml volumetric flask in order, the mixture was shaken thoroughly and allowed to stand for 2 min at room temperature ( $25 \text{ }^{\circ}\text{C}$ ). And then, the mixture was transferring into 3 ml of sodium carbonate solution added in another 25 ml volumetric flask. The volume was shaken thoroughly and stood for 5 min, which was then filled to the mark with distilled water. Absorbance was measured in 1,00 cm quartz cells at 500 nm against reagent blank, which was prepared in the same manner without nitrite.

## Results and discussion

*Molecule structure design of the self-coupling diazotizing reagent.* The treatment of primary aromatic amine with nitrous acid produces a diazonium salt in a reaction is diazotization. Diazonium salts undergo coupling reactions to form azo compounds. The great importance of the self-coupling diazotizing reagents in molecule structure design lies in the coupling reactions that occur with an activated aromatic ring, such as that in phenols or aromatic amines. Moreover, a sulfo group is always introduced to increase water solubility, Last but not the least, the azo compounds tend to be brightly colored due to the increased extended conjugated systems. Consequently, the most simple molecular should be aminonaphthol sulfonic acid. Considering the amino activation and group space steric hindrance of sulfonic, it was appropriate that use 1,8 (or 1,6 or 1,7)-amino naphthalene sulfonic acid for the self-coupling diazotizing reagent in weak acid solution. On the contrary, coupling with phenols the phenoxide ion is the reactive species, and slightly basic solution is used. Reaction gives azo compounds with an arylazo group at the position para or ortho to hydroxy. To prevent yielding more isomers, hydroxy and amino should be designed in naphthalene on both sides. And the sulfonic group introduced avoided at the

position of ortho and para. Considering all of it, we regarded J-acid, H-acid, 1-amino-2-naphthol-4-sulfonic acid, 1,8-amino naphthalene sulfonic acid, 2-naphthylamine-1-sulfonic acid and sulfanilic acid as the diazo self-coupling reagents and made experimental research. The molecular structure of the azo compound, J-acid was as example (Fig. 1).

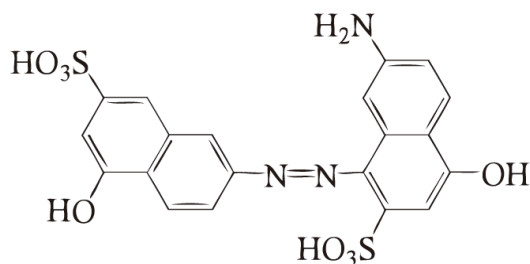


Fig. 1. Azo compound molecular structure of J-acid.

*Comparison of the self-coupling diazotizing reagents.* Self-coupling diazotizing reagent was compared in both acidic and alkaline aqueous solution respectively. Table 1 showed that the highest molar absorptivity was of J-acid azo compound in alkaline medium, H-acid azo compound took the second place, the color rendering of 1,8-amino naphthalene sulfonic acid azo compound was weak. Sulfanilic acid was not colored, the solution of 1-amino-2-naphthol-4-sulfonic acid azo compound showed brown color, which suggested that the spectrums of the by-products were different from each other.

Table 1. Comparison of diazo self-coupling reagents

Diazo coupling reagents	$\lambda_{\max}$ , nm	pH	Molar absorptivity, $L \cdot mol^{-1} \cdot cm^{-1}$
1, 8-Amino naphthalene sulfonic acid	500	3,4	$4,21 \cdot 10^3$
		9,8	$3,08 \cdot 10^2$
2 - Naphthylamine - 1 - sulfonic acid	450	3,4	$2,14 \cdot 10^2$
		9,8	0
Sulfanilic acid	—	3,4	0
		9,8	0
J-acid	500	3,4	$1,52 \cdot 10^4$
		9,8	$3,82 \cdot 10^4$
H-acid	520	3,4	$1,65 \cdot 10^3$
		9,8	$1,72 \cdot 10^4$
1-2-4-Acid	—	3,4	—
		9,8	—

The larger the molar absorptivity was, the more sensitive the reaction was. The wavelength of maximum absorption of the color formed was obtained at 500 nm for J-acid and 1,8-amino naphthalene sulfonic acid and 520 nm for H-acid with negligible absorbance for the corresponding reagents blank (Fig. 2).

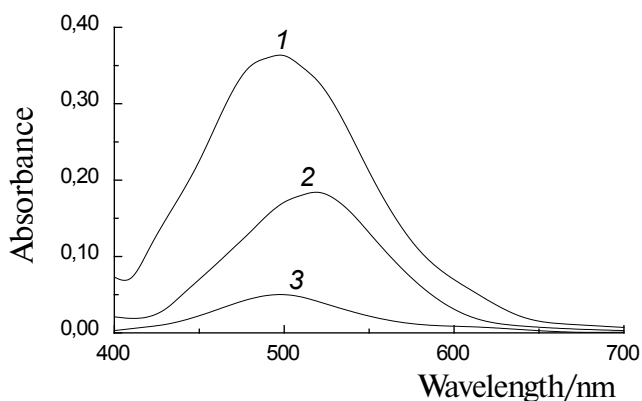


Fig. 2. The absorption spectrum of azo compounds: J-acid (1); H-acid (2); 1,8 – amino naphthalene sulfonic acid (3).

*Effect of acidity on the diazo reaction.* According to the method of experiment, we tested the effect of acidity on the J-acid (H-acid) diazo reaction. The results showed, when the pH of solution was between 1,0 – 2,0, namely the concentration of hydrochloric acid was in 0,30 – 0,60 mol · L<sup>-1</sup>. The absorbance was maximum and stable (Fig. 3) the concentration of nitrite was 1,0 · 10<sup>-5</sup> mol · L<sup>-1</sup>.

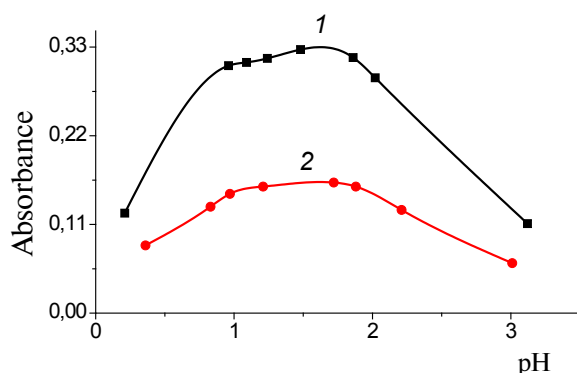


Fig. 3. Effect of acidity on the diazo reaction: J-acid (1), H-acid (2).

Hence, 1 ml of  $1,5 \text{ mol} \cdot \text{L}^{-1}$  hydrochloric acid was chosen in the experiment. The acidity was rather strong so that the reaction rate was faster and the color intensity and stability was improved.

*Effect of time on the diazo reaction.* Because the sulfonic group was the electrophilic group so that weakening the alkalinity of aromatic amine, the time of diazotization was often very short. According to the method of experiment, we test the effect of time on the J-acid (H-acid) diazotization. The results showed, the most stable and highest absorbance was within 1 – 5 min, thus, 2 min was selected for the reaction in the experiment.

*Effect of temperature on the diazo reaction.* With the rise of temperature, diazotization speeded up. However, in theory, the diazonium salt was usually relatively stable at low temperature and it was easy to decompose at high temperature. This study tested the effect of temperature on the diazo reaction between 0 – 25°C. It was developed that in the range of 5 – 20°C, the absorbance of J-acid (H-acid) was stable, so diazotization was carried out at room temperature.

*Effect of potassium bromide concentration.* In order to speed up the diazotization, we used potassium bromide as the catalyst. As the method of experiment, adding 1 ml potassium bromide solution of different mass fraction in a series of fixed nitrite concentration in the flask to observe and study the effect of different dosage of potassium bromide on the rate of the reaction. The results showed when the concentration of potassium bromide was  $0,8 \text{ mg} \cdot \text{mL}^{-1}$ , diazotization completed quickly in 2 min. Hence, 1 ml of 1% potassium bromide was added in the determination.

*Effect of pH on the coupling.* According to the method of experiment, pH on the coupling reaction was chosen. From Fig. 4 (the concentration of nitrite is  $1,0 \cdot 10^{-5} \text{ mol} \cdot \text{L}^{-1}$ ), it was found that the absorbance increased with the rise of the pH. When the pH rose at the range of 8,2 – 10,2, the absorbance of J-acid (H-acid) was maximum and stable.

Coupling in alkaline medium helped to form lively anion of hydroxyl in the naphthalene of J-acid. However, when the alkalinity was too strong, the diazoate would convert to inert trans-diazoate so that did not couple. Therefore, 3 ml of  $1 \text{ mol} \cdot \text{L}^{-1}$  sodium carbonate solution was chosen in the determination.

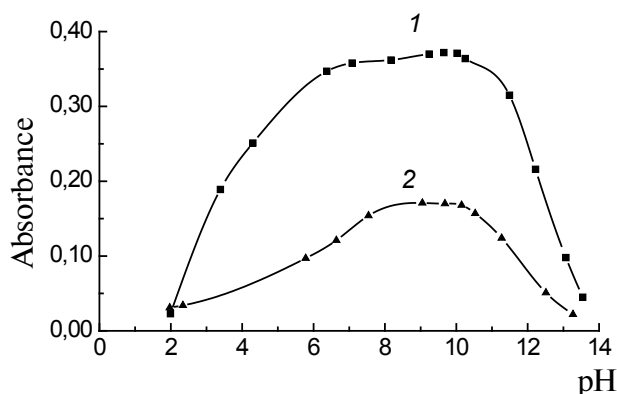


Fig. 4. Effect of pH on the coupling: J-acid (1), H-acid (2).

*Effect of time on the coupling.* From the method of experiment, we researched the effect of time on the coupling reaction. It was found that when the coupling time was between 5 – 30 min, the absorbance was at maximum and stable, as the result, 5 min was selected in the determination.

*Effect of self-coupling diazotizing reagent concentration.* Only when the concentration of J-acid (H-acid) was far greater than the sodium nitrite content, it could ensure that sodium nitrite would completely react in diazo-coupling reaction. As the method of experiment, the effect of varying concentrations of J-acid on the color intensity and stability had been studied by adding different dosage of J-acid (H-acid) to a series of fixed nitrite concentration in the flask (1,0 ml of  $2,49 \cdot 10^{-4} \text{ mol} \cdot \text{L}^{-1}$  sodium nitrite working solution.). It was found that, the maximum intensity and stability of the color formed was obtained with the concentration of J acid (H-acid) within  $5,00 \cdot 10^{-5} - 4,00 \cdot 10^{-4} \text{ mol} \cdot \text{L}^{-1}$ . Thus, 1 ml of  $1,00 \cdot 10^{-2} \text{ mol} \cdot \text{L}^{-1}$  J-acid (H-acid) was used in the experiment.

*Interference studies.* The selectivity of the proposed method was studied by the effect of possible interferents on the determination of nitrite. At the concentration of  $0,688 \text{ mg} \cdot \text{L}^{-1}$  sodium nitrite, (unit:  $\text{mg} \cdot \text{L}^{-1}$ )  $\text{K}^+$ (200),  $\text{Na}^+$ (200),  $\text{Ca}^{2+}$ (550),  $\text{Mg}^{2+}$ (550),  $\text{Al}^{3+}$ (0,2),  $\text{Fe}^{3+}$ (0,3),  $\text{Pb}^{2+}$ (0,1),  $\text{Cu}^+$ (1,0),  $\text{Cd}^{2+}$ (0,01),  $\text{Ag}^+$ (0,05),  $\text{Cr}^{3+}$ (0,1),  $\text{Hg}^{2+}$ (0,001),  $\text{Ni}^{2+}$ (0,02),  $\text{Zn}^{2+}$ (2,0),  $\text{NO}_3^-$ (10),  $\text{SO}_4^{2-}$ (250),  $\text{H}_2\text{PO}_4^-$ (0,4),  $\text{F}^-$ (1,5),  $\text{Cl}^-$ (250),  $\text{CN}^-$ (0,2) was added. These data were the upper limit of environment quality standard of the basic material limit value in surface water [13]. The results showed that the concentration of added ion causing less than  $\pm 5\%$  relative error for the nitrite determination.

**Calibration.** According to the general procedures, a calibration graph was plotted for nitrite. A straight line passing through the origin had been obtained. The results were shown in Table 2.

*Table 2. Calibration graph*

Method	Regression equation <sup>a</sup>	Correlation coefficient	Liner range, mol · L <sup>-1</sup>	Molar absorptivity, L · mol <sup>-1</sup> · cm <sup>-1</sup>
J-acid	$A = 0,852 c + 3,0 \cdot 10^{-3}$	0,998	0,02 – 0,60	$3,93 \cdot 10^4$
H-acid	$A = 0,375 c - 8,5 \cdot 10^{-3}$	0,998	0,03 – 1,5	$1,73 \cdot 10^4$

**Determination of nitrite in water samples.** In order to assess the suitability of the general method, it was applied for the determination of nitrite in real water. The samples were collected from the tributaries of Huangpu River and campus pond respectively and without adding any preservative in polyethylene bottles and analyzed within 4h. In the samples, nitrite analysis was performed as follow. Firstly, the sample was stand for 30 min, and 25 ml of supernatant was taken for use later. Then, 1,0 ml supernatant of samples was transferred into a 25 ml volumetric flask. After that 1,0 ml of potassium bromide aqueous solution, 1,0 ml of J-acid (or H-acid) solution and 1,0 ml of hydrochloric acid solution were added into the 25 ml volumetric flask in order, the mixture was shaken thoroughly and allowed to stand for 2 min at room temperature (25°C). And then, the mixture was transferring into the 3 ml of sodium carbonate solution added in another 25 ml volumetric flask. The volume was shaken thoroughly and kept aside for 5 min, which was then made up to the mark with distilled water. Absorbance was measured in 1,00 cm quartz cells at 500 nm for J-acid and at 520 nm for H-acid against reagent blank, which was prepared in the same manner without nitrite.

Finally, the results were statistically evaluated in terms of Student's test and variance ratio F-test and the values calculated to compare with national standard method [14] (GB/T 75750.5-2006). The experimental results were listed in Table 3.



Table 3. The results of sample analysis

Method	Sample	Nitrite found, mg·L <sup>-1</sup>	R.S.D, %	Nitrite added, mg·L <sup>-1</sup>	Total nitrite found <sup>a</sup> , mg·L <sup>-1</sup>	Recovery, %
J-acid	Huangpu River <sup>b</sup>	0,955	1,80	0,344 0,688	1,303 1,634	101,2 98,7
	Pond <sup>c</sup>	0,588	1,59	0,344 0,688	1,933 1,267	100,3 99,1
H-acid	Huangpu River <sup>b</sup>	0,950	2,20	0,344 0,688	1,286 1,633	97,7 99,3
				0,344 0,688	1,900 1,269	98,3 102,8
	Pond <sup>c</sup>	0,562	2,04			
GB/T	Huangpu River <sup>b</sup>	0,975	1,61	0,344 0,688	1,321 1,650	100,6 98,1
5750.5-2006 <sup>d</sup>	Pond <sup>c</sup>	0,593	1,96	0,344 0,688	0,945 1,278	102,3 99,6

<sup>a</sup> Student's test and variance ratio F-test ( $n = 8$ ); <sup>b</sup> Water of Huangpu River, Shanghai, China;

<sup>c</sup> Water of campus pond, University of Shanghai for Science and Technology; <sup>d</sup> China national Standard examination methods for drinking water.

### Conclusion

In the sodium carbonate solution, J-acid diazonium salt coupled with excess J-acid yielding red colored derivatives. At 500 nm, the molar absorptivity was  $3,92 \cdot 10^4 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ . R.S.D. was 1,8%. The recoveries were in the range of 98,7% – 101,2%.

The new self-coupling diazotizing reagent J acid made the procedure cost-effective and versatile. It was superior to the national standard method for the determination of nitrite which necessarily involved multiple steps and was not environmentally friendly. Moreover, J-acid is also better than H-acid for its higher sensitivity. The proposed method has distinct advantages of simplicity, rapid, sensitivity, selectivity and generated lesser amounts of toxic waste, which could be applied for the determination of trace nitrite in environmental water.

### Acknowledgements

The author would like to thank Chinese Natural Science Foundation (No. 51208299), Science and Technology Commission of Shanghai Municipality

(No. 11JC1408700), "Chen Guang" project supported by Shanghai Municipal Education Commission and Shanghai Education Development Foundation (No. 11CG52) for financial support.

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Recieved 25.03.2013