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STUDY ON NITRITE DETERMINATION BY SPECTROPHOTOMETRIC METHOD AND ITS APPLICATION IN ANALYSIS OF MEKOMG RIVER WATER IN LAOS

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ABSTRACT

Spectrophotometric method using Griess – Ilosvay reagent was established for determination of nitrite in water samples. The standard equation was determined. It is $A = (0,71 \pm 0,03).C_N + (0,02 \pm 0,01)$ with $R^2 = 0.99842$. By this equation the values of LOD, LOQ of this method were calculated, where: LOD = 0.018 mg/L; LOQ = 0.062 mg/L. This method was used to determining nitrite concentration in Mekong river at Vientiane, Laos.

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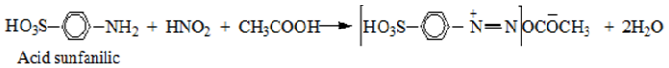
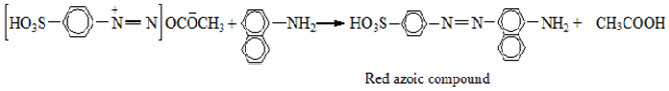
INTRODUCTION

Nitrite is known as a precursor in the formation of N-nitrosamines, many of which have been reported to carcinogenic agents (Lijinsky, 1970). So drinking water contaminated with elevated nitrite levels is known to be harmful to human health due to the role of nitrite in the formation of N-nitrosamines. Nitrite ion also plays a role in "blue baby" syndrome and gastric cancer (Kemper, 2010; Scaher *et al*., 2010; Dutt, 2002).This is particularly harmful for infants due to their limited physical size. On the other hand, the presence of nitrite is always indicating the level of organic pollution in water samples (Gabbay, 1977). The level of nitrite concentration in water depends on the sorts of water sources. In surface water, (NO_2^- - N) concentration is 0.01 mg/L (QCVN 08:2008/BTNMT). From these reasons the study to establish a method determining exact concentration in water is always desirable and needed.

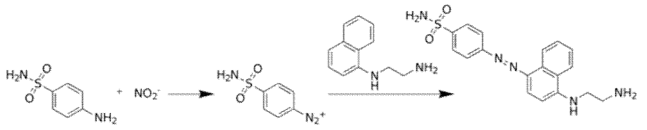
There are many methods for determination of nitrite in water (Abbas Afkhami, 2004; Kazemzadeha, 2001). The main methods determining nitrite consist of UV-Visible, fluorescence, chemiluminescence, and electrochemical techniques (Green *et al*., 1982; Pasquali *et al*., 2010). The most of these methods is based on the Griess reaction as following:





The mechanism shows there is the formation of azo dyes by the Griess reagent. Sulfanilic acid reacts with nitrous acid in the presence of another acid (CH_3COOH) to produce the diazonium salt and then diazo form and water. The diazo form

is coupled with an amine (alpha- naphthylamine) for forming a red azo dye and the acid is regained. Besides, the diazon form resulting then may coupled to N- (1-naphthyl) ethylenediamine forming an azo dye that can be spectrophotometrically quantitated based on its absorbance. The overall reactions are following:



The Mekong is a trans-boundary river in Southeact Asi with the length of 4,350 km and with discharging 475 km^3 of water annually (10). From the Tibetan Plateau the Mekong river runs through China's Yunnan Province, Myanmar, Laos, Thailand, Cambodia, and Vietnam, Fig.1.

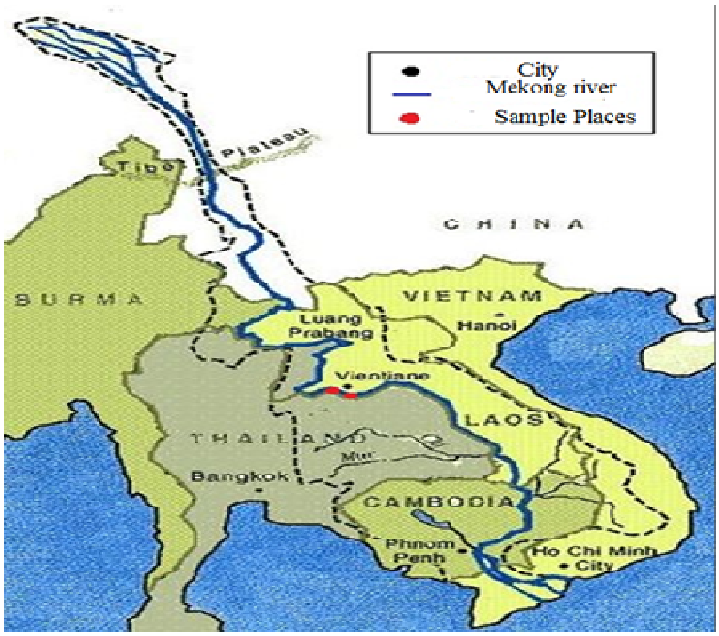


Fig.1. The collected places of water samples in Mekong river Basic data on country share of Mekong Basin territory and water flows are listed in the table 1.

Table 1. Basic data of Mekong Basin (Mekong River Commission, *2005*)

	China	Myanmar	Lao PDR	Thailand	Cambodia	Vietnam	Total
Area in Basin (km^2)	165,000	24,000	202,000	184,000	155,000	65,000	795,000
Catchment as % of MRB	21	3	25	23	20	8	100
Flow as % of MRB	16	2	35	18	18	11	100

Table 2. The chemicals used for experiments

N0.	Chemicals	Sources
1	Sodium nitriet $NaNO_2$	Germany
2	Acid acetic CH_3COOH	Germany
3	Acid boric H_3BO_3	Germany
4	Acid sulfanilic HO_3S-C_6H_4-NH_2	Germany
5	Alpha -naphtylamine $C_{10}H_9N$	Germany
7	Sodium hydroxit NaOH	China
8	Acid sulfuric H_2SO_4	China

The data in the Table 1 showed that the part of Mekong river in Laos is the biggest than other countries. Therefore the study to analyze the nitrite present of Mekong river here manifests definitely most practical significance. In this article the spectrophotometric method based on the reaction of nitrite ions with Griess- Ilosvay reagents would be studied in detail including main factories influencing on the absorbance of azo dye resulting. The values of Limit of Detect (LOD), and Limit of Quantification (LOQ) were determined too. The obtained method was applied to determining nitrite present in Mekong river in Laos territory.

Experimental method

Chemicals

All chemicals used for this experiment were analytical reagent grade and listed in the Table 2. following.

Preparation of solutions

Stock solution of $NaNO_2$ (100 mg NO_2^-/L): Dissolve 0.1493 g of dried sodium nitrite in 500 mL of twice distilled water, then dilute to 1000 mL.

The solution would be used for three onths. From this solution, the working solution of sodium nitrite 1mg (NO_2^- - N/L) would be already prepared.

Griess-Ilosvay reagent

Sulfanilic acid solution (reagent A): Dissolve 0.5 g of dried sulfanilic acid in 150 mL of acetic acide (30 %).

- Alpha naphthylamine solution (reagent B): Dissolve 0.2 g of alpha naphthylamine in 20 mL of distilled water and 150 mL of acetic acid (15 %).
- Mixt reagent A with reagent B with the ratio of (1:1) to obtain Griess- Ilosvay reagent before using.
- Universal buffer solution: Take 33,30 mL of H_3PO_4 0.04 M + 33.30 mL of CH_3COOH 0,04 M + 33,30 mL of H_3BO_3 0.04 M + 3.00 ÷ 6.5 mL of NaOH 1M. The pH of this buffer are from 1.81 to 2.09.
- 0.1 M H_2SO_4 solution: Take 0.02803 mL H_2SO_4 (98%) to mix with 49.7197 mL of twice distilled water.
- 0.04 M acetic acid solution: Dilute 0.0761 mLof CH_3COOH (30 %) in 33,239 mL of twice distilled water.
- 0.04 M H_3BO_3 solution: Dissolve tan 0.82358 g H_3BO_3 in 33.30 mLof twice distilled water.
- 0.2M approximate NaOH solution: Dissolve 0.2 NaOH in 25 mL of twice distilled water.

Apparatus

Spectrophotometer model of UV-Vis Biochrom S60 (2013) (USA) and others instruments available in the Lab.

Experimental procedure

Study on the absorption spectrum of azo dye compound

The azo dye compound formed under the reaction of nitrite ions with Griess- Ilosvay reagent is carried out as following
Take three 50 mL volumetric flasks denoted DD1, DD2 and DD3.

DD1: Take 12 mL of nitrite working solution and 2 mL of universal buffer into a volumetric flask, then adding twice distilled water to reach 50 mL.

DD2: Take 2 mL of Griess- Ilosvay reagent solution and 2 mL of universal buffer flask, then adding twice distilled water to reach 50 mL.

DD3: Take 12 mL of nitrite working solution, 2 mL of Griess-Ilosvay reagent solution and 2 mL of universal buffer flask, then adding twice distilled water to reach 50 mL.

All flasks leave for 24 minutes before measuring absorbance at wavelength, λ from 400 to 600 nm to confirm again the formation of azo dye.

Experiments on factors influencing the formation of azo dye compound

- Influence of pH: Take 12 volumetric flasks (50 mL) involving 15.00 mL of nitrite working solution, 2 mL of of Griess- Ilosvay reagent solution and twice distilled water. The pH of solutions were adjusted by H_2SO_4 0.1M or NaOH 0.2M to reach from 1.75 to 4.50.
- Influence of the ratio (v/v) of reagents of Griess- Ilosvay / NO_2^- :Take 9 volumetric flasks (50 mL) involving 15.00 mL of nitrite working solution, 2 mL of buffer to keep pH = 2.00 ÷ 2.75 and twice distilled water. The volumes of reagent are taken such as 0.5; 1.0; 1.5; 2.0; 2.5; 3.0; 3.50; 4.00; 4.50 mL,
- Influence of time: Take 15,00 mL of nitrite working solution, 2 mL of buffer, 2 mL of Griess-Ilosvay reagent and twice distilled water into 50 mL volumetric flask. Leave the flask for a certain interval of time then measuring the absorbance.

Establishment of standard plot

Take 7 volumetric flasks (50 mL) involving in turn 6.00; 8.00; 10.00; 12.00; 16.00; 20.00; 30.00mL of $NaNO_2$ solution, 2.0 mL of beffer 2.0 mL of of Griss-Ilosvay reagent, and twice distilled water to reach 50 m, wait for 24 minutes then measuring absorbance.

Water samples collected from Mekong river in Laos territory

The spetrophotometric method determining nitrite using Griess –Ilosvay was applied to analyze nitrite in water samples collected from Mekong river in Laos territory. The samples were taken in two periods listed in Table 3. Before analyzing, all water samples were filtered to remove dirty and suspended particles. Take about from 30 to 45 mL of sample, 2 mL of griess- Ilosvay reagent, 2 mL buffer into 50 mL volumetric flask adding twice distilled water to reach mark, waiting for 24 minutes, then measuring repeated three times to have got average value. The nitrite concentration was determined basing standard plot.

Table 3. Water samples from Mekong river

No.	Places	Data	Denoted	Coodinates
1	Pump station Chinaimo, Khoknin, Sixattanark distreet, Vientiane city	Period 1: 18/07/2017 Period 2: 3/08/2017	N_{MK1}	17°54'18.7164'' N 102°37'0.714'' E
2	Pump station Kaolieu Kaolieu, Sikhot distreet, Vientiane city	Period 1: 18/07/2017 Period 2: 3/08/2017	N_{MK2}	17°97'25,18'' N 102°55'23,42''E

RESULTS AND DISCUSSION

The Absorbance spectrum of azo dye compound

The samples DD3 indicated there is an absorbance spectrum of azo dye compound, whils DD1 and DD2 do not give any absorbance. The maximum wavelength of azo dye compound is 526 nm Fig. 2. which is close to the literature (12). The wavelength λ = 526 nm was chosen for determination of nitrite in the samples.

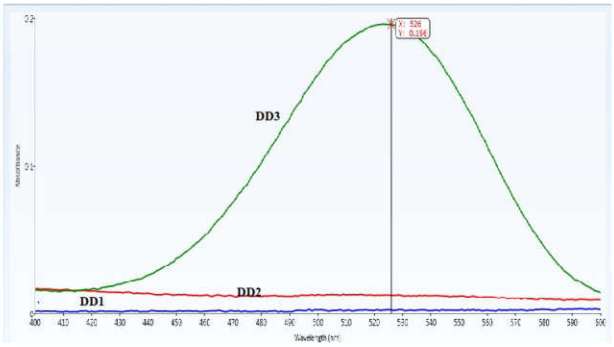


Fig. 2. Absorbance spectrum of azo dye compound

Factors influencing maximum absorbance of azo dye compound

pH – influence

The experimental data of pH –influence on maximum are presented in Fig.3.

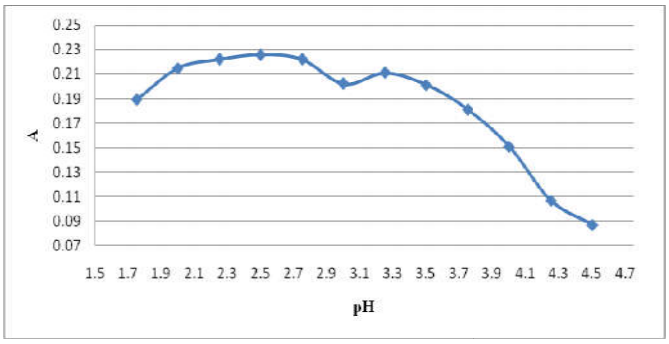


Fig. 3. Plot of pH influence on absorbance of azo dye

From this plot, the pH interval from 2.00 to 2.75 is chosen as optimal value used for experiments.

Influence of ratio of Griess- Ilosvay / NO_2^-

The experimental data of influence of ratio of Griess- Ilosvay / NO_2^- on maximum absorbance are presented in Fig. 4.

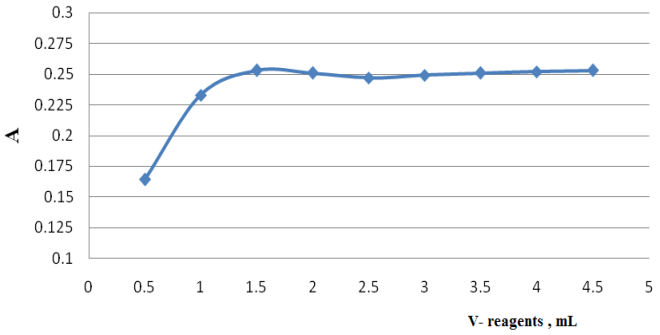


Fig.4. Influence of ratio Griess- Ilosvay / NO_2^- on maximum absorbance

From the obtained plot, the ratio of Griess- Ilosvay / NO_2 equals 2/15 (v/v), which can be used for experiment as optimal value.

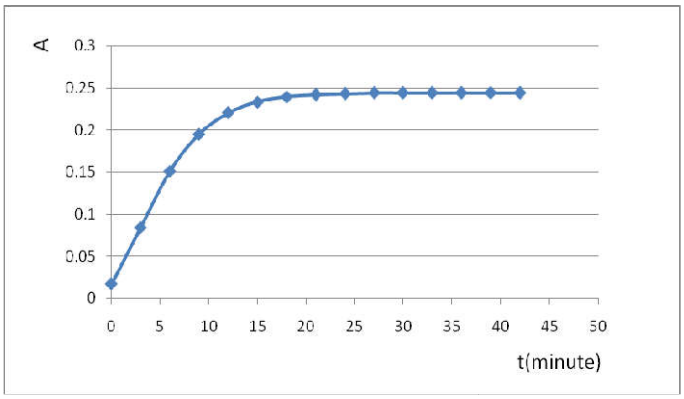


Fig. 5. Influence of the reaction time

Influence of time of azo dye formation

The reaction forming azo dye involves three steps that must need an enough time, so that study on influence of the reaction time is necessary. The experimental data of the influence on the reaction time are presented in Fig. 5.

From these data, reaction time from 24 to 42 minutes may be chosen for experiments.

Standard plot

The nitrite concentrations in the samples were determined using the following standard plot, Fig. 6.

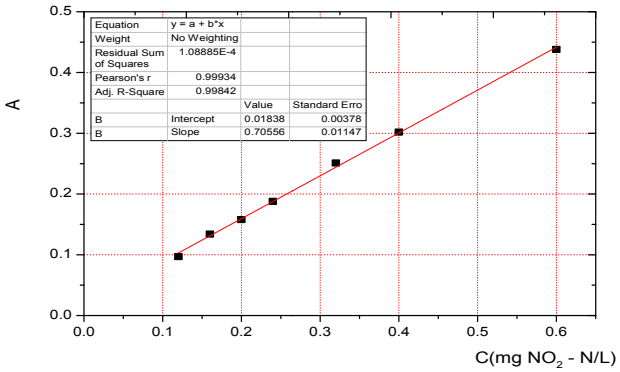


Fig. 6. The standard plot for determination of (NO_2^- – N)

The standard plot is corresponding the equation :

$A = (0,71 \pm 0,03).C_N + (0,02 \pm 0,01)$ with $R^2 = 0.99842$.
By this equation and standard derivation the values of LOD, LOQ were calculated, here:

LOD = 0.018 mg/L; LOQ = 0.062 mg/L

Table 4. Nitrite concentration in Mekong river Vientiane Laos

Sampl	Period	Times measur.	A_i	C_i (mg NO_2-N/L)	$C_N = \overline{C} \pm \varepsilon$ (mg NO_2-N/L)
N_{MK1}	1	1	0.035	0.0211	0.017±0,016
		2	0.030	0.0141	
		3	0.031	0.0155	
	2	1	0.079	0.0831	0.080±0,015
		2	0.074	0.0761	
		3	0.077	0.0803	
N_{MK2}	1	1	0.036	0.0225	0.018±0,018
		2	0.030	0.0141	
		3	0.033	0.0183	
	2	1	0.070	0.0704	0.0718±0,006
		2	0.071	0.0718	
		3	0.072	0.0732	

Application in determination of nitrite in Mekong River in Vientiane – Laos

The nitrite concentrations in the water samples collected from Mekong river presented in Table 4.

The obtained results showed that the nitrite concentrations in Mekong river water are about from 0.017 ÷ 0.08 mg/L (NO_2^- N), higher than Vietnam standard (QCVN 08:2008/BTNMT). The Mekong river water in Vientiane Laos is polluted by nitrite, it must be treated before using for domestic purpose.

Conclusion

Nitrite is known as a precursor in the formation of N-nitrosamines reported as carcinogenic agent. It must be analyzed and removed from water before use. The analysis of nitrite using Griess − Ilosvay was studied in detail including experimental condition of Azo dye formation, factors influencing its absorbance and finally a standard plot with the equation of $A = (0.70556 \pm 0.01147).C_N + (0.01838 \pm 0.00378)$ with $R^2 = 0.99842$ established. This method was

applied for analyzing nitrite concentration in Mekong river water in Vientiane city, Laos. The obtained nitrite in studied samples is higher than Vietnam standard (QCVN 08:2008/ BTNMT).

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