DETERMINATION OF NITRATE AND NITRITE SPECTROPHOTOMETRICALLY BY NITRITE AZOTATION AND USING HYDRAZINE AS REDUCING AGENT

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ABSTRACT

Nitrate and nitrite determination by spectrophotometry through nitrite azotisation reaction was evaluated. Nitrate was reduced into nitrite by hydrazine sulfate in basic solution using $CuSO_4$ as catalyst. Nitrite was then reacted with sulfanilic acid and N-1-naphthyl amine in acid solution forming colored solution. The best reduction conditions were obtained at hydrazine concentration of 0.03 ml/L, $CuSO_4.5H_2O$ concentration of 0.03 g/L and NaOH concentration of 0.5 mol/L, heated at 45 °C for 60 second. Azotation reaction was optimum at sulfanilic concentration of 0.3 %, HCl concentration of 6 %, naphthyl amine concentration of 0.3 %. Maximum absorbance was obtained at 520 nm and color was stabile for 3 hours. Relative standard deviations for six replications were 1.68 % for nitrate and 1.36 % for nitrite. Detection limits of the method were 0.015 mg/L for $N\text{-}NO_3$ and 0.0067 mg/L for $N\text{-}NO_2$. Recoveries obtained by spike samples were 101.2 % for nitrate and 105.7 % for nitrite.

INTRODUCTION

Nitrate is the most abundant form of inorganic nitrogen. It is formed during nitrification step. Nitrite is well known as intermediate product in the nitrogen cycle, which is produced during the biodegradation of organic compound containing nitrogen. Nitrite is strong carcinogen, however it is used as food preservative. Both nitrate and nitrite are studied parameter for air pollution, acid rain and eutrophication of lakes and marshes in environmental studies[1,2]. Therefore, the determination of nitrate and nitrite is very important in various fields such as environmental studies, biology, food and clinical chemistry.

Various methods have been developed for nitrate and nitrite determination. General method for nitrate determination is direct UV spectrophotometry at 225 nm. UV absorption by other organic component is corrected by absorbance measurement at 275 nm[1,3]. Tanaka *et al.*[4] developed a method for nitrate determination in vegetable product, based on reaction with 2-sec-buthyl phenol in sulfuric acid and extracted to toluene. Nitrate is subsequently extracted to alkaline solution to form a yellowish color (sodium salt of nitro phenol) and then measured spectrophotometrically. Osibanjo and Ajayi[5] developed nitrate determination by spectrophotometry using 3,4-xylenol as reagent. Nitration was conducted at 0 °C in 80 % sulfuric acid and then extracted into toluene. Toluene layer was treated by NaOH for color formation. Kawagami and Igarashi[2] reported spectrophotometric determination of nitrite using porphyrin. Water-soluble porphyrin having the aminophenyl group, 5,10,20-tetrakis(4-aminophenyl) porphine, react with nitrite ion, and produced a large spectral changed ($\Delta\varepsilon = 3.0 \times 10^5$ L mol^{-1} cm^{-1} at 434 nm).

Anderson[6] developed simultaneous determination of nitrate and nitrite. Nitrate was reduced to nitrite by cadmium coated on copper and nitrite formed was reacted with sulfanilic acid and N-(1-naphthyl ethylene diammine). Measurement was conducted spectrophotometrically by flow injection analysis. Nitrite concentration was obtained without reduction and nitrate concentration was calculated by difference. Masden[7] reported the reduction of nitrate to nitrite or ammonium. Aluminum, chromium and titanium reduced nitrate to ammonium, while zinc, cadmium and hydrazine sulfate reduced nitrate to nitrite. Nitrite was then diazotised for spectrophotometric determination of nitrate. Determination of nitrate through photo-induced reduction to nitrite was reported by Motomizo

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and Sanada[5]. The method utilized an activator, made of low resistant mercury lamp, to reduce nitrate to nitrite. Nitrate solution was injected into buffer carrier and pass through PTFE tubing coiled around mercury lamp, and then followed by diazotation coupling reaction.

Characterization and reduction of interferences in flow injection Analisis for *in situ* determination of nitrate and nitrite was reported by Daniel *et al.*[9]. Dual wavelength spectrophotometry was performed to eliminate the refractive index provoked by heterogeneous flow in FIA and errors induced by temperature, salinity and pressure variation.

Sequential injection analysis of nitrite and nitrate was reported by Onis *et al.*[10]. Nitrite determination was based on Griess-Illosvay reaction, while nitrate was previously reduces by hydrazine in basic medium and determined as nitrite.

Nikonorov and Moskin[11] utilized 4-iodo-N,N-dimethyl aniline for spectrophotometric determination of aniline. The reaction is based on nitrozation of p-halogen derivative of N-N-dimethyl aniline forming colored compound. Tarafder and Rathore[12] reported another method for determination of nitrite in water. Nitrite reacted with p-aminophenylmercapto acetate in the present of hydrochloric acid to form diazonium cation and then followed by N-1(naphthyl)ethylene diamine dihydrochloride in acid medium to form pink azo compound.

In this work, colored azo compound was formed by reaction of nitrite with sulfanilic acid and N-1(naphthyl)amine for nitrite determination. Total nitrate and nitrite was determined by reducing nitrate to nitrite using hydrazine sulfate and nitrate was calculated by difference.

EXPERIMENTAL

Material and Equipment

All chemical used (KNO_3, $NaNO_2$, N-1(naphthyl)amine, sulfanilic acid, hydrazine sulfate) are high purity grade obtained from Merck. KNO_3 and NaNO2 were used for preparation of nitrate and nitrite solutions. Spectronic 20D Spectrophotometer (Milton Roy) was used for absorbance measurement.

Color Development

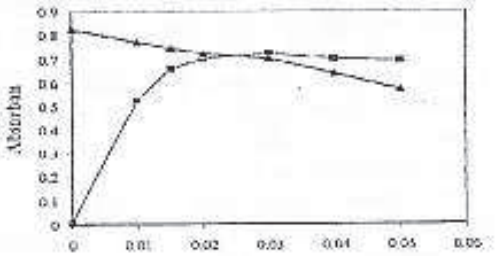
10.0 mL of nitrate solution (standard or sample) is pipetted into volumetric 20 mL volumetric flask. Then, 1.0 mL of 0,03 ml/L hydrazine sulfate, 1,0 ml of 0.03 g/L $CuSO.5H_2O$ and 1.0 ml of 0.5 mol/L NaOH were added into the flask. The flask was heated in water bath at temperature of 45 °C for 60 seconds. 1.0 ml of 0.3 % sulfanilic acid and 1.0 ml of 0.3 % N-1(naphthyl) amine were added, mix and diluted to the mark. Absorbance of the colored solution was then measured at 520 nm after 15 minutes. The concentrations of the above reagents were obtained after optimization by varying concentration of each reagent.

Application to Natural Sample

The method obtained was applied for determination of nitrate and nitrite concentration in natural water samples. Recovery of the method was studied by spiking natural samples with nitrate and nitrite standard solution to concentration of 0.02 mg/L.

RESULT AND DISCUSSION

Spectrum scan of azo colored solution was performed between 400 to 600 nm. Absorption maximum appeared at 520 nm. Effect of hydrazine sulfate concentration as reducing agent was studied for by varying the concentration from 0 to 0.05 mol/L. The study was conducted for both nitrate and nitrite. The results were showed in Figure 1.



Hydrazine sulfate concentration (mol/L)

Figure 1. Effect of hydrazine sulfate concentration on absorbance signal of nitrate (■) and nitrite (▲). Nitrate and nitrite concentration was 0.10 mg/L, $CuSO_4$ concentration was 0.03 g/L. NaOH concentration was 0.50 mol/L. Sulfanilic acid concentration was 0.6 %. Naphthyl amine concentration was 0.6 %. Temperature was 35 °C and reducing time was 50 s.

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Figure 1 shows that absorption signal increase by increasing hydrazine concentration and level off beyond 0.03 mol/L. At higher concentration, excess hydrazine sulfate might reduce nitrite further to nitrogen and reduced absorption signal. It can also be seen that absorption signal of nitrite alone in the presence of hydrazine sulfate was slightly reduced. Hydrazine sulfate concentration of 0.03 mol/L was chosen as optimum concentration.

Effect of $CuSO_4$ concentration, used as catalyst during reduction process, was studied by varying from 0.01 – 0.05 g $CuSO_4$. 5 H_2O/L. The result is shown in Figure 2.

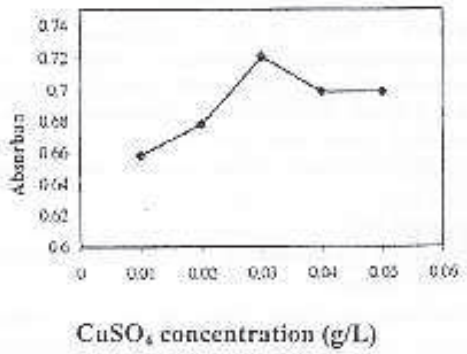

Figure 2. Effect of $CuSO_4$ concentration in catalyzing nitrate reduction by hydrazine sulfate. Nitrate concentration was 0.10 mg/L, hydrazine sulfate concentration was 0.03 mol/L, NaOH concentration was 0.5 mol/L, sulfanilic acid concentration was 0.6 % naphthyl amine concentration was 0.6 %, temperature was 35 °C, and reducing time was 50 s.

Figure 2 shows that $CuSO_4$ concentration of 0.03 g/L gave highest signal and was chosen as optimum concentration in the method. At higher concentration, $CuSO_4$ formed precipitate with NaOH, since reduction took place in basic medium, and further affected azo color formation.

NaOH was used to adjust basic condition of reduction process. The effect of NaOH concentration was studied by varying the concentration from 0 to 0.7 mol/L. The result is shown in Figure 3. Absorption increased as NaOH concentration increase up to 0.5 mol/L. Absorption signal decreased at higher NaOH concentration because NaOH would affect the next azotation reaction that took place in acidic medium. NaOH concentration of 0.5 mol/L was selected as optimum concentration.

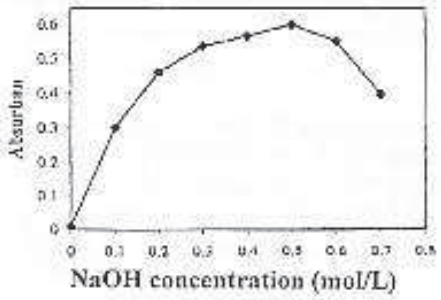

Figure 3. Effect of NaOH concentration on absorption signal. Nitrate concentration was 0.10 mg/L, $CuSO_4$ concentration was 0.03 g/L, hydrazine sulfate concentration was 0.03 mol/L, sulfanilic acid concentration was 0.6 %, naphthyl amine concentration was 0.6 %, temperature was 35 °C and reduction time was 50 s.

Effect of temperature of reduction process on absorption signal was studied by varying temperature from room temperature (25 °C) to 60 °C either for nitrate or nitrite. The results are shown in Figure 4.

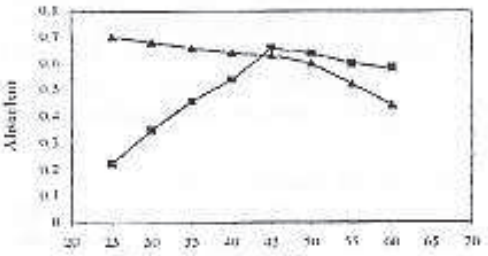

Figure 4. Effect of temperature on absorption signal of nitrate (■) and nitrite (▲). Nitrate and nitrite concentration was 0.10 mg/L., hydrazine sulfate concentration was 0.03 mol/L, $CuSO_4$ concentration was 0.03 g/L. NaOH concentration was 0.5 mol/L, sulfanilic acid concentration was 0.6 %, naphthyl amine concentration was 0.6 %, and reduction time was 50 s.

Absorption signal of nitrate increased by increasing temperature from 25 to 45 °C. At higher temperature, the absorption signal decreased, since nitrite was reduced to nitrogen at higher temperature. Nitrite signal however was slightly reduced at temperature between 25 °C to 45 °C. Temperature of 45 °C was selected for optimum reduction process.

Effect of soaking time was studied by varying from 0 to 90 s. The result is shown in Figure 5.

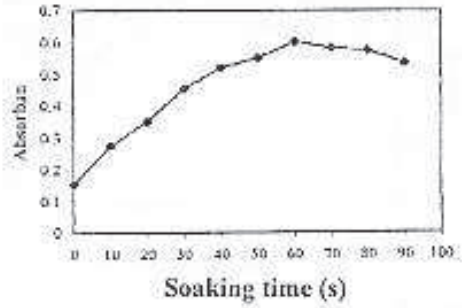

Figure 5. Effect of soaking time during reduction process on absorption signal. (▲). Nitrate concentration was 0.10 mg/L., hydrazine sulfate concentration was 0.03 mol/L, $CuSO_4$ concentration was 0.03 g/L. NaOH concentration was 0.5 mol/L, sulfanilic acid concentration was 0.6 %, naphthyl amine concentration was 0.6 %, and reduction temperature was 45 °C.

It can be seen from Figure 5 that optimum signal was obtained at 60 s. Soaking time longer that 60 s might reduce nitrite further to nitrogen so that absorption signal decreased.

Effect of sulfanilic acid concentration in formation of colored azo compound was studied by varying the concentration from 0.1 to 0.6 %. The result is shown in Figure 6. Figure 6 shows that optimum concentration of sulfanilic acid was 0.3 %.

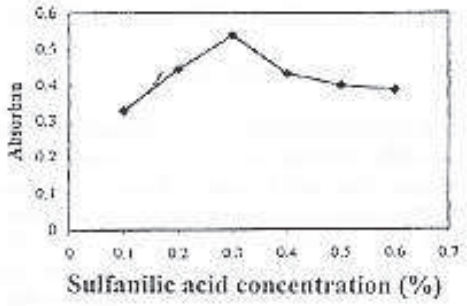

Figure 6. Effect of sulfanilic acid on absorption signal. Nitrate concentration was 0.10 mg/L., hydrazine sulfate concentration was 0.03 mol/L, $CuSO_4$ concentration was 0.03 g/L. HCl concentration was 6 %, naphthyl amine concentration was 0.6 %, reduction temperature was 45 °C and reduction temperature was 45 °C.

The effect of HCl concentration during color formation process was studied by varying HCl concentration from 2 to 12 % (v/v). The result is shown in Figure 7. It can be seen from Figure 7 that optimum concentration of HCl was 6 % v/v.

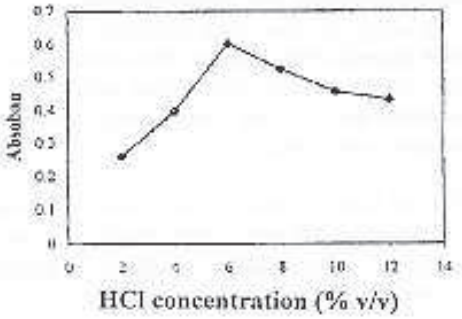

Figure 7. Effect of HCl concentration during color formation on absorption signal. Hydrazine sulfate concentration was 0.03 mol/L, $CuSO_4$ concentration was 0.03 g/L. sulfanilic acid concentration was 0.3 %, naphthyl amine concentration was 0.6 %, reduction temperature was 45 °C and reduction temperature was 45 °C

Effect of N-1-naphthyl amine concentration on color formation was studied by varying the concentration from 0 to 0.6 %. The result is shown in Figure 8. Optimum concentration of N-1-naphthyl amine was found at 0.3 %.

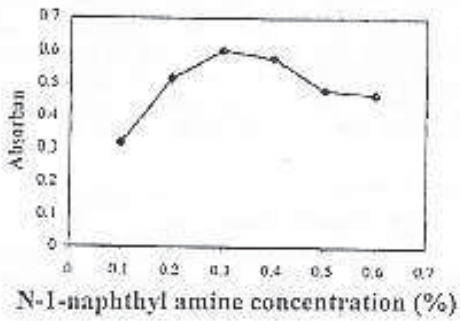

Figure 8. Effect of N-1-naphthyl amine concentration on absorption signal. Hydrazine sulfate concentration was 0.03 mol/L, $CuSO_4$ concentration was 0.03 g/L. HCl concentration was 6 %, sulfanilic acid concentration was 0.3 %, reduction temperature was 45 °C and reduction temperature was 45 °C

The result of color stability study for both nitrate and nitrite are shown in Figure 9. It can be seen from Figure 9 that the azo color was stable for 3 hours.

At optimum conditions, calibration curves were linear up to concentration of 0.06 mg/L, with r^2

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of 0.997 for nitrate and 0.005 for nitrite. Detection limits of the method evaluated from calibration curve for 3σ were 0.07 mg/L for nitrate and 0.2 mg/L for nitrite.

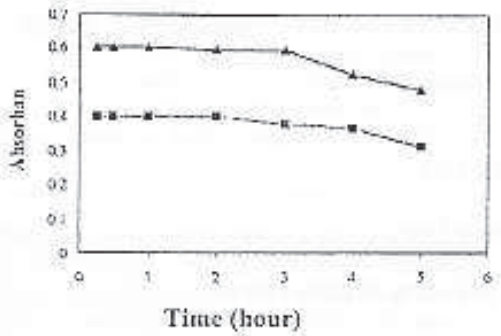

Figure 9. Variation of absorption signal of nitrate (■) and nitrite (▲) versus time. All parameters were the same as previously mentioned.

The developed method was utilized for determination of nitrate and nitrite in pool water for fishery. The result found was 0.021 mg/L for nitrate and 0.0082 mg/L for nitrite. Relative standard deviations for six replications of determination were 1.68 % for nitrate and 1.36 % for nitrite. Recovery of the method was studied by spiking water sample with 0.02 mg/L standard nitrate and nitrite. The results obtained are 101.2 % for nitrate and 105.7 % for nitrite.

CONCLUSION

Nitrate and nitrite concentration can be determined spectrophotometrically through nitrite azotisation reaction and using hydrazine as reducing agent. The best reduction conditions were obtained at hydrazine concentration of 0.03 ml/L, $CuSO_4.5H_2O$ concentration of 0.03 g/L and NaOH concentration of 0.5 mol/L, hented at 45 °C for 60 second. Azotation reaction was optimum at sulfanilic concentration of 0.3 %, HCl concentration of 6 %, naphthyl amine concentration of 0.3 %. Maximum absorbance was obtained at 520 nm and color was stabile for 3 hours. Relative standard deviations for six replications were 1.68 % for nitrate and 1.36 % for nitrite. Detection limits of the method were 0.015 mg/L for $N\text{-}NO_3$ and 0.0067 mg/L for $N\text{-}NO_2$. Recoveries obtained by spike samples were 101.2 % for nitrate and 105.7 % for nitrite.

REFERENCES

1. M. Karlsson, B. Karlberg and R.J.O. Orlson, Determination of nitrate in municipal water by UV spectroscopy, *Anal. Chim. Acta*, 1996, **312**, 107-113.
2. T. Kawakami and S. Igarashi, Highly sensitive spectrophotometric determination of nitrite ion using 5,10,15,20-tetrakis(4-aminophenyl)porphine for application to natural waters, *Anal. Chim. Acta*, 1996, **333**, 175-180.
3. A.E. Greenberg, L.S. Clessery and A.D. Eaton (Editors), *Standard Methods for the Examination of Water and Wastewater*, 18th Ed., American Public Health Association, Washington DC 1992.
4. A. Tanaka, N. Nose and H. Iwasaki, Spectrophotometric determination of nitrate in vegetable products using 2-sec-buthylphenol, *Anal. Chim. Acta*, 1982,**107**,190-194.
5. O. Osibanjo and S.O. Ajayi, Rapid sensitive spectrophotometric method for the determination of nitrate in rain water using 3,4, xylenol, *Analyst*, 1980, **102**, 908-911.
6. L. Anderson, Simultaneous spectrophotometric determination of nitrite and nitrate by flow injection analysis, *Anal. Chim. Acta*, 1979, **110**, 123-128.
7. B.C. Madsen, Utilization of flow injection analysis with hydrazine reduction and photometric detection for determination of nitrate in rain water, *Anal. Chim. Acta*, 1981, **124**, 437-441.
8. S. Motomizo and M. Sanada, Photo-induced reduction of nitrate to nitrite and its application to the sensitive determination of nitrite in natural water, *Anal. Chim. Acta*, 1995, **308**, 406-412.
9. A. Daniel, D. Birot, M. Lehaire and J. Poncin, Characterization and reduction interferences in flow injection analysis for *in situ* determination of nitrate and nitrite in sea water, *Anal. Chim. Acta*, 1995, **308**, 413-424.
10. M.T. Oms, A. Carda and V. Carda, Sequential injection analysis of nitrite and nitrate, *Anal. Chim. Acta*, 1995, 308-312.
11. V.V. Nikonorov and L.N. Moskin, Spectrophotometric determination of nitrite with 4-iodo-N,N-dimethyl aniline, *Anal. Chim. Acta*, 1995, **306**, 357-360.
12. P.K. Tarafder and D.P.S. Rathore, Spectrophotometric determination of nitrite in water, *Analyst*, 1988, **113**, 1073-1076