Chem. Anal. (Warsaw), **48**, 759 (2003)

Spectrophotometric Determination of Trace Amounts of Nitrites in Water and Soil Samples

by H.D. Revanasiddappa* and T.N. Kiran Kumar

Department of Chemistry, University of Mysore,
Manasagangothri Mysore 570 006, India

Key words: spectrophotometry, nitrite determination, *p*-nitroaniline, citrazinic acid

A simple and accurate spectrophotometric method for the determination of trace amounts of nitrites in water and soil has been described. The method is based on the diazo-coupling reaction between *p*-nitroaniline and citrazinic acid. Under aqueous alkaline conditions the azo dye is formed, which absorption reaches maximum at 530 nm and molar extinction coefficient equals 2.8×10^4 L mol^{-1} cm^{-1}. The Beer's law is obeyed over the concentration range of nitrites of 0.5–14 µg in the final volume of 10 mL. The influence of ionic interferences on the determination of nitrite has been also described. The proposed method has been successfully applied to the determination of nitrite in the real water and soil samples. The results were compared to those obtained with the standard method involving sulfanilamide-N-(1-naphthyl) ethylenediamine dihydrochloride. Statistical analysis of the results confirms the higher precision and accuracy of the proposed method.

Opisano prostą i dokładną, spektrofotometryczną metodę oznaczania śladowych ilości azotynów w wodzie i glebie; w metodzie wykorzystano reakcję sprzęgania diazowego między *p*-nitroaniliną i kwasem cytrazonowym. W wodnym, alkalicznym roztworze powstaje barwnik azowy wykazujący maksimum absorpcji przy dł. fali 530 nm i molowy współczynnik ekstynkcji równy $2{,}8 \times 10^4$ L mol^{-1} cm^{-1}. Prawo Beera jest spełnione w zakresie stężeń azotynów od 0,5 do 14 µL na 10 mL. Zbadano również wpływ obcych jonów na oznaczanie azotynów. Opracowaną metodę zastosowano z powodzeniem do oznaczania azotynów w rzeczywistych próbkach wody i gleby. Otrzymane wyniki porównano z wynikami uzyskanymi standardową metodą stosującą sulfaniloamido-N-(1-naftylo)etylenodiaminodihydrochlorek. Statystyczna ocena wyników potwierdza wyższą precyzję i dokładność proponowanej metody.

---

* Corresponding author. E-mail: hdrevanasiddappa@ yahoo.com

Since nitrites participate in the formation of N-nitrosoamines, they are considered as potential carcinogens and their level in organic pollution of water must be monitored [1], which requires a sensitive, fast and handy analytical procedure for environmental, agricultural and ecological needs.

So far many spectrophotometric methods involving various reagents [2–6] have been reported, among which the one utilising the modified Griess reaction has been adopted as standard for the determination of nitrites in natural waters [2]. Very recently, Moorcroft *et. al.* [7] has proposed new strategies towards detection, determination and monitoring of nitrates and/or nitrites in a variety of matrices. However, these methods quite often require a precise control of pH and temperature conditions during the diazotisation, as well as the coupling step usually lasts relatively long. In this paper the influence of a new coupling agent citrazinic acid (2,6-dihydroxyisonicotinic acid) for nitrite determination in water and soil samples has been studied.

EXPERIMENTAL

Apparatus

A Jasco, Model UVIDEC–610 and Elico, Model CL–27 spectrophotometers with 1 cm quartz cells were used for all the absorbance measurements.

Reagents

All chemicals used were of analytical grade and distilled water was used throughout.

The standard nitrite solution (1 mg mL^{-1}) was prepared by dissolving 0.150 g of the dried sodium nitrite in 100 mL of water. A pellet of sodium hydroxide and a small amount of chloroform were added to prevent the liberation of nitrous acid and to inhibit the bacterial growth, respectively. Working solutions were prepared by the appropriate dilution of the standard.

0.05% *p*-nitroaniline solution in 2.5 mol L^{-1} HCl was prepared by dissolving 0.1250 g of *p*-nitroaniline in 55.3 mL HCl, and diluting up to 250 mL with water.

0.1% citrazinic acid solution was freshly prepared by dissolving 0.1 g of citrazinic acid in 2 mL of 4 mol L^{-1} NaOH and diluting up to 100 mL with water.

Other solutions used were as follows: sodium hydroxide solution (4 and 1 mol L^{-1}), EDTA (0.2 mol L^{-1}) and sodium carbonate (1%).

Procedures

General procedure for the determination of nitrites. The appropriate volumes of the sample solution containing from 0 to 14 μg of nitrite were transferred into a series of 10 mL calibrated flasks. To each of them 0.5 mL of 0.05% *p*-nitroaniline was added and the solutions were shaken thoroughly for 2 min to complete diazotisation. After that 2 mL of 0.1% citrazinic acid and 3 mL of 4 mol L^{-1} NaOH solutions were added and the contents were diluted to the mark with distilled water and mixed well again. After 5 min, the absorbance of the coloured azo-dye was measured at 530 nm against the blank.

Determination of nitrites in water samples. An aliquot (≤ 3 mL) of the sample containing maximally 14 μg of nitrites was treated with 0.5 mL of 1 mol L^{-1} NaOH and 0.5 mL of 0.2 mol L^{-1} EDTA. The solution was mixed and centrifuged to remove any formed precipitate. The centrifugate was transferred to a 10 mL calibrated flask, and analysed for the nitrite content according to the general procedure described above.

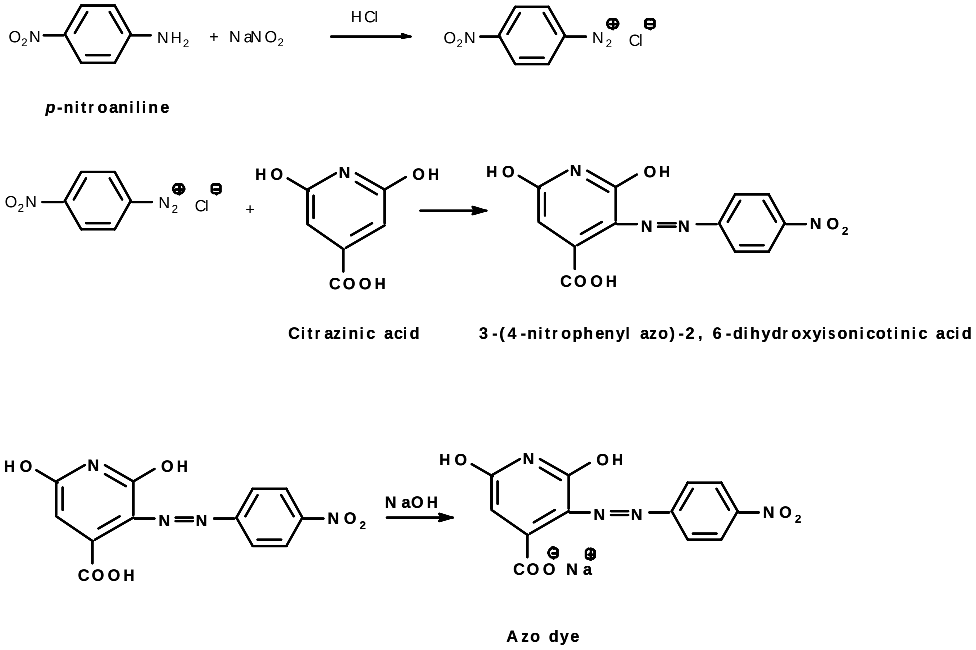
Determination of nitrites in soil samples. The known mass of the soil sample was transferred into 50 mL beaker and triply extracted with 5 mL of 1% Na_2CO_3. The extract was filtered and the filtrate was diluted with distilled water up to 25 mL. A volume of ≤ 3 mL containing not more than 14 mg of nitrites was taken for the determination according to the procedure for the water samples.

RESULTS AND DISCUSSION

Preliminary studies were carried out with 10 μg of standard solutions in the final volume of 10 mL. The absorption spectra of the coloured azo-dye exhibit maximum absorption at 530 nm. Under the recommended conditions the formed dye was stable for 2 h. The diazotisation was carried out at the room temperature (*ca* 25±5°C), and cooling to 0–5°C was not necessary. The effect of acidity on the diazotization reaction was studied in the range of 0.1–0.5 mol L^{-1} hydrochloric acid, and constant absorbance was observed in this range. At higher concentration of HCl, a decrease of the absorbance was observed. The optimum acidity for diazotisation and the minimum time required were 0.125 mol L^{-1} and 2 min, respectively. The use of 0.5 mL of 0.05% *p*-nitroaniline, 2 mL of 0.1% citrazinic acid and 3 mL of 4 mol L^{-1}NaOH solutions provided a constant and maximum absorption.

The reaction scheme

There are two reaction steps, which precede the formation of the coloured azo-dye. In the first one nitrites undergoes diazotisation with amino group of *p*-nitroaniline in the presence of hydrochloric acid to form a diazonium chloride ion. In the second step, the formed diazonium ion couples with citrazinic acid to form an azo-dye in the alkaline medium. The proposed reaction pathway is presented in Scheme 1.



Scheme 1. The reaction pathway of diazo-coupling process

Analytical data

Calibration graph exhibited linearity (r = 0.999) for the nitrite amount ranging from 0.5 to 14 μg in the final volume of 10 mL. The detection limit (DL = 3.3 σ/S) and quantitation limit (QL = 10 σ/S) [where 'σ' is the standard deviation of the blank, n = 10 and 'S' is the slope of the calibration curve] of the determination were 0.03 and 0.08 μg mL^{-1}, respectively. Molar extinction coefficient and Sandell's sensitivity were found as 2.8×10^4 L mol^{-1} cm^{-1} and 0.00164 μg cm^{-2}, respectively. The precision and accuracy of the method were established by the analysis of the standard solutions containing 4, 6 and 10 μg of nitrites in the final volume of 10 mL using the recommended procedure. Ten repetitive determinations of each concentration gave relative standard deviations (RSD) of 2.2, 1.2 and 0.7% respectively, and the values of Student's t-test were ≤ 0.3 (the tabulated t – value at the 95% confidence level and for n = 5, is 2.78), which confirms the reliability of the proposed method.

The influence of interfering ions

Since nitrites were determined in the polluted water and soil samples, one should study the possibility of interferences originating from the common foreign ions.

Despite the tendency of some metal ions to form hydroxides in alkaline medium, they were masked with EDTA. The following tolerance limits (µg mL^{-1}) for the foreign ions that affected the measured absorbance values at most by ±2% during the determination of the fixed amount of nitrites (1 µg mL^{-1}) were found 3000 for acetate, iodate, citrate, fluoride, carbonate, sulfite, chloride, nitrate and sulfate; 2000 for phosphate and tartrate; 500 for barium, magnesium and aluminium; 100 for nickel(II), mercury(II) and chromium(VI); less than 20 for copper(II), cobalt(II), calcium(II) iron(II) and iron(III); and 80 for sulfide.

Applications

The proposed method was applied to the determination of nitrites in the real water and soil samples. Freshly collected water samples from various sources were filtered through the Whatman filtering paper No. 41, stored at 4°C to retard bacterial growth, and analysed within 24 h.

Since the concentration of common pollutants in natural waters is usually far below the tolerance levels for interfering agents, the method was applied directly to the determination of nitrites. Similarly, the manured garden soil sample was analysed. In Table 1 the results for the river and lake water, as well as for soil shows the analytical results for river, lake waters, and soil samples obtained with the recommended procedure and the standard sulfanilamide–N-(1-naphthyl) ethylenediamine dihydrochloride method [2] are presented. The reliability of the procedure was confirmed by standard additions. Having determined the nitrite content with the direct and reference methods, the appropriate amounts of the standard nitrite solution were added to the fresh water and soil samples of same water and soil, and determination of nitrites was repeated. The results are presented in Table 1.

Table 1. The results of the determination of nitrites in natural water and soil samples

Sample	Amount taken mL	Nitrite added µg mL^{-1}	Proposed method			Standard method [2]		
			Nitrite found[1] µg mL^{-1}	Recovery %	RSD %	Nitrite found[1] µg mL^{-1}	Recovery %	RSD %
River water	1.5	–	0.12 ± 0.003	–	2.5	0.13 ± 0.004	–	3.0
	2.0	–	0.11 ± 0.002	–	1.8	0.12 ± 0.003	–	2.5
	3.0	–	0.13 ± 0.001	–	0.8	0.10 ± 0.002	–	2.0
		Average =	0.120	–	–	0.117	–	
	2.0	0.40	0.518 ± 0.004	99.6	0.8	0.516 ± 0.005	99.8	1.0

(continuation on the next page)

Table 1 (continuation)

Lake water	1.5	0.40	0.24 ± 0.007	0.40	2.9	0.24 ± 0.006	0.40	2.5
	2.0	0.40	0.22 ± 0.005	0.40	2.3	0.23 ± 0.005	0.40	2.2
	2.5	0.40	0.22 ± 0.004	0.40	1.8	0.22 ± 0.003	0.40	1.4
		Average =	0.226	–	–	0.230	–	
	2.0	0.40	0.625 ± 0.006	99.8	1.0	0.628 ± 0.005	99.7	0.8
Soil (*g*)	1.00	–	2.75 ± 0.04[2]	–	1.4	2.75 ± 0.03[2]	–	1.1
	1.25	–	2.74 ± 0.03	–	1.1	2.76 ± 0.02	–	0.7
	1.50	–	2.76 ± 0.02	–	0.7	2.77 ± 0.01	–	0.4
		Average =	2.75			2.76		
	1.5	3.0 μg g^{-1}	5.74 ± 0.03	99.6	0.5	5.74 ± 0.02	99.2	0.3

[1] Mean value ± standard deviation (n = 5).

[2] μg g^{-1}.

CONCLUSIONS

For the first time one used citrazinic acid as the new coupling agent for the determination of trace nitrites in water and soil samples. The proposed method is simple, fast, accurate, selective, and does not require any complex conditions. High tolerance limit for a large number of foreign ions and elimination of the extraction steps are especially advantageous. Low relative standard deviations and t-values confirm the precision and high accuracy of the method, which can be considered as alternative towards other methods for the determination of nitrites.

Acknowledgements

T.N.Kiran Kumar thanks the University Grants Commission, New Delhi and Department of Collegiate Education, Government of Karnataka for provision of a Teacher Fellowship.

REFERENCES

1. Gabbay J., Almog Y., Davidson M. and Donagi A.E., *Analyst*, **102**, 371 (1977).
2. *Standard Methods for the Examination of Water and Wastewater,* 19th edn., American Public Health Association, Washington, DC 1995, pp. 4–83, 4–84.

3. Kaveeshwar R., Cherian L. and Gupta V.K., *Analyst,* **116**, 667 (1991).
4. Shanthi K. and Balasubramanian N., *J. AOAC. Int'l.*, **77**, 1639 (1994).
5. Satake M. and Wang G.F., *Fresenius' J. Anal. Chem.,* **357**, 433 (1997).
6. Kawakavi T. and Igarashi S., *Anal. Chim. Acta*, **333,** 175 (1996).
7. Moorcroft M.J., Davis J. and Compton R.G., Talanta, **54**, 785 (2001).

Received August 2002
Accepted January 2003