

Micro Technique for Determination of Nitrite in Water

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A new micro technique for determination of nitrite in water has been standardized. Potassium ferrocyanide (1.2 mg mL^{-1}) forms a pale-green colour with nitrite (0.2 to 1.0 ppm) in presence of dilute HCl (12%). The colour reaches to peak intensity at 90 min and is stable for at least up to 150 min following acidification. The colour is monitored at 430 nm, is linear over the test range with regression estimate in units of absorbance value per μg total nitrite, $b \pm \text{S.E.}$ as 0.091 ± 0.002 ($n = 30$). Metallic ions (Zn, Cu, Mn, Cd, Fe) that interfere with the test are removed from the water sample with treatment of NaOH and the residual hydroxide ions removed by addition of MgCl_2 solution. The extraction does not significantly affect nitrite recovery ($P > 0.1$). Nitrate ions do not interfere. The reaction is unlikely due to oxidation of ferrocyanide to ferricyanide by nitrous acid. The technique can be used as an alternative to the diazotization technique with advantage of requiring cost-effective reagents.

Key Words: Potassium ferrocyanide, Nitrite, Water, Micro technique.

INTRODUCTION

Nitrite is an ubiquitous environmental pollutant, an established toxicant and a therapeutic agent. For safety, its levels in drinking water should not exceed 3 ppm for human¹ and 30 ppm for livestock and poultry². The only spectrophotometric technique recommended for its determination in water is a diazotization procedure, that requires^{3, 4} 50 mL or 90 mL water sample, and a costlier coupling agent, N-(1-naphthyl) ethylenediamine dihydrochloride (NEDA). Its application is also limited in presence of some metallic ions in water³.

The present investigation is an example of serendipity. While checking purity of freshly synthesized cupric nitrite, addition of potassium ferrocyanide solution in acidic medium produced an intense pale-green colouration. The colour was distinct from reddish brown colouration or precipitation expected from copper ions. Further studies revealed that the colour was due to interaction of nitrous acid and potassium ferrocyanide. Therefore, investigations were designed to standardize the technique for determination of nitrite in water.

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EXPERIMENTAL

The chemicals used were of high purity. The experiments were carried out at $24.7 \pm 0.4^\circ\text{C}$. Double distilled water was used wherever required. The experiments were executed in the following order:

Experiment 1. Aliquots of 0.1, 0.2, 0.3, 0.4 and 0.5 mL of 10 ppm nitrite solution in water (diluted freshly from 100 ppm nitrite in water containing 0.02% chloroform as preservative) were each made 4 mL with water. To each was added 0.4 mL of potassium ferrocyanide reagent (1.5% in 1% Na_2CO_3 in water—the reagent is stable for at least a month at room temperature if protected from air and light) and then to each was added 0.6 mL of concentrated HCl. The pale-green colour was monitored at 430 nm at 30, 60, 90 and 150 min following acidification, each sample having six observations.

Experiment 2. Recovery of nitrite in presence of metallic ions known to interact with the reagent potassium ferrocyanide (*viz.*, zinc, copper, manganese, cadmium and iron) or in their absence as affected by the extraction protocol was studied. Each mL of water containing 30 μg nitrite with or without containing 1 mL metal cocktail was diluted to 8 mL with water; added 1 mL 0.125 N NaOH, mixed and allowed to stand for 2–3 min (to precipitate metallic hydroxides), and then added 1 mL 2.5% MgCl_2 solution, mixed and allowed to stand for 2–3 min (to precipitate out residual hydroxide ions). The samples ($n = 6$ each) were either filtered (Whatman or Merck Grade 1 papers) or centrifuged 6000 rpm for 10 min. Each mL filtrate/centrifugate was made 4 mL with water and assayed for nitrite at 90th to 100th min following acidification as per Experiment 1.

Metal cocktail contained appropriate amounts of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; FeCl_3 ; $\text{CdCl}_2 \cdot \text{H}_2\text{O}$ and $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ in water so as to provide at least per mL 500 μg Zn, 55 μg Mn, 50 μg Cu, 30 μg Fe (Fe^{2+} and Fe^{3+} 1 : 1) and 5 μg Cd, the concentrations being nearly 100 times more than the maximum permissible levels (MPL) of these in drinking water for human and animal use^{1,2}.

Experiment 3. Nitrate ions 1 and 3 mg (KNO_3 or HNO_3) in 4 mL water were treated like Experiment 1 to find whether or not the nitrate would interact with potassium ferrocyanide under test conditions ($n = 6$ each).

Experiment 4. Absorbance of potassium ferricyanide at 430 nm was monitored using 0, 150, 450 and 1500 μg in 4 mL water and treated like Experiment 1; 450 μg concentration was compared with identical concentrations in 5 mL water with or without containing 5 μg nitrite ($n = 6$ each).

Experiment 5. Effect of standard H_2O_2 (concentration estimated titrimetrically with standard potassium permanganate technique³) on oxidation of potassium ferrocyanide was evaluated. For this, to 0.4 mL of potassium ferrocyanide reagent were added 0, 0.2, 0.6 and 2.0 mL of standard H_2O_2 ($680 \pm 2 \mu\text{g mL}^{-1}$) and then added 0.6 mL HCl, and volume of each made 5 mL with water ($n = 6$ each). Colour was monitored at 430 nm at 90th to 100th min following acidification.

The results obtained were analyzed statistically using regression analysis and Students' t-test.

RESULTS AND DISCUSSION

As evident from the results (Table-1), the technique is linear over 0.2 to 1.0 ppm final reaction volume; absorbance reaches to peak at 90th min, and is thereafter stable for 1 h observation period. The values observed at 60th min are significantly higher than those observed at 30th min at all test concentrations ($P < 0.01$) and significantly lower than 90th min values at 3 to 5 μg levels ($P < 0.05$) but not at lower levels of nitrite ($P > 0.05$). There is no significant difference between the corresponding values at 90th and 150th min ($P > 0.10$). Regression coefficient also shows improvement from 30th to 90th min and there after remains stable. Therefore, it is recommended to read optical density between 90 to 150 min following acidification.

TABLE-1
LINEAR RELATION BETWEEN ABSORBANCE VALUES AND NITRITE
CONCENTRATIONS WITH RESPECT TO TIME

Nitrite concentration ($\mu\text{g}/5\text{ mL}$)	Optical density at 430 nm			
	30 min	60 min	90 min	150 min
1	0.033 \pm 0.001	0.041 \pm 0.001	0.047 \pm 0.003	0.049 \pm 0.004
2	0.112 \pm 0.003	0.147 \pm 0.004	0.149 \pm 0.004	0.154 \pm 0.003
3	0.204 \pm 0.006	0.243 \pm 0.005	0.262 \pm 0.005	0.261 \pm 0.005
4	0.270 \pm 0.005	0.320 \pm 0.004	0.334 \pm 0.004	0.343 \pm 0.004
5	0.334 \pm 0.008	0.381 \pm 0.006	0.410 \pm 0.008	0.413 \pm 0.008
r	0.991	0.991	0.992	0.992
b \pm S.E.	0.076 \pm 0.002	0.085 \pm 0.002	0.091 \pm 0.002	0.092 \pm 0.002

The values are Mean \pm S.E. of six observations each for absorbance values.

Extraction procedure (Experiment 2) did not affect nitrite recovery; per cent recovery was found to be as 99.1 ± 1.1 and 99.8 ± 1.7 , respectively, in presence and absence of the metallic ions ($P > 0.10$). Each mL of 0.125 N NaOH is capable of removing 2 to 7 mg of metallic ions, quantity varying with the nature of metal, whereas maximum permissible limit for the total ions does not exceed 7 ppm in drinking water^{1,2}. Failure of nitrate to react under test conditions would suggest that nitrite could be determined in presence of high concentrations of nitrate (Experiment 3).

Experiments 4 and 5 were designed to test whether or not the colour produced by nitrous acid was the result of an oxidation phenomenon. The investigations revealed that oxidation of ferrocyanide to ferricyanide by nitrous acid was an unlikely mechanism to account for colour development :

(i) Theoretically 1 to 5 μg nitrite (m.w. 46) would maximally generate 7.15 to 35.75 μg of potassium ferricyanide (m.w. 329.25) by oxidation of potassium ferrocyanide; however, practically potassium ferricyanide remained undetectable up to 50 μg at 430 nm whereas 1 μg nitrite yielded a distinct colour (Table-1).

(ii) Potassium ferricyanide under test conditions yielded absorbance values as 0.073 ± 0.002 , 0.179 ± 0.001 and 0.508 ± 0.001 , respectively at 150, 450 and 1500 μg concentrations in 5 mL reaction volume with b \pm S.E. as 0.000320 ± 0.000002

($n = 18$), which is about 1/280th of that observed with nitrite (Table-1). The values at 450 μg concentration under test conditions were not different from those observed at identical concentrations in water with or without containing 5 μg nitrite ($P > 0.1$) being respectively as 0.180 ± 0.001 and 0.179 ± 0.001 ($n = 6$ each).

(iii) Stronger oxidizing agent hydrogen peroxide produced colour intensity and regression coefficient comparable to those obtained with potassium ferricyanide. Absorbance values with 136, 408 and 1360 μg H_2O_2 (Experiment 5) were, respectively, 0.095 ± 0.002 , 0.215 ± 0.003 and 0.474 ± 0.004 , with $b \pm \text{S.E.}$ as 0.000299 ± 0.000008 ($n = 18$). In contrast nitrous acid is a weaker oxidizing agent, but yields nearly 280 times better colour intensity per μg basis. These observations may suggest that colour intensity to be presumably due to nitroso-potassium ferrocyanide formation rather than due to oxidation. Further studies are required to confirm the mechanism.

The studies indicate that potassium ferrocyanide technique can be employed as a sensitive spectrophotometric alternative technique to diazotization technique for determination of nitrite in water with lower detection limit of 0.3 ppm nitrite in test sample. Samples containing higher nitrite should be appropriately diluted to fall within the linear detection range (Table-2). The technique requires^{3, 4} smaller sample volume of 8 mL in contrast to 50 mL or 90 mL needed for diazotization technique. Besides, it obviates the use of costlier reagent NEDA required for diazotization technique. Metallic ions in water, viz., Bi^{3+} , Pb^{2+} , Ag^+ , Hg^{2+} , Fe^{3+} , Cu^{2+} and Au^{3+} , rendering diazotization technique inapplicable³ are unlikely to limit the application of potassium ferrocyanide technique due to precipitation of these and other ions by the extraction step.

TABLE-2
THE ALIQUOT VOLUMES FOR DIFFERENT DETECTION RANGES
FOR NITRITE IN WATER

Sample volume (mL)	Filtrate volume (mL)	Detection range (NO_2 ppm sample)
8.0	4.0	0.3–1.5
2.5	4.0	1.0–5.0
5.0	1.0	2.0–10.0
1.0	1.0	10.0–50.0
0.2	1.0	50.0–250.0

Formula : Minimum detection limit = $(10)/(\text{sample vol.})(\text{filtrate vol.})(\text{ppm})$

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