

# Development of a Field Kit Using The 'Mixed Reagents Azo-Dye Method' For The Determination of Nitrite In Water And Environmental Samples

S.O. Ajayi, B.O. Odesanya, A.A. Fashina & J.O. Ajayi

**Abstract** - The transient nature of the nitrite ion and its toxicity make on-site determinations preferable to ensure actual values are obtained for analytical data integrity. A simple, compact and cheap field kit for the on-site determination of nitrite in water and environmental samples is presented using a method of colour development based on mixed reagents – (N-(1-naphthyl) ethylenediamine dihydrochloride and sulphanilamide) with standard nitrite solutions used to calibrate the field kit before determination of the nitrite in water samples. Results indicate the method is sensitive, selective and reproducible and would provide a reliable method for the determination of nitrite in water and environmental samples on-site.

**Key Words:** Field Kit (development), mixed reagent, (N-(1-naphthyl) ethylenediamine dihydrochloride, sulphanilamine, calibration graph, on-site determination of nitrite.

## INTRODUCTION

Nitrite ion,  $\text{NO}_2^-$ , an intermediate in the nitrogen cycle, is formed during the decomposition of organic matter. Nitrite is present in wastewaters, effluents from sewage treatment plants, water distribution systems and natural waters and has been shown to be highly toxic to all aquatic organisms<sup>22,20,34</sup>. Dutt and Davis<sup>10</sup> have shown that concentrations between 0.1 – 0.2 ppm are enough to poison fishes.

The World Health Organization (WHO) gives 0.2ppm as the highest desirable limit and 3.0ppm as maximum permissible limits<sup>17</sup> while the United States public health service puts 0.06 ppm as the maximum permissible limits<sup>27,28</sup> for nitrite concentrations in potable water.

Methods exist for the determination of nitrite in water, blood plasma, food and soil<sup>29,31,11,15,24,25,27,36,6,16,2,12,30,19,5,28,10,26,35,33,32,13</sup>. The Greiss-Ilosvic azo-dye method is the most popular. It is a sensitive and standard method which is adequate for monitoring nitrite concentrations in aquaculture, potable water production and environmental management. The laboratory procedure for the method is well developed<sup>3</sup> only that the sample requires effective sample preservation. The field option for the method has not received extensive research.

Due to the toxicity of the nitrite ion<sup>7,8,23,9,22,4,21,14,1</sup> and its transient nature as a result of air oxidation or bacterial activity<sup>10</sup>, there is a need to develop a field kit to determine

nitrite levels on-site for environmental management and safety. Recently, a simple and portable colorimeter using a red-green-blue light emitting diode was developed for on-site determinations<sup>33</sup>. More work still needs to be done in this direction.

This investigation aimed at a simple, portable, reliable and sensitive field kit for the determination of nitrite in water on-site based on the well established Greiss-Ilosvic standard method. The thrust of the investigation is to minimize equipment and reagents requirements for field work without compromising the method, its accuracy and sensitivity.

## EXPERIMENTAL

The developed field kit consists of a tungsten light source with three filters for red (710nm), yellow (580nm) and green (520nm) regions for wavelength selection and a Light Dependent Resistor (LDR) which serves as a sensor preceding the 1cm<sup>2</sup> sample compartment. The LDR converts the light that emerges from the sample compartment into analog-digital voltage with the resistance of the LDR varying with the concentration of the sample solution. This variation of the LDR's resistance is measured as absorbance.

### Reagents ( APHA Method 4500, 2000)<sup>3</sup>

2.5g of sulphanilamide was weighed and dissolved in a mixture of 25mL hydrochloric acid and 150mL deionised water. The solution was then made up to the mark in a 500mL standard flask.

*Prof S.O. Ajayi & Dr B.O. Odesanya are of the College of Pure and Applied Sciences, Caleb University, Imota, Lagos State while Mr. A.A. Fashina & Mr. J.O. Ajayi are of the University of Ibadan, Nigeria. All correspondence to skipaodes@yahoo.com*

0.5g of N-(1-naphthyl) ethylenediamine dihydrochloride was weighed and dissolved in 500mL deionised water in a standard volumetric flask.

1.232g of sodium nitrite was weighed into a 1000mL standard flask, 1mL of chloroform was added to preserve the solution before it was made up to the mark. The ensuing nitrite solution was standardized with 0.05M  $\text{KMnO}_4$  using sufficient ferrous ammonium sulphate solution as reductant.

#### Traditional laboratory procedure for Nitrite analysis by azo-dye method using sequential addition of reagents

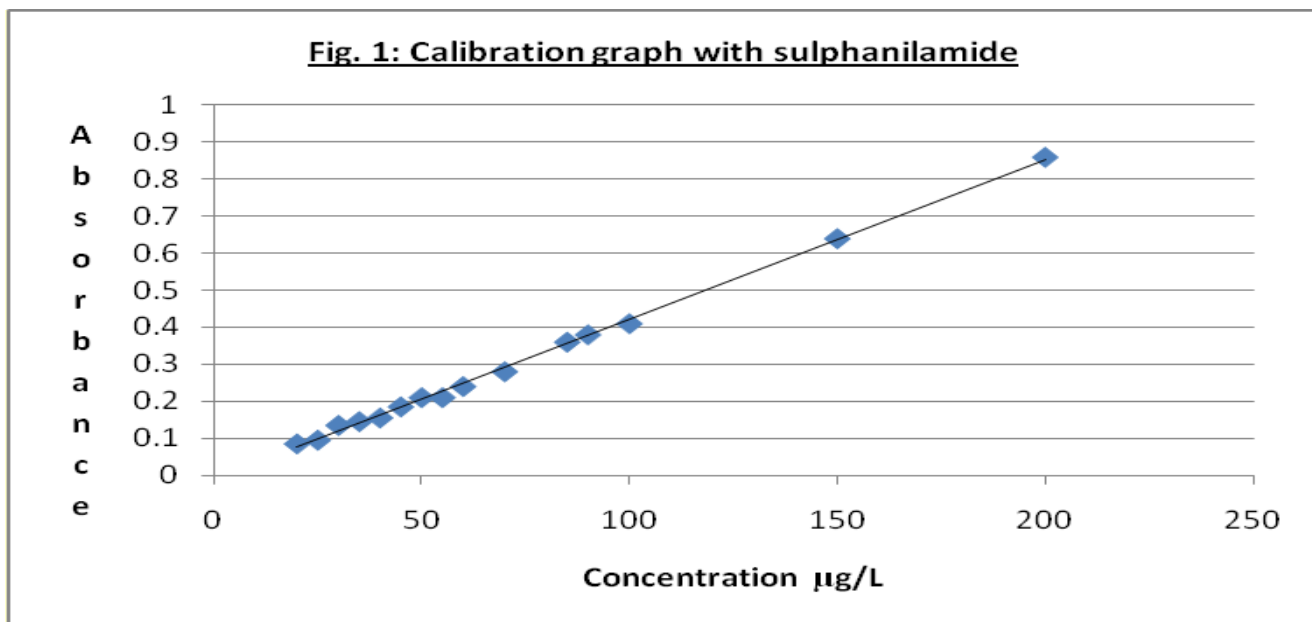
50mL of standard nitrite solution was measured and the pH of the solution was brought to 7.0 by the addition of a phosphate buffer solution of pH 7.0. 1mL of sulphanilamide was added to this solution and left for 8 minutes for complete reaction after which 1mL of N-(1-naphthyl) ethylenediamine dihydrochloride solution was added and mixed thoroughly. The solution was left to stand for 10 minutes before the absorbance was taken with a UV-Vis spectrophotometer at 520nm with the mixed reagent as blank.

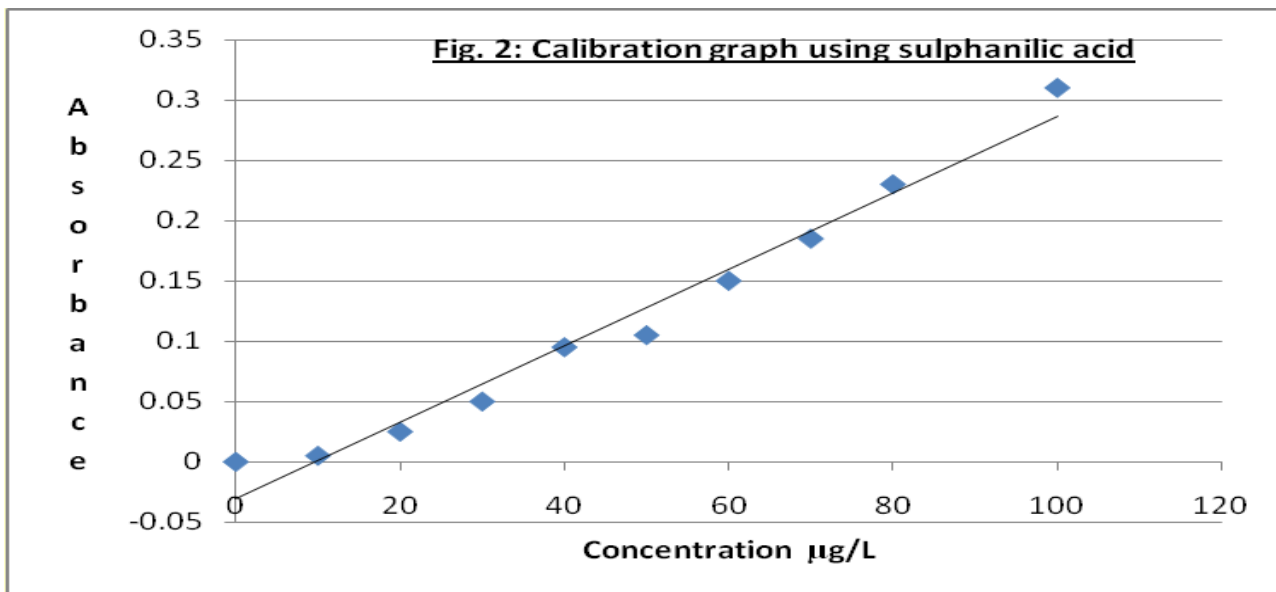
#### Mixed Reagent Method

In developing the field kit, two methods of colour development were considered: (i) normal and (ii) mixed reagents. The normal method involved the sequential addition of sulphanilamide to nitrite samples for diazotization before N-(1-naphthyl) ethylenediamine dihydrochloride is added to get the highly coloured azo dye chromophore. However, the reagents for the mixed reagent method (sulphanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride) were mixed in a 1:1 ratio to obtain one reagent. Analytical procedure is essentially the same except that 1 mL of mixed reagent was used for each 50 mL sample.

#### RESULTS AND DISCUSSION

There are many combinations of reagents for the azo-dye colour development in the determination of the nitrite ion. This work considered two of the foremost combinations: (a) sulphanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride and (b) sulfanilic acid and N-(1-naphthyl) ethylenediamine dihydrochloride. Figures 1 and 2 show the calibration graphs obtained using the two sets of reagents. The results show that the sulphanilamide- N-(1-naphthyl) ethylenediamine dihydrochloride combination with standard nitrite solutions gave a linear calibration graph with a regression coefficient of 0.997. The sulfanilic acid- N-(1-naphthyl) ethylenediamine





dihydrochloride-standard nitrite solutions also gave a fairly linear graph but the absorbance values were much lower. The better linearity, intensity and stability of colour formed made us adopt the sulphanilamide- N-(1-naphthyl) ethylenediamine dihydrochloride combination as the reagents of choice for colour formation in this study. A

comparison of the absorbance values obtained using the method of sequential addition (Normal) and mixed reagents are given in Table 1.

Concentration of nitrite, (ppb)	Absorbance values at 520nm Normal method	Mixed reagents method
25	0.090	0.090
50	0.145	0.150
75	0.250	0.230
100	0.300	0.280
R <sup>2</sup>	0.979	0.992

Table 1: Absorbance values using Normal and Mixed reagents methods

The F-test value of 0.8364 indicates a 2-tail probability that the variances between the two sets of absorbance values are not statistically different. A 2-tailed, homoscedastic T-test gave a value of 0.8954 implying an insignificant difference between the 2 sets of absorbance values. Therefore, in a bid to minimize the number of glassware required for field

measurements, reduce the volume of reagents required for colour development and achieve the objective of producing a field kit that is simple and portable, the mixed reagent method is adopted for these determinations.

**Sensitivity test for adopted method**

In order to test the sensitivity of the adopted mixed reagent method and reproducibility of analytical data, 0.025 ppm and 0.05 ppm nitrite solutions were prepared and the

absorbance values determined using the mixed reagent with different volumes but same ratio of mixed reagent to standard nitrite solution.

The results are given in Tables 2 and 3.

Volume of mixed reagent plus standard nitrite solution	Absorbance at 520nm*
2.0ml of mixed reagent plus 2.5ml of 0.025ppm nitrite solution made up to 50ml in std. flask	0.080
1.0ml of mixed reagent plus 1.25ml of 0.025ppm nitrite solution made up to 25ml in std. flask	0.075
0.5ml of mixed reagent plus 0.625ml of 0.025ppm nitrite solution made up to 12.5ml	0.080

**Table 2: Absorbance values for sensitivity tests (0.025ppm)**

\*values are averages of triplicate readings

Volume of mixed reagent plus standard nitrite solution	Absorbance at 520nm*
2.0ml of mixed reagent plus 5.0ml of 0.05ppm nitrite solution made up to 50ml in std. flask	0.155
1.0ml of mixed reagent plus 2.5ml of 0.05ppm nitrite solution made up to 25ml in std. flask	0.145
0.5ml of mixed reagent plus 0.125ml of 0.05ppm nitrite solution made up to 12.5ml	0.155

**Table 3: Absorbance values for sensitivity tests (0.05ppm)**

\*values are averages of triplicate readings

Table 2 shows the absorbance values obtained with different volumes of 0.025 ppm standard nitrite solutions and mixed reagent. Reduction in volume of the standard nitrite solution and volume of reagent made insignificant difference in absorbance values. A similar trend was observed with standard 0.05ppm nitrite solution (Table 3). Although this relative consistency of absorbance values is expected due to the same dilution factor being used, it is also an indication that the mixed reagent method gives

reproducible results and can be a sensitive method of colour development. In a bid to confirm the sensitivity of the method, the volume of the mixed reagent was reduced to one and two drops respectively. The drop(s) of the mixed reagent were fed directly into the 1.0ml cuvette using a 2ml syringe with the volume of standard nitrite or sample solution added afterwards before stirring. The absorbance values are taken at least in triplicates. The results of absorbance taken at 520nm are presented in Table 4:

Concentration of std. nitrite solution (ppm)	1 drop of mixed reagents plus 1.5ml std. nitrite solution*	2 drops of mixed reagents plus 1.5ml std. nitrite solution*
0.5	0.95,0.96,1.02,0.95 (0.97)	1.10,1.05,1.10,1.00 (1.06)

0.4	0.95,0.95,1.0 (0.97)	1.02,1.02,1.0 (1.05)
0.3	0.78,0.80,0.77,0.83 (0.80)	0.78,0.80,0.83,0.83 (0.81)
0.2	0.58,0.60,0.58,0.60 (0.59)	0.56,0.62,0.62,0.60 (0.60)
0.1	0.36,0.37,0.35,0.35 (0.36)	0.40,0.41,0.38,0.39 (0.395)
0.075	0.16,0.175,0.20 (0.18)	0.18,0.19,0.175 (0.182)

**Table 4: Absorbance at 520nm for different concentrations of nitrite solutions** \* Average values in brackets.

Plots (figures 3 and 4) indicate a linear relationship up to 0.4ppm solution. A linear regression plot gave R2 values of 0.9707 and 0.9728 for one and two drops of mixed reagent up to 0.4 ppm also giving values of 0.9193 and 0.9377 respectively up to 0.5 ppm. The results indicate that the method is reproducible and of enough sensitivity for use as an analytical method. Statistical examination of the results using a 2-tail homoscedastic T-test gave a value of 0.8519 showing that using 1 or 2 drops of mixed reagent does not

significantly affect the absorbance values. However, for the purpose of this study, 2 drops of mixed reagent was used.

**Adoption of Field Procedure**

A field procedure was adopted for this investigation thus: Cuvette in colorimeter, add 1 mL sample, 2 drops pH 7.0 buffer solution plus 2 drops mixed reagent from 2 mL syringe. Mix with syringe and measure at 520nm.

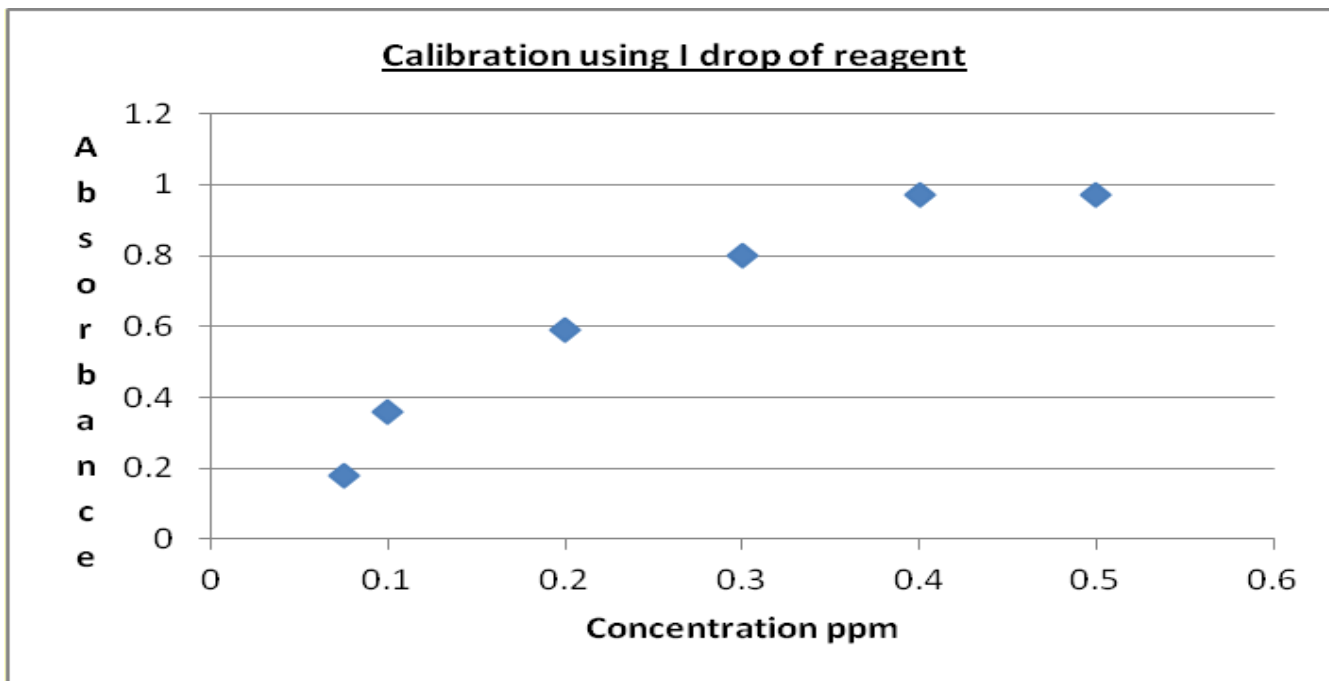


Figure 3: Calibration using 1 drop of reagent

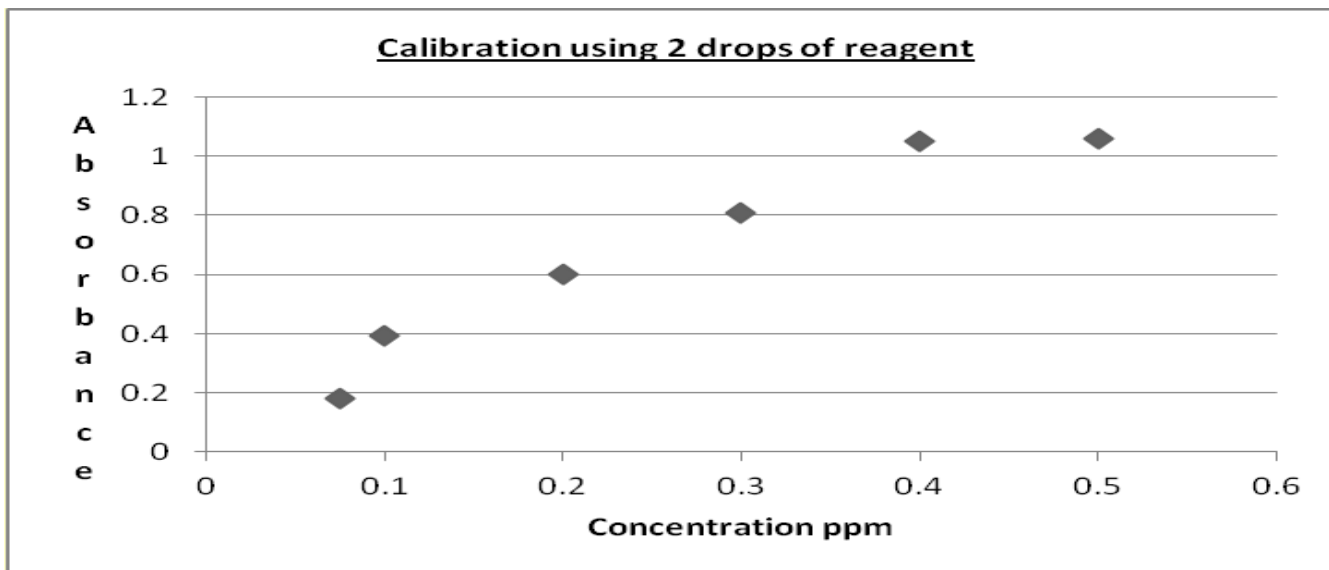


Figure 4: Calibration using 2 drops of reagent

#### Stability test for the Mixed Reagent

In a bid to determine the effects of environmental factors on the reagents before use, calibration graphs were prepared using:

- Mixed reagent preserved in a refrigerator before use
- Mixed reagent kept at room temperature before use
- Sulphanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride kept separately in a refrigerator and mixed just before use and
- Sulphanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride preserved

(e) separately at room temperature and mixed just before use.

Absorbance readings were taken for each concentration and category of reagents for 25 days. A comparison of the values for (a) and (b) for 25 days showed no significant difference. A similar pattern was observed for categories (c) and (d). There was a very low standard deviation ( $n = 25$ ) making us to conclude that the mixed reagent is stable for nitrite determinations. The results are presented in Tables 5 and 6.

This established the feasibility of the method for nitrite determinations.

<u>Concentration (ppm)</u>	<u>Reagent Conditions</u>	<u>Absorbance</u>
0.03	Mixed Reagent at room Temperature (A)	0.06
	Mixed Reagent preserved in Refridgerator (B)	0.06
	Sequential addition of reagents at room Temperature ( C )	0.05
	Sequential addition of reagents preserved in Refridgerator (D)	0.07
0.06	Mixed Reagent at room Temperature (A)	0.245
	Mixed Reagent preserved in Refridgerator (B)	0.24
	Sequential addition of reagents at room Temperature ( C )	0.265
	Sequential addition of reagents preserved in Refridgerator (D)	0.245
0.09	Mixed Reagent at room Temperature (A)	0.27
	Mixed Reagent preserved in Refridgerator (B)	0.25
	Sequential addition of reagents at room Temperature ( C )	0.26
	Sequential addition of reagents preserved in Refridgerator (D)	0.26
0.12	Mixed Reagent at room Temperature (A)	0.405
	Mixed Reagent preserved in Refridgerator (B)	0.43
	Sequential addition of reagents at room Temperature ( C )	0.415
	Sequential addition of reagents preserved in Refridgerator (D)	0.43
0.15	Mixed Reagent at room Temperature (A)	0.435
	Mixed Reagent preserved in Refridgerator (B)	0.44
	Sequential addition of reagents at room Temperature ( C )	0.435
	Sequential addition of reagents preserved in Refridgerator (D)	0.435
0.18	Mixed Reagent at room Temperature (A)	0.47
	Mixed Reagent preserved in Refridgerator (B)	0.47
	Sequential addition of reagents at room Temperature ( C )	0.485
	Sequential addition of reagents preserved in Refridgerator (D)	0.495
0.21	Mixed Reagent at room Temperature (A)	0.6
	Mixed Reagent preserved in Refridgerator (B)	0.625
	Sequential addition of reagents at room Temperature ( C )	0.61
	Sequential addition of reagents preserved in Refridgerator (D)	0.605
0.24	Mixed Reagent at room Temperature (A)	0.685
	Mixed Reagent preserved in Refridgerator (B)	0.675
	Sequential addition of reagents at room Temperature ( C )	0.685
	Sequential addition of reagents preserved in Refridgerator (D)	0.665
0.27	Mixed Reagent at room Temperature (A)	0.785
	Mixed Reagent preserved in Refridgerator (B)	0.785
	Sequential addition of reagents at room Temperature ( C )	0.765
	Sequential addition of reagents preserved in Refridgerator (D)	0.805
0.3	Mixed Reagent at room Temperature (A)	0.93
	Mixed Reagent preserved in Refridgerator (B)	0.94
	Sequential addition of reagents at room Temperature ( C )	0.935
	Sequential addition of reagents preserved in Refridgerator (D)	0.965

Table 5: Effects of temperature variation on Absorbance for method

\* All readings are averages of duplicate readings

<b>Concentration (ppm)</b>	<b>Reagent Conditions</b>	<b>Absorbance*</b>	<b>Standard deviation</b>
0.03	Mixed Reagent at room Temperature (A)	0.059	0.0147
	Mixed Reagent preserved in Refridgerator (B)	0.0562	0.0136
	Sequential addition of reagents at room Temperature ( C )	0.063	0.0417
	Sequential addition of reagents preserved in Refridgerator (D)	0.053	0.0135
0.06	Mixed Reagent at room Temperature (A)	0.2488	0.0123
	Mixed Reagent preserved in Refridgerator (B)	0.2434	0.0494
	Sequential addition of reagents at room Temperature ( C )	0.2484	0.0202
	Sequential addition of reagents preserved in Refridgerator (D)	0.244	0.0156
0.09	Mixed Reagent at room Temperature (A)	0.2736	0.0109
	Mixed Reagent preserved in Refridgerator (B)	0.2607	0.051
	Sequential addition of reagents at room Temperature ( C )	0.2766	0.0207
	Sequential addition of reagents preserved in Refridgerator (D)	0.267	0.0141
0.12	Mixed Reagent at room Temperature (A)	0.3826	0.0253
	Mixed Reagent preserved in Refridgerator (B)	0.3822	0.034
	Sequential addition of reagents at room Temperature ( C )	0.3731	0.0426
	Sequential addition of reagents preserved in Refridgerator (D)	0.3718	0.0479
0.15	Mixed Reagent at room Temperature (A)	0.4416	0.0137
	Mixed Reagent preserved in Refridgerator (B)	0.4446	0.0118
	Sequential addition of reagents at room Temperature ( C )	0.4418	0.0184
	Sequential addition of reagents preserved in Refridgerator (D))	0.444	0.0166
0.18	Mixed Reagent at room Temperature (A)	0.476	0.0168
	Mixed Reagent preserved in Refridgerator (B)	0.4762	0.0124
	Sequential addition of reagents at room Temperature ( C )	0.4802	0.0303
	Sequential addition of reagents preserved in Refridgerator (D)	0.4774	0.033
0.21	Mixed Reagent at room Temperature (A)	0.5618	0.0232
	Mixed Reagent preserved in Refridgerator (B)	0.5594	0.0241
	Sequential addition of reagents at room Temperature ( C )	0.5606	0.0438
	Sequential addition of reagents preserved in Refridgerator (D)	0.5508	0.0295
0.24	Mixed Reagent at room Temperature (A)	0.6612	0.015
	Mixed Reagent preserved in Refridgerator (B)	0.6616	0.0257
	Sequential addition of reagents at room Temperature ( C )	0.6732	0.0389
	Sequential addition of reagents preserved in Refridgerator (D)	0.6728	0.0202
0.27	Mixed Reagent at room Temperature (A)	0.769	0.0194
	Mixed Reagent preserved in Refridgerator (B)	0.7704	0.023
	Sequential addition of reagents at room Temperature ( C )	0.7796	0.0411
	Sequential addition of reagents preserved in Refridgerator (D)	0.772	0.0206
0.3	Mixed Reagent at room Temperature (A)	0.9286	0.0167
	Mixed Reagent preserved in Refridgerator (B)	0.9194	0.0232
	Sequential addition of reagents at room Temperature ( C )	0.9266	0.0189
	Sequential addition of reagents preserved in Refridgerator (D)	0.9254	0.0153

Table 6 : Stability ckeck for method over 25 days.

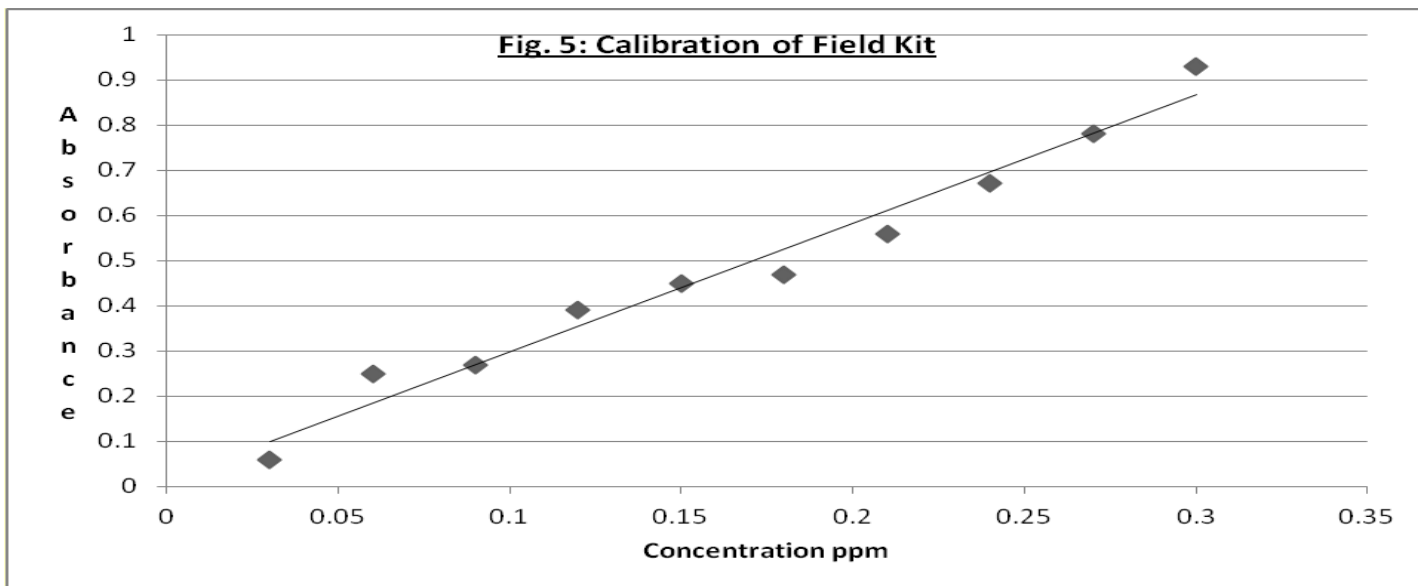
(\* Absorbance readings are average values over 25 days.)

**Translation of method to Field Kit Calibration of Field Kit and Determination of Nitrite concentration in Water samples**

To enable the field kit be used, a calibration graph using Various nitrite concentrations - 0.03, 0.06, 0.09, 0.12,

0.15, 0.18, 0.21, 0.24, 0.27 and 0.3 ppm- was prepared. The resulting calibration curve is given in figure 5. A correlation between concentration of nitrite and absorbance was established with a regression coefficient ( $R^2 = 0.989$ ) indicating its suitability for use for calibration.





The procedure was adopted for use in the determination of nitrite concentration in samples obtained from Awba dam and along the river flowing into it. The mixed reagents method was also applied for nitrite determinations in

different fish ponds. The results are shown compared with those obtained using the sequential method and are given in Tables 7 and 8.

Location along the river and Awba Dam	Absorbance at 520nm*(Normal method)	Absorbance at 520nm* (Mixed Reagents method)	Concentration of Nitrite µg/mL
Point 1	0.11	0.11	0.035
Point 2	0.11	0.11	0.035
Point 3	0.16	0.16	0.052
Awba Dam	0.095	0.095	0.030

**Table 7: Nitrite concentrations in Awba Dam and along its river**

(\*Average of triplicate readings)

Samples	Absorbance at 520nm* (Normal method)	Absorbance at 520nm* (Mixed Reagents)	Concentration of Nitrite µg/mL
Surface fish pond	0.072	0.075	0.0165
Concrete fish pond	0.066	0.065	0.0129
Concrete fish pond with recycling facility	0.035	0.035	0.0024

**Table 8: Nitrite concentrations from various fish ponds**

(\* Average of triplicate readings)

**CONCLUSION**

A field kit and mixed reagent method for the determination of nitrite has been developed. It has been successfully applied for the on-site determination of nitrite in Awba

Dam, along the river flowing into the dam and in some selected fish ponds.

The method is sensitive, reproducible and stable and can be used in the determination of nitrite in water samples for routine analysis.

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