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# Determination of Trace Nitrite Amounts in Soil by Developed Cloud Point Extraction-Spectrophotometry Method

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## Abstract

A new and simple method for the selective and sensitive determination of trace amounts of nitrite in soil samples is developed by a combination of cloud point extraction (CPE) and spectrophotometry technique. The procedure is based on the Griess reaction on 3,3`-dimethyl benzidine with nitrite in the presence of 1-naphthol in acidic media to form a yellow diazo dye, which shows absorption at lambda max 455nm. The formation of colored diazo dye is followed by extraction into the Triton X-114-rich phase. Cloud point extraction method parameters such as surfactant amount, temperature, and incubation time are evaluated and optimized. The results obtained showed a very good linear range of 10-1250 µgL$^{-1}$, with good regression coefficients ($R^2$) $\geq$ 0.9997 and a preconcentration factor of 12.5. The limit of detection (LOD) describes as 3SD$_{\mathbf{blank/m}}$, and the quantification limit (LOQ) describes as 10SD$_{\mathbf{blank/m}}$, where m is the slope of the calibration curve and they are equal to 7.50 and 22.71 µgL$^{-1}$ respectively. The recommended method can apply to determine the ultra-trace amount of nitrite in soil samples.

*Keywords: Nitrite; Spectrophotometric method; CPE; Griess reaction*

## 1. Introduction

A major percentage of the total nitrogen in the soil exists as organic compounds, which are mostly unavailable directly to plants. On the other hand, a tiny fraction of this total nitrogen exists as inorganic forms. The conversion of inorganic species (ammonium, nitrite, and then nitrate) occurs via biological processes involving soil microorganisms [1].

Many scientists focused their research on the estimation of pollutants that present a dire concern in different environments. Determination of nitrite is also essential because it results in a widespread hazardous inorganic pollutant that exists in the environment, food additives, industrial, and physiological systems [2-5]. Nitrite ion is highly poisonous to human and animal health at high levels as it integrates with the blood pigments to produce meta-hemoglobin, which is resulting in the exhaustion of the oxygen in the tissue [6,7].

Numerous researchers zeroed in on their examination on the assurance of toxins that present a desperate worry in various conditions. Assurance of nitrite is likewise significant in light of the fact, which is a boundless risky inorganic toxin that exists in the climate, food added substances, modern, and physiological frameworks [2-5]. Nitrite particle is profoundly noxious to the human and creature wellbeing at significant levels as it coordinates with the blood shades to deliver methemoglobin, which is brought about by the weariness of the oxygen in the tissue [6,7].

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about by the weariness of the oxygen in the tissue [6,7].

The spectrophotometric approach is most frequently used for the determination of nitrite [8-11], which is based on the simple reaction of nitrite named Griess reaction [12] to form colored azo dye, this approach can also determine nitrate (after nitrate reduction). Griess reaction reported by a German scientist Johann Peter Griess (1829-1888), detailed in the original work the synthesis of the azo dye "Dia-zobenzolamidonaphtol" starting from diazobenzene and naphthylamine [13]. Spectrophotometry pushes along to enjoy wide popularity because of the current availability of the instrumentation and simplicity of procedures as well as low cost. Moreover, it provides high sensitivity when convenient chromogenic reagents are available.

Cloud point extraction (CPE) has become one of the more discriminating extraction techniques, which used a minimum amount of solvents and reduces the extraction time with high enrichment factors for analysts. Watanabe and Tanaka first developed CPE for the preconcentration of metal ions from aqueous samples [14]. Cloud point extraction principles are based on the separation of the homogeneous micelles aqueous solution into two phases [15-19], a surfactant-rich phase, and a dilute aqueous phase [20]. The surfactant-rich phase, of a small volume, including a surfactant analyst composed of a complexing agent with a small amount of water, and the aqueous phase (water-rich phase), contains a low concentration of the surfactant.

The aim of this work is the determination of ultra-trace amounts of nitrite in soil by a more straightforward and sensitive cloud point extraction followed by a spectrophotometric method. Griess reaction applied for a sensing trace amount of nitrite in the presence of 3,3`-dimethylbenzidine and 1-naphthol to form colored diazo dye and then followed by preconcentration and detecting.

## 2. Material and Methods

### *2.1 Standard Solutions and Reagents*

All reagents were of analytical grade and used without any further purification. All solutions were prepared using double distilled water; all glassware used in the experiments was treated at first with dilute nitric acid, and hydrochloric acid solutions. A stock nitrite and nitrate solutions of 1000 μgL$^{-1}$ were prepared by dissolving appropriate amounts of sodium nitrite and potassium nitrate (Sigma) in double-distilled water, to each of these solutions, a 0.1 g of sodium hydroxide and 1 mL of chloroform were added. The solutions of nonionic surfactant Triton X-114 6.0% (v/v) (Sigma), 0.05% of 3,3`-dimethyl benzidine (Merck), 5% of 1-naphthol (Sigma), 2M hydrochloric acid, and solutions of various metal ions and anions were prepared using double-distilled water. A buffer solution (0.2M of $Na_2HPO_4$ and $NaH_2PO_4$) was prepared to adjust the pH-value of solutions, and to dilute a surfactant phase a saturated solution of 0.15M ascorbic acid in ethanol was used.

### *2.2 Apparatus*

A UV-Visible spectrophotometer (PG Instruments Limited, model, T80, EMC-LAB) with a quartz cell with a path length of 1 cm was used for the measurements of absorbance. The pH values were measured by Lovibond SD 300 pH meter supplied with a combined electrode. A thermostat bath was used for the cloud point experiments to maintain temperatures at the desired. A Mistral-2000 centrifuge was used to accelerate the surfactant phase separation.

### *2.3 Procedure*

The diazo coupling reaction was achieved by a reported method in the literature with some modification [8]. A nitrite stock solution was pipetted out into standard flasks, each containing 1.0 mL of 0.05% 3,3`-dimethylbenzidine and 1.0 mL of 2M hydrochloric acid. Then, the content was mixed thoroughly for 3 to 4 minutes, followed by adding 2.0 mL of 5.0% 1-naphthol and 2.0 mL of 2M sodium hydroxide; then, the volume completed up to 25.0 mL using double distilled water so that its final concentration would be in the range of 5.0 to 100.0 μgL$^{-1}$. The diazo coupling (Griess reaction) showed in Fig. 1. The diazo dye formed has maximum absorption at λmax=455 nm against the reagent blank.

Cloud point extraction experiments were carried out by mixing 3.0 mL of aliquots samples with 1.0 mL of ($Na_2HPO_4$/$NaH_2PO_4$) buffer solution for adjusting pH-value to 9.2, 1.0 mL of 25.0% (w/v) sodium chloride, 1.0 mL of 6.0% (v/v) Triton X-114 were added in a 25.0 mL centrifuge tube and made up to the mark by double-distilled water. All solutions were left in a thermostatic water bath at 50.0 ºC for 10.0 minutes to improve turbidity, and during this time, the solution became cloudy, followed by separation of two phases by centrifugation for 2 minutes at 3000 rpm. The aqueous phase was decanted by simply inverting the tube.

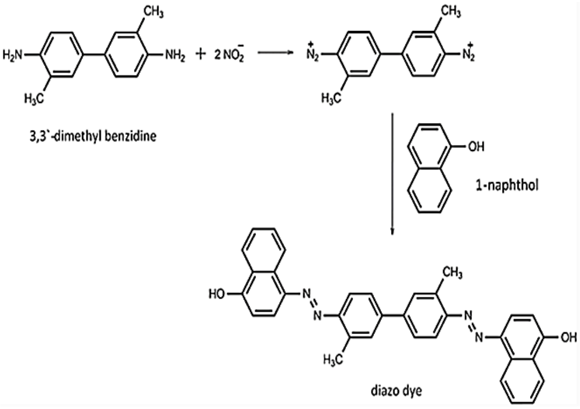

Fig. 1. Griess reaction of 3,3`-dimethylbenzidine followed by coupling with 1-naphthol.

To decrease the viscosity of the obtained surfactant-rich phase, the micellar phase was diluted to 2.0 mL with 0.15M ascorbic acid in ethanol. Then, the resultant solution was directly introduced into an ultraviolet spectrophotometer for the determination of nitrite. The same procedure was repeated using the standard solution prepared in the diazo coupling step instead of the aliquots samples.

*2.4 Application*

Our new approach was applied to the real soil samples taken from Basra city-Iraq. The work started by sifting soil samples through a 20-mesh sieve (US Standard Screen, 850 μm) and mechanical shaking to 10.0 g soil with 50.0 mL of Morgan's reagent (100 g of sodium acetate + 30 ml of glacial acetic acid, diluted to one liter with distilled water and adjusted to pH 4.8) and 0.25 gm of activated charcoal. The solution was filtered through Whatman No. 1 filter paper. If some turbidity is seen, the filtrate could be transferred to the funnel and re-filtered [21].3. Results and Discussion

*3.1 Effect of surfactant amount*

Triton X-114 is a non-ionic surfactant widely used in CPE because it is commercially available with high purity, non-toxic, eco-friendly reagent as well as gives high separation efficiency as it provides high surfactant-rich phase density. The effect of its different concentrations (1.0-10.0) % (v/v) is shown in Figure 2. A sharp increase in absorbance is observed when Triton X-114 concentration increased from 1.0 to 6.0% (v/v), while over the range from 6.0 to 10.0% (v/v) absorbance diminution was found. This result can be attributed to the maximum quantitative recovery of nitrite obtained with 6.0% (v/v) Triton X-114. Whereas, at the lower surfactant concentration (<6.0% v/v), the complex preconcentration was lower than the optimum concentration (6.0% v/v), at the same time the decrease in the absorbance by increasing the concentration of Triton X-114 (>6.0% v/v) is associated with an increase in the volume of the formed micellar phase.

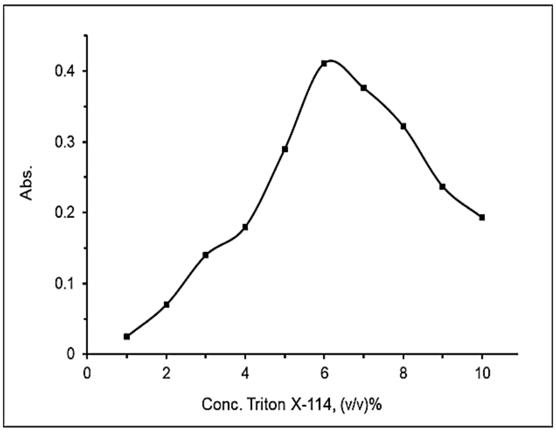

Fig. 2. Effect of Triton X-114 amount on CPE.

*3.2 Effect of temperature and incubation time*

To reach ease phase separation and high preconcentration efficiency, CPE temperature and incubation time optimization were studied. Figure 3 demonstrated the effect of the cloud point extraction temperature on the signal enhancement, the temperature ranging from 20 to 70oC, in the same way, the incubation time effect is shown in Figure 4, the range of times was (5-55) minutes, the results showed that the optimum CPE temperature and incubation time were 50oC and 25.0 minutes respectively.

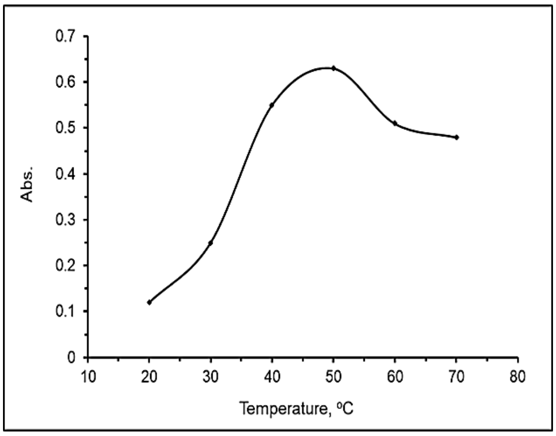

Fig. 3. Effect of temperature amount on CPE.

### *3.3 Effect of foreign ions*

The effect of foreign ions on the determination of nitrite by the proposed method was investigated. The tolerance limit was described as the concentration of added ions that caused a higher than ±5.0% relative error. Table 1. illustrates the tolerance limits of ions of alkaline metals, alkaline earth, and some other ions in the determination of nitrite. It can be seen that the major foreign ion did not interfere; thus, this method can be considered selective for the determination of nitrite.

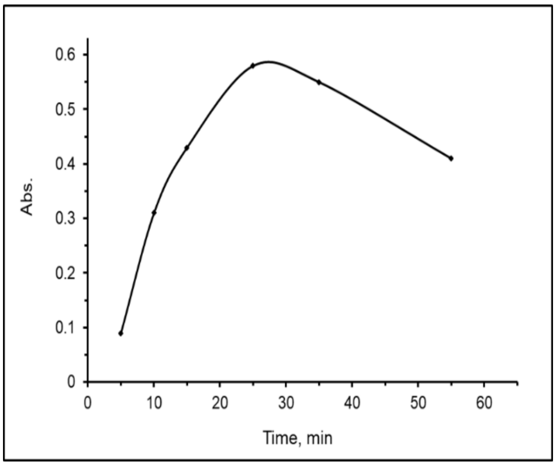

Fig. 4. Effect of time on CPE.

Table 1. Tolerance limits of some foreign ions in the determination of Nitrite.

| Foreign Ions | Foreign Ion: Nitrite |
|---|---|
| K (I), Na (I), $NH_4$ (I) | 500 |
| Ba (II) , Ca (II), Mn (II), Mg (II) | 1500 |
| Co (II), Pb (II), Cu (II), Ni (II) | 1500 |
| Fe (III), Al (III) | 1000 |
| Acetate, Citrate | 250 |
| Sulfate, Oxalate | 500 |
| Phosphate | 500 |

### *3.4 Analytical parameters and comparison with some reported methods*

The analytical characteristics of the proposed method for the determination of nitrite were also studied under optimal conditions. After applying the optimum condition, a calibration curve was obtained by the proposed method. The analytical parameters were calculated, such as the limit of detection (LOD) which defined as $3SD_{blank}/m$ and the limit of quantification (LOQ) defined as $10SD_{blank/m}$ (where $SD_{blank}$ is the standard deviation of 10 replicate measurements of the blank and 'm' is the slope of the calibration curve [22]. The limits of detection and quantification (LOD and LOQ) are essential parameters in the validation process. Estimation of these parameters is especially important when trace and ultra-trace quantities of analyte are to be detected [23]. The analytical parameters of the proposed method are summarized in Table 2.

Table 2. Analytical characteristics of the method

| Parameter | Value |
|---|---|
| Regression Equation (*n=10*) | Y = 1.3484X + 0.0174 ,<br>Y=A , X=[$NO_2^-$] , μgL$^{-1}$ |
| Linearity Range, μgL$^{-1}$ | 10 -1250 |
| Correlation Coefficient, $R^2$ | 0.9997 |
| Preconcentration factor$^*$ | 12.5 |
| Limit of Detection (*LOD*), μgL$^{-1}$ | 0.75 |
| Limit of Quantification (*LOQ*) , μgL$^{-1}$ | 22.7 |

### *3.5 Methodology validation*

The developed method in this work was validated by applying it in the determination of nitrite in soil samples. The recovery experiments were carried out by spiking the real samples with different four concentrations of nitrite, as illustrated in Table 3. the results showed that the mean recoveries 101.05 % this indicating that the developed procedure approach is free from matrix effects, and that led to considering the proposed method could be reliable in a wide range of samples.

Table 4 summarized comparison between the proposed method and some other methods of nitrite determination reported in the literature (8, 24-28). The method of this work shows a reasonable limit of detection and linearity range; it can be seen the proposed method less complex and much faster and sensitive.

Table 3. Recoveries s for determination of the nitrite in soil. (n=4), nitrite=0.25 μgL$^{-1}$.

| Added (μgL$^{-1}$) | Found (μgL$^{-1}$) | Recovery ± SD |
|---|---|---|
| 0.25 | 0.51 | 102.0±0.92 |
| 1.0 | 1.30 | 102.4±1.02 |
| 1.50 | 1.74 | 99.4±0.76 |
| 2.50 | 2.76 | 100.4±0.87 |

- mean %R=101.05

Table 4. Comparison of the proposed approach with some references

| Method | Detection | Principle | Linear Range μgL$^{-1}$ | LOD μgL$^{-1}$ | Ref. |
|---|---|---|---|---|---|
| Spectrophotometric | UV-Vis | based on the tetrazotization of benzidine with nitrite, to form orange-colored azo dye shows an absorption maximum at 463.5 nm | 200-2800 | 4.9 | [8] |
| Spectrophotometric | UV-Vis | based on the oxidation of brilliant cresyl blue (BCB) by nitrite in an acidic medium | 20-800 | 4.0 | [24] |
| CPE and microplate assay | UV-Vis | based on the Griess reaction and the reaction product is extracted into nonionic surfactant Triton-X114 using the CPE technique | 10 - 400 | 2.5 | [25] |
| CPE and HPLC | ion-pair HPLC | based on precolumn derivatization using the Griess reaction and cloud-point extraction (CPE) | 10 - 1000 | 1.0 | [26] |
| Indirect CPE preconcentration | UV-Vis | based on the well known oxidation–reduction reaction of nitrite by iodide ion in acidic medium and cloud point extraction of I3− formed from aqueous solution using Triton X-100 | 1.5 - 150 | 0.42 | [27] |
| Spectrophotometric | UV-Vis | based on the diazotization-coupling reaction of nitrite by 3- nitroaniline and 1-naphthylamine in hydrochloric acid solution at pH 1.0 (Griess reaction). | 10 – 1700 | 0.50 | [28] |
| CPE | UV-Vis | based on the diazo coupling of benzidine with nitrite to form azo dye and preconcentration by CPE | 10 - 1250 | 0.55 | present study |

## 4. Conclusion

The ultra-trace amount of nitrite was determined in a soil sample by a new approach based on the combination of spectrophotometric and cloud point extraction methods; the method gave excellent sensitivity and allowed us to prevent interference. The diazo dye was rapidly formed with very good reproducibility with staple color for about one day, followed by preconcentration of cloudy phase in the CPE procedure. The comparison of the results with other reported references showed the present method was easy, fast, and pleasant with accuracy and sensitivity. This kind of analytical approach can provide confidence in inexpensive and reduced time methods in the determination of nitrite in a wide range of soil samples.

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