Smart Ammonia Analyzer for Detecting Nitrogen-Ammonium Content in Fertilizer

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Abstract. Nitrogen is an essential nutrient required by majority of plants to enhance metabolic processes for growing. Insufficient supply of nitrogen leads to severe plant disorders in flowering and fruiting; however, there are many false fertilizers available in markets. This project is focused on developing a smart method as a means for quality detection of nitrogen fertilizer. The smart ammonia analyzer was constructed on a normal smartphone which filled with Qpython3 software functions as an information processing application to analyze the level of nitrogen-ammonium and Color Grab application to process the RGB image of coloured product reaction of nitrogen-ammonium to be measured. The image of blue indophenol as the coloured product of the nitrogen was developed on a paper containing phenate reagent by releasing nitrogen ammonia from sample. The measurement process using the constructed “Smart Ammonia Analyzer” involving taking image by smartphone camera, measuring image intensity on position with RED readings, and analyzing process to change the RED value to concentration of nitrogen-ammonium. The chemicals used for colour formation of blue indophenol were 0.2 M NaOCl, 0.5 M HCl, 0.003 M MnSO4 M, and 2 M phenate with 0.1 M of NaOH for releasing agent of nitrogen fertilizer as ammonia. The developed smart ammonia analyzer resulted linear relation between the absorbance of blue indophenol image to concentration of nitrogen-ammonium (y = 0.0181x + 0.2834, R² = 0.9726) and has been successfully applied to nitrogen fertilizer. Development of smart ammonia analyzer has been achieved to provide an easy, inexpensive, and fast device for detecting quality of nitrogen fertilizer with satisfactory results.

1. Introduction
Nitrogen is the main component of various important substances in plants, where about 40-50% of the protoplasm content, which is the living substance of plant cells consists of nitrogen compounds [1]. Nitrogen fertilizer is commonly added to soil to improve the availability of nitrogen in the form of nitrate or ammonium in order to give high rates of photosynthesis and plant growth. Meanwhile, there are many false fertilizers available in markets which become hot issue of concern to farmers in buying fertilizer. Therefore the availability of simple and smart method for monitoring the nitrogen content is demanded. The common determination of nitrogen content including Kjeldahl method, UV-Vis spectrophotometric method based on Nessler's reagent and phenate [1-5]. Other method was also reported based on reaction of NH3- o-phthalaldehyde -Na2SO3 [6] reaction. However, these methods have a complex analysis stage and not applicable for field analysis. An approach to simplify this
method was done using paper analytical device (PAD), but this method required scanning, and detection by employing a computer with Image-J software [7]. Phenate reagent has been proven to be selective for nitrogen-ammonia determination spectrophotometrically. Therefore, phenate has been used as a basis for developing of nitrogen analyzer. The principle of this method is that the sample solution containing ammonium is converted to ammonia by the addition of NaOH solution; then the released ammonia is captured with paper moistened with phenate reagents, consisting of NaOCl, HCl, MnSO₄ and phenate or phenol in alkaline conditions [8–9] producing blue colour on the paper. Smartphone has been reported as an easy detection device for analytical measurements [10]. Therefore in this work, a smartphone was explored to be used as a means for detection. The image of blue indophenol as the coloured product of the nitrogen was monitored with a normal smartphone which filled with Qpython3 software functions as an information processing application to analyze the level of nitrogen-ammonium. The smart ammonia was applied to analyze nitrogen content of N-ammonia fertilizer sample and validated by comparing the results with the results obtained from standard UV-Vis spectrophotometric-distillation method.

2. Materials and Method

2.1 Materials and chemical
The materials used are ammonium chloride, (NH₄Cl, Merck), pheno (Merck), NaOH (Sigma), HCl (37%) (Sigma), MnSO₄ (Sigma), H₂SO₄ (98%) (Sigma), NaOCl (Aldrich)15%, Whatman filter paper and NPK fertilizer as samples.

The equipment used was a set of distillation apparatus, 5-50 µL micromile pipettes (Acura 821 adjustable micropipette), 100-1000 µL micromile pipettes (Assipette No. 115/100), UV-Vis spectrophotometer (Shimadzu), and smartphone.

2.2. Smartphone Ammonia Analyzer (SAA)
The smart ammonia analyzer was constructed on a normal smartphone which filled with Color Grab application to process the RGB (RED, GREEN, BLUE) image of the coloured product reaction of nitrogen-ammonium to be measured to become RGB value and Qpython3 software functions as an information processing application to analyze the level of nitrogen-ammonium by converting RGB value to absorbance value which is then correlate to concentration of N-ammonium.

2.3. Principle of Detection Procedure
The detection of nitrogen (N-ammonium) in the sample solution was conducted by inserting sample solution containing N-ammonium into a test tube, followed by the addition of 1 M NaOH, then the test tube is covered with Whatman filter paper moistened with phenate reagents by sequential droppings of 0.003 M MnSO₄, 0.2 M NaOCl, 0.5 M HCl, and 0.2 M phenate solution. After each drop, the paper was allowed to dry before the next drip. The test tube was heated over a Bunsen burner for 5 minutes, and the paper was dripped with 0.2 M NaOCl and allowed to stand until a blue color appeared on the Whatman paper, and the colour image was photographed and analyze with the smartphone ammonia analyzer (Figure 1).
2.4. Optimization of NaOH
Optimization of NaOH was conducted by inserting 0.15 ml of 1% N-ammonium chloride solution into a test tube, followed by the addition of various volume of 1, 3, 5, 7 and 10 mL 1 M NaOH, then covered with Whatman filter paper moistened by sequential droppings of 50 μL of 0.003 M MnSO₄, 50 μL of 0.2 M NaOCl, 50 μL of 0.5 M HCl, and 20 μL of 0.2 M phenate. After each drop, the paper was allowed to dry before the next drip. The test tube was heated over the Bunsen burner for 5 minutes, and the paper was dripped with 50 μL of 0.2 M NaOCl and allowed to stand until a blue color appeared on the Whatman paper. The colour images were photographed and analyze with the SAA. The optimum condition obtained was applied to set other optimizations and the similar procedure was repeated for conducting other optimizations, including NaOCl; MnSO4; phenate; and HCl solutions with volume variation range from 10-35 μL.

2.5 Standard Colour Calibration
Standard colour calibration for N-ammonium was made by preparing a series of concentrations, representing 1 to 15 % N, into test tubes; then conditioned as the optimum conditions obtained from procedure 2.4 to produce series intensity of blue colors which are then photographed and analyzed using the smartphone ammonia Analyzer.

2.6 Method Validation
The smart ammonia analyser was validated by comparing the results obtained in the nitrogen-ammonia analyser with the results obtained from the standard distillation-spectrophotometric UV-Vis method for determining the N content (%) in the NPK fertilizer sample.

3. Results and Discussion
The principle of nitrogen determination is based on the formation of blue indophenol, followed by detection using the developed Smartphone Ammonia Analyzer, SAA. The reagents were optimized in order to obtained optimum volume of each reagents producing maximum intensity of the blue indophenol as the product reaction of nitrogen with phenate reagent. All measurements were done by capturing the colour reaction products of blue indophenol formed on the Whatman paper followed by analysis process using the developed smartphone ammonia analyser. Optimizations were carried out for each of all reagents of NaOH, NaOCl, HCl, MnSO₄, and phenate solutions which correspond to the principle of maximum formation of indophenol blue compounds.

3.1. Optimization of NaOH
The optimization was done in order to find out the adequate volume of NaOH to release ammonia from N-ammonium sample. The result of the optimization of NaOH is shown in Figure 2, where increasing the addition of NaOH increased the blue colour intensity of indophenol with maximum colour obtained at 7 mL of 1 M NaOH.
Figure 2: The effect of NaOH addition on indophenol absorbance

It can be seen from Figure 2 that the addition of 1 mL of NaOH 1M gave a low absorbance with each absorbance intensity of RED 0.233; GREEN 0.230; and BLUE 0.239. This indicates that the available NaOH was not sufficient to free the ammonium ion sample as ammonia, consequently, only a very small amount of indophenol compound was formed. The more additions of NaOH (3-7 mL) gave significant increases in the liberation of ammonia from ammonium with the highest intensity on the volume of 7 mL giving absorbance values of RED 0.549; GREEN 0.365; and BLUE 0.385. The more addition of 10 mL NaOH, the resulting absorbance intensity was relatively constant indicating that all ammonium has been converted into ammonia. Therefore, the optimum volume of 7 mL NaOH 1M was used for further experiments.

3.2 Optimization of NaOCl

The solution of NaOCl functions to the formation chloramine compounds after reacting with ammonia. Figure 3 shows that the addition of 0.05–0.25 mL of NaOCl 0.2 M increased the absorbance with the highest absorbance at the addition of 0.25 mL of NaOCl with absorbances at the intensity of RED 0.650; GREEN 0.462; and BLUE 0.433. The decrease in colour intensity in the addition of 0.3–0.35 mL of NaOCl was due to the fact that NaOCl is an oxidizing agent so that if the volume is excessive, the colour product blue indophenol will be reduced and the colour becomes fade. The optimum NaOCl was found at 0.25 mL which gave the highest absorbance and with this optimum condition was used for further experiments.

Figure 3. The optimization of NaOCl
3.3. Optimization of HCl
The addition of acid serves to condition the formation of chloramine as well as to change the colour of indophenol from yellow to blue which is used as the basis for determining nitrogen. The addition of 0.05 - 0.3 mL HCl showed an increase absorbance of the indophenol blue compound with the highest absorbance at a volume of 0.3 mL with absorbance values of RED 0.487; GREEN 0.377; and BLUE 0.369, however; further addition of HCl did not increase the absorbance of the blue indophenol (Figure 4). This shows that the addition of 0.3 mL of HCl was sufficient as a source of hydrogen (H\(^+\)) ions for the formation of chloramine and consequently the formation of indophenol blue and these conditions are used for further experiments.

![Figure 4. The optimization of HCl](image)

3.4. Optimization of MnSO\(_4\)
The MnSO\(_4\) reagent acted as a catalyst that speeds the reaction between chloramine and phenol to form an intermediate product of p-quinone-chloramine. The optimization of MnSO\(_4\) showing that the addition of 0.003 M MnSO\(_4\) gave no significant increase on the formation of blue indophenol. However, the absorbance of blue indophenol increase with optimal intensity obtained under the addition of 0.20 mL, giving absorbance values of RED 0.549; GREEN 0.389; and BLUE 0.353 (Figure 5). Further addition, 0.25 - 0.35 mL MnSO\(_4\), there was a decrease in the absorbance of the blue indophenol; therefore, 0.20 mL 0.003 M MnSO\(_4\) was used for further experiments.

![Figure 5. Optimization of MnSO\(_4\)](image)
3.5. Optimization of Phenate (Alkane Phenol Solution)
Phenate works as reagent to react with monochloramine to form p-quinone-chloramine which reacts further with the remaining phenol to form blue indophenol. The results of phenate optimization showed that the addition of phenate reduces the absorbance of the blue indophenol compound with the optimum absorbance in the addition of 0.10 mL as shown in Figure 5. Therefore, 0.1 mL of 0.2 M phenate was used for further experiments.

![Figure 6: Optimization of Phenate reagent using RGB readings](image)

3.6. Standard Calibration Curve for N 1-15%
Standard colour chart of nitrogen (N) 1-15 % was made under optimal conditions obtained from previous experiments, 7 ml 1M NaOH; 0.25 mL 0.2 M NaOCl; 0.3 mL0.5 M HCl; 0.2 mL 0.003 M MnSO4 , and 0.2 mL 0.2 M phenate. The standard colour chart was photographed using the smartphone ammonia analyser, read their intensity (RED, GREEN, BLUE) followed by direct changing to absorbance values. This absorbance values were used to draw the calibration curve to obtain linear relationship between concentration of ammonia and absorbance. The obtained calibration curve (Figure 7) shows that the higher nitrogen concentration (%) resulting higher absorbance of blue indophenol. The calibration curve showed the best sensitivity using RED readings, supported by the highest slope value compared to those obtained from GREEN and BLUE readings. The curve performed linear correlation between concentration and absorbance from 1-15 % N with linear equation of: y = 0.0181x + 0.2834 and coefficient determination, R² , close to 1 (0.9726) from the RED readings. Therefore, RED reading was suggested to be used for the analysis of nitrogen ammonia (% N) in fertilizers samples.

![Figure 7: Standard calibration curve of N-ammonia](image)
3.7. Method Validation

In order to investigate the validity of the method, smart ammonia analyser was applied to analyse the nitrogen content in three different fertilizers (A, B, and C). The results are shown in Table 1. The same fertilizer samples were also analysed the nitrogen-ammonia content by standard distillation procedure followed by spectrophotometry for detection. Table 2 demonstrates the measurements results of the three samples using both SAA and the standard methods, where both methods show very good agreement by giving insignificant different results for all of the three samples.

Table 1: Analysis of three fertilizers (A, B, C) using the developed SAA

| Sample | Intensity | Absorbance | Concentration (%) |
|--------|-----------|------------|-------------------|
|        | R         | G          | B                 | R       | G       | B       |       |
| A      | 127       | 133        | 129               | 0.30    | 0.28    | 0.30    | 1.07   |
| B      | 106       | 133        | 142               | 0.38    | 0.28    | 0.25    | 5.41   |
| C      | 88        | 127        | 126               | 0.46    | 0.30    | 0.31    | 9.87   |

Table 2. Validation Test of Smart Ammonia Analyzer to Standard Distillation-Spectrophotometry

| Sample | N Content (%) | Accuracy of The SAA Method (%) |
|--------|---------------|--------------------------------|
|        | Spectrophotometry Visible | SAA  |
| A      | 1.06          | 1.07                           | 100.9 % |
| B      | 5.29          | 5.41                           | 102 %   |
| C      | 10.31         | 9.87                           | 95.73 % |

Data from Table 2 shows that the Smart Ammonia Analyzer (SAA) has high accuracy for analysis of nitrogen-ammonia content in N-ammonium fertilizers.

4. Conclusion

Smart Ammonia Analyzer analytical technique can be used as a new innovation for practical test of nitrogen-ammonium fertilizer content. This technique is inexpensive, simple, and fast for detecting the quality of nitrogen fertilizer with satisfactory results.

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