Spectrophotometric determination of ammonia using ninhydrin assay and kinetic studies

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ARTICLE INFORMATION

ABSTRACT

A spectrophotometric method for the determination of ammonia in aqueous media was investigated. This newly developed method for the reaction of ammonia with ninhydrin found to be simple, accurate and precise. A deep red color product was formed in the presence of sodium hydroxide at wavelength of 508 nm. The effects of variables such as temperature, time, acidity and reagents concentration have been evaluated to select the appropriate conditions for this reaction. Beer's law is obeyed; the calibration curve is linear over the range of 2×10^{-4}-1.4×10^{-3} M of ammonia with regression coefficient (r^2) of 0.994 (n = 7). The calculated molar absorptivity and Sandell's sensitivity values are 1.069×10^3 L/mol.cm and 0.015 $\mu g/cm^2$, respectively. The limits of detection (LOD) and quantification (LOQ) are calculated to be 1.2×10^{-5} and 3.6×10^{-5} M, respectively. The intra-day accuracy expressed as relative error was less than 0.5% with precision (RSD) ranging from 0.49 to 1.55%. The inter-day accuracy ranged (RSD) from 0.49 to 1.90 % with a relative error was less than 1.50 %. The stoichiometry of this reaction was determined to be of 1:2 between ammonia and ninhydrin with first order reaction.

1. Introduction

Ammonia (NH_3) is an important nitrogen species in the natural environment. As a dominant gaseous base in the air, it plays a very important role on the acid-base chemistry of the atmosphere and greatly influences the atmospheric sulfur cycle in the remote marine boundary. The main anthropogenic sources of nitrates in the environment are municipal and industrial wastes and artificial fertilizers. Nitrogen oxides present in the air and originating from natural and anthropogenic sources (Combustion or transportation) after the reactions with water come back to the earth surface in the form of acid rains, other sources of ammonia are the reduction of nitrites and nitrates by hydrogen sulfide, iron(II), humus substances (or other reducing compounds) and, first of all, municipal wastes, industrial wastes and animal farm wastes [1,2]

Ammonia can easily dissolve in water and become ammonium ion (NH_4^+). Determination of ammonia-nitrogen is a critical parameter to evaluate the drinking water safety and possible environmental pollution. High concentrations of nitrogen ions in drinking water and other food products can lead to serious problems and diseases. Methods for the determination of total ammonia (including ammonium) were reviewed in a paper by Seale Nessler's method was used but this method lacks sensitivity and requires the use of toxic mercury compound [3,4].

Kinetic spectrophotometric methods are becoming of great interest in chemical analysis. The application of these methods offered some specific advantages such as improved selectivity, avoiding the interference of the colored and/or turbidity background of the samples, and possibility avoiding the interference of the other active compounds present in the commercial product if they are resisting the reaction conditions established for the proposed kinetic method. The initial rate and fixed time methods were adopted for determination of ammonia in aqueous media [5,6].

The main aim of this research is to develop a simple, analytical procedure. In this work, we try to use ninhydrin for determination of ammonia in synthetic and real samples by optimizing the experimental variables to develop a simple sensitive, specific and selective spectrophotometric method for quantification of NH_3/NH_4^+ in aqueous solution and in wastewater. To the best of the authors knowledge, the use of ninhydrin (2,2-Dihydroxy-1,3-indanedione) as a complexing reagent for the spectrophotometric determination of ammonia in aqueous media has not yet been studied. The ninhydrin was used as a chromogenic reagent for this spectrophotometric method.

2. Experimental

2.1. Material

All chemicals are analytical grade and were used as received. Doubly distilled water was used throughout the study. Doubly distilled water was prepared through a reverse osmosis system then redistilled immediately before use in the presence of potassium permanganate to eliminate organic matter. It was used for the preparation of all needed solutions.

2.2. Selection of optimum wavelength

Standard solution of ammonia of 2×10^{-4} M was taken and mixed with 2×10^{-2} M of ninhydrin, the pH was 10 using NaOH solution and the mixing has done at room temperature with final volume of 25 mL. The reaction was found to be complete when the reaction was allowed to stand for 20 min forming a red colored product. The absorbance was measured by the spectrophotometer. The absorbance was measured at range of wave length of 400-800 nm using UV/Vis spectrometer (UNICAM 900). The absorbance has a good absorption in the visible region and the absorbance reading was not changed after 1 h. It was found that the maximum absorbance is at 508 nm as shown in Figure 1.
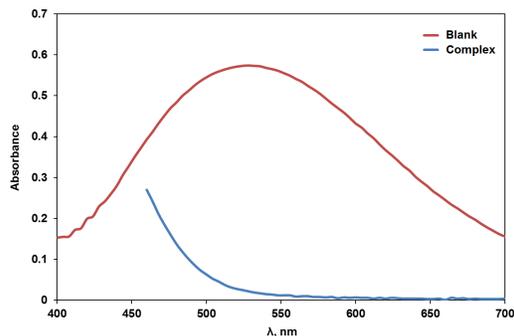

Figure 1. The absorption spectra of (a) Ammonia-Ninhydrin complex, (b) blank.

2.3. Effect of different parameters

In order to develop a method for this study, different parameters were investigated such as initial concentration of ninhydrin, time, temperature, pH, initial concentration of NH₃ and initial concentration of NaOH. Also, the kinetic studies are investigated at optimum conditions.

2.3.1. Effect of ninhydrin initial concentration

Different concentrations of ninhydrin ranged from 8.0×10^{-3}-2.6×10^{-2} M was added to a fixed ammonia concentration of 2×10^{-4} M at pH = 10 using NaOH solution.

2.3.2. Effect of time

A concentration of 8×10^{-3} M from ninhydrin reacts with different concentration of ammonia separately in the range of 2×10^{-4}-1.4×10^{-3} M in sodium hydroxide medium to form a red colored product. The absorbance values were obtained after 10 min. Different time intervals were chosen as follows 2, 4, 6, 8, 10, 12, 14, 16, ..., 60 min. The reactions done at room temperature.

2.3.3. Effect of solution temperature

The investigation of the effect of temperature on the stability of the complex of ammonia with ninhydrin was done,

the following temperature values were selected: room temperature, 25, 30, 35, 40, 45, and 50 °C. Several experiments were carried out while remaining the other conditions constant such as: reaction time 20 min, pH = 10.0, NH₃ = 2×10^{-4} M, Ninhydrin = 8×10^{-3} M, NaOH = 1.6×10^{-2} M.

2.3.4. Effect of pH on the complex stability

Several experiments were carried out to study the effect of pH in the range of 1.0-10.0 using different buffer solutions, while keeping the other conditions constant such as: reaction time 20 min, NH₃ = 2×10^{-4} M, ninhydrin = 8×10^{-3} M, NaOH = 1.6×10^{-2} M and at room temperature.

2.3.5. Effect of initial concentration of NaOH

Different concentrations of NaOH were used in the range of 4.0×10^{-3} to 3.2×10^{-4} M while keeping the other parameters constant to study the effect of the initial concentration of NaOH on the stability of the complex.

2.3.6. Effect of initial concentration of NH₃

Under the optimum conditions of all of the previous parameters, the studying of the effect of initial concentration ammonia in the range of 2.0×10^{-4} to 2.6×10^{-3} M was investigated.

3. Results and discussion

3.1. Optimization of reaction condition

In developing this method, systematic study of the effects of various parameters were undertaken by varying one parameter at a time and controlling all others fixed.

3.2. Effect of ninhydrin initial concentration

The absorbance was measured at room temperature. The results indicate that absorbance increases with the increasing concentrations of ninhydrin solution. But when the added concentration of the ninhydrin up to 2×10^{-2} M and above it was found that the absorption remained constant indicating ninhydrin was sufficient enough to form a complex with 2×10^{-4} M ammonia solution as shown in Figure 2.
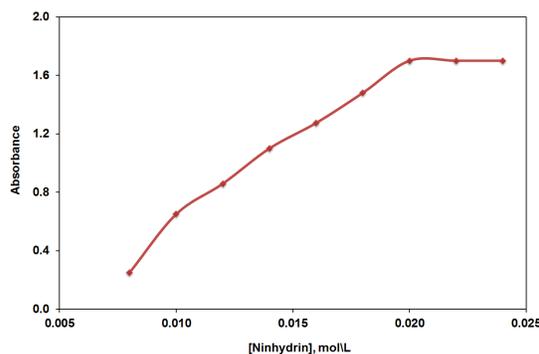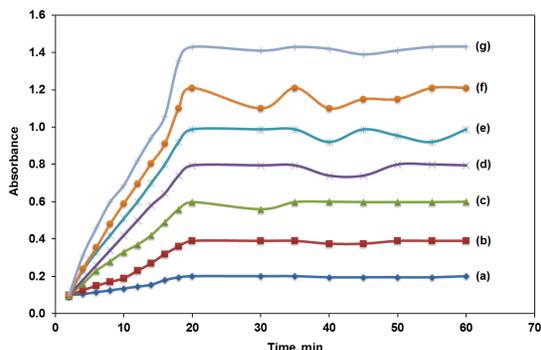

Figure 2. Effect of ninhydrin concentration on the absorbance at λ_{max} of the colored product in NH₃ = 2×10^{-4} M, pH = 10, total volume 25 mL, at room temperature.

3.3. Effect of time

The absorbance values increased with time up to 20 min and red color developed instantaneously with increased intensity and remained stable for the remaining time [7]. The optimum time for measuring of absorbance for all concent-
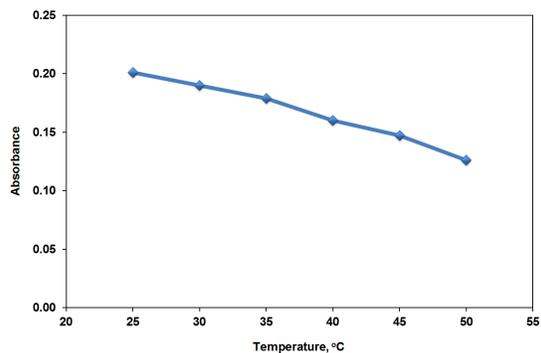
rations of ammonia was found to be about 20 min. The results are illustrated Figure 3.

Figure 3. The effect of time on absorbance at λ = 508 nm of the colored product at different concentrations of ammonia. Conditions: Ninhydrin= 8×10^{-3} M, pH= 10, total volume = 25 mL, at room temperature. Concentrations of ammonia were (a) 2×10^{-4}, (b) 4×10^{-4}, (c) 6×10^{-4}, (d) 8×10^{-4}, (e) 10×10^{-3}, (f) 1.2×10^{-3} and (g)1.4×10^{-3} M.

3.4. Effect of solution temperature

The spectrophotometer has controlled-heating facility so that the absorbance could be simply taken at the desired temperature. From the results that shown in Figure 4, we can see that the absorbance of the colored complex decrease with increasing temperature that may be because of the dissociation of the complex, and this indicated that the reaction is an exothermic one [8].

Figure 4. Effect of temperature on the absorbance at λ = 508 nm of the colored product. Condition: NH_3 = 2×10^{-4}, Ninhydrin = 8×10^{-3} M, pH = 10, Total volume = 25 mL.

3.5. Effect of pH on the complex satiability

It was shown from Figure 5 that the pH of 10 is the most suitable and desired pH for the complexation of ammonia with ninhydrin, because this pH > pKa for ammonia. It was also seen that in acidic media, the complex was less stable. The buffer solutions (Carbonate-bicarbonate buffer) used for pH maintenance did not interfere in actual analysis and provided the desired pH for complex formation [9], so this pH was fixed in all experiments later.
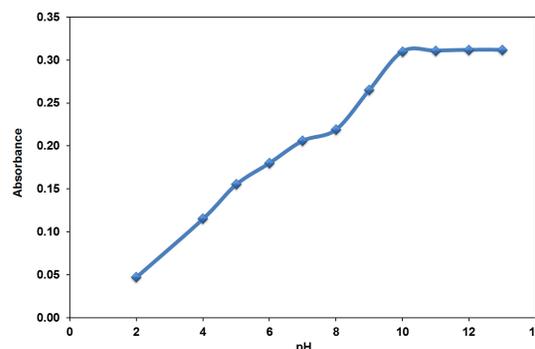
3.6. Effect of initial concentration of NaOH

Different concentrations of NaOH were used in the range of 4.0×10^{-3} to 3.2×10^{-4} M while keeping the other parameters constant to study the effect of the initial concentration of NaOH on the stability of the complex. As shown in Figure 6, the concentration of the maximum absorption of the complex was at concentration of 1.6×10^{-2} M of NaOH, then slight decrease in
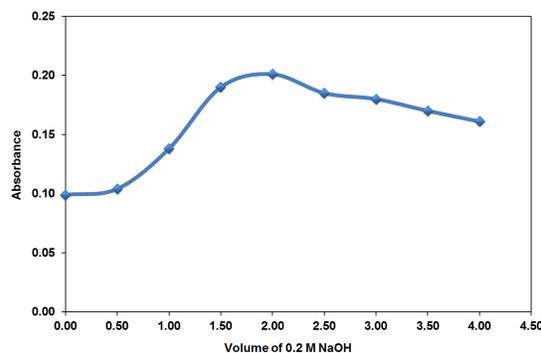
absorbance for concentration larger than this value [10]. Thus, the optimum concentration for NaOH is 1.6×10^{-2} M.

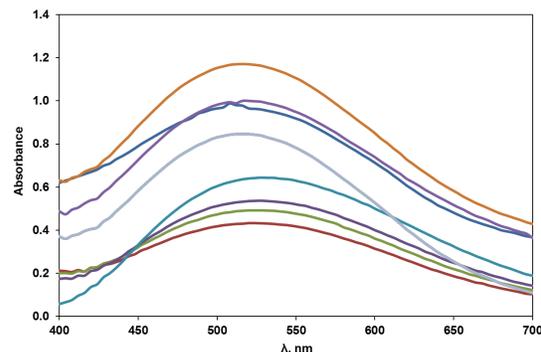### 3.7. Effect of initial concentration of NH_3

Under the optimum conditions of all of the previous parameters, the results of the studying of the effect of initial concentration ammonia are illustrated in Figure 7 and 8. As expected, a more intensely colored solution of sample and reagent yielded a higher absorbance and, in turn, a higher apparent ammonia concentration [11] as shown in Figure 7. From Figure 8, it can be seen that the values of absorbance increase by increasing the concentration of ammonia until reaching the platue for values of concentration larger than 2×10^{-4} M.

Figure 5. Effect of pH on the absorbance value of the reaction product at λ = 508 nm. Conditions: NH_3 = 2×10^{-4}, Ninhydrin = 8×10^{-2} M, t = 20 min, Total volume = 25 mL, at room temperature.

Figure 6. Effect of sodium hydroxide concentration on the reaction product at 508 nm. Conditions: Ammonia 2×10^{-4} M and 2×10^{-2} M ninhydrin measured at room temperature.

Figure 7. Absorption spectra for ammonia concentration on the absorbance at λ = 508 nm of the colored product in Ninhydrin 2×10^{-2} M, pH = 10, t = 20 min, at room temperature, and (a) 2×10^{-4} (b) 4×10^{-4} (c) 6×10^{-4} (d) 8×10^{-4} (e) 1×10^{-3} (f) 1.2×10^{-4} M ammonia.

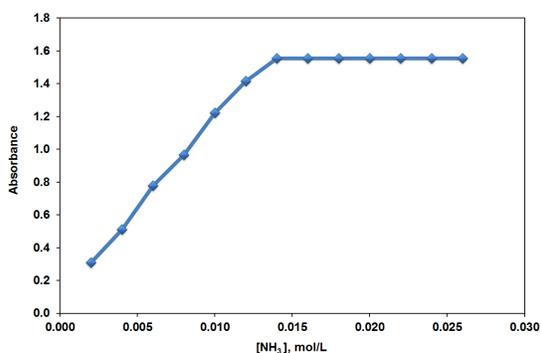**Table 1.** Analytical parameters for the developed spectrophotometric method *.

Parameter	Value
Color	Deep red
λ max, nm	508
Linearity range, M	3.6×10^{-5} - 1.4×10^{-3}
ε, L/mol.cm	1.1×10^{3}
Color stability	20 min
pH	10.00
Temperature, °C	Room temperature
Sandell's sensitivity ($\mu g.cm^2$)	0.01589
Slope (b) ± RSD%	1069.821±3.24
Intercept (a) ± RSD%	0.109±0.01
Standard deviation for the blank (sbl)	3.9×10^{-3}
Standard deviation about regression (Sy)	±0.037
Standard deviation of slope (Sb)	±34.584
Standard deviation of intercept (Sa)	±0.031
Correlation co-efficient (r^2)	0.994
Limit of detection (LOD, M)	1.2×10^{-5}
Limit of quantification (LOQ, M)	3.6×10^{-5}

* The absorbance measured in a glass cuvette of-section is 1 cm^2.

Table 2. Evaluation of intra-day and inter-day accuracy and precision *.

Method	$[NH_3]_{Taken}$, mol/L	$[NH_3]_{Found}$, mol/ L	SD	RSD%	R.E.%
Intra-day accuracy and precision	2.00×10^{-4}	1.99×10^{-4}	3.08×10^{-6}	1.55	-0.5
	4.00×10^{-4}	4.02×10^{-4}	3.39×10^{-6}	0.84	0.5
	6.00×10^{-4}	5.96×10^{-4}	2.91×10^{-6}	0.49	-0.6
Inter-day accuracy and precision	2.00×10^{-4}	2.03×10^{-4}	3.77×10^{-6}	1.9	1.5
	4.00×10^{-4}	4.01×10^{-4}	2.74×10^{-6}	0.68	0.25
	6.00×10^{-4}	5.95×10^{-4}	2.93×10^{-6}	0.49	-0.83

* Main value of five determinations; Tabulated t-value at the 95% confidence level is 2.13; Tabulated F-value at the 95% confidence level is 2.39.

Figure 8. Effect concentration of ammonia on complex product at λ = 508 nm. Ccondition: Ninhydrin = 2×10^{-2} M, NaOH = 1.6×10^{-2} M, pH = 10, at room temperature.

3.8. Method validation

3.8.1. Linearity, detection and quantification limits

The calibration curve was constructed by plotting absorbance against concentration of NH$_3$ under the optimum conditions which are reaction time 20 min, at room temperature, initial concentration of ninhydrin 2×10^{-2} M, initial concentration ammonia range from 2.0×10^{-4} to 1.4×10^{-3} M. The regression analysis of Beer's law data using the method of least squares was made to determine the slope, intercept and the correlation coefficient (r^2) as shown in Table 1. The limits of detection (LOD) and quantification (LOQ), sensitivity parameters such as molar absorptivity, and Sandall's sensitivity are also contained in Table 1.

Nessler method is applied for the analysis of nitrogen in water, it can be detected low concentrations (i.e., 0.4 - 1.0 mg/ L), in our method it was found that the lowest concentration for detection is 0.5 mg/L. Comparing with Nessler method it can be noticed that using ninhydrin for determination of ammonia is good method [12].

3.8.2. Precision

Precision of the method was calculated in terms of intermediate precision (Intra-day and inter-day). Three different concentration of NH$_3$ were analyzed in five replicates during the same day (intra-day precision) and five consecutive days (inter-day precision) [12]. The RSD (%) values of intra-day and inter-day studies showed good precision Table 2. The repeatability within one day (Intra-day) for each concentration of ammonia in terms of RSD% were obtained in the range of (0.49-1.55), with respect to different day (inter-day) for the same concentration of ammonia is RSD% values were obtained (0.49-1.90).

3.8.3. Accuracy

Accuracy of an analytical method is the closeness between the reference value and the found value. Accuracy was evaluated as percentage relative error between the measured concentrations and actual concentrations for NH$_3$ (Bias %) [13]. The results obtained are shown in Table 2, good accuracy for the method was obtained. Statistical analysis using Student's t-test for accuracy (1.83) and *F*-test (1.9) for precision revealed no significant difference between the proposed methods and the t-Table values at the 95% confidence.

3.8.4. Effect of interfering ions

Under the optimized experimental conditions; the effect of foreign ions on the absorbance of the complex of ammonia with ninhydrin, the experiment was done using 2×10^{-4} M of ammonia which was prepared in 25 mL volumetric flasks. 2.0 mL of 0.2 M NaOH and 0.1 M ninhydrin were added to the flask, different salts were used such as NaCl, NaSCN, NaNO$_3$, KCl, KNO$_3$, KI, MgCl$_2$.6H$_2$O, AlCl$_3$ and CaCl$_2$; all of the concentration of ions is 0.1 M, after 10 min ionic salts solution was added successively to complete the volume to 25 mL at room temperature. The contents of flask was mixed well, and then measured the absorbance at $\lambda_{selected}$ the 508 nm.

Table 3. Effect of foreign ions on the amount of ammonia.

Added salt	Tolerance (μg/mL)
NaCl	None
NaNO$_3$	9.4
NaSCN	9.7
KCl	None
KNO$_3$	6.5
KI	1.6
MgCl$_2$	6.5
CaCl$_2$	10.0
AlCl$_3$	12.9

Table 4. Regression equation for NH$_3$ at different fixed time over the range 2 x 10^{-4} to 1.2x10^{-3} M at 508 nm.

Δ A	Time (min) interval	Regression equation	Correlation coefficient (r^2)	LOD, M	LOQ, M
A4-A2	2-4	ΔA = 52.42C-0.000	0.989	2.46×10^{-4}	7.37×10^{-4}
A6-A2	2-6	ΔA = 109.2C-0.000	0.991	1.18×10^{-4}	3.54×10^{-4}
A8-A2	2-8	ΔA = 180.4C-0.004	0.996	7.13×10^{-5}	2.14×10^{-4}
A10-A2	2-10	ΔA = 250.1C-0.010	0.995	5.15×10^{-5}	1.54×10^{-4}
A12-A2	1-12	ΔA = 329.8C-0.016	0.975	3.90×10^{-5}	1.17×10^{-4}
A14-A2	2-14	ΔA = 403.8C-0.027	0.989	3.19×10^{-5}	9.56×10^{-5}
A16-A2	2-16	ΔA = 506.8C-0.042	0.998	2.54×10^{-5}	7.62×10^{-5}
A18-A2	2-18	ΔA = 533.4C-0.008	0.989	2.41×10^{-5}	7.24×10^{-5}
A20-A2	2-20	ΔA = 608.7C-0.003	0.991	2.11×10^{-5}	6.34×10^{-5}

The results shown in Table 3, it was noticed from the table that the negative and positive ions do not make interferences for the absorbance reading and the determination of the complex.

3.9. Stoichiometry of the reaction

The stoichiometric ratio between ammonia and ninhydrin was evaluated by limiting logarithmic method. In this method, two sets of experiments were performed, in the first set the concentration of NH$_3$ was varied keeping a constant concentration of ninhydrin, while in the second set, the concentration of NH$_3$ was kept constant and the ninhydrin concentration was varied. Log absorbance *vs* log [NH$_3$] and [Ninhydrin] were plotted separately to evaluate the slope of the respective line to determine the order of reaction of the ammonia with respect to ninhydrin or vice versa. The obtained slope from the linear regression equation from limiting logarithmic plot for ammonia reaction is 0.962±0.015. It was noticed that the slope was approximately equal one. From limiting logarithmic plot for ninhydrin reaction, the slope was found to be 1.999±0.353 which is also approximately equal two, which confirmed the molar combining ratio of 1:2 between ammonia and ninhydrin [14].

3.10. Kinetic studies

3.10.1. Initial rate of reaction

The initial rate constant (K) of the reaction were determined from absorbance-time plot by measuring the slopes of the initial tangent to the absorbance-time curves, the linear range from each curve was obtained to get the slope [15], as shown in Table 3. The initial rates of the ammonia reaction would follow a pseudo-first order, and were found to obey the following equation:

$$\log K = \log k' + n\log C \qquad (1)$$

where *K* is the reaction rate, k′ the apparent rate constant, C the molar concentration of ammonia, and n (slope of the regression line) which represents the order of the reaction, a straight line was obtained from the plot of the logarithm of the initial rate (log K) of the reaction *vs* logarithm of molar concentration of ammonia (log C). The slope value of 1.005 (≈1) was obtained which confirming the first order reaction. At 95% confidence level, the slope is (1.005±0.13) and the intercept (1.507±0.41) and r^2 of 0.946 [16,17]

3.10.2. The fixed-time method

In this method, the reaction rates were determined for different concentrations of NH$_3$ over the range of 2.0×10^{-4}-1.4×10^{-3} M at a pre-selected fixed-time, which was accurately determined, the absorbance was measured. Calibration curves of absorbance (ΔA = A$_t$-A$_2$) versus initial concentration of ammonia were established at fixed times of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 min. The regression equations assembled in Table 4 and it is clear that, the slope increases with time and the most acceptable values of the correlation coefficient (r^2) was obtained for time interval measurement at 2-16 min. Also it was noticed that the highest slope was obtained when the calibration graphs in interval (A$_{20}$-A$_2$) min and also the lowest detection limits were obtained with fixed times (A$_{20}$-A$_2$) but the detection limit is not required to be part of the validation, for that the recommended fixed time will be 20 min. The calibration graph was linear in the range of 2×10^{-4} to 1.2×10^{-3} M. Validation of the proposed method was evaluated by statistical evaluation of the data, it was found that the standard deviation of the residual (Sy/x) is 2.13×10^{-2}, the standard deviation of the intercept (S$_a$) is 2.15×10^{-2} and standard deviation (S$_b$) of the slope is 27.64 [17,18].
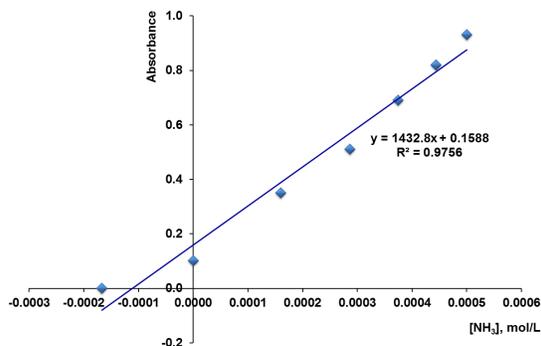
3.11. Field application

In order to assess the validity of the method, water samples were collected from the different cleansing agents industries in Jordan. The ammonium samples were preserved by adding several drops of chloroform. The samples were analyzed by the standard addition method. It was found that industrial wastewater did contained ammonia; therefore, recovery of ammonia was checked by adding known concentration of ammonia to a fixed volume of test solution. The method of standard addition is an important alternative to the typical Beer's Law method when the unknown sample contains a complex matrix that influences the sensitivity of the analyte. In the standard addition version of the calibration curve, each of the standards is spiked with the same quantity of unknown [19] A plot of absorbance *vs.* concentration added to the unknown is produced, and the concentration of the unknown is given by the x-intercept was found to be 1.3×10^{-4} M as shown in Figure 9. The accuracy and validity of the proposed method were further ascertained by performing recovery studies. Pre-analyzed for industrial wastewater was spiked with pure NH$_3$ at three concentration levels (2×10^{-4}, 4×10^{-4} and 6×10^{-4} M) and the total was found by the proposed method.

Table 5. Results of recovery study by standard-addition method.

Study No	Concentr. NH_3 (M) for industrial water	Concentr. NH_3 (M) added	Concentr. NH_3 (M) found *	Recovery % Percent* ±SD
1	1.3×10^{-4}	2.0×10^{-4}	3.0×10^{-4}	89.00±1.81
2	1.3×10^{-4}	4.0×10^{-4}	5.1×10^{-4}	95.00±0.78
3	1.3×10^{-4}	6.0×10^{-4}	7.0×10^{-4}	96.60±0.39

* Mean value of three determinations.

Figure 9. Calibration curve of concentration added to the unknown aliquot *vs*. absorbance.

In all cases, the added NH_3 recovery percentage values ranged between 89 and 96.6% with relative standard deviation of 2.61%. The results of this study given in Table 5 indicated good recovery and no interference in the determination of the co-formulated substances [20].

4. Conclusions

The proposed method is simple, fast and inexpensive, sensitive, both precise and accurate for determination of NH_3 under optimum conditions like (concentration of ammonia, concentration of ninhydrin, concentration of NaOH, pH, time, and temperature). Kinetic spectrophotometric method for the determination of NH_3 has been successfully developed and validated. This proposed method is selective, because it involves measurements in visible region, with low detection limit and relatively high working range. It is sensitive kinetic spectrophotometric method using different approaches: the reaction rate method, rate constant and fixed time methods to select the best interval time for measurement. These advantages encourage the application of the proposed method for the determination of ammonia from real field.

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