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# APPLICATIONS OF NINHYDRIN: SPECTROPHOTOMETRIC DETERMINATION OF HYDRAZINE AND HYDROXYLAMINE

## (Aplikasi Ninhidrin: Penentuan Spektrofotometri bagi Hidrazin dan Hidroksil Amina)

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### Abstract

A rapid, accurate, and sensitive spectrophotometric method for the determination of micro amounts of hydroxylamine and hydrazine is described. The proposed method is based on the interaction of ninhydrin with the amino group of hydroxylamine and hydrazine. Hydroxylamine reacted with ninhydrin as a chromogenic reagent in phosphate buffer at pH 9.14, forming a violet complex with a maximum absorbance at 375 nm after heating for 10 min at 70 °C. Hydrazine reacted with ninhydrin upon heating for 10 min at 85 °C in the presence of phosphate buffer (pH 9), giving a red-brown complex with a maximum absorbance at 425 nm. The optimum conditions affecting the method, such as pH and buffer, sequence of addition, time, temperature, organic solvent, and surfactant, were studied. The complexes of ninhydrin with hydroxylamine and hydrazine were formed in a molar ratio of 1:2. The hydroxylamine–ninhydrin and hydrazine–ninhydrin complexes obeyed Beer's law in a concentration range of 0.033-3.3 and 0.130-3.25 µg mL$^{-1}$, respectively. Molar absorptivity and Sandell's sensitivity were $0.21508 \times 10^{2}$ L mol$^{-1}$ cm$^{-1}$ and 1.53 µg cm$^{-2}$, respectively, for hydroxylamine and $0.8583 \times 10^{2}$ L mol$^{-1}$ cm$^{-1}$ and 1.51 µg cm$^{-2}$, respectively, for hydrazine. The limit of detection and quantification was calculated. The interference effect of various foreign ions and substances was also studied. The proposed method was applied for the determination of micro amounts of hydroxylamine in urine and tap water and hydrazine in tap water.

**Keywords:** spectrophotometric method, ninhydrin, hydroxylamine, hydrazine, urine

### Abstrak

Kaedah spektrofotometri yang cepat, tepat dan sensitif untuk penentuan jumlah mikro hidroksilamin dan hidrazin diterangkan. Kaedah yang dicadangkan adalah berdasarkan interaksi ninhidrin dengan kumpulan amino yang terdapat dalam hidroksilamin dan hidrazin. Hidroksilamin bertindak balas dengan ninhidrin sebagai reagen kromogenik dalam medium beralkali pada pH = 9.14 menggunakan penimbal fosfat membentuk kompleks ungu dengan penyerapan maksimum pada 375 nm selepas dipanaskan selama 10 minit pada 70 °C. Hidrazin bertindak balas dengan ninhidrin apabila dipanaskan selama 10 minit pada 85 °C dengan kehadiran penimbal fosfat (pH = 9) memberikan kompleks merah-coklat dengan penyerapan maksimum pada 425 nm. Keadaan optimum yang mempengaruhi kaedah dikaji seperti pH dan penimbal, jujukan penambahan, masa, suhu, pelarut organik, dan surfaktan. Dua kompleks yang terbentuk bagi ninhidrin dengan hidroksilamin dan hidrazin terbentuk dengan nisbah molar (1:2). Kompleks hidroksilamin–ninhidrin dan hidrazin–ninhidrin mematuhi hukum Beer dalam julat kepekatan 0.033-3.3 dan 0.130-3.25 µg mL$^{-1}$, masing-masing. Penyerapan molar dan kepekaan Sandell masing-masing ialah $0.21508 \times 10^{2}$ L mol$^{-1}$ cm$^{-1}$ dan 1.53 µg cm$^{-2}$, untuk hidroksilamin dan $0.8583 \times 10^{2}$ L mol$^{-1}$ cm$^{-1}$ dan 1.51 µg cm$^{-2}$ masing-masing, untuk hidrazin. Had pengesanan dan kuantifikasi telah dikira. Kesan gangguan pelbagai ion dan bahan asing turut dikaji. Kaedah yang dicadangkan digunakan untuk penentuan jumlah mikro hidroksilamin dalam air kencing dan air paip dan hidrazin dalam air paip

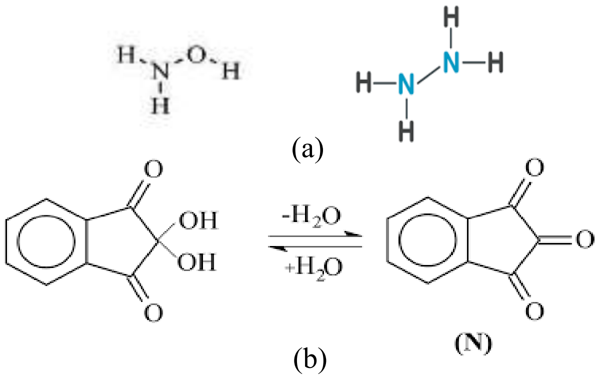
**Kata kunci:** kaedah spektrofotometri, ninhidrin, hidroksilamin, hidrazin, urin

## Introduction

Ninhydrin (2,2-dihydroxyindane-1, 3-dione), a white solid soluble in ethanol and acetone, is generally used to determine ammonia or primary and secondary amines via the formation of Ruhemann's purple as a result of the interaction ninhydrin with these free amines. Ninhydrin has been applied in various spectrophotometric methods for the determination of famotidine in drug formulations [1], cobalt [2], amino acids, peptides, and proteins in agricultural and biomedical fields [3], trace cyanide [4], neomycin sulfate [5], gabapentin [6], and amlodipine besylate [7] in pharmaceutical forms, and glyphosate [8] and ammonia [9].

Hydroxylamine is produced during nitrification and anaerobic ammonium oxidation, which are both essential microbial processes in the nitrogen cycle [10-11]. Hydroxylamine is frequently used as a starting material for the production of pharmaceutical intermediates and drugs [12]; however, hydroxylamine is a well-known mutagen that is toxic to humans, animals, and plants [13], even at low concentration [12]. Many methods have been developed for the determination of hydroxylamine, including spectrophotometry, gas chromatography (GC), and voltammetry methods. For instance, a spectrophotometric method for the determination of hydroxylamine involves its oxidation to nitrite by using sodium arsenate in alkaline conditions. Then the diazo coupling reaction between *p*-nitroaniline and *N*-(1-naphthyl) ethylenediamine dihydrochloride is used to determine the amount of nitrite produced [14]. The use of ferric ammonium sulfate as an oxidant and 1,10-phenanthroline as a chromogenic agent was reported for the determination of the concentration of hydroxylamine in biological wastewater treatment processes [15]. Meanwhile, an indirect spectrophotometric determination of hydroxylamine based on its reaction with iodate was also reported [16]. Another hydroxylamine determination method based on its oxidation to nitrite uses *N*, *N*-dimethyl aniline and *p*-nitroaniline accompanied by micelle-mediated extraction of the produced azo dye for nitrite determination [17]. As examples of GC and voltammetry methods, the conversion of hydroxylamine to nitrous oxide ($N_2O$) allows for the GC indirect determination of hydroxylamine [18], and a carbon-paste electrode modified with $TiO_2$ nanoparticles and quinizarine was reported for the voltammetric determination of hydroxylamine [19].

Hydrazine is an important inorganic base of formula $N_2H_4$, which is a colorless and flammable liquid highly toxic and dangerously unstable in solution [20]. There are a variety of spectrophotometric methods for the determination of hydrazine such as the condensation of veratraldehyde with hydrazine in acidic medium [21]. Hydrazine can be also determined by reducing ferric ion to ferrous ion, which then reacts with 2,2'-bipyridyl at 60 °C to form a stable red-pink complex [22]. Other method for detecting hydrazine is based on its inhibitory effect on the oxidation of Victoria Blue 4- R by $KBrO_3$ [23]. Moreover, hydrazine can be detected using the Firordt method, which consists in its preliminary derivatization with 5-nitro-2-furaldehyde [24]. A flow injection spectrometric method for the determination of hydrazine consists of its reaction with *p*-dimethylamino-benzaldehyde to produce aldazine [25]. The chemical structures of hydroxylamine, hydrazine, and ninhydrin are shown in Figure 1(a, b), respectively.



(a)

(b)

Figure 1. (a) chemical structure of hydroxylamine and hydrazine, (b) ninhydrin

## Materials and Methods

### Reagents and solutions

All the chemicals and solvents utilized were of analytical grade and were used without further purification. The materials used were of the highest quality. Ninhydrin (purity 99.00%) was obtained from BDH Company. Hydroxylamine (purity 99.00%) and hydrazine sulfate (purity 99.00%) were obtained from Sigma-Aldrich. Ethanol (purity 99.99%) was obtained from Elnaser Company, Egypt. Ninhydrin stock solutions ($1 \times 10^{-2}$ M) were prepared by dissolving 0.20 g of ninhydrin in 100 mL ethanol 96%. Hydroxylamine stock solutions ($1 \times 10^{-2}$ M) were prepared by dissolving 0.033 g hydroxylamine in appropriate quantities of distilled water, followed by dilution to 100 mL in a measuring flask. A hydrazine sulfate ($1 \times 10^{-2}$ M) solution was prepared by adding 0.13 g in a 100 mL measuring flask and diluting up to the mark with distilled water.

### Instrumentation

A UV/VIS double beam spectrophotometer (T80 Spectrophotometer, PG instruments Ltd. Company, United Kingdom) was used to measure the absorbance using matched quartz cells with a path length of 10 mm and a width of 2 mm. An Adwa pH meter, model AD 1030, from Romania was used to determine the pH change.

### Buffers

Different types of buffers were prepared according to standard methods, including acetate buffer (pH 3.5-6) universal buffer (pH 1.81-11.98) [26], borate buffer (pH 7.6-10) [27], citrate buffer (pH 3-6), phosphate buffer (pH 7-11) and acetate buffer (pH 3.5-6) [28].

### Surfactant solutions

Different surfactants such as anionic sodium laurate sulfate (SLS), nonionic Triton-X 100, and cationic cetyl trimethyl ammonium bromide (CTAB) were prepared at a concentration of $1 \times 10^{-2}$ M by dissolving the appropriate amounts in 100 mL of deionized water and used without further purification.

### General procedure

In a 10 mL measuring flask, 2.00 mL of hydroxylamine ($1 \times 10^{-2}$ M) or 1.00 mL hydrazine ($1 \times 10^{-2}$ M) was added, followed by 2.00 mL of ninhydrin ($1 \times 10^{-2}$ M), and 2.00 mL of phosphate buffer at pH 9.14 for hydroxylamine and at pH 9 for hydrazine. The measuring flask was heated in a water bath for 10 min at 70 °C for hydroxylamine but at 85 °C for hydrazine, and the absorbance was then measured by scanning the wavelength from 200 to 700 nm against a blank reagent containing 2.00 mL of both ninhydrin and phosphate buffer and 6.00 mL of distilled water.

### Beer's law

Aliquots of hydroxylamine in a concentration range of 0.033-3.3 μg $mL^{-1}$ and hydrazine in a concentration range of 0.0130-3.25 μg $mL^{-1}$ hydrazine were transferred into a series of 10 mL measuring flasks containing 2.00 mL of phosphate buffer at pH 9.14 and 9 for hydroxylamine and hydrazine, respectively, and 2.00 mL of ninhydrin was then added. The absorbance was recorded at 375 and 425 nm for hydroxylamine and hydrazine, respectively. The calibration graph was obtained by plotting the absorbance against the concentration of the studied materials in μg $mL^{-1}$.

### Applications of hydroxylamine and hydrazine: Spectrophotometric determination of hydroxylamine and hydrazine in tap water

Tap water (250 mL) was heated gently until the volume was reduced to 25 mL. In two 10 mL volumetric flasks, 2.00 mL of concentrated tap water was added, followed by 2.00 mL of ninhydrin ($1 \times 10^{-2}$ M) and 2.00 mL of phosphate buffer at pH 9.14 for hydroxylamine and pH 9 for hydrazine, and the mixture was heated in a water bath for 10 min at 70 °C for hydroxylamine and at 85 °C for hydrazine. The absorbance was measured against the blank solution.

### Spectrophotometric determination of hydroxylamine in urine

A urine sample was obtained from two patients, i.e., an adult man (32 years old) and a woman (24 years old), who did not suffer from kidney problems. 50 mL of urine sample was concentrated to 7 mL by gentle heating and then filtrated. The filtrate (0.5 mL), 2.00 mL of ninhydrin (1 × $10^{-2}$ M) and 2.00 mL of phosphate buffer (pH 9.14) were placed in a 10 mL measuring flask using a microburette. The measuring flask was heated in a water bath for 10 min at 70 °C, and the absorbance was measured against the blank at 375 nm.

## Results and Discussion

### Absorption curve

Ninhydrin was used as a new spectrophotometric reagent for the determination of hydroxylamine and hydrazine sulfate on the basis of the reaction of ninhydrin with the amino group of the studied compounds, which produced a violet complex with hydroxylamine and a red-brown complex with hydrazine showing maximum absorbance at 375 and 425 nm, respectively, as shown in the absorption curve depicted in Figure 2**.**

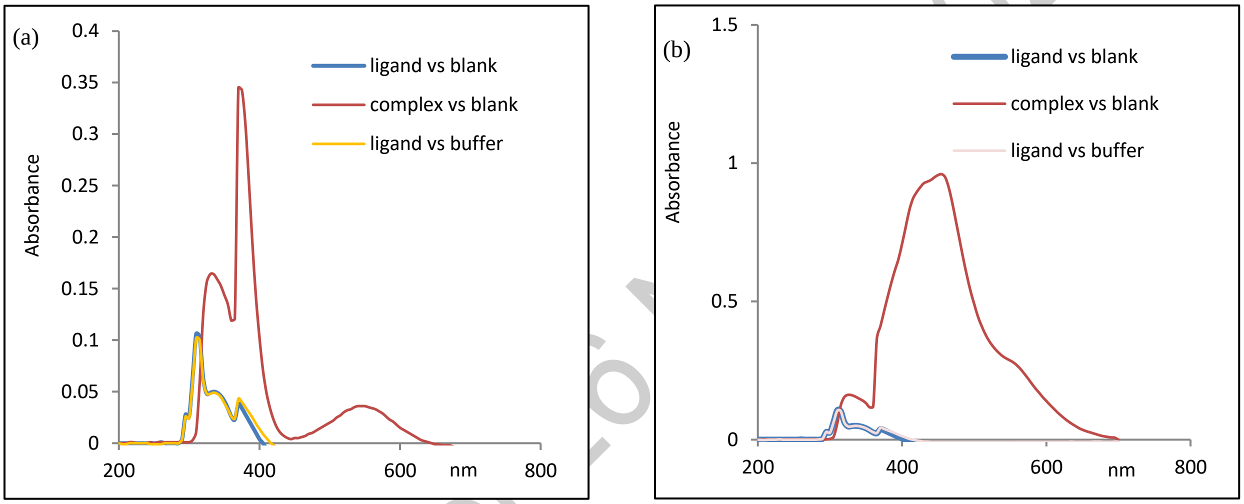


Figure 2. Absorption curves for the complexes of ninhydrin with (a) hydroxylamine and (b) hydrazine

### Effect of pH and buffer solutions

The influence of pH on the formation of complexes between ninhydrin and hydroxylamine or hydrazine was studied in a pH range of 1.26–11 using 0.1 M NaOH and 0.1 M HCl solutions. The pH required for complete complex formation ranged between 9 and 11. To adjust the pH, the influence of different buffer solutions (universal, phosphate, and borate buffers) was studied. The maximum absorbance and color Intensity of the complex was attained using the phosphate buffer at pH 9.14 for hydroxylamine and at pH 9 for hydrazine, and 2.00 mL of phosphate buffer gave the maximum absorbance. Figure 3 illustrates the results.

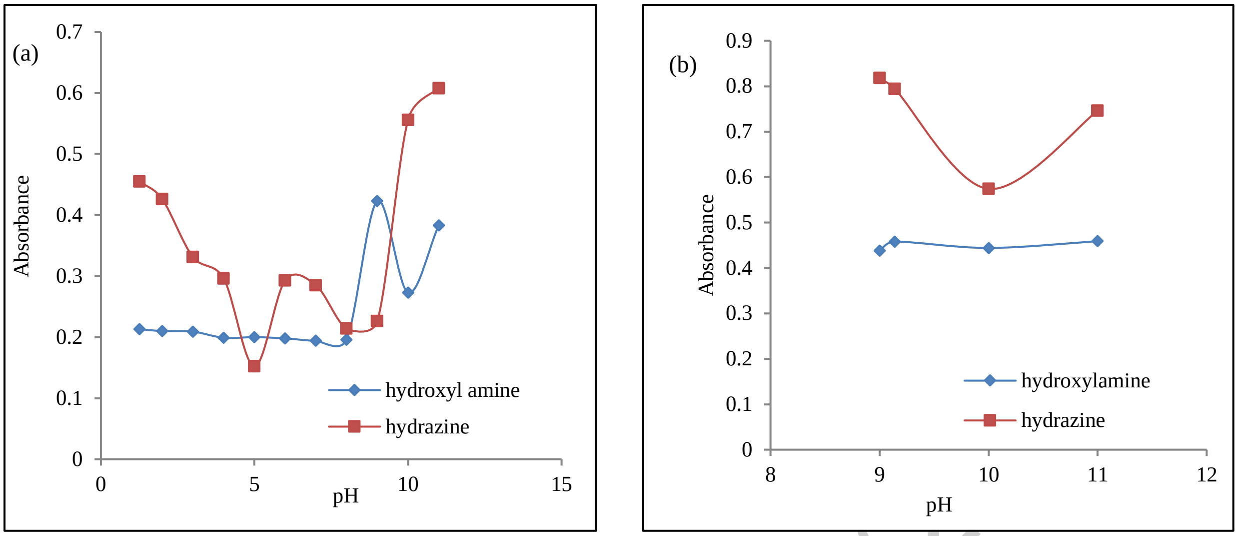


Figure 3. Effect of (a) pH and (b) phosphate buffer on the formation of hydroxylamine and hydrazine complexes with ninhydrin

**Influence of the sequence of addition**

To determine the optimum sequence of addition, the absorbance of different samples prepared by changing the order of addition of hydroxylamine or hydrazine, ninhydrin, and phosphate buffer was recorded. The total volume of the samples was 10 mL which comprised about 2.00 mL of hydroxylamine or hydrazine, 2.00 mL of ninhydrin, 2.00 mL of phosphate buffer, and 4.00 mL of distilled water. The results showed that the most suitable sequence of addition was hydroxylamine or hydrazine – buffer – ninhydrin, as shown in Table 1.

Table 1. Effect of the sequence of addition on hydroxylamine and hydrazine with ninhydrin

| **Sequence of addition** | **Absorbance of Hydroxylamine** | **Absorbance of Hydrazine** |
|---|---|---|
| Hydrazine or hydroxylamine + ninhydrin + buffer | 0.414 | 0.835 |
| Hydrazine or hydroxylamine + buffer + ninhydrin | 0.422 | 0.838 |
| Buffer + ninhydrin + hydrazine or hydroxylamine | 0.420 | 0.816 |
| Ninhydrin + buffer + hydrazine or hydroxylamine | 0.394 | 0.822 |

**Effect of time**

The effect of time on the color intensity of the ninhydrin complexes was studied by measuring the absorbance of the colored solution at different times (2 to 120 min). The results showed that the absorbance of both complexes reached its maximum at two min time and then decreased slightly with time, which indicates that time had no significant effect on the absorbance of the produced complexes, as shown in Figure 4.

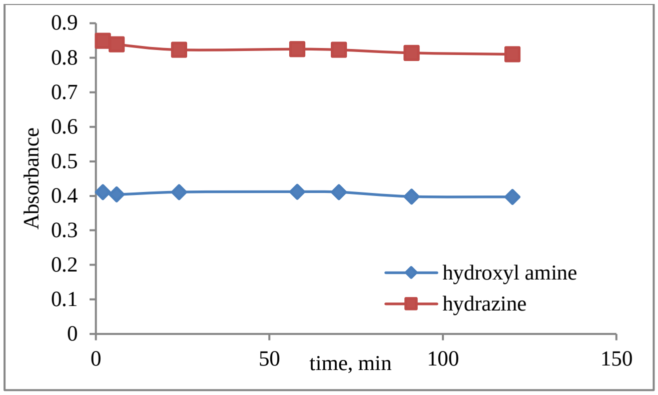


Figure 4. Effect of time on the hydroxylamine and hydrazine complexes with ninhydrin

## Effect of temperature

Different samples were prepared by heating in a water bath at different temperatures to investigate the effect of temperature on the hydroxylamine and hydrazine complexes with ninhydrin, and the maximum absorbance was measured against the blank. The results showed that the hydroxylamine complex reached its maximum absorbance at 70 °C, whereas that of the hydrazine complex was observed at 85 °C (Figure 5).

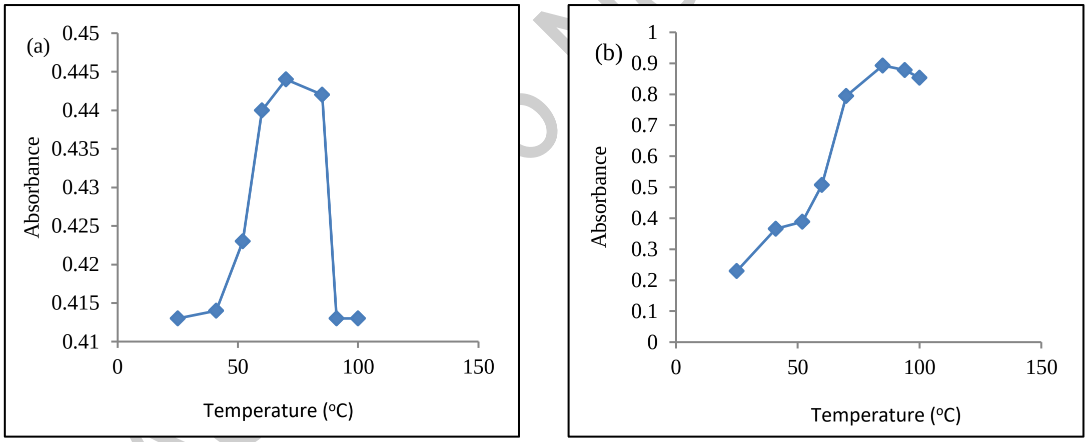


Figure 5. Effect of temperature on the ninhydrin complexes with (a) hydroxylamine and (b) hydrazine

## Effect of the addition of an organic solvent

To evaluate the effect of the addition of an organic solvent, 2.00 mL of various organic solvents were added to the prepared samples and the absorbance was measured against the blank. The results depicted in Figure 6 shows that ethanol, ethylene glycol, and glycerin shifted the absorbance to higher values in the case of the complex with hydroxylamine, whereas the addition of the organic solvents decreased the absorbance of the hydrazine complex.

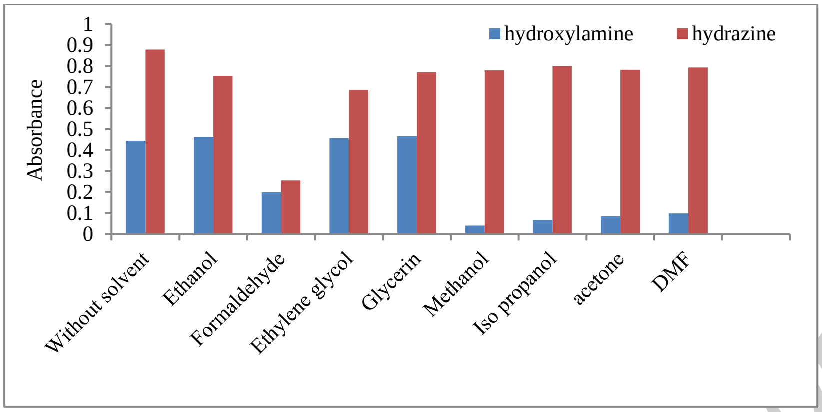


Figure 6. Effect of organic solvent on hydroxylamine and hydrazine complex with ninhydrin

**Effect of surfactant**

To study the effect of a surfactant on the formed complex, 1.00 mL of cationic surfactant (CTAB), nonionic surfactant (Triton X-100), and anionic surfactant (SLS) was added to a sample solution containing 1.00 mL of hydroxylamine or hydrazine (1 × $10^{-2}$ M), 2.00 mL of ninhydrin (1 × $10^{-2}$ M), and 2.00 mL of phosphate buffer at pH 9.14 or 9. Then, the absorbance was measured versus the blank. As shown in Table 2 and Figure 7, the presence of the three surfactants decreased the absorbance.

Table 2. Effect of surfactants on the hydroxylamine and hydrazine complexes with ninhydrin

| **Surfactant** | **Absorbance of Hydroxylamine** | **Absorbance of Hydrazine** |
|---|---|---|
| Without surfactant | 0.444 | 0.878 |
| SLS | 0.136 | 0.792 |
| Triton X-100 | 0.040 | 0.765 |
| CTAB | 0.099 | 0.758 |

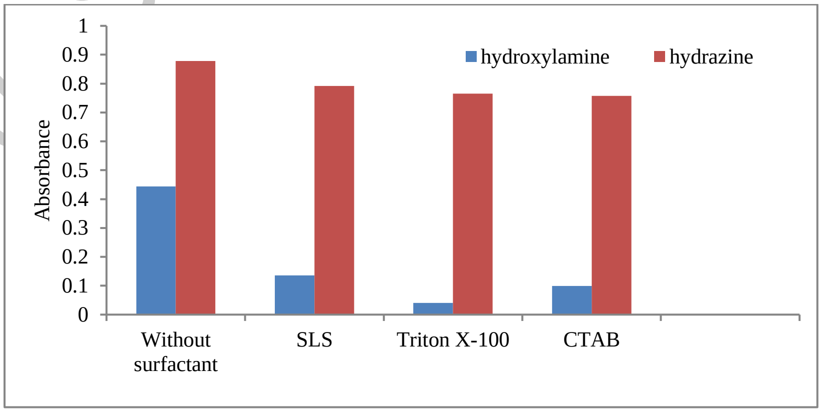


Figure 7. Effect of surfactants on the hydroxylamine and hydrazine complexes with ninhydrin

### Determination of the stoichiometric ratio: The molar ratio method

To determine the precise stoichiometry of the complexes formed between hydroxylamine or hydrazine and ninhydrin, a series of solutions were prepared by varying the amount of 1 × $10^{-2}$ M solution of ninhydrin from 1.00 to 5.00 mL while keeping the amount of hydroxylamine or hydrazine sulfate constant (2.00 mL of 1×$10^{-2}$ M solution). The absorbance of the samples was then measured and plotted against the molar ratio [ninhydrin] / [hydroxylamine or hydrazine sulfate] [29], as shown in Figure 8 revealing that the complexes were formed in a molar ratio of 1:2.

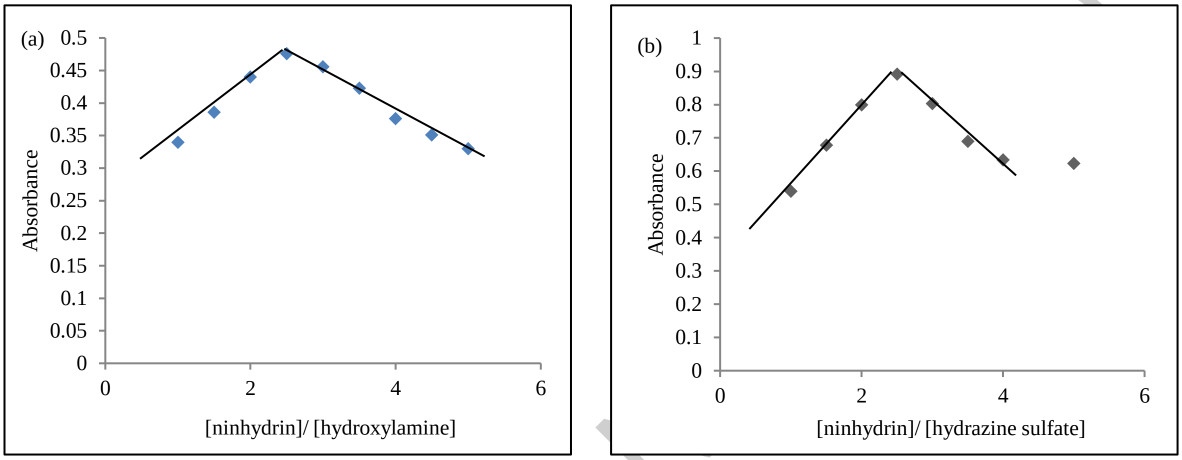


Figure 8. Effect of molar ratio of ninhydrin complexes with (a) hydroxylamine and (b) hydrazine

### The continuous variation method

A series of solutions were prepared by combining an equimolar amount of hydroxylamine or hydrazine with 1 × $10^{-2}$ M ninhydrin in various proportions (0.10–0.90 mL) while keeping the total molar concentration constant, and the absorbance was measured and plotted against the mole fraction [30], as shown in Figure 9.

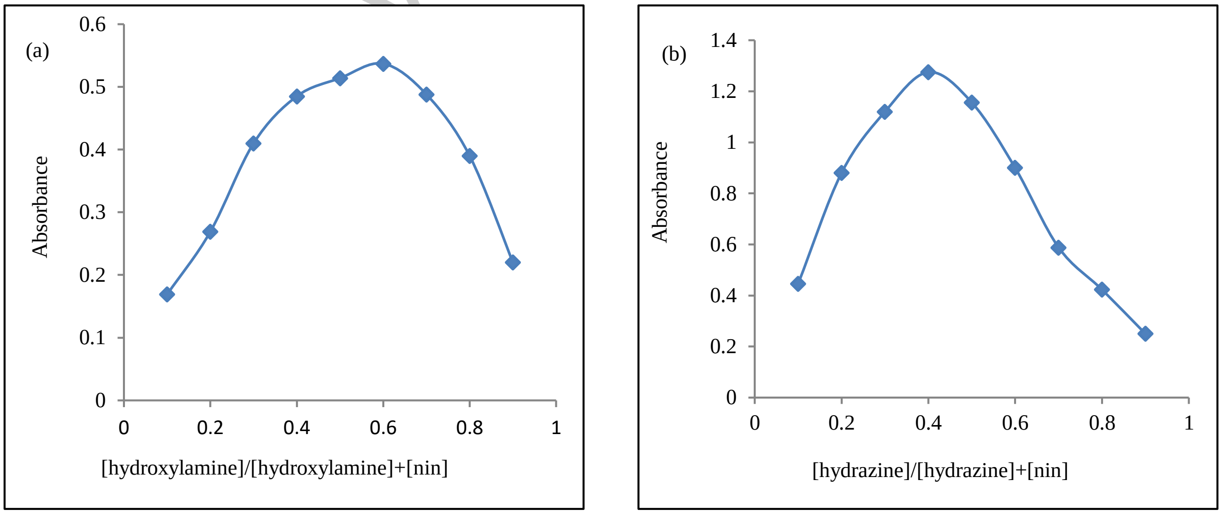


Figure 9. Continuous variation of the ninhydrin complexes with (a) hydroxylamine and (b) hydrazine

### Calibration curve (Beer's law)

To determine whether Beer's law was obeyed in the present system, several solutions were prepared by varying the concentration of hydroxylamine and hydrazine while maintaining the concentration of ninhydrin constant at 1 × 10$^{-2}$ M. The results depicted in Figure 10 show that Beer's law was obeyed in the concentration range 0.033-3.3μg mL$^{-1}$ for hydroxylamine and 0.130-3.25 μg mL$^{-1}$ for hydrazine.

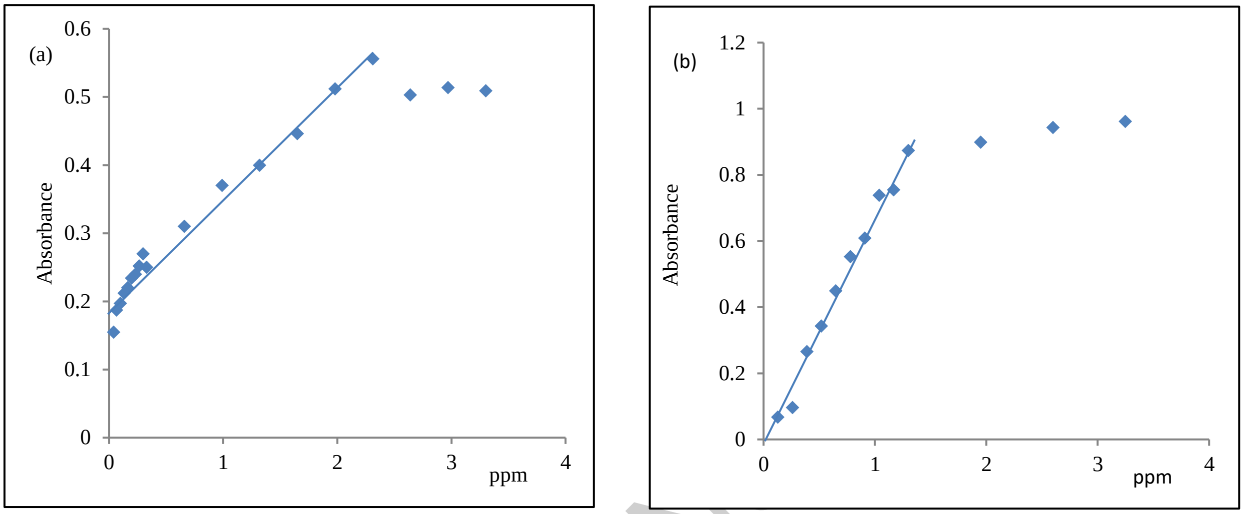


Figure 10. Calibration curves of the ninhydrin complexes with (a) hydroxylamine and (b) hydrazine

### Effect of interferences

A variety of interfering materials such as thiourea, urea, ascorbic acid, sodium nitrate, ammonium sulfate, sodium nitrite, fructose, trisodium citrate, sodium benzoate, magnesium sulfate, and hydroxide ammonia at a concentration of 1 × 10$^{-1}$ M were added to 1 × 10$^{-2}$ M of hydroxylamine or hydrazine to study their interference effect. The results showed that sodium nitrite and hydroxide ammonia caused interference with hydroxylamine and magnesium sulfate caused interference with hydrazine.

### Statistical treatment

Seven measurements were conducted for concentrations of 0.33 and 0.91 μg mL$^{-1}$ of hydroxylamine and hydrazine sulfate, respectively, under the optimum conditions to evaluate the reproducibility of the method. Table 3 summarizes a series of statistical parameters, such as standard deviation, molar absorptivity, Sandell's sensitivity, Student's t-test, correlation coefficient, limit of detection, and limit of quantification. The results indicate that the proposed method exhibited excellent linearity, high sensitivity, and good accuracy and precision. Analytical properties such as pH and buffer, time, temperature, and Beer's law are also listed in Table 3.

Table 3. Statistical treatment and analytical properties for the determination of hydroxylamine and hydrazine using ninhydrin as a chromogenic reagent in the presence of phosphate buffer

| **Parameter** | **Hydroxylamine** | **Hydrazine** |
|---|---|---|
| Molar absorptivity L $\text{mol}^{-1}$ $\text{cm}^{-1}$ | $0.21508\times10^{2}$ | $0.8583\times10^{2}$ |
| Standard deviation (SD) | 0.003134 | 0.004887 |
| (RSD %) | 0.930246 | 0.53136 |
| Standard error of the mean (SE) | 0.0011845 | 0.001847 |
| Student t-test | 5.487 | 5.25144 |
| Intercept | 0.2075 | 0.2477 |
| Correlation coefficient | 0.9427 | 0.8572 |
| Slope | 0.11466 | 0.289801 |
| Limit of detection (µg / mL ) | 0.09019 | 0.0556488 |
| Limit of quantification (µg / mL) | 0.27331 | 0.168632 |
| Complex color | Violet | Reddish-brown |
| Beer's law range (mg $\text{mL}^{-1}$) | (0.033─3.3) | (0.130─3.25) |
| pH and buffer | Phosphate (pH 9.14) | Phosphate (pH 9) |
| Time (min) | 2 | 2 |
| Temperature °C | 70 | 85 |

**Applications of hydroxylamine and hydrazine**

The suggested method was used to determine hydroxylamine in urine, hydroxylamine and hydrazine in tap water. The results in a Table 4 were in good agreement with the recommended method, indicating that the proposed method could be used.

Table 4. Determination of hydroxylamine and hydrazine in urine and tap water

| Sample | **Taken (mg)** | **Found (mg)** | **Recommended Method** | **% Recovery** | **% RSD** |
|---|---|---|---|---|---|
| Hydroxylamine in urine | 2.1 | 2.202 | 2.04 | 99.998 | 0.0022 |
| Hydroxylamine in tap water | 0.32 | 0.31 | 0.317 | 99.9999 | 0.00007 |
| Hydrazine sulfate in tap water | 0.25 | 0.27 | 0.24 | 99.9997 | 0.0003 |

Recommended method according to Watt [31] and Frear [32]

## Conclusion

A simple, accurate, cost-effective, and sensitive method for the determination of microamounts of hydroxylamine and hydrazine has been demonstrated. The method is based on the use of ninhydrin as a chromogenic reagent, which reacts with the amino group of the substrates. The color of the formed complex between ninhydrin and hydroxylamine or hydrazine remains stable for a long time. The use of organic solvents or high temperature is not required. However, some foreign substances interfere with hydroxylamine and hydrazine, leading to a decrease or increase in the absorbance. The feasibility of the method for determining hydroxylamine and hydrazine sulfate in real samples, i.e., tap water and urine, is demonstrated.

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