Research Article

A Spectrophotometric Flow Injection Method for Determination of Ultratrace Amounts of Phenylhydrazine by Its Inhibition Effect on the Reaction of Thionin and Nitrite

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A simple flow injection colorimetric procedure for determining phenylhydrazine was established. It is based on the reaction of phenylhydrazine in sulfuric acid with thionin and sodium nitrite. Reaction was monitored spectrophotometrically by measuring thionin absorbance at $\lambda_{\max} = 602 \text{ nm}$. A standard or sample solution was injected into the sulfuric acid stream, which was then merged with sodium nitrite stream and thionin stream. Optimum conditions for determining phenylhydrazine were investigated by univariate method. Under the optimum conditions, a linear calibration graph was obtained over the range $0.05\text{--}0.60 \mu\text{mol L}^{-1}$, and the detection limit was $0.027 \mu\text{mol L}^{-1}$ ($s/n = 3$). The proposed method has been satisfactorily applied to the determination of phenylhydrazine in human serum and water samples.

1. Introduction

Phenylhydrazine has many side effects. Its absorption through skin causes erosion, burns, and contact dermatitis and if exposures be in toxic dosage, skin contact can produce symptoms in other organ systems. Its inhalation in the form of combustible solid, liquid, and vapor produces cough and dyspnea. Its swallowing causes vomiting, faint, jaundice, and dizziness. Phenylhydrazine too can breakdown red blood cells (RBCs) and produce hemolytic anemia and consequential involvement of other tissues, such as spleen, liver, and kidney injury. Chronic exposure causes adverse effects in Bone Marrow (BM), liver, kidney, and it also increases the cancer risk [1].

Several methods have been reported for the determination of phenylhydrazine. These include spectrophotometry [2–5], chromatography [6], H-point standard addition method [7, 8], kinetic methods [9–12], and electrochemical methods [13–16]. According to our best knowledge, this is the first flow injection (FI) method proposed for the determination of phenylhydrazine. This proposed method

does not require a solvent extraction step; hence, the use of organic solvent is avoided.

This paper studies a rapid, sensitive, and cost-effective flow injection method for the determination of phenylhydrazine based on the spectrophotometric detection by the reaction of phenylhydrazine standard in sulfuric acid, thionin, and sodium nitrite as a sensitizer. Thionin and phenylhydrazine have the following structure (Figure 1). Measurements were made at 602 nm.

2. Experimental

2.1. Apparatus. The 8-channel peristaltic pump (ismatec, MCP process, IP 65,) was fitted for pumping solutions. Silicon rubber tube with 0.8 mm i.d. was used for delivery of the solutions. A mixed solution of thionin, nitrite, and sulfuric acid as a carrier stream was delivered through silicon rubber tubing (at 35°C). The thermostatic water bath (Gallen Kamp Griffin, BGL 240 V) was used at a given temperature of $35 \pm 0.1^\circ\text{C}$. The standard solution of phenylhydrazine

FIGURE 1: Structure of (a) thionin and (b) phenylhydrazine.

was injected into a carrier stream with a sample injector (Rhedyne, model 9125). An UV-Visible spectrophotometer (2501 CECIL) equipped with a flow-through cell with 10 mm path length connected a recorder was used for monitoring the variation in the absorbance spectrum.

2.2. Reagents and Solutions. All chemicals were of analytical reagent grade and were used without further purifications. A $1.0 \times 10^{-3} \text{ mol L}^{-1}$ stock standard solution of phenylhydrazine was prepared by dissolving 0.0147 g of phenylhydrazine chloride (Merck, $M = 144.6 \text{ g/mol}$) in distilled water and diluting it to 100 mL. Solutions of the desired concentrations were obtained by diluting the stock solution to volume with distilled deionize water. A 100 mL of $2.0 \times 10^{-3} \text{ mol L}^{-1}$ sodium nitrite solution was prepared by dissolving 0.0138 g of NaNO_2 (Merck, $M = 69.0 \text{ g/mol}$) in distilled water and diluting it to mark in a 100 mL volumetric flask. A $4.0 \times 10^{-4} \text{ mol L}^{-1}$ thionin solution was directly prepared by dissolving 0.0115 g of thionin (Merck, $M = 287.34 \text{ g/mol}$) in distilled water and diluting it to mark in a 100 mL volumetric flask. Sulfuric acid solution (2.0 mol L^{-1}) was prepared by diluting a known volume of its concentrated solution (Merck). All laboratory glasswares were cleaned by soaking in a detergent solution and acidified solution, followed by washing with concentrated nitric acid and rinsing several times with distilled deionize water.

2.3. Recommended Procedure. Using of this the three channels manifold as shown in Figure 2, a $200 \mu\text{L}$ sample or standard solution containing phenylhydrazine was injected into the reagent stream consisting of 2.0 mol L^{-1} of sulfuric acid and $0.9 \times 10^{-4} \text{ mol L}^{-1}$ of sodium nitrite, which were then merged with $2.5 \times 10^{-4} \text{ mol L}^{-1}$ thionin at the same optimum flow rate of 0.3 mL min^{-1} . Subsequently, the sample zone flowed through the mixing coils no. 1 and 2 with 75 and 125 cm in reaction coil length, respectively, where the reagents to be mixed and flowed through the detection unit. The signal was monitored by the spectrophotometric detector at 602 nm, and the FI signal was recorded on a chart recorder.

2.4. Sample Preparation. Mineralization of 2.0 mL of the human serum was carried out for 1.0 h at 100°C with the addition of 4 mL of concentrated nitric acid [17]. Then

FIGURE 2: Proposed FI manifolds: R_1 , sulfuric acid; R_2 , sodium nitrite; R_3 , thionin; PP, peristaltic pump; MC_1 , mixing coil no. 1; MC_2 , mixing coil no. 2; D, detector; R, recorder; W, waste.

samples were analyzed directly after neutralization with sodium hydroxide solution and dilution with doubly distilled water to a suitable volume.

The water samples were collected in 1.0 L polyethylene bottles from Zayandeh Rood River, Isfahan province and analyzed immediately after sample digestion. The water samples were filtered through Whatman no. 41 filter paper, then 5 mL of each filtered water sample was accurately transferred into a 25 mL round bottom flask and made up to the mark with distilled deionize water, mixed, subsequently analyzed by the proposed FI method.

3. Results and Discussion

The proposed flow system was undertaken for the development of FI procedure for analysis of phenylhydrazine based on the phenylhydrazine with thionin in sulfuric acid and sodium nitrite resulting in having an absorption maximum at 602 nm. The present work was developed and optimized by an univariate method. The variable by variable methods was applied to select the appropriate conditions for the flow injection spectrophotometric determination of phenylhydrazine. To have more signals, the effect of reagent concentrations and manifold variables on the analytical signal was studied.

3.1. Choice of FI Manifold. Preliminary investigation has been carried out to obtain the most suitable FI manifold for phenylhydrazine thionin in a sodium nitrite/sulfuric acid. Three FI manifold have been designed namely a three-channel FI manifold two channels designed FI various and a single-channel FI manifold.

They were tested for determining phenylhydrazine in standard solutions. It was preferable to use the three-channel FI manifold for phenylhydrazine determination since it provided a greatest sensitivity, reproducibility, and relative high sample throughput.

3.2. Effect of Sulfuric Acid, Thionin, and Sodium Nitrite Concentration. The effect of varying concentration of H_2SO_4 solutions between 0.2 – 5.0 mol L^{-1} was examined. The highest peak height was recorded when the concentration H_2SO_4 solution was 2.0 mol L^{-1} and was therefore chosen as optimum concentration. Further increasing of the sulfuric acid

FIGURE 3: Effect of sulfuric acid, thionin, and sodium nitrite concentrations on the mean of peak height ($n = 5$) of $0.1 \mu\text{mol L}^{-1}$ phenylhydrazine standard solution.

concentration makes the peak height decreased gradually up to 5.0 mol L^{-1} (Figure 3).

The concentration of thionin solution was optimized. Various concentrations over the range 0.5×10^{-4} – $4.0 \times 10^{-4} \text{ mol L}^{-1}$ were investigated. It was found that the peak height increased with increasing thionin concentration and reached a maximum peak height at $2.5 \times 10^{-4} \text{ mol L}^{-1}$, above which the peak height decreased. Thus, $2.5 \times 10^{-4} \text{ mol L}^{-1}$ of thionin was used subsequently (Figure 3).

The effect of various concentrations of sodium nitrite solution (0.1×10^{-4} – $1.5 \times 10^{-4} \text{ mol L}^{-1}$), on the absorption of the sodium nitrite (as peak height) was examined. The sodium nitrite concentration which exhibited the greatest peak height was found to be $0.9 \times 10^{-4} \text{ mol L}^{-1}$ and was therefore chosen as optimum concentration. Further increasing of the sodium nitrite concentration makes the peak height decreased gradually up to $1.5 \times 10^{-4} \text{ mol L}^{-1}$ (Figure 3).

3.3. Effect of Mixing Coil Length, Injection Loop Volume, and Flow Rate. These studies were carried out at various mixing coil lengths between 50 and 275 cm for mixing coil 1 (H_2SO_4 , sodium nitrite) and mixing coil 2 (H_2SO_4 , sodium nitrite, thionin), injection loop volumes between 50 and $400 \mu\text{L}$ were investigated. It was found that the peak height increased with the mixing coil 1 and mixing coil 2 lengths up to 75 and 125 cm, respectively. Both mixing coil lengths of 50, 75, 125, 175, 225, and 275 cm provided the peak height (ΔA) of 1.35, 1.90, 1.81, 1.76, 1.74, 1.70 and 1.42, 1.69, 2.09, 1.88, 1.86, 1.81, respectively. The optimum mixing coil lengths of no. 1 and no. 2 for subsequence studied were 75 and 125 cm, respectively.

The influence of the sample/standard volume on the absorbance was recorded by injecting volumes in the range 50 and $400 \mu\text{L}$ of phenylhydrazine standard solution ($0.1 \mu\text{mol L}^{-1}$). It was shown that the peak height (ΔA) increased from 1.51 to 2.33 on increasing the injection

volume from 50 to $400 \mu\text{L}$. It was found that the peak height increased with the injection volumes up to $200 \mu\text{L}$, and the injection volume of 50, 100, 200, 250, 300, and $400 \mu\text{L}$ produced the peak height (ΔA) of 1.51, 1.92, 2.87, 2.32, 2.29, and 2.25, respectively. The appropriate peak height was reached at $200 \mu\text{L}$. The most suitable injection loop volume value for further use was $200 \mu\text{L}$.

The effects of sulfuric acid, thionin, and sodium nitrite solutions flow rates were investigated on the determination of phenylhydrazine standard solution ($0.1 \mu\text{mol L}^{-1}$). The peak heights were monitored from the flow rate of 0.2 – 0.6 mL min^{-1} for all solution streams. The peak height increased with increasing flow rate of each stream up to 0.3 mL min^{-1} for sulfuric acid and sodium nitrite solutions, which were then merged with the flowing stream of thionin solution with the flow rate of 0.3 mL min^{-1} above which the peak height slightly decreased. Thus, 0.3 mL min^{-1} of sulfuric acid, thionin, and sodium nitrite solutions was regarded as the optimum flow rates.

3.4. Analytical Characteristics. Analytical characteristics for the determination of phenylhydrazine were studied under the optimum conditions (Table 1).

3.5. Calibration Curve. Using this the proposed FI manifold for the determination of phenylhydrazine under the optimum conditions, the linear calibration graph over the range of 0.05 – $0.6 \mu\text{mol L}^{-1}$ of phenylhydrazine standard solution was established, which could be expressed by the regression equation $y = 1.0929x + 3.0029$ ($r^2 = 0.9990$) where y represents the peak height (ΔA) and x was phenylhydrazine concentration in $\mu\text{mol L}^{-1}$ after subtraction of blank. Thus, the amounts of phenylhydrazine in samples can be quantified according to the above regression lines of equation. The detection limit was defined as the concentration of analyte that gives the signal that is different from the blank by an amount equal to three times the standard deviation of the blank signal ($s/n = 3$). It was found to be $0.027 \mu\text{mol L}^{-1}$. The quantitation limit (defined as ten time standard deviation) was studied and found to be $0.091 \mu\text{mol L}^{-1}$. It is shown that the present method was very suitable for determining relatively large amounts of phenylhydrazine in human serum and Zayandeh Rood water.

3.6. Reproducibility and Accuracy. The relative standard deviation of the proposed method (peak height (ΔA)) calculated from 5 replicate injections of 0.2 and $0.4 \mu\text{mol L}^{-1}$ of phenylhydrazine was 0.62% and 0.56%, respectively. The recoveries were determined with the standard addition method, in which phenylhydrazine (0, 0.1, 0.2, 0.4, and $0.5 \mu\text{mol L}^{-1}$) was added and mixed with human serum and Zayandeh Rood water samples the human serum and water samples were analyzed using the proposed method. The percentage recoveries of 0.1, 0.2, 0.4, and $0.5 \mu\text{mol L}^{-1}$ ($n = 5$) of phenylhydrazine were found to be between 92.50%–108.00%, showing that the proposed method could provide acceptable

TABLE 1: Variable ranges study and optimum conditions for the determination of phenylhydrazine.

Parameter studied	Range studied	Optimum level
Wavelength (nm)	200–700	602
Sulfuric acid concentration (mol L ⁻¹)	0.2, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0	2.0
Thionin concentration (×10 ⁻⁴ mol L ⁻¹)	0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0	2.5
Sodium nitrite concentration (×10 ⁻⁴ mol L ⁻¹)	0.1, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5	0.9
Flow rate (mL min ⁻¹)	0.2, 0.3, 0.4, 0.5, 0.6	0.3
Mixing coil [I] (cm) [phenylhydrazine + H ₂ SO ₄ + sodium nitrite]	50, 75, 125, 175, 225, 275	75
Mixing coil [II] (cm) [phenylhydrazine + H ₂ SO ₄ + sodium nitrite + thionin]	50, 75, 125, 175, 225, 275	125
Injection volume (μL)	50, 100, 200, 250, 300, 400	200

TABLE 2: Determination of phenylhydrazine in real samples.

Sample ^a	Concentration of phenylhydrazine added (μmol L ⁻¹)	Concentration of phenylhydrazine found (μmol L ⁻¹)	Recovery %
Human serum	0.000	—	—
	0.100	0.108 ± 0.002	108.00
	0.200	0.185 ± 0.003	92.50
	0.400	0.378 ± 0.001	94.50
	0.500	0.527 ± 0.004	105.40
River water (Zayandeh Rood)	0.000	—	—
	0.100	0.105 ± 0.005	105.00
	0.200	0.193 ± 0.002	96.50
	0.400	0.389 ± 0.005	97.25
	0.500	0.521 ± 0.007	104.20

^a Average from five determinations.

TABLE 3: Effect of foreign ions on the determination of 8 nM phenylhydrazine.

Foreign species	Tolerated ratio $W_{\text{Species}}/W_{\text{Phenylhydrazine}}$
NO ₃ ⁻ , SO ₃ ²⁻ , Cl ⁻ , BO ₃ ³⁻ , ClO ₃ ⁻ , Br ⁻ , IO ₄ ⁻ , SCN ⁻ , S ₂ O ₈ ²⁻ , CO ₃ ²⁻ , PO ₄ ³⁻ , I ⁻ , CH ₃ COO ⁻ , IO ₃ ⁻ , Na ⁺ , K ⁺ , Ba ²⁺ , Fe ²⁺ , Cr ³⁺ , Mn ²⁺ , Hg ²⁺ , Cu ²⁺ , Al ³⁺ , Ag ⁺ , Zn ²⁺	1000
Mg ²⁺ , Mo ⁶⁺ , V ⁵⁺ , Rh ³⁺ , S ₂ O ₃ ²⁻ , Pd ²⁺ , Ru ³⁺	800
Ni ²⁺ , Se ⁴⁺	500
Pb ²⁺ , Co ²⁺	200
Cd ²⁺	100
N ₂ H ₄	50

method efficiency, and recoveries of the added phenylhydrazine by this analysis method were satisfactory (Table 2).

3.7. Interferences. In this stage, the influence of contaminant species presented in various samples for the determination of phenylhydrazine, 0.1 μmol L⁻¹, was investigated. The tolerance limit was defined as the concentration of added ions causing a relative error less than 3% (Table 3). The results show that the developed method is very selective.

4. Conclusions

The proposed FI spectrophotometric method has proven to be simple and sensitive for phenylhydrazine determination. The linearity of the calibration graph is in the useful concentration range for quantitation of phenylhydrazine in human serum and water samples. The method developed simple, economic, rapid, providing a good sample frequency of 40 h⁻¹ and is especially suitable for routine analysis.

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