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Spectrophotometric Determination of Trace Amounts of Phenylhydrazine in Water and Biological Samples After Preconcentration by the Cloud Point Extraction Method

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A cloud point extraction method for preconcentration of ultra-trace quantities of phenylhydrazine as a prior step to its determination by spectrophotometry has been developed. The method is based on the extraction of phenylhydrazone, the colored product of the reaction of phenylhydrazine with *p*-nitrobenzaldehyde, in the presence of electrolyte as an inducing phase separation factor. The anionic surfactant sodium dodecyl sulfate (SDS) and nonionic surfactant Triton-X-114 were used as mixed micelle-mediated. The phenylhydrazone concentrated in surfactant rich phase was then determined spectrophotometrically at 427 nm. The optimal extraction and reaction conditions (*e.g.* surfactant, reagent and electrolyte concentrations and centrifuge time) were studied and the analytical characteristics of the method (*e.g.* limit of detection, linear range, preconcentration, and improvement factors) were obtained. Linearity was obeyed in the range of 4.0-600 ng mL^{-1} of phenylhydrazine and the detection limit of the method is 1.80 ng mL^{-1}. The interference effect of some cations, anions, and organic compounds was also tested. The method was successfully applied to the determination of phenylhydrazine in water and biological samples.

Key Words: Cloud point extraction, Spectrophotometry, Phenylhydrazine, Mixed micelle, Preconcentration.

INTRODUCTION

Phenylhydrazine is the hydrazine derivatives with the formula $C_6H_5NHNH_2$, that is used mainly in the preparation of indoles which are intermediates in the synthesis of various pharmaceutical, dyes, agrochemical and chemical industries. Phenylhydrazine is toxic by single exposure *via* the oral route (LD_{50} 80-188 mg/kg body weight) and by the inhalation and dermal routes[1-3]. Phenylhydrazine reacts readily with carbonyl group, -C=O, which is common among biological molecules. It also interacts with haemoglobin and cytochrome P-450 in an oxidation reaction, resulting in the generation of destructive free radicals, which are responsible for subsequent haemolysis, therefore, exposure to phenylhydrazine cause damage to red blood cells, potentially resulting in anaemia and consequential secondary involvement

of other tissues, such as the spleen, liver and kidney injury[4-7]. The National Institute for Occupational Safety and Health (NIOSH) and the Occupational Safety and Health Administration (OSHA) recommended that the permissible exposure limit of phenylhydrazine in workplace air should not be exceeded from 0.14 μg mL^{-1} at any time[8,9]. Because of the environmental and toxicological significance of phenylhydrazine, sensitive and reliable analytical methods are necessary for preconcentration and determination of phenylhydrazine in samples.

Several instrumental methods for determination of phenylhydrazine by titrimetry[10], gas chromatography[11] and spectrophotometry[12-20] have been reported.

Spectrophotometric methods offer many appealing characteristics, including simple instrumentation, rapid response times and easy operation. These properties are highly desirable to the future design and development of portable analytical devices capable of quickly responding to trace levels of hazardous compounds in the field. The lowest determination limit of 0.05 μg mL^{-1} has been reported for phenylhydrazine by spectrophotometric method based on inhibition effect of phenylhydrazine on the reaction of bromate with hydrochloride acid in presence of methyl orange[18]. Therefore, for spectrophotometic determination of trace amounts of phenylhydrazine a suitable enrichment procedure prior to its determination is necessary. To the best of our knowledge, there is no report on the preconcentration of phenylhydrazine.

Aqueous solutions of non-ionic surfactants become turbid when they are heated above the temperature known as the cloud point[21]. The solution is then separated into two isotropic phases, *i.e.* a surfactant-rich phase and a bulk aqueous phase. The hydrophobic solutes can be enriched into the surfactant-rich phase. The small volume of the surfactant-rich phase obtained with this methodology permits the design of extraction schemes that are simple, cheap and have lower toxicity than extraction with organic solvents[22].

The mixed micelle-mediated extraction (mixed-MME) is becoming an important and practical application of the use of surfactants in analytical chemistry[23,24]. Cloud point phenomenon is generally observed in nonionic surfactant micellar solutions when the temperature of the system is raised to a certain value. It was reported that the cloud point of Triton X-114 increased on adding small amounts of either cationic surfactant cethyl trimethylammonium bromide (CTAB) or anionic surfactant sodium dodecyl sulfate (SDS)[25]. Mixed surfactants of different charges in order to accomplish both ideal hydrophobic and non-ideal electrostatic interactions within the same extraction system. The use of cationic surfactants in combination with non-ionic surfactant has been documented with an increase in the extraction efficiency of polar organic compounds[26,27]. Moreover, the cloud point of a mixed solution of Triton-X-114 and ionic surfactants decreased when small amounts of inorganic salts were added. The decreasing effect depended on the nature and concentration of the salt and salting out effect[28,29].

The purpose of this study is to propose a method for the spectrophotometric determination of phenylhydrazine after preconcentration in a simple cloud point extraction process. The method is based on the condensation reaction of phenylhydrazine with *p*-nitrobenzaldehyde and phenylhydrazone formation and cloud point extraction of phenylhydrazone product in the presence of NaCl electrolyte in mixed surfactant media. To the best of my knowledge, there is no report in literature on the preconcentration of phenylhydrazine using mixed-micelle-mediated extraction.

EXPERIMENTAL

A Hitachi model 3310 UV-Vis spectrophotometer with 1 cm quartz cells (1.0 mL) was used for recording absorbance spectra. A centrifuge with 10 mL calibrated centrifuge tubes (Hettich, Germany) was used to accelerate the phase separation process.

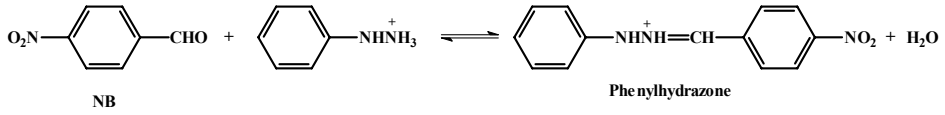
All the solutions were prepared using reagent grade substances and triply distilled water. The surfactants, Triton-X-114 and sodium dodecyl sulfate (SDS) (Merck) were used without further purification. A stock solution of phenylhydrazine (1000 μg mL^{-1}) was prepared by dissolving 0.1338 g of phenylhydrazine hydrochloride (Merck) in water and diluting to the mark in a 100 mL volumetric flask. A 2.0 % (w/v) SDS was prepared by dissolving 2.0 g SDS (Merck) in water and diluting to the mark in a 100 mL volumetric flask. A 0.0250 M *p*-nitrobenzaldehyde solution was prepared by dissolving 0.3775 g *p*-nitrobenzaldehyde in 2.0 % (w/v) SDS and diluting to the mark with SDS solution in a 100 mL volumetric flask.

Procedure: An aliquot of the solution containing 40-6000 ng (0.37-55.56 nmol) of phenylhydrazine and 1.0 mL of 0.025 M *p*-nitrobenzaldehyde solution were transferred into a 10 mL tube. The solution was diluted to *ca*. 7 mL with water and allowed to stand for 10 min at room temperature. Then 1.0 mL of 2.0 % (w/v) of Triton-X-114 solution and 2.0 mL of 25 % (w/v) NaCl solution were added. The solution was taken up to the mark with triply distilled water. Separation of two phases was accelerated by centrifugation for 5 min at 3500 rpm. The mixture was cooled in an ice-salt bath to increase the viscosity of the surfactant-rich phase, and the aqueous phase was easily decanted by simply inverting the tube. The surfactant rich phase of this procedure was dissolved and diluted to 1.0 mL with ethanol and transferred to 1.0 mL quartz cell for absorbance measurement at 427 nm.

Preparation of biological samples: Human serum was separated from blood by centrifugation at 3500 rpm for 10 min. A 1 mL of human serum was transferred into 100 mL volumetric flask and diluted to the mark with distilled water and stored in a refrigerator in the dark. For preparation of human urine, 1 mL urine was transferred into 100 mL volumetric and diluted to the mark with distilled water. The 1 mL aliquots of serum and urine were subjected to the cloud point extraction methodology as described above.

RESULTS AND DISCUSSION

In the SDS micellar media, the condensation reactions of aromatic aldehydes with hydrazine derivatives produce color products. Condensation of *p*-nitrobenzaldehyde with phenylhydrazine affording phenylhydrazone product, proceed according to stoichiometric equation given below:



SDS micellar media strongly enhance the rate and equilibrium constants of the above reactions[16]. Phenylhydrazone product shows an absorption spectrum with maximum absorbance at 427 nm. It was observed that addition of the neutral surfactant Triton-X-114 in the presence of NaCl salt makes the solution turbid. Therefore, the phenylhydrazone product can be extracted by cloud point extraction method. The absorption spectrum of phenylhydrazone in surfactant-rich phase shows a maximum absorbance at 427 nm. After separation of surfactant-rich phase, the absorbance was measured in 427 nm against a reagent blank as the reference (Fig. 1).

Optimization of the system: To take full advantage of the procedure, the reagent concentrations and reaction conditions must be optimized. Various experimental parameters were studied in order to obtain optimized system. These parameters were optimized by setting all parameters to be constant and optimizing one each time. This optimization procedure may not lead to the actual optimum, although it certainly leads to an improvement of the analytical method.

The effect of *p*-nitrobenzaldehyde concentration on the extraction and determination of phenylhydrazine was investigated in the range $2.5 \times 10^{-4} - 4.0 \times 10^{-3}$ M. As Fig. 2 shows, the absorbance increased by increasing *p*-nitrobenzaldehyde concentration up to 2.5×10^{-3} M and remained nearly constant at higher concentrations. Therefore, a concentration of 2.5×10^{-3} M *p*-nitrobenzaldehyde was selected as the optimum.

The effect of pH on the reaction of phenylhydrazine with *p*-nitrobenzaldehyde was studied in the range 1.0-10. As Fig. 3 shows, for the reaction of phenylhydrazine with *p*-nitrobenzaldehyde the absorbance increased by increasing pH up to 7.0 and decreased slowly at higher pH. Therefore, pH 7 was used as optimum concentration for the reaction of phenylhydrazine with *p*-nitrobenzaldehyde.

The effect of SDS concentration on the extraction and determination of phenylhydrazine was studied in the range 0.03.0.4 % (w/v). As Fig. 4 shows, sensitivity of method increased by increasing SDS concentration up to 0.2 % (w/v) and decrease at higher concentrations. Therefore, a concentration of 0.2 % (w/v) SDS was selected as optimum.

It was observed that Triton-X-114 concentration as a non-ionic surfactant can be affect the extraction of phenylhydrazone. The effect of Triton-X-114 concentration on the absorbance of the extracted phase was investigated. As Fig. 5 shows, the

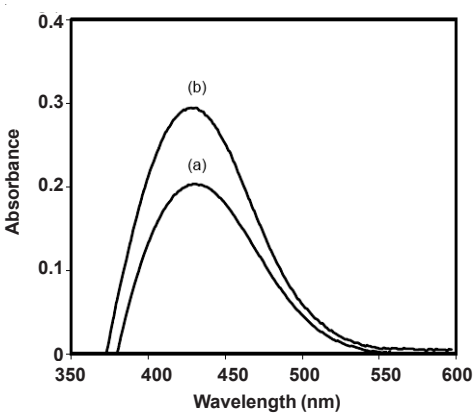


Fig. 1. Absorption spectra of phenylhydrazone (a) 1000 ng mL^{-1} phenylhydrazine before CPE (b) 100 ng mL^{-1} phenylhydrazine after CPE, Conditions: NB, 2.5×10^{-3} M; SDS, 0.2 % (w/v); Triton-X-114, 0.2 % (w/v); NaCl, 5 % (w/v) and pH 7.0

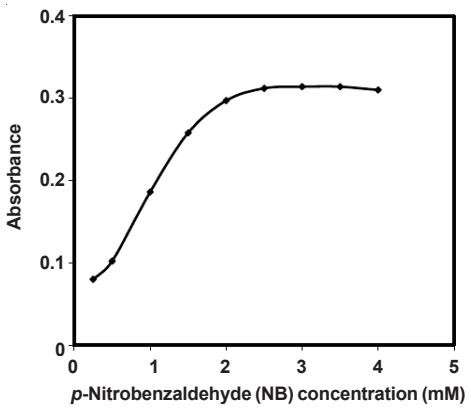


Fig. 2. Effect of NB concentration on the absorbance system after CPE, Conditions: Phenylhydrazine, 100 ng mL^{-1}; SDS, 0.2 % (w/v); Triton-X-114, 0.2 % (w/v); NaCl, 5 % (w/v) and pH 7.0

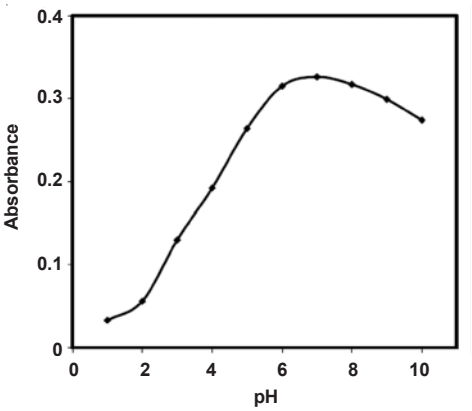


Fig. 3. Effect of pH on the CPE preconcentration efficiency of the system, Conditions: Phenylhydrazine, 100 ng mL^{-1}; NB, 2.5×10^{-3} M; SDS, 0.2 % (w/v); Triton-X-114, 0.2 % (w/v) and NaCl, 5 % (w/v)

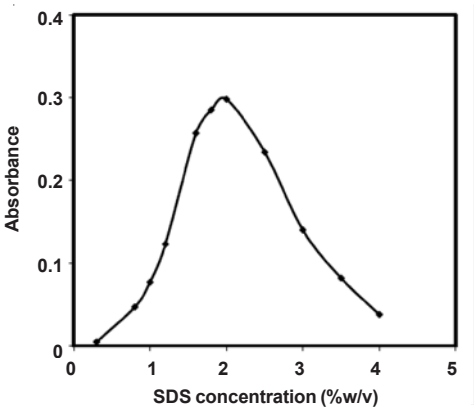


Fig. 4. Effect of SDS concentration on the CPE preconcentration efficiency of the system, Conditions: Phenylhydrazine, 100 ng mL^{-1}; NB, M; Triton-X-114, 0.2 % (w/v); NaCl, 5 % (w/v) and pH 7.0

absorbance of the surfactant-rich phase increased by increasing Triton-X-114 concentration between 0.01 and 0.2 % (w/v) and remained nearly constant at higher concentrations. Therefore, a concentration of 0.2 % (w/v) Triton-X-114 was used as optimum concentration.

The electrolytic effect on the cloud point from mixed nonionic-ionic surfactant systems plays an important role. When small amounts of inorganic salts are added to the system, a decrease in the cloud point temperature was noted[30,31]. As pointed out by Gu and Galera-Gomez[25], if the concentration of the added electrolyte is high enough, the cloud points of some mixed systems could be even lower than those of the pure non-ionic surfactant solution. In this work, the addition of NaCl electrolyte to the Triton-X-114/SDS system reduces drastically the cloud point, thus allowing phase separation at room temperature. As Fig. 6 shows, the cloud point extraction efficiency increased by increasing NaCl concentration up to 5 % (w/v) and decreased at higher concentration. Therefore, 5 % (w/v) NaCl was used as optimum concentration.

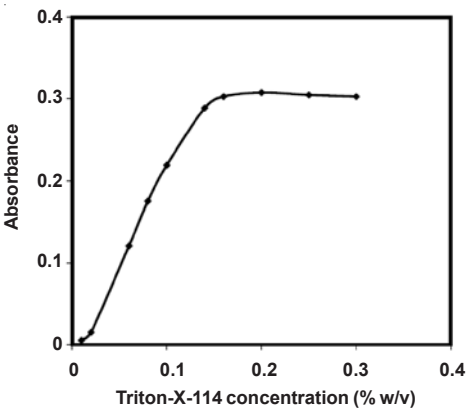


Fig. 5. Effect of Triton-X-114 concentration on the CPE preconcentration performance of the system, Conditions: Phenylhydrazine, 100 ng mL^{-1}; NB, 2.5×10^{-3} M; SDS, 0.2 % (w/v); NaCl, 5 % (w/v) and pH 7.0

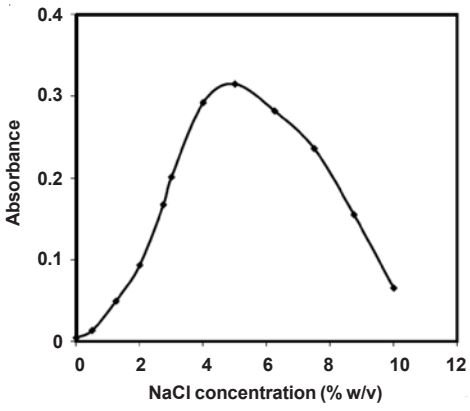


Fig. 6. Effect of NaCl concentration on the CPE preconcentration performance of the system, Conditions: Phenylhydrazine, 100 ng mL^{-1}; NB, 2.5×10^{-3} M; SDS, 0.2 % (w/v); Triton X-114, 0.2 % (w/v) and pH 7.0

In general, centrifugation time hardly ever affects micelle formation but accelerates phase separation in the same sense as in conventional separations of a precipitate from its original aqueous environment. Therefore, a centrifugation time of 5 min at 3500 rpm was selected as optimum, since complete separation occurred for this time and no appreciable improvements were observed for long time.

Because the surfactant-rich phase was very viscous, ethanol was added to the surfactant-rich phase after cloud point extraction to facilitate its transfer into spectrophotometric cell.

Analytical characteristics: Table-1 summarizes the analytical characteristics of the optimized method, including regression equation, linear range, limit of detection, reproducibility and preconcentration and improvement factors. The limit of detection, defined as $C_L = 3S_B/m$ (where C_L, S_B and m are the limit of detection, standard

TABLE-1
ANALYTICAL CHARACTERISTICS OF THE PROPOSED METHOD

Regression equation (n = 15)	A = 0.0029C + 0.021, r = 0.9998
Regression equation (n = 15) before preconcentration	A = 0.0002C + 0.0132, r = 0.9995
Linear range (ng mL^{-1})	4.0-600 (300–6000)[a]
Limit of detection (ng mL^{-1})[b]	1.80
Reproducibility (RSD, %)[c]	1.26
Preconcentration factor[d]	10.00
Improvement factor[e]	14.50

[a]Linear range before preconcentration.
[b]For seven replicate measurements of blank (n = 7).
[c]For seven replicate measurements of 100 ng mL^{-1} phenylhydrazine.
[d]Ratio of phenylhydrazine concentration before and after the CPE method.
[e]Ratio of the slope of the calibration graph for the CPE method to that of the slope of the calibration graph in micellar media without preconcentration.

deviation of the blank, and slope of the calibration equation, respectively), was 1.80 ng mL^{-1}. Because the amount of phenylhydrazine in 10 mL of sample solution is measured after preconcentration by cloud point extraction in a final volume of nearly 1 mL, the solution is concentrated by a factor of 10. The improvement factor, defined as the ratio of the slope of the calibration graph for the cloud point extraction method to that of the slope of calibration graph in micellar media without preconcentration, was 14.5.

The relative standard deviation (RSD) for seven replicate measurements of 100 ng mL^{-1} phenylhydrazine was 1.26 %.

Selectivity: To study the selectivity of the proposed methods, the effect of various species on the determination of 100 ng mL^{-1} phenylhydrazine by the proposed method was tested under the optimum conditions. The tolerance limit was defined as the concentration of added ion that caused less than ± 3 % relative error. The results showed that 1000 µg mL^{-1} Na^{+}, K^{+}, NH_4^{+}, Ba^{2+}, As^{3+}, Co^{2+}, Sn^{4+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Fe^{2+}, Ca^{2+}, CO_3^{2-}, PO_4^{3-}, SO_4^{2-}, NO_3^{-}, Cl^{-}, Br^{-}, F^{-}, CH_3COO^{-}, tartrate, citrate, 10 µg mL^{-1} urea, hydrazine, acetylhydrazine, semicarbazide and ammonia did not interfere on the determination of phenylhydrazine.

Application: In order to evaluate the analytical applicability of the proposed method, it was applied to the determination of phenylhydrazine in water and biological samples. The results are given in Table-2. The recoveries for the addition of different concentrations of phenylhydrazine to samples were in the range of 96-105 %. The results show that the proposed method is suitable for determination of trace amounts of phenylhydrazine in the real samples.

Conclusion

The proposed method gives a simple, sensitive and low-cost spectrophotometric procedure for determination of phenylhydrazine that can be applied to biological and water samples. The surfactant has been used for preconcentration of phenylhydrazine

TABLE-2
DETERMINATION OF PHENYLHYDRAZINE IN WATER AND BIOLOGICAL SAMPLES BY PROPOSED METHOD[a]

Samples	Phenylhydrazine (ng mL^{-1})		
	Added	Found	Recovery (%)
Drinking water	5	5.18± 0.32	103.6
	20	19.8 ± 0.12	99.0
	40	40.6 ± 0.24	101.5
	100	98.9 ±.0.11	98.9
	400	398.1 ± 0.68	99.5
River water	10	9.79± 0.42	97.9
	30	31.1 ± 0.87	103.7
	50	49.2 ± 0.66	98.4
	80	82.2 ± 0.43	102.8
	200	204.4 ± 0.22	102.2
Human serum	20	20.7± 0.35	104.0
	50	52.3 ± 0.74	104.6
	100	97.4 ± 0.68	97.4
	200	196.5 ± 0.74	98.3
	500	497 ± 0.41	99.4
Human urine	50	48.3 ± 1.12	96.6
	100	103.3 ± 0.58	103.3
	200	204.8 ± 0.49	102.4
	500	504.1 ± 0.31	100.8

[a]Average of three determinations.

in samples and thus toxic solvent extraction has been avoided. A comparison of the proposed method with the previously reported methods for preconcentration and spectrophotometric determination of phenylhydrazine (Table-3) indicates that the proposed method is faster and simpler than the existing methods and that it provides a wider dynamic range and a lower limit of detection. The results of this study clearly show the potential and versatility of this method, which could be applied to monitoring of phenylhydrazine spectrophotometrically in various samples.

TABLE-3
COMPARISON OF THE PERFORMANCE OF THE PROPOSED METHOD WITH THAT OF OTHER REPORTED METHODS FOR THE SPECTROPHOTOMETRIC DETERMINATION OF PHENYLHYDRAZINE

Analytical method	Linear range (μg mL^{-1})	Detection limit (μg mL^{-1})	Reference
Resin based detection and spectrophotometry	10-300	10	13
Modular stopped flow-diode-array detection system	8.0-2200	-	14
Kinethic method in the micellar media	1.0-35	-	16
Inhibitition effect of phenylhydrazine in the reaction between bromate and hydrochloric acid	0.050-8.0	0.020	18
Reaction with *p*-nitrobenzaldehyde in micellar media	0.20-10	0.050	19
Cloud point extraction-Spectrophotometry	0.0040- 0.60	0.0018	Present method

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