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Research Article

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Disposable sensors for the determination of diphenylamine with ninhydrin as a choromogenic reagent

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ABSTRACT

The proposed method describes the use of paptode for determination of diphenylamine. The method describes chemo sensors developed for the determination of diphenylamine. The method is based on the reaction of diphenylamine with ninhydrin gives an iminium salt, on heating which formed orange color dye in acidic medium. The color system formed particular pattern on an inert support. Scanner detects the product on TLC strips and obtained pattern analyzed with program written in visual basic 6.0 (VB6). The proposed sensor is linear in concentration range 0.3-5.1 µg/ml. All the analytical parameters and optimum reaction conditions was evaluated. The sensors are used to detect diphenylamine in real sample i.e. fruits and milk.

INTRODUCTION

Diphenylamine is the organic compound with the formula $(C_6H_5)_2NH$. It is colourless solid, but samples are often yellow due to oxidizing impurities [1]. Diphenylamine is used as a pre-or postharvest scald inhibitor for apples. Its anti-scald activity is the results of its antioxidant properties, which protect the apple skin from the oxidation products of alpha-farnesene during storage [2].Diphenylamine (DPA) is the most common stabilizer used in smokeless powders, especially in single base powder [3].Diphenylamine derivatives, are also useful i.e. ring alkylated derivatives of diphenylamine are used as antiozinates in the manufacture of rubber product, reflecting the antioxidant nature of aniline derivatives. With sulphur, it gives phenothiazine, a precursor to certain pharmaceuticals[4].The threshold limit value for diphenylamine by ACGIH and recommended exposure limit by NIOSH is 10mg/m^3 (TWA)[5,6].

Several methods have been described in the literature for the determination of diphenylamine using different analytical techniques such as Gas Chromatography [7], Tandom mass spectrometric method [8], Gas Chromatography Mass Spectrometry [9], and spectrophotometery [10]. Spectrophotometric methods are more useful for the determination of diphenylamine at low concentration level, but these methods suffer from poor linear dynamic ranges and some of method requires expensive instruments.

Various method is based on spot test analysis for qualitative determination of materials on an absorbent paper or other inert support has been found in litracher [11].According to Narayanswamy and Sevilla for a quantitative and reproducible analysis transmittance spectroscopy is considered whereas for a qualitative and non-reproducible analysis, the reflectance spectroscopy will be used due to the effect of the inhomogeneous media [12].

Test paper developed for detection and semi-quantitative determination of hydrazine in water and air [13, 14]. Abbaspour et al. introduced paptode for speciation of Iron (II) and Iron (III), construction of a pH sensor [15], which are similar to optode in many features. In paptode, ionophore immobilizing on a simply a paper or other ordinary porous material such as cotton or clay and even TLC can be used as a substrate support for reagent, But in optode

ionophore immobilizing on a hydrophobic or hydrophilic polymer. They have also developed paptode for determination of hydrazine [16] further Sharma and Amlathe developed paptode for determination of arsenic [17].

The proposed method describes the use of paptode for determination of diphenylamine. The proposed method describes chemo sensors developed for the determination of diphenylamine. The method is based on the reaction of diphenylamine with ninhydrin gives an iminium salt, on heating which formed orange colour dye in acidic medium. The colour system formed particular patter on an inert support. The product on TLC strips are detected by scanner and obtained pattern was analyzed with program written in visual basic 6.0 (VB6).

EXPERIMENTAL SECTION

APPARATUS AND SOFTWARE

In the spot test analysis commercially available flatbed-scanner is used for obtaining the images of colour spots. The obtained images have been transferred to computer for analyzing and determining the intensity of colour-spots using DIP technique through MATLAB.

A specific area has been selected for analysis of colour spot. The number of pixels that can be indicated by this area was about 10000–300000 and this program can average these pixels. Therefore, the signal to noise ratio can be increased dramatically. Furthermore, the problem of inhomogeneous media, which was problematic in reflectance spectroscopy, is not important here due to averaging the intensity of each colour spot.

Advantage of this method is that, not only is it possible to save experimental data, but it is also possible to save images of results (e.g. the colourful complex) in a computer, then they could be reviewed for applying more powerful software on it in the future. In addition, the porosity of support-based materials in these sensors causes shorter response times compared with the typical response times in optodes. Other significant feachers are portability of the proposed method and ease of reagent immobilization is considerable.

In this proposed method the scanner (HP SCANJET G2410) was used for scanning the TLC strips. Area of the spots, which were used to measure the colour intensity, was a square with 40000 dpi (200-200 dpi).The spots perfectly homogeneous. Resolution of the scanner was regulated at 200 dpi for analyzing the colour for analyzing colour values in RGB (red, green, blue) system. The software, which was written in visual basic media, was used. A micro-lit micropipette was used for injecting samples on TLC strips.

CHEMICAL AND REAGENT

All chemical used wear of A.R. grade. Double distilled water was used throughout the experiment**.**
Diphenylamine solution: 1% (w/v) stock solution of diphenylamine was prepared in ethanol.
Ninhydrin: 5 % (w/v) solution was prepared in ethanol.
Hydrochloric acid: 4% (v/v) solution was prepared in double distilled water

CHEMICAL REACTION

The secondary amine react with ninhydrin gives iminium salt, which is coloured and generally yellow orange in colour[18,19].This proposed method is fallowed above reaction and diphenylamine react with ninhydrin on heating gives iminium salt in acidic medium. (Fig: 1)

PREPARATION OF PAPTODE

Diphenylamine forms orange colour product (iminium salt) with ninhydrin in acidic medium. The sensor strips for diphenylamine, constructed by ninhydrin solution and dried at 70-80˚C for 5 minutes and then dipped in hydrochloric acid to again for 5 minutes drying at 70-80˚C temperature. Aliquots of 30µl of diphenylamine solution containing 1.8µg/ml were injected on these TLC stripes, then after dried at 70-80˚C for 10 minutes formed orange color product (iminium salt). The stripes were scanned and images of spots were analyzed by software system for finding their R, G and B values. The RGB colour model is an additive colour model in which red, green and blue light are added together in various ways to produce a broad array of colours. Any colour can be analyzed to obtain its corresponding R, G and B value. Effective intensity for any colour values of colour spot was calculated as

$Ar = -Log (Rs/Rb)$
$Ag = -Log (Gs/Gb)$
$Ab = -Log (Bs/Bb)$

Ar, Ag and Ab are effective intensities for red, green and blue colour. Rs, Gs, Bs and Rb, Gb and Bb refer to R, G and B values of sample and blank respectively. To obtain calibration curves, effective intensities of R, G and B values were plotted with respect to the analyte concentrations.

RESULTS AND DISCUSSION

The reaction of diphenylamine with ninhydrin in acidic medium gives an iminium salt by Schiff base transfer. The proposed sensors are linear in concentrations range of 0.3-5.1 µg/ml of diphenylamine.

OPTIMIZATION OF THE REACTION CONDITIONS

Injection volume: The influence of volume of the analyte solution which must be injected onto the TLC strip was investigated. The optimum sample volume was obtained to be 30µl. If higher volume was injected then spot spreading occurs due to diffusion and consequently the intensity of colour was decreased.

Effect of ninhydrin: The TLC strips were prepared containing ninhydrin solution in different concentration to dried at 70-80˚C within 5 minutes. Then 30µl of standard solution containing 1.8µg/ml of diphenylamine was injected on each TLC strips with micro-lit micropipette and effective intensity of the R, G and B values were plotted with respect to concentration of ninhydrin. (Fig 2).The maximum colour intensity was observed at 5% solution of ninhydrin.

Effect of hydrochloric acid concentration: To study the effect of hydrochloric acid for maintaining acidic medium different concentration of hydrochloric acid were prepared, each solution immobilized on a TLC strips then was allowed to dry at 70-80˚C for 5 minutes. After drying 30µl of standard solution containing 1.8µg/ml of diphenylamine was injected on each TLC strips and corresponding effective intensity of the R, G and B values were plotted (Fig 3).The maximum colour\ intensity was observed at 4% solution of hydrochloric acid and this concentration was selected for further experimental work.

Effect of temperature: The effect of temperature on the color reaction is studied (fig 4).The optimum condition for complete color reaction was found to be 70-80 ˚C. Below this reaction was slow and response time was found to be increases and above the color spot is not to be found. After immobilization of reagent onto the TLC Strips, the strips need to be dried. Some methods such as drying at room temperature, oven and hot air were applied and no change in signal was observed. However, for increasing the speed of analysis, using an oven is recommended. After immobilization of reagent onto the TLC Strips, the strips need to be dried. Some methods such as drying at room temperature, oven and hot air were applied and no change in signal was observed. However, for increasing the speed of analysis, using an oven is recommended. The TLC stripes dried on 70˚-80 ˚C temperature.

Calibration curves

The effective intensity with respect to the concentration of diphenylamine has been studied. Calibration graph of diphenylamine at optimum condition ($C_{ninhydrin=5\%}$and $C_{hydrochloric\ acid=4\%}$) shows linear relationship between effective intensity and concentration of diphenylamine is 0.3 µg/ml-5.1 µg/ml (fig 4). The Calibration in different concentration of diphenylamine as shown by photograph (fig 5)

Reproducibility, response time, stability and detection limit of the system

Reproducibility of the proposed system investigated at five different sensors and three replicate analyses under optimum condition for various concentrations of diphenylamine. Reproducibility of the propose method as shown by Table 1.

The response time of the system was evaluated under optimum experimental condition for 1.8 µg/ml concentration of diphenylamine. It was calculated by measuring the time required to achieve 95% values of colour intensity of the spot. The response time of 10 min was achieved.

To study the stability of colour spots, 1.8 µg/ml of diphenylamine was injected under optimum experimental condition on the sensors. Scanning of the strips was done in the time period of 5, 10, 15, 20, 30, 60 120, 180 min. No change in colour intensity was observed for a period up to 60 min that shows the sensors are stable for 1 hour after injection of the diphenylamine.

In order to study the stability of the sensors, after immobilization ninhydrin on the TLC strips it was used periodically each day, the signal did not show any significant change within 27 days of experiment .After 27 days a response time was found to be increased from 10 min. to 20 min. This reveals that the prepared strips are stable at least for 27 days

Theoretical DL (detection limit) of this method was 0.25µg/ml for B value. As the G value miner change so this is negligible and R value does not vary considerably by changing the concentration of diphenylamine, we calculated DL only for B values. We can also determine the detection limit by practical experiment. Practical DL is the lowest concentration that would give a colour on the TLC strip. Practical DL was about 0.18µg/ml.

EFFECT OF INTERFERENTS

To check the validity of method the effect of various interferences study on under optimum experimental conditions. The method was found to be free from most of the interference's including organic compound and metal ion in water. The tolerance limits for various interferences test are shown by Table 2.

Fig: 1 Chemical reaction between diphenylamine and ninhydrin

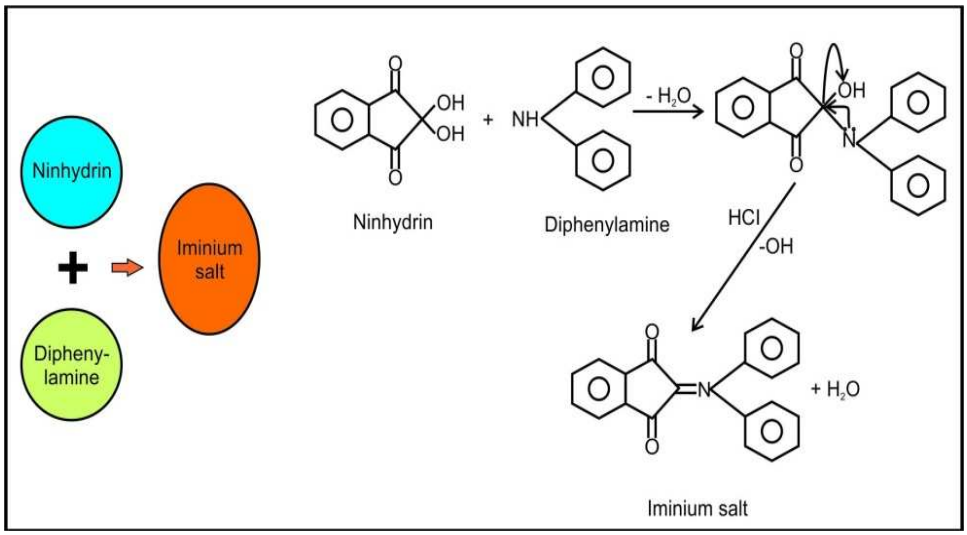
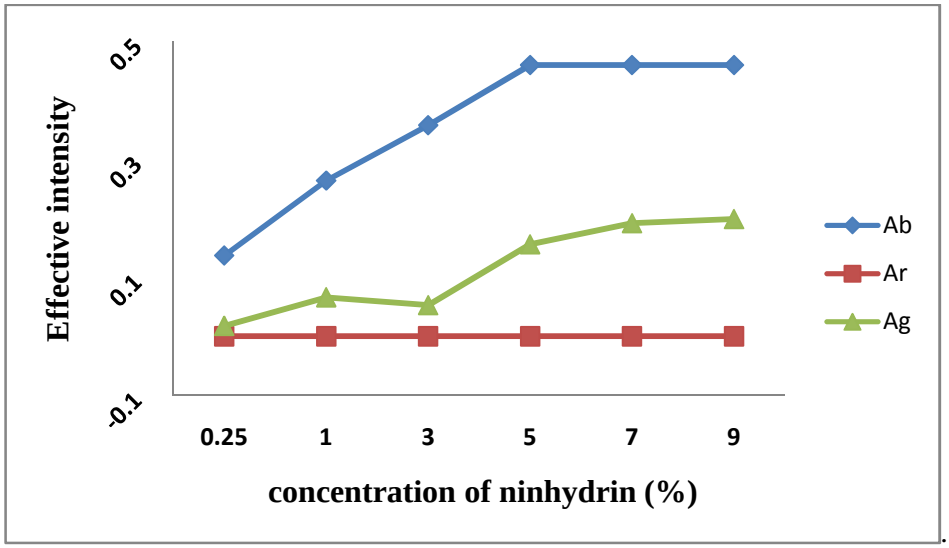


Fig: 2 Effect ninhydrin concentrations on effective intensity of color spot.



Where:
Effective intensity is the antilog of RGB values of sample to RGB values of blank.
Ar=Effective intensity for R values
Ag=Effective intensity for G values.
Ab= Effective intensity for B values.

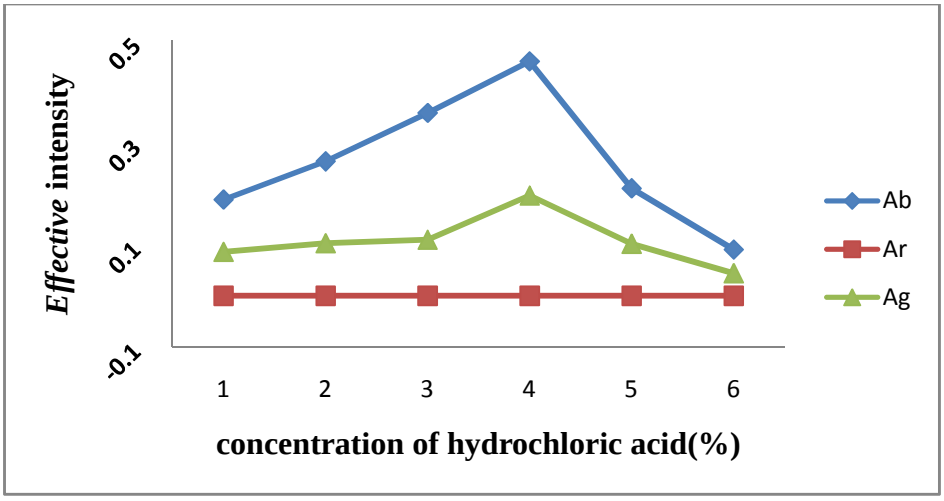
APPLICATION

This proposed method for the determination of diphenylamine is successfully applied in biological samples i.e. apple, pears and gout milk (Table 3).

(A) In apple (Red delicious and granny smith from Reliance fresh): 20 gram of apple blended with acetone and filtered. Diphenylamine residue wear extracted from the aqueous acetone with hexane and then derivatized by tricloroacetic anhydride in butane. [20]

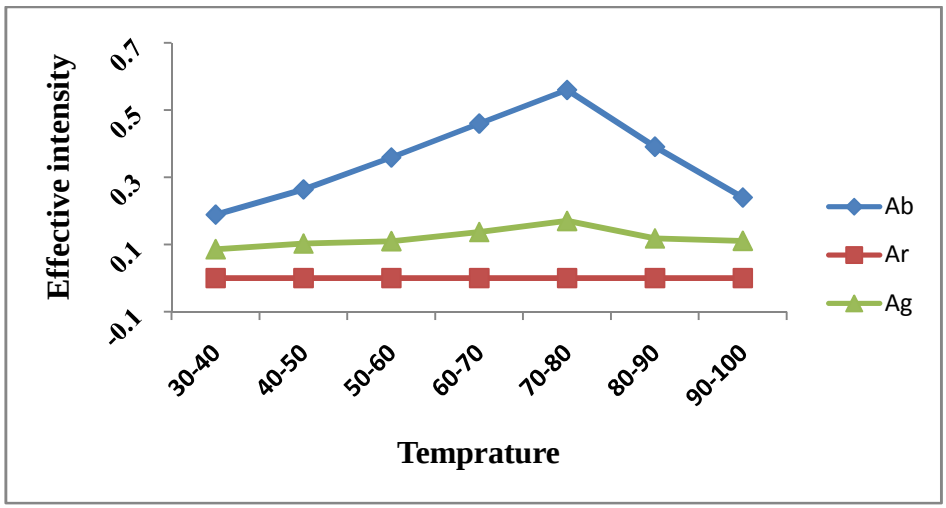
(B) In pears: the samples extracted with acetone. The residue is filter, filtered is the final solution was analysed. [20]
(C)In goat milk: In the method of analysis for diphenylamine residue in goat milk. The milk is extract with acetonitrile, which was mix with hexane to remove fate. The acetonitrile extract was evaporated by dryness, redissolved in hexane and analyzed. [20]

Fig 3: Effect of hydrochloric acid on effective intensity of spot



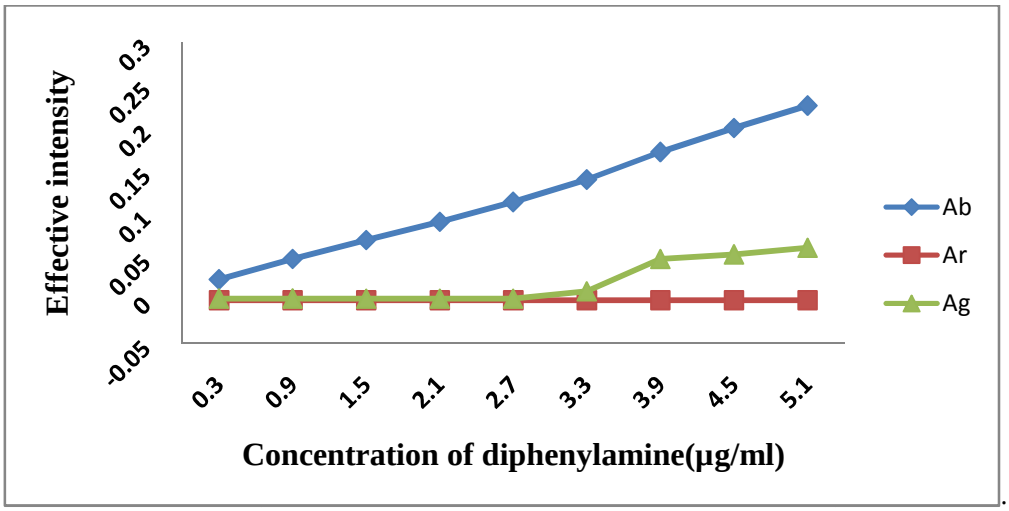
Where:
Effective intensity is the antilog of RGB values of sample to RGB values of blank.
Ar=Effective intensity for R values
Ag=Effective intensity for G values.
Ab= Effective intensity for B values.

Fig 4: Effect of temperature on effective intensity of spots



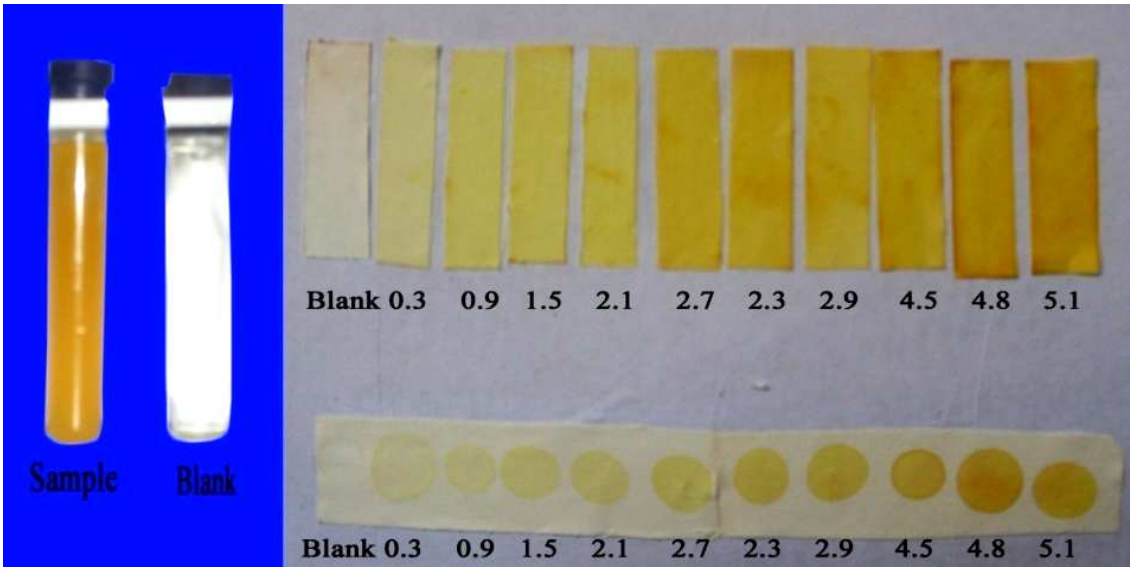
Where:
Effective intensity is the antilog of RGB values of sample to RGB values of blank.
Ar=Effective intensity for R values
Ag=Effective intensity for G values.
Ab= Effective intensity for B values.

Fig. 4: Calibration graph of diphenylamine at optimum condition ($C_{ninhydrin=5\%}$and $C_{hydrochloric\ acid=4\%}$).



Where:
Effective intensity is the antilog of RGB values of sample to RGB values of blank.
Ar=Effective intensity for R values
Ag=Effective intensity for G values.
Ab= Effective intensity for B values.

Fig: 5 (A) photograph of orange color change upon the addition of diphenylamine in solution of ninhydrin and hydrochloric acid and (B) calibration of diphenylamine (µg/mi) in different concentration on test paper



A **B**

Table:1 Reproducibility of the system at optimum condition

concentration of diphenylamine in µg/µl	A_B		
	Ave[a]	SD[b]	RSD[c]
5.1	1.31	0.003	0.23
3.6	0.16	0.002	1.27
1.8	0.08	0.002	2.30
0.3	0.02	0.001	6.30

a Average of eight measurement on different TLC
b Standard deviation
c Relative standard deviation

Table 2: Effect of foreign species on the determination of diphenylamine

Interferences	Masking agent	Tolerance[a] limit(ppm)
Benzidine	–	500
Benzene	-	700
Pyridine	-	1200
1-napthylamin	-	4000
Hydrazine	-	200
Phenol	–	7000
Formaldehydes	–	2000
Ascorbic acid,semicarbazides	–	1500
Ammonia, FAS,	–	120
Nitrobenzene	–	500
$Cl^-SO_4^{2}$,CO_3^{2},Na^+,K^+Ca^{2+}, Mg^{2+}	10%EDTA solution(1ml)	10000
Cd^{2+},Cu^{2+}	10%EDTA solution(1ml)	3000
Fe^{3+} /Al^{3+}	10%sodium potassium tartrate(1ml)	10000

[a]*Causing an error of ±2% or less*

Table: 3 Analysis of diphenylamine in biological samples**

No	Samples*	Diphenylamine added(µg)	Diphenylamine found (µg)	Recovery (%)
1	Apple (a)red delicious	2.0	2.15	102
		2.5	2.5	100
		3.0	3.5	105
	(b)granny smith	2.0	1.80	97
		2.5	2.5	100
		3.0	2.98	99
2	Pears	2.0	1.75	96
		2.5	2.0	98
		3.0	2.97	99
3	Milk	2.0	1.90	90
		2.5	2.30	98
		3.0	2.98	98

**Amount of samples 1 ml*
***mean of three replicate analyses*

Table 4: Comparison of the propose diphenylamine sensors with some reported method

Techniques	Reagent	Linear range	Detection limit	Remark
Gas chromatography (7)	Methylene chloride	–	1.6µg/l	Reagent is carcinogenic
Tandom mass spectrophotomety (8)	–	5.0-200ng/ml		Instrument is highly expensive
Gas chromatograMass spectrophotomety (9)	Acetonile and Acetone			
Spectrophotometry (10)	p-Dimethyamino cinnamaldehyde	–	2.5ng/ml	Less sensitive
Proposed method	ninhydrin	–	10ppb	–
		0.3-5.1µgml	0.18µg/ml	Highly sensitive, simple, rapid, quantitative, less interferences.

CONCLUSION

The proposed chemo-sensor is highly sensitive and free from most of the interfering species. The proposed method was found to be superior to other method (Table 4). This method is simple and rapid and it does not need any expensive apparatus.

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REFERENCES

[1] Jean-Pierre Shirmann, Paul Bourdauducq " Hydrazine" in Ullamann's Encyclopedia of Industrial Chemistry, **2001**.
[2] Occupational Safety and Health Act, Commerce Clearing House Inc., U.S.A.,**1984,** 3825-3827.
[3] Kumarawickrama RAD. The characterization and identification of organic GSR, University of Strathclyde, UK, M Sc Thesis **1985**.
[4] Leithe W., The Analysis of Air Pollutant, Ann Arbor Science Publishers, **1977**, 174-175.
[5] DIPHENYLAMINE-Centers For Disease Control And Prevention, American Conference Of Governmental Industrial Hygienists (Acgih),**2011**.
[6] Center For Disease Control And Prevention Niosh Pocket Guide To Chemical Hazads November ,**2010**
[7] J.S.Warner, T. M. Engel, P. J. Moudron, National Service Center For Envioonmental Publication(NSCEP), **1983**,EPA Method 620, 1-39.
[8] Yan Tong, Zhuping Wu, Chengdui Yang, Jianyuan Yu, Xinrong Zhang, Shijie Yang, Xinyuan Deng, Yucai Xu and Yuxiu Wen , *Analyst*, **2001**, 126, 480-484
[9] .Lei Yu,Royal Schoen, Arlene Dunkin, Michael Firman, and Holly Cushman,*J. Agric. Food Chem.*, **1997**, *45* (3), 748–752
[10] Anees Ahmad, Mohsin Qureshi, Iftekhar Ahmad Khan,Khwaja Zuber Alam,Analytial letter, 23, **1990**, 1139-1157.
[11] F. Feigl and V. Anger, Spot Test in Inorganic Analysis, Elsevier,Amsterdam, 6th ed., **1972**
[12] R. Narayanaswamy and F. Sevilla, *Microchim. Acta*, 97,**1989**,293.
[13] S. Amlathe and V. K.Gupta , *Analyst*, 113,**1988**, 1481-1483.
[14] S.Amlathe and V.K.Gupta, *Microchemical Journal*, 42,**1990**, 331-335.
[15] A. Abbaspour, M. A. Mehrgardi, a. Noori, m. A. Kamyabi,a. Khalafi-nezhad and M. N. Soltani rad, sens. *Actuators*, 113,**2006**, 857.
[16] Abdolkarim, abbaspur ,ehsan mirchandani and abdolreza khajehzadah,*Anal Method* , 2, **2010**, 349-353.
[17] R. D. Sharma, S. Joshi and S. Amlathe, *Anal methods*, 3, **2010**,452-456.
[18] Basic chemistry laboratory febrvery **2010**,2-8.
[19] NINHYDRIN-wekipedia,the free encyvlopedia,1-3.
[20] Diphenylamine(030),www.fao.org/ag/AGP/AGPP/Pesticide/...../07%20diphenylamine. pdf.