Simple Method for Spectrophotometric Determination of Benzidine in Aqueous Solutions by Coupling with B-Naphthol

Ameera .H. Hamed
Department of Chemistry, College of Science for Women, University of Baghdad, Baghdad-Iraq.

Abstract

Anew simple and rapid colorimetric method has been developed for the determination of benzidine in aqueous solutions. The method is based on the formation of a pink colored azo dye by diazotization of benzidine, followed by a diazo-coupling reaction between the resulting product and β-naphthol. The maximum absorbance of azo dye at 525 nm. Beer's law was found to be obeyed in the concentration range of 0-12 µg. ml^{-1} with molar absorptivity 12360 L. mol^{-1}. cm^{-1}. The optimum reaction conditions and other analytical parameters were evaluated.

Keywords: Determination Benzidine, Diazo Coupling Reaction, β-naphthol.

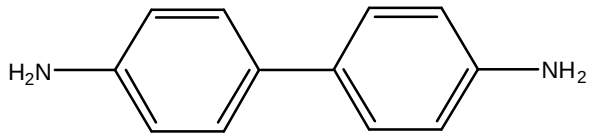
Introduction

Benzidine is a white to slightly reddish crystalline powder turns dark on exposure to air and light; melting point of 128 C^{0}[1]. In the past, industries used large amounts of benzidine to produce dyes for cloth, paper, and leather.

Benzidine is no longer used in medical laboratories or in the rubber and plastics industries [2].

Benzidine is very toxic to animals and men, affecting chiefly the blood, urinary system, liver, and skin. It is carcinogenic to animals and produces bladder cancer in occupational exposures. It is mutagenic in the Ames test [3].

Benzidine is used commercially as a starting material in the production of azo dyes and in the analytical laboratory as a good chemical reagent [3]. The molecular weight is 184.23 g/mole and it s chemical structure [3].



In literature there are many works about the detection and determination of benzidine, most of them are dealing with chromatographic techniques[4-8] , which involving extraction into methylene chloride or chloroform followed by separation without derivatization and detection with a mass spectrometer which see to be expensive and use chlorinate organic solvent[9-10] Spectrophotometic determination method used on diazotization and coupling of diazonium ion with α-naphthol in acidic medium [11].

In this work a new Spectrophotometic method is developed which based on coupling the oxidation product of benzidine with ß- naphthol in alkaline medium.

Experimental Parts

Apparatus

All spectral and absorbance measurements were by using a Computerize UV-Visible, shimadzu T60U Spectrophotometer, with 1cm matched quartz cells.

Materials and Reagents

All chemicals were of analytical reagent grade. Benzidine of (98% purity) was obtained from (BDH) and stander solution of 100 µg. ml^{-1} benzidine was freshly prepared by dissolving 0.01gm of benzidine in 20 ml of absolute ethanol and further diluted with distilled water to 100 ml. ß-naphthol of (99.8% purity) was obtained from (RDL) a stander solution of 100 $\mu g.ml^{-1}$ of β-naphthol was freshly prepared by dissolving 0.01gm of β-naphthol in 20 ml absolute ethanol and then diluted with distilled water to 100 ml, Sodium nitrite (99.8 purity) from(BDH) and stander solution of 1% was prepared. Sulphamic acid of (98.5%) from (BDH) and stander solution of 3% in distilled water was prepared. Sodium hydroxide of (98% purity) from (RDL), solution of 1M was prepared by dissolving 4.0 gm in 100 ml distilled water, 100 µg. ml^{-1}

of varies interferences and 1 M both of Hydrochloric acid, sulfuric acid and phosphoric acid were used.

Recommended Procedures

General procedure

1.0 ml of standard solution of benzidine 100 µg. ml^{-1} and 0.70 ml of 1% sodium nitrite and 0.50 ml of 1M Hydrochloric acid were mixed in volumetric flask of 10 ml and shacked for 2 minutes, then 0.75 ml of 3% sulphamic acid was added to remove any excess of nitrite ions, The 2.0 ml of ß-naphthol and 1.0 ml of 1M Sodium hydroxide solutions were added with shaking and cooling for 3 minute, after 5 minutes the color is completely developed the absorbance measurement was carried out at a wavelength at 525 nm, against a blank solution prepared in the same method but without benzidine.

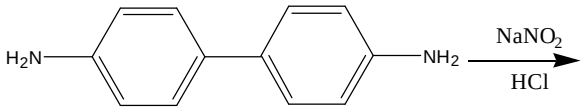
Mole –Ratio procedure

Transfer exactly (1 ml) of 1×10^{-3}M benzidin to 8 ml volumetric flacks add exactly (0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4) ml of 1×10^{-3}M β-naphthol solution to each flask. Diluted to the mark with distilled water and mixed the absorbance was measured at 525 nm as function of mole ratio.

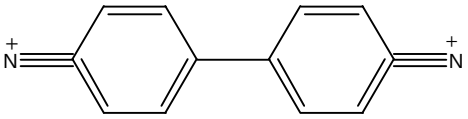
Results and Discussion

Oxidation of Benzidine

Benzidine was oxidized by sodium nitrite 1% in acidic medium of HCl in order to obtain a reactive species according to the following reaction:



Benzidine



Benzidine diazonium ions

Fig.(1) shows the absorption spectrum of 10 µg.ml^{-1} of benzidine in distilled water, which shows maximum absorption wavelength at 283 nm.

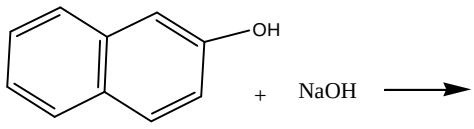
Fig.(2) shows the absorption of the oxidation product of benzidine in acidic medium at wavelength of 312 nm being more sensitive and selective than benziden.

The effect of sodium nitrite was studied and the obtained results showed in Table (1) demonstrate that maximum absorption can be obtained on using 0.70 ml of 1% sodium nitrite.

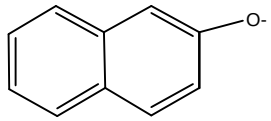
The effect of mineral acids on the oxidation of benzidine was also studied and the Table (2) shows that the absorption of 10 µg.ml^{-1} benzidine which demonstrates quite obviously that 0.5 ml of 1M HCl gave maximum absorbance at wavelength 312 nm.

Alkaline hydrolysis of β-naphthol

ß-naphthol was subjected to alkaline hydrolysis in order to obtain reactive nucleophilic species according to the following equation:



β -naphthol



β- naphthoxide

The nucleophilic ß-naphthonate accelerates substitution reactions by activating the 1-position. Thus couples with diazonium salt in the 1- position to form colored species.

Fig.(3) shows the U.V absorption spectrum of 20 µg.ml^{-1} β-naphthol in distilled water the maximum absorption wavelength being at 273 and 327 nm.

Fig.(4) shows the absorption spectrum of 20 µg. ml^{-1} β–naphthol in alkaline medium as which shows absorption at 346 nm.

Developing the indodye

The sensitivity and stability of indodye depends on many analytical parameters which were subjected under insensitive experiments shows in next items.

Effect of sulphamic acid

Usually sulphamic acid is used to remove any excess of nitrous ion $(NO_2)^-$ which may affect on the coloured species and different volumes from (0.25 -1.5) ml of 3% sulphamic acid were studied Table (3) shows that (0.75 ml) at mentioned acid gave high absorbance.

Effect of sodium hydroxide concentration

The effect of sodium hydroxide on the absorbance of indodye was studied .Table (4) shows that 1.0 ml of NaOH gave high absorbance.

Effect of β-naphthol concentration

The effect of varying volume of coupling agent was studied and Table (5) shows that the best volume of β-naphthol is 2.0 ml which provides sufficient quantity of this reagent that quantitatively react with benzidine.

Study of order addition

The order of adding reagents were studied and the absorbance results show that the addition of benzidine, sodium nitrite, sulphamic acide, Hydrochloric acid, ß-naphthol, and Sodium hydroxide gave absorbance at 0.698.

Stability of the developing colored indodye

On using the optimum parameters and the absorption measurents were carried out at the wavelength at 525 nm as function of time (5 -180) minute as shown the absorbance is stable more than 2 hours and hence gives enough time for measuring absorbance.

Calibration curve

The effects of parameters were evaluated under the best conditions found for the determination of benzidine such concentration 1% sodium nitrite (0.70 ml) of 3% sulphamic acid (0.75 ml) as well as 1M sodium hydroxide (1.0ml). A calibration curve obtained for series of standard solutions of benzidine from (0 -12) µg. ml^{-1} using the above parameters provide atypical calibration line with following analyticale regression features

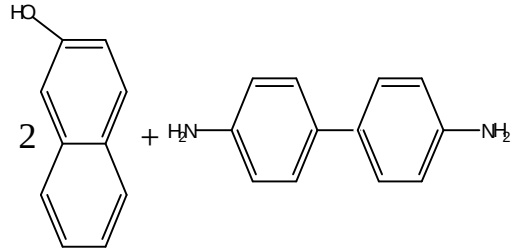
A_{525} = - 0.00014 + 0.06808 (C in µg $ml^{-1}$of benzidine).

with regression coefficient of 0.999 The limited of detection for three replicates of 5.0 µg. ml^{-1}. Obtained in these conditions was 8.317×10^{-4} µg. ml^{-1} and the relative standard deviation for three replicates measurements of 5 µg.ml^{-1} benzidine was 0.832 %.

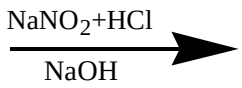
Studying the nature of indodye

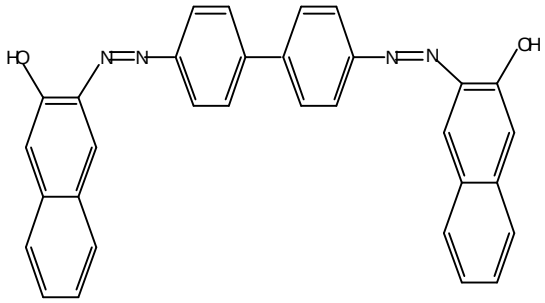
In this method the concentration of benzidine is held constant and the consternation of β-naphthol was increased stepwise Fig.(6) shows that on the graph of absorbance vs. moles reagent added .the intersection of the extrapolated linear segments determines the ratio moles of reagent /moles of benzidine.

The reaction between benzidine and β-naphthol is 1:2 according to the following suggested reaction:



β-naphthol Benzidine





Azo dye

Effect of organic solvents

The effect of organic solvents suchus methanol, ethanol, ether and distilled water were studied by using in the dilution and measuring the absorbance were found (0.883, 0.733, 0.536, 0.631) respectively. Which shows that methanol provides high absorbance.

Effect of organic compounds

The effect of phenolic and carboxylic compound on absorbance of the indodye was studied using 10 µg ml^{-1} benzidine and 2ml of 100 µg ml^{-1} of β-naphthol Table (6) shows that the phenolic and carboxylic organic companied indicated in the Table (6), demonstrate clear interference which means that this method can not be used for the determination of benzidine samples in the presence of the indicate phenolic and carboxylic compounds.

Study of stability constant of pigment

For the calculation of stability constant of pigment, various solutions were prepared which contain relative quantities of substrate and analytical reagent one mole each material), and the absorbance was measured (As) in other step solution was prepared which the same concentration of substrate with excess of analytical reagent that of give high absorbance (Am), then the dissociation degree of reaction (α) was calculated. The results are given in Table (7).

Analysis of real water sample

In order to evaluate the applicability of the developed method for the determination of benzidine in water samples, three water samples (distilled, river and wall water) were fortified with a known quality of benzidine and using the optimum parameters and the absorbance measurement were carried out at the wavelength at 525 nm. Table (8) shows the recovers and standard deviation of the obtained result.

The recoveries demonstrate this method can be used for determination of benziden without any interference which many are caused by the presence of inorganic.

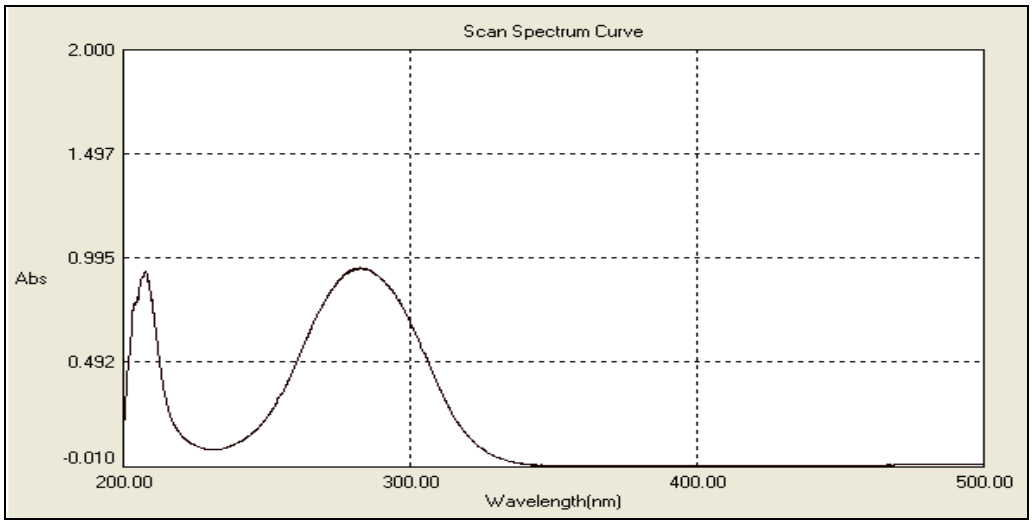


Fig.(1) Absorption spectrum of 10 µg.ml^{-1} of benzidine in distilled water.

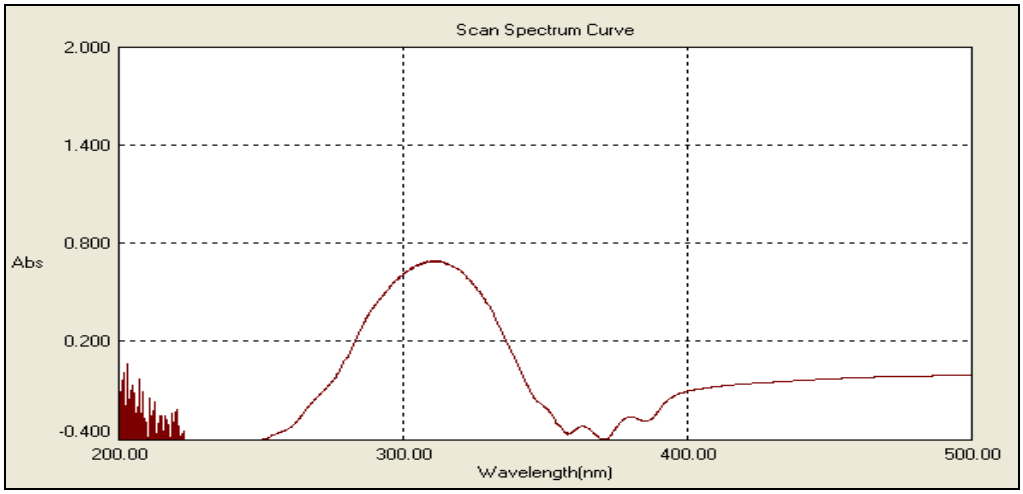


Fig.(2) Absorption spectrum of oxidation product of 10 µg.ml^{-1} benzidine in acidic medium.

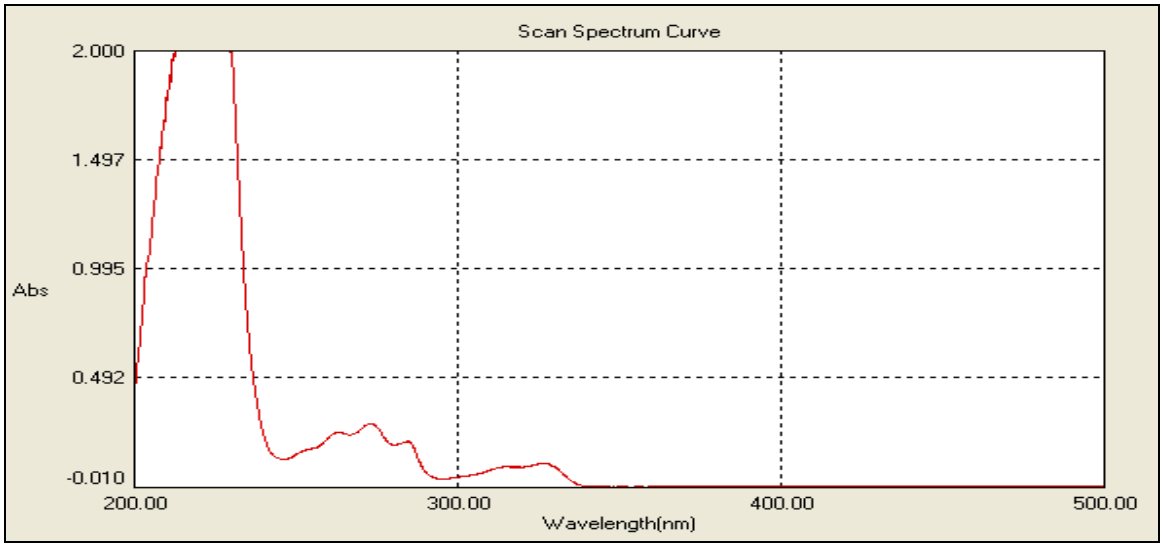


Fig.(3) Absorption spectrum of 20 µg. ml^{-1} of ß- naphthol in distilled water.

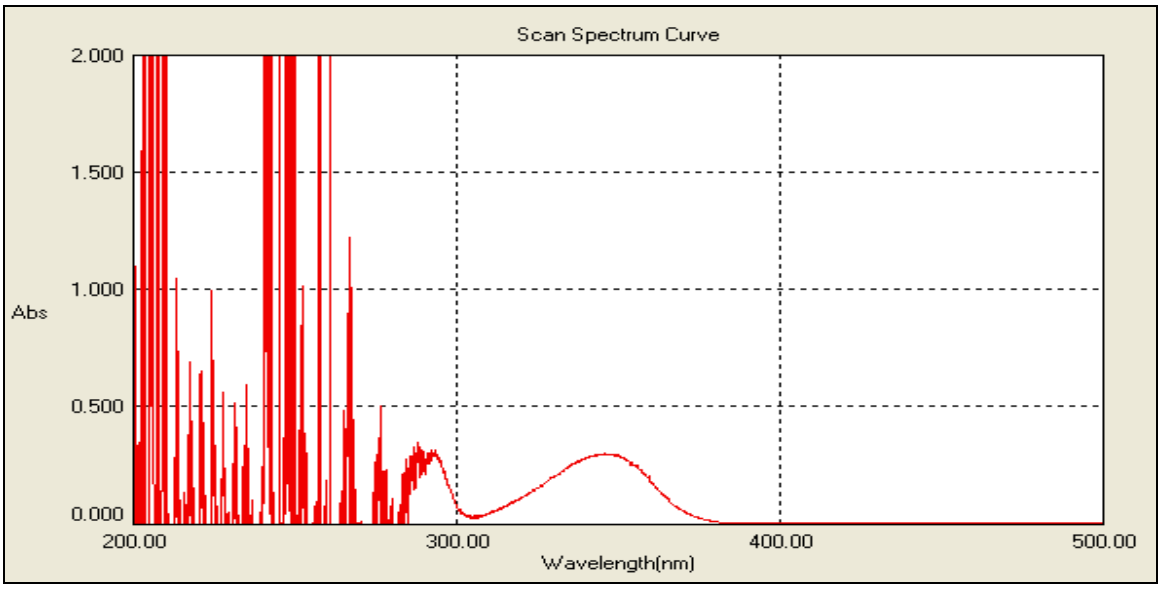


Fig.(4) Absorption spectrum of the alkaline hydrolysis product of 20 µg. ml^{-1} ß- naphthol of the some concentration.

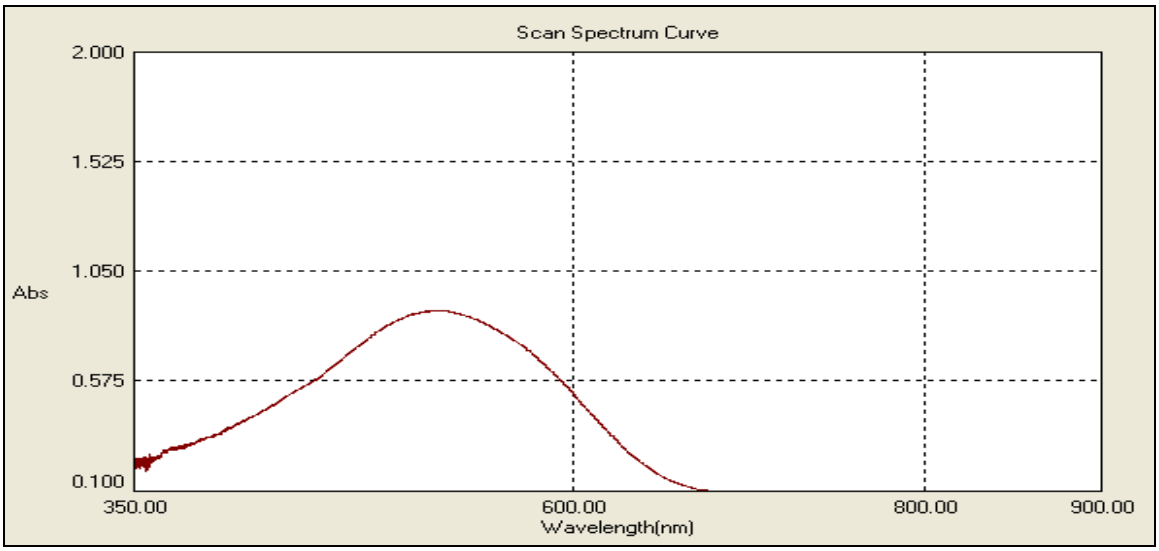


Fig.(5) Absorption spectrum of the azo dye at 525 nm of 10 µg. ml^{-1} benzidine and 20 µg. ml^{-1} ß- naphthol.

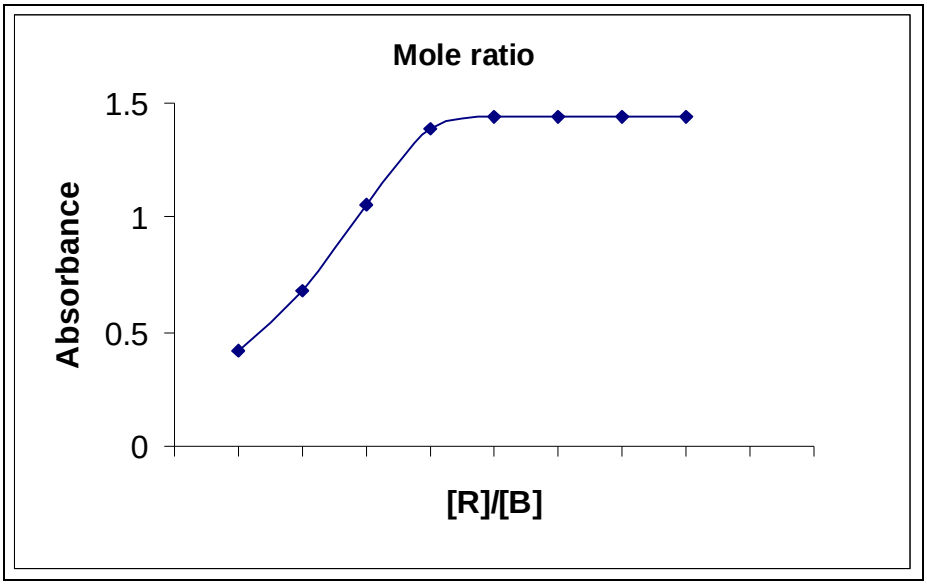


Fig. (6) The mole ratio of azo compounds production.

Table (1)
Effect of 1%sodium nitrite on the absorbance.

V ml of 1% $NaNO_2$	0.1	0.2	0.3	0.4	0.5	**0.7**	1.0
Absorbance	0.244	0.355	0.37	0.401	0.44	**0.633**	0.59

Table (2)
Effect of mineral acid.

	Absorbance							
(1M) acid	***V (ml)***							
	0.0	0.25	**0.5**	1.0	1.5	2.0	2.5	3.0
HCl	–	0512	**0.643**	0.428	0.402	0.308	0.270	0.25
H_3PO_4	–	0.556	0.589	0.602	0.510	0.407	0.116	0.085
H_2SO_4	–	0.471	0.564	0.226	0.180	0.106	0.086	0.053

Table (3)
The effect of sulphamic acid concentration on the absorbance.

V ml of Sulphamic acid (3%).	0.25	0.5	**0.75**	1.5	2
Absorbance	0.373	0.429	**0. 659**	0.322	0.244

Table (4)
Effects of 1M sodium hydroxide on the absorbance.

V ml of 1 M NaOH	0.25	0.5	0.75	**1.0**	1.25	1.5
Absorbance	0.555	0.611	0.640	**0.669**	0.505	0.450

Table (5)
Effect of β-naphthol concentration.

V ml of 100 µg.ml^{-1} β-naphthol	0.5	1	1.5	**2**	2.5
Absorbance	0.486	0.611	0.588	**0.673**	0.535

Table (6)
Effects of organic compounds on the absorbance.

Interference	***β-naphthol only***	***resosenol***	***phenol***	***benzoic acid***	***α-naphthol***
Absorbance	0.698	0.06	0.58	0.42	0.69

Table (7)
The value of stability constant for pigment.

As	***Am***	***α***	***K, M^{-1}***
0.239	0.812	0.766	6.67×10^{7}

Table (8)
The recovers and standard deviation of the obtained result.

Water sample	***Benzidine add µg.ml^{-1}***	***Benzidine found µg.ml^{-1} ± RSD***	***Recovery %***
Distilled water	4	3.9 ± 0.019	97.5
	8	7.8 ± 0.019	97.5
	12	11.5 ± 0.0	95.8
River water	4	3.9 ± 0.202	97.5
	8	7.8 ± 0.027	97.5
	12	11.6 ± 0.019	96.7
Wall water	4	2.6 ± 0.019	65
	8	4.5 ± 0.019	56.3
	12	7.2 ± 0.019	60.0

Conclusions

The proposed method was found to be simple, economic, and fast consume unharmful reagents. The statistical feature and recovery data indicate that this method can be used for rotine analysis of benzidine in aqueous solution.

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الخلاصة

تم تطوير طريقة لونية جديدة بسيطة لتعيين تركيز البنزدين في المحاليل المائية ان الطريقة مستندة على تكوين صبغة الازو الناتجة من تكوين ملح الديازونيوم للبنزيدين ثم ازدواج الناتج مع ß- نفثول والحصول على صبغة الازو عند 525 نانوميتر, قانون بير وجد مطاوعا في مدى تراكيز 12- 0 ملغم/لتر وامتصاصية مولارية 12360 لتر /مول.سم وقد قدرت الظروف المثلى للتفاعل وكذلك العوامل التحليلية الاخرى.