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Spectrophotometric Determination of Barbituric Acid by Coupling with Diazotized Nitroanilines

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ABSTRACT

A simple spectrophotometric method for the trace determination of barbituric acid (BA) has been established. The method is based on the coupling reaction of barbituric acid with diazotized nitroaniline in basic medium to form an intense yellow-water soluble and stable mono azodyes which shows maximum absorption at 418, 380, and 370 nm with diazotized o-, m- and p-nitroanilines, (DONA, DMNA, and DPNA), respectively. A plot of absorbance versus BA concentration was linear to a certain limit, indicating that Beer's law was adhered to over the range 5-300 µg of BA/25 ml final volume (i.e., 0.2-12 ppm) with a molar absorptivity of 1.998×10^4, 2.328×10^4, and 2.184×10^4 l mol^{-1} cm^{-1} for DONA, DMNA, and DPNA, respectively. Sandell sensitivity indexes were 0.0064, 0.0055, and 0.0059 µg cm^{-2}, while the relative errors were -3.365 to 0.904%, -3.325 to 0.138%, and -2.053 to 2.679% for DONA, DMNA, and DPNA, respectively. The relative standard deviation (RSD) were 0.489 to 3.330, 0.897 to 2.209 and 0.059 to 3.689 for DONA, DMNA and DPNA, respectively. The optimum conditions for the color reaction, nature of dye, stability constant and the interference of variety of organic compounds had been investigated. The method has been successfully applied to the determination of barbituric acid in Tigris water river. The proposed method for the determination of barbituric acid is simple, sensitive, very low cost, has a wide analytical range and without the need for heating or solvent extraction techniques.

keywords: Azo-dye, Barbituric acid, Diazotized Nitroanilines, Spectrophotometric determination.

التقدير الطيفي لحامض الباربيتيورك باقترانه مع نايتروانيلين المؤزوت

الملخص

تم تثبيت طريقة بسيطة لتقدير كميات متناهية في الصغر من حامض الباربيتيوريك، تعتمد الطريقة على تفاعل اقتران حامض الباربيتيوريك مع كواشف نايتروانيلين المؤزوت في محيط قاعدي لتكوين صبغة أحادية صفراء ذائبة في الماء والتي تعطي أعلى امتصاصية عند 418 و380 و370 نانومتر للمعوضات

DONA وDMNA وDPNA وعلى التوالي. أظهر منحني الامتصاصية خطاً مستقيماً والذي يؤكد على أنه يتبع قانون بير عند مدى تركيز حامض الباربيتيوريك 5-300 مايكروغرام/25 مللتر أي أن(0.2 -12.0) جزء بالمليون وبمعامل امتصاص مولاري 1.998×10^4، 2.328×10^4و 2.184×10^4 لتر.مول$^{-1}$.سم$^{-1}$ للمعوضات DONA وDMNA وDPNA وعلى التوالي. وكانت دلالة ساندل للحساسية 0.0064, 0.0055 و 0.0059 مايكروغرام سم$^{-2}$، بينما بلغ الخطأ النسبي من -3.365 إلى 0.904% و -3.325 إلى 0.138% و -2.053 إلى -2.679% للمعوضات DONA وDMNA وDPNA وعلى التوالي. بالإضافة إلى أن الطريقة كانت بسيطة وسريعة ولا تحتاج إلى التحكم في درجة الحرارة ولا تقنية الاستخلاص بالمذيبات، كما وتمت دراسة الظروف المثلى للتفاعل وتركيب الصبغة وثابت الاستقرارية والمتداخلات. ولقد طبقت الطريقة بنجاح لتقدير حامض الباربيتيورك في ماء نهر دجلة.

الكلمات الداالة: صبغة الآزو، حامض الباربيتيوريك، نايتروانيلين، التقدير الطيفي.

INTRODUCTION

Barbituric acid {2,4,6(1H,3H,5H)-pyrimidinetrione}is widely used in the preparation of barbiturates, dyes and polymerization catalysts (Hawley, 1981), pharmaceutical preparation and indicators (Acheson, 1967), textile (Brown, *et al.*, 1970) and also been identified as an intermediate in many processes. It is well known that barbituric acid itself has no affect on the central nervous system (Wesson and Smith, 1977) however it is used as a precursor to medical barbiturates which can be lethal in excessive amounts (Matther, 1971). Therefore, the determination of trace amounts of barbituric acid is very important in studies for both biological and industrial processes. Different methods such as chromatography (Cela *et al*., 2000), mass spectrometry (Van Langenhove *et al*., 1982) capillary electrophoresis (You, 2000) infra-red spectrophotometry (Pawelczyk, *et al*., 1972) spectrophotometry (Medien, 1996), (Medien and Zahran, 2001) and (Bartzatt, 2002) and colorimetry (Nematollahi and Hesari, 2001) also have been reported for determination of barbituric acid. Some of these methods are time consuming and suffer from lack of selectivity or good sensitivity and/or have short linear dynamic range or have higher limit of detection and/or use reagents not commercially available.

Recently, (Nematollahi and Hesari, 2001) used controlled potential colorimetric technique for barbituric acid analysis in the range of 1-200 mmol. The method is not very sensitive and has several interferences. (Medien and Zahran, 2001) used 1,4-naphthoquinone as a spectrophotometric reagent for barbituric acid determination, but the method has a high limit of determination 2.7-61.5 mg ml^{-1} and has many interfering substances for barbiturate determination. (Bartzatt, 2002) used sodium nitrite as a suitable reagent for colorimetric analysis of barbiturate, with linear range of 18.7-225 mg ml^{-1}. Therefore, the need for a fast, low cost and sensitive method is obvious, especially for routine quality control analysis. This spectrophotometric procedure is suitable for determination of barbituric acid at trace level.

EXPEREMINTAL

Apparatus

Spectral measurements were made on a Shimadzu UV-1601 recording spectrophotometer. All the pH measurements were done on Elico pH meter (LI-10T).

Reagents and Chemicals

All chemicals used were of highest purity.

Standard barbituric acid solution (100 µg/ml). A 0.1000 g of barbituric acid dissolved in 1L distilled water. This solution was prepared weekly.

Diazotized nitroanilines reagents solution, (5×10^{-3} M). A 0.0691g of o-, m-, and p-nitroanilines (Fluka) were dissolved in about 8 ml of distilled water. Then 1.6 ml of 5 M hydrochloric acid solution was added (the solution was heated in case of o-nitroaniline only), the clear mixture was then transferred to a 100- ml volumetric flask and cooled to 0-5^0C in an ice bath. A 3.5 ml of 1% of sodium nitrite ($NaNO_2$) solution was added and the mixture was stirred vigorously. After 5 min., the solution was made up to volume in 100-ml volumetric flask with cold distilled water. The solution was kept in a brown bottle in refrigerator and was stable for five days at least.

Hydrochloric acid solution (5 M). This solution was prepared by appropriate dilution of concentrated hydrochloric acid (Fluka) with distilled water.

Na_2Co_3 solutions 2%. Sodium carbonate solution were prepared by appropriate dilution of concentrated ampoule solution (Fluka) with distilled water and then transferred to a plastic bottle. The other bases (sodium hydroxide, potassium hydroxide, sodium acetate, sodium bicarbonate, sodium formate) 2% solutions were prepared by dissolving 2.0 g of base in 100-ml volumetric flask with distilled water and then transferred to plastic bottle.

Surfactant solutions (1×10^{-3} M). These solutions were prepared by dissolving 0.0365 g of SDS and 0.0289 g of CTAB each in 20 ml distilled water and the volume was made to 100 ml in volumetric flask with distilled water.

Triton X-100 (1%). This solution was prepared by completing 1.00 ml Triton X-100 in 100-ml volumetric flask with distilled water.

Foreign compounds (Fluka) solutions (100 µg/ml). These solutions were prepared by dissolving 0.1000 g of the foreign compound in 1L distilled water.

River water: This water taken from the middle of Tigris river in forest place in Mosul city was filtered and then 25, 50, 75 and 100 µg barbituric acid were added and the recommended procedure was applied without any further treatment.

Procedure

Transfer aliquots of aqueous sample solutions containing (5-300µg) of barbituric acid into a series of 25-ml calibrated flasks. To each flask, 1ml of reagent (DONA, DMNA, and DPNA) solutions and 1ml of 2% KOH, 1 ml of 2% CH_3COONa and 0.5 ml of 2% NaOH solutions were added, respectively. The solutions were mixed and diluted to the mark with distilled water. The absorbance of each colored solution at 418, 380, and 370 nm were

measured against the reagent blank, (DONA, DMNA, and DPNA) respectively, using 1-cm cells.

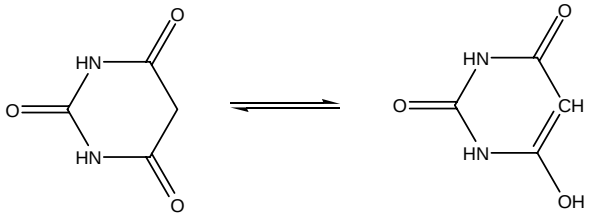
For the subsequent experiments, 100µg of barbituric acid was taken and the final volumes were 25 ml.

RESULTS AND DISCUSSION

In this work, the reaction occurs in two steps: in the first step the reaction of o, m, and p-nitroaniline with sodium nitrite occurs in an acid medium producing the diazo compound. In the second, the diazo compound in alkaline medium coupled with the barbituric acid and produced azo compound that was monitored at 418, 380 and 370 nm.

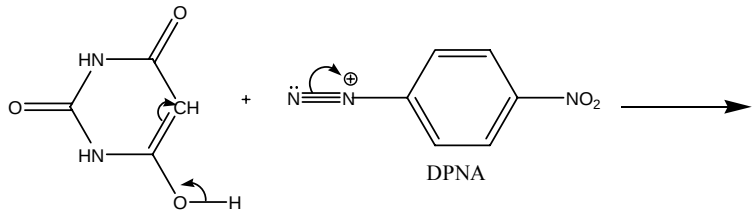
Diazotized nitroaniline reagents had been selected in this study for the following reasons: (1) the strongest diazonium electrophile ever used . (2) the strongest color observed in its azo dye production. (3) the solution of the diazotized nitroaniline reagents are stable for long time (>1 week) if kept in cold and dark conditions, and (4) the color of the diazotized reagents are faint yellow thus giving lower blank values (Al-Abbasi, 2009, Al-Kass and Younis, 2004, Bashir and Mansour, 2007 , Othman, 2004, Rahim *et al*., 1986,)

The reaction involving diazotized nitroanilines as the chromogenic reagent for the determination of barbituric acid is shown below (for example DPNA). Diazotization and coupling reactions were found to be temperature dependent. Diazotization was carried out at 0-5 ºC and coupling reactions was carried out at room temperature 20-25 ºC. However, comparison between the absorption of the three different substituent is not possible since the determination of the spectrum have been done in different pHs. The choose of three different substituted nitroaniline reagents was in order to explore the reaction, i.e. to see whether coupling occur with all substituents o, m, p- nitroanilines.



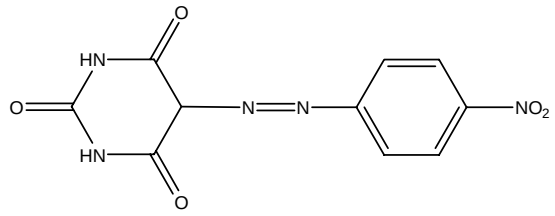
Barbituric acid
Keto-form

Barbituric acid
Enol-form



DPNA

Barbituric Acid
Enol-form



Azo dye

Optimum Reaction Conditions :

The effects of various parameters on the absorption intensity of the colored complex (azo-dye) were studied and the reaction conditions were optimized.

Effect of Base

To produce the colored azo-dye with barbituric acid upon coupling with diazotized substituted nitroanilines reagents, only basic medium can form the azo-dye with useful analytical properties. Therefore, different amounts (0.5-10.0 ml of 2% solutions) of various bases had been tried for the purpose of producing intense colored dye, and lower blank value. Ammonia solution, potassium hydroxide, sodium acetate, sodium bicarbonate and sodium hydroxide had been tried for this purpose. The experimental data had shown that potassium hydroxide solution (1.0 ml, pH=12.25), sodium acetate solution (1.0 ml, pH=5.00) and sodium hydroxide solution (0.5 ml , pH=12.30) produced intense yellow color azo-dye with low blank value at 418,380 and 370 nm for DONA, DMNA and DPNA reagent respectively. Consequently, 1.0 ml of 2% KOH solution, 1.0 ml of 2% CH_3COONa solution and 0.5 ml of 2% NaOH solution had been recommended for the subsequent experiments for DONA, DMNA and DPNA reagent, respectively.

Effect of Diazotized Nitroanilines Reagents Amount

The effect of the DNAs concentration on the color intensity of the maximum absorbance of the dye formed was studied using the proposed procedure and adding volumes from 0.5 - 5.0 ml of 5.0×10^{-3} M solutions of DNAs to a series of barbituric acid solutions (5-300 μg of BA in final volume 25ml). The results showed that a 1.0 ml of 5.0×10^{-3} M of DNAs solution was sufficient for complete color development from both correlation as well as sensitivity points of view. Higher concentration did not enhance the absorbance further, and lower concentration did not give good results.

The absorbance of the dye formed at corresponding maximum wavelength due to varying the concentration of coupling agent were illustrated in Table 1.

Table 1: Effect of diazotized nitroaniline reagent amount on absorbance of the dye formed.

Reagent 5×10^{-3} M	ml of reagent	Absorbance, μg/ml Barbituric Acid							R^2	A_1
		0.2	0.4	1.0	2.0	4.0	8.0	12.0		
DONA	0.5	0.031	0.051	0.145	0.291	0.369	0.955	1.442	0.991	0.024
	1.0	0.027	0.060	0.142	0.289	0.624	1.018	1.681	0.997	0.041
	1.5	0.034	0.063	0.150	0.305	0.571	1.135	1.754	0.999	0.060
	2.0	0.034	0.060	0.127	0.291	0.615	1.211	1.816	0.999	0.086
	3.0	0.033	0.057	0.173	0.322	0.651	1.231	1.866	0.999	0.099
	4.0	0.021	0.059	0.156	0.277	0.569	1.060	1.847	0.992	0.108
	5.0	0.045	0.046	0.135	0.316	0.616	1.171	1.754	0.999	0.120
DMNA	0.5	0.034	0.076	0.220	0.428	0.718	1.219	1.786	0.993	0.029
	1.0	0.039	0.082	0.226	0.421	0.727	1.220	1.898	0.995	0.026
	1.5	0.034	0.079	0.213	0.422	0.723	1.380	2.008	0.998	0.027
	2.0	0.041	0.094	0.198	0.426	0.711	1.392	2.219	0.997	0.025
	3.0	0.034	0.082	0.202	0.430	0.678	1.393	2.128	0.998	0.028
	4.0	0.034	0.084	0.205	0.401	0.666	1.361	2.072	0.998	0.035
	5.0	0.043	0.092	0.231	0.433	0.666	1.347	2.105	0.997	0.038
DPNA	0.5	0.020	0.054	0.163	0.317	0.623	1.084	1.769	0.996	0.041
	1.0	0.030	0.073	0.163	0.336	0.682	1.197	1.888	0.995	0.093
	1.5	0.037	0.067	0.168	0.344	0.542	1.198	1.76	0.998	0.147
	2.0	0.030	0.065	0.115	0.254	0.593	1.250	1.762	0.998	0.208
	3.0	0.045	0.091	0.126	0.225	0.59	0.989	1.397	0.992	0.195
	4.0	0.060	0.100	0.194	0.336	0.645	1.201	1.612	0.995	0.280
	5.0	0.027	0.036	0.179	0.252	0.629	1.084	1.694	0.996	0.300

A_1 = absorbance of reagent blank against distilled water.

Order of Addition.

The order of addition for reagent (ml) and corresponding volume of base to the sample solution (100μg/25ml of BA) had been examined (Table 2).

Table 2: Effect of order of addition on absorbance of the dye.

Reagent	Order of addition	A_1	A_2
DONA	BA + R + B	0.612	0.049
	BA + B + R	0.194	0.040
DMNA	BA + R + B	0.701	0.025
	BA + B + R	0.126	0.020
DPNA	BA + R + B	0.672	0.099
	BA + B + R	0.375	0.030

A_1 = absorbance of the dye against reagent R (1 ml)

A_2 = absorbance of reagent against DW.

B = base (recommended volume)

The order BA + R + B for DONA, DMNA, and DPNA reagents were recommended due to their higher absorbance value since the order BA + B + R gave very low absorbance which probably due to conversion of the active diazonium salt ($Ar\text{-}N{\equiv}N^{+}$) into inactive diazohydroxide species ($Ar\text{-}N{=}N\text{-}O^{-}$, in basic medium).

Effect of Time on Formation of the Azo-Dye

The effect of time after the addition of the reagents was evaluated for different time intervals (3-70 min). The absorbance of the azo-dye was found to be constant after 10 min (DONA and DMNA reagent) and after 45 min (DPNA reagent) and remain constant after that. The reaction proceeded very quickly at room temperature at (20-25 ºC) and provided satisfactory results. The absorbance values were constant at this temperature, and no change was observed. Hence, heating was not needed for completion of the color development. The effect of time on the formation of the color of the azo-dye product is shown in Table 3.

Table 3: Effect of time on the formation of the azo-dye product from BA concentration 4.0µg/ml.

Reagent	Time, min.	Absorbance /4µg BA	A_1
DONA	3	0.619	0.073
	10	0.621	0.071
	25	0.622	0.072
	45	0.626	0.070
	70	0.622	0.070
DMNA	3	0.721	0.070
	10	0.725	0.074
	25	0.724	0.074
	45	0.722	0.075
	70	0.725	0.075
DPNA	3	0.450	0.091
	10	0.577	0.092
	25	0.658	0.092
	45	0.686	0.092
	70	0.684	0.092

A_1= absorbance of reagent against DW

Effect of Surfactants

In order to verify the importance of the electrostatic attraction in the detection of surfactant aggregates, the effects of cationic surfactant CTAB, anionic surfactant SDS and nonionic surfactant Triton X-100 was studied on the UV–visible spectra of BA. The effect of several types of surfactants on color intensity of the dye had been investigated Table 4.

Table 4 : Effect of surfactants on absorbance of dye formed.

Reagent	Surfactant used	Absorbance* /5ml of surfactant added	Absorbance without surfactant
DONA	CTAB (10^{-3}M)	0.580	0.624
	SDS (10^{-3}M)	0.555	
	TritonX-100(1%)	0.467	
DMNA	CTAB (10^{-3}M)	0.768	0.727
	SDS (10^{-3}M)	0.768	
	TritonX-100(1%)	0.805	
DPNA	CTAB (10^{-3}M)	0.696	0.682
	SDS (10^{-3}M)	0.700	
	TritonX-100(1%)	0.713	

* The concentration of BA was 100µg/25ml.

The addition of surfactants did not show a remarkable reduction of the UV-visible-absorption intensity of the longest wavelength absorption band of the dye formed between barbituric acid and DONA in the aqueous solution. Also, there is no enhancement in case of the other two species. Therefore, we excluded the surfactants from our experiments because of the small effect of surfactants on the absorption intensity maxima of the dye formed.

Absorption Spectra

When very dilute aqueous solutions of BA and diazotized nitroaniline reagent solution are mixed in the presence of base, an intense yellow dye forms immediately. The intense dye formed shows a maximum absorption at 418, 380, and 370 nm, in contrast to the reagent (DONA, DMNA and DPNA) blanks , which shows absorbance 0.041, 0.026, and 0.093 at the above λ_{max} against distilled water, respectively.

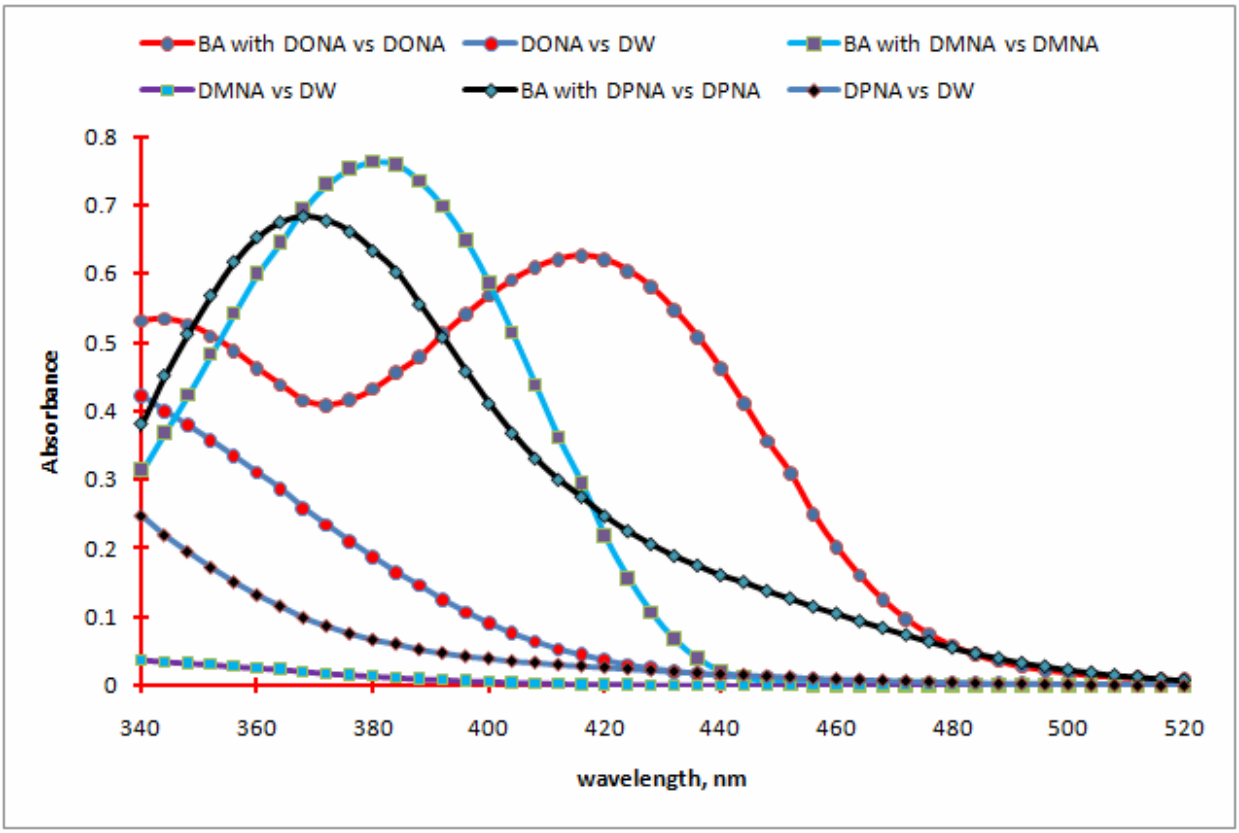


Fig. 1: Absorption spectra of 100 µg/25ml BA, treated as described under procedure and measured against a reagent blank; and reagent blank measured against distilled water.

The wavelength of maximum absorption of dye formed at 418 nm for DONA, 380 nm for DMNA, and 370 nm for DPNA was used in all subsequent experiments. Resonance effects, varying the polarity of the solvent, hydrogen-bonding and pH variations may explain the differences in the wavelength of maximum absorption of the individual substituted (Bashir and Mansour , 2007; Krystal, 2007).

Recommended Procedure and Validity of Beer's Law

The agreement of Beer's law was studied by measuring the absorbance values of solutions and varying barbituric acid amount. A straight-line calibration curves were obtained, indicating that Beer's Law is obeyed over the concentration range 5-300 µg of barbituric acid in 25 ml final volume, i.e., 0.2-12 ppm as shown in Fig 2. The molar absorptivity were 1.998×10^4, 2.328×10^4, and 2.184×10^4 l mol^{-1} cm^{-1} for DONA, DMNA, and DPNA, respectively, and Sandell`s sensitivity index calculated as 0.0064, 0.0055, and 0.0059 µg cm^{-2} for DONA, DMNA, and DPNA, respectively .

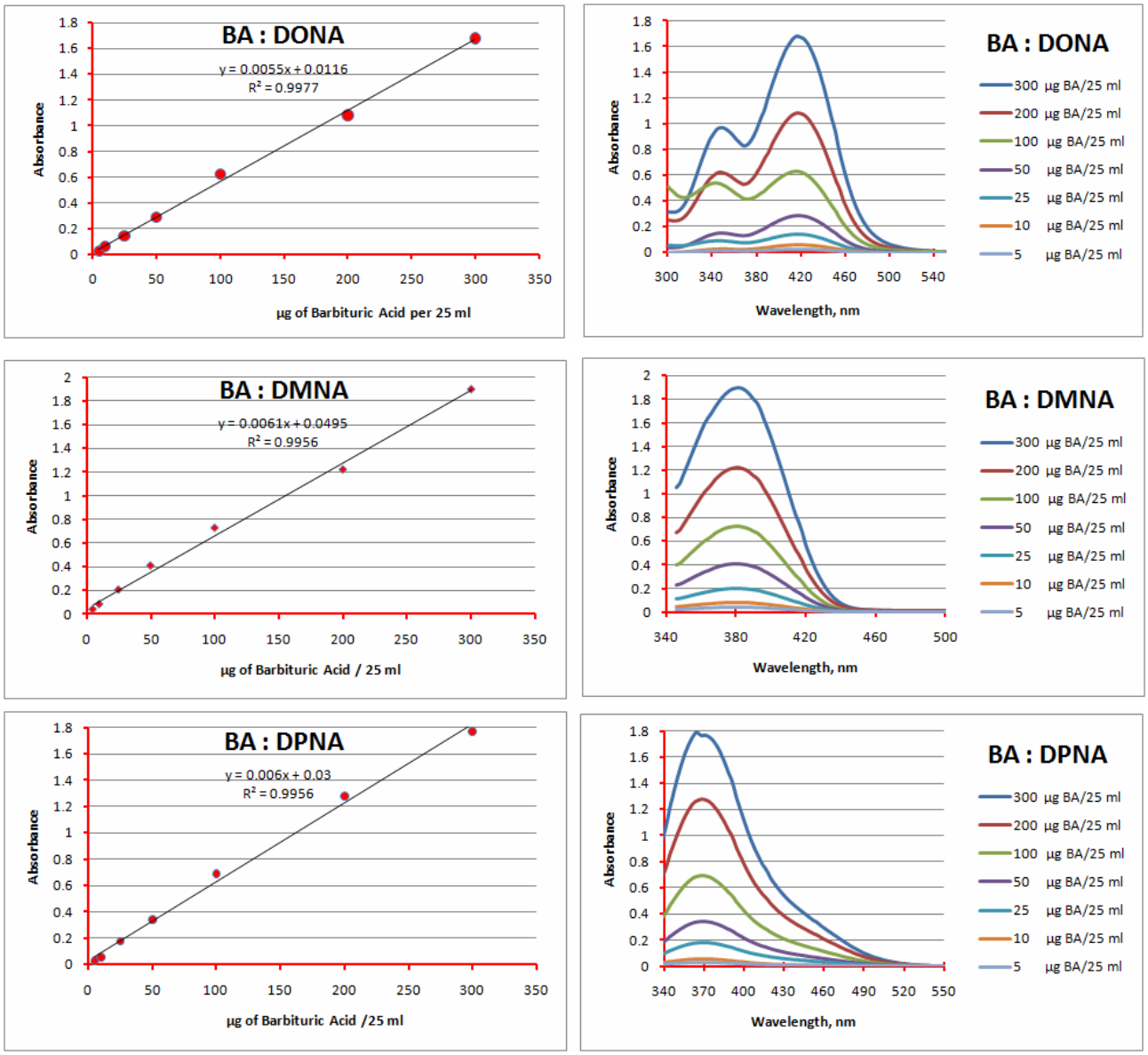
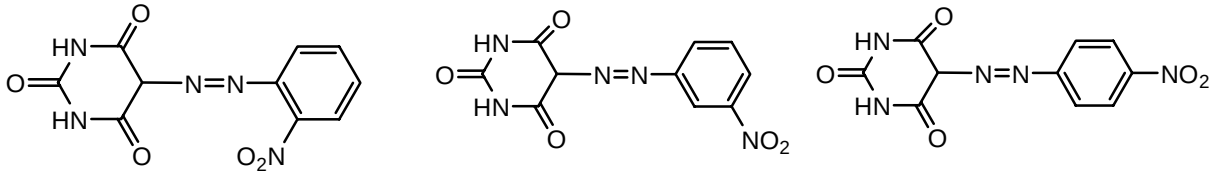


Fig 2 : Calibration curves of barbituric acid with different diazotized reagents.

Nature of the Dye

To establish the composition (ratio of barbituric acid to diazotized nitroaniline reagents) of the yellow azo dye formed, Job's method of continuous variations had been used (Hargis,1988). The resulting data reveal that the dye had been formed by the reaction of barbituric acid with diazotized nitroaniline reagent (DONA, DMNA and DPNA) in a 1:1 ratio, indicating a mono azo dye with probably of the following structures:



BA:DONA BA:DMNA BA:DPNA

The apparent stability constant of azo dye in aqueous solution, under the conditions of the experimental procedure, had been calculated, and found to be $2.4126x10^4$, $2.5349x10^5$ and $5.7968x10^5$ M^{-1}, respectively.

Accuracy and Precision

In order to determine the accuracy and the precision of the method, standard solutions containing three different concentrations of BA were analyzed in five replicates. The mean results obtained are summarized in Table 5. The results indicate that the method was satisfactory.

Table 5: Accuracy and precision of the method.

Reagent	µg BA	Relative error % *	RSD % **
DONA	2	-1.384	±0.489
	4	-3.365	±2.012
	8	0.904	±3.330
DMNA	2	-3.325	±0.897
	4	0.138	±1.453
	8	-1.098	±2.209
DPNA	2	2.679	±0.059
	4	-2.053	±1.164
	8	0.042	±3.689

* Average of five determinations.
** RSD Relative standard deviation.

Study of Interferences

The effect of interferences was studied for the proposed system, because the system was developed for the analysis of barbituric acid. Interference with variety of organic compounds was studied in the absence and presence of known amounts of variety of ions added to a solution containing 100 µg/ml of barbituric acid per 25 ml final volume. Each solution was treated according to the proposed procedure. The tolerance limits of the foreign organic compounds are given in Table 6 as the amounts that caused not more than ±3% changes in the values of absorbance during the determinations. These organic compounds were tolerated to a great extent without any pretreatment.

Table 6: Effect of foreign compounds on barbituric acid determination.

Reagent	Substance added	Tolerance amount added(µg)	Relative error, %
DONA	Acetonitrile	100	1.78
	Ethanol	10	-0.70
	Ethyl acetate	100	-2.92
	Formic acid	100	0.64
	Malonic acid	10	-1.75
	Oxalic acid	100	-0.34
	Phenyl barbital	100	-0.36
	Sucrose	1000	1.61
	Saccharine	10	-1.02
	Urea	1000	-0.72
DMNA	Acetonitrile	100	-1.24
	Ethanol	10	0.14
	Ethyl acetate	1000	0.00
	Formic acid	100	-1.38
	Malonic acid	1000	0.52
	Oxalic acid	100	0.52
	Phenyl barbital	100	-0.52
	Sucrose	100	-0.13
	Saccharine	100	-1.50
	Urea	100	-1.82
DPNA	Acetonitrile	1000	0.48
	Ethanol	1000	1.18
	Ethyl acetate	1000	-0.39
	Formic acid	100	0.78
	Malonic acid	10	-0.53
	Oxalic acid	1000	-1.02
	Phenyl barbital	100	2.01
	Sucrose	1000	-1.18
	Saccharine	10	-0.2
	Urea	10	-1.73

Application of the Method

Determination of Barbituric Acid in Water River

The proposed method was successfully applied to the determination of barbituric acid in water river samples. The water samples were collected from Tigris river and were filtered before analysis. The tested water was found to be free from barbituric acid and so synthetic samples were prepared by adding known amounts of barbituric acid to the water samples. The reliability of the method to analyze real samples was checked by recovery experiments, which gave acceptable results. The recoveries are close to 100% and indicate there is no serious interference in such water samples. The results are shown in Table 7.

Table 7: Determination of (BA) added to river water samples by the recommended procedure.

Reagent	µg of BA added	Recovery % of BA per ml of river water used		
		1ml	3ml	5ml
DONA	25	101.86	100.31	104.64
	50	100.45	100.91	98.04
	75	101.73	99.24	104.35
	100	101.62	102.16	98.15
DMNA	25	99.81	100.93	100.37
	50	101,03	101.96	101.5
	75	100.43	100.87	100.06
	100	100.09	100.51	100.61
DPNA	25	97.63	95.26	100.24
	50	96.49	100.98	96.69
	75	100.24	93.70	97.70
	100	104.28	104.13	104.62

Comparison with the other Method

In comparison with the other methods, it had been found that proposed method seem to be more reliable for application from analytical point of view (Table 8).

Table 8: Comparison of the methods.

Analytical parameters	Present method			Literature method (Ibraheem, 1984)
	o-NA	m-NA	p-NA	
pH	12.25	5.00	12.35	Basic medium
Temperature (C°)	RT	RT	RT	RT
Development time (min.)	3	3	3	1
λmax (nm)	418	380	370	355
Medium of reaction	Aqueous	Aqueous	Aqueous	Aqueous
Reagent	DONA	DMNA	DPNA	DAA
Beer`s law range (ppm)	0.2-12.0	0.2-12.0	0.2-12.0	0.4-8.0
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	1.998×10^4	2.328×10^4	2.184×10^4	2.36×10^4
Relative error (%)	(-3.365 to 0.904)	(-3.325 to 0.138)	(-2.053 to 2.679)	(-2.0 to 0.8)
RSD (%)	±0.489 to ±3.330	±0.897 to±2.209	±0.059 to±3.689	± 0.10 to ± 3.30
Color of the dye	Yellow	Yellow	Yellow	Orange
Nature of dye	(1:1)	(1:1)	(1:1)	....
Stability constant	2.4126×10^4	2.5349×10^5	5.7968×10^5	....

RT= Room temperature.
DAA= Diazotized Anthranilic Acid

CONCLUSION

A new spectrophotometric method for the assay of micro amounts of barbituric acid in aqueous solution had been developed. The method was based on the coupling of barbituric acid with diazotized nitroanilines in basic medium. The azo-dye formed was water-soluble, stable, and shows maximum absorption at 418, 380, and 370nm for DONA, DMNA, and DPNA, respectively. The proposed method for the determination of barbituric acid is simple, sensitive, very low cost, has a wide analytical range and without the need for extraction or heating. The reagent proposed has the advantage of high sensitivity and low

absorbance of reagent blank. The developed method does not involve any stringent reaction conditions and offers the advantages of high color stability (more than 1 hour). The proposed method has been successfully applied to the determination of trace amounts of barbituric acid in Tigris water river.

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