J. Edu. Sci., Vol. (18), No. (2), 2006

# Spectrophotometric Determination of Aniline by Azo-Dye Formation with 2, 6-Dihydroxybenzoic Acid–Application to waters

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**Received** **Accepted**
**21/9/2005** **23/1/2006**

## ABSTRACT

A simple and sensitive spectrophotometric method for the determination of aniline in aqueous medium is described. The method is based on the formation of a yellow coloured azo product from the diazotisation of aniline followed by a coupling reaction with 2,6-dihydroxybenzoic acid in the presence of sodium carbonate. Absorbance of the resulting yellow azo dye is measured at 432 nm and is stable for 1 hour at least. Beer's law is obeyed in the concentration range of 5-80μg of aniline in a final volume of 25ml, with a molar absorptivity of $4.2\times10^4$ l.mol$^{-1}$.cm$^{-1}$, a relative error of +1.13 to+ 4.40 % and a relative standard deviation of ±0.30 to ±0.76%, depending on the concentration level.

The method is successfully employed for the determination of aniline in river and waste waters.

## الخلاصة

يتضمن البحث طريقة طيفية بسيطة وحساسة لتقدير الأنيلين في الوسط المائي. تعتمد الطريقة على أزوتة الانيلين ثم اقتران الانيلين المؤزوت مع 2،6- ثنائي هيدروكسي حامض البنزويك وباستخدام كاربونات الصوديوم لتكوين صبغة صفراء. الصبغة الناتجة مستقرة لمدة ساعة على الأقل وتعطي أعلى امتصاص عند الطول الموجي 432 نانوميتر.تراوحت حدود تطبيق قانون بير في مدى التركيز 5-80 مايكروغرام انيلين/25 مل حيث بلغت الامتصاصية المولارية $4.2\times10^4$ لتر.مول$^{-1}$.سم$^{-1}$، والخطأ النسبي من 1.13+الى 4.40+ والانحراف القياسي النسبي بين ±3.60 و ±0.76 اعتماداً على مستوى التركيز. تم تطبيق الطريقة بنجاح في تقدير الانيلين في مياه النهر ومياه الفضلات.

## INTRODUCTION

Aniline is an organic chemical, extensively used in the rubber, dye, pharmaceutical, and many other industries(1,2).

Many spectrophotometric methods for the determination of aniline are based on diazotisation of aniline and coupling of diazotised aniline with different coupling agents, such as 1-naphthol(3), 2-naphthol(4),1-amino-8-naphthol-2,4-disulphonic acid(5),2-naphthol-3,6-disulphonic acid(6), N-(1-naphthyl)ethylendiamine(7-9),N-sulphatethyl-*m*-toluidine and 3-hydroxy-2-naphthonic acid(10),N-diethyl-N-naphthylpropylene-diamine(11),8-amino-1-hydroxynaphthalene-3,6-disulphonic acid(12), pyrogallol(13),phloroglucinol(14),resorcinol(15),*p*-nitroaniline(16)and orcinol(17).

Another spectrophotometric method for the determination of aniline includes the oxidative coupling with promethazine-HCl in the presence of hypochlorite(18).

Another spectrophotometric method for simultaneous determination of aniline and cyclohexylamine, is based on the reactions involving aniline and/or cyclohexylamine with bis(acetylacetoneethylenediamine) tributylphosphine cobalt (III) perchlorate as a complexing reagent(19).

From the above literature survey it may be stated that some of the above methods have not been applied to any extent for the determination of any real sample(7,13,18) and another needs extraction in to organic medium (9).Therefore, it may seem desirable to develop a method that may satisfy more of the analytical requirements.

The present method involves the diazotisation of aniline and followed by coupling with 2,6-dihydroxybenzoic acid to form a highly coloured dye that has been proved successfully for the assay of aniline in a river and waste water.

## EXPERIMENTAL

### Instrument

A Shimadzu UV-Visible Recording Spectrophotometer UV-160 with 1-cm matched silica cells is used for optical measurements.

### Reagents

All reagents and solvents used are of analytical grade.

***Aniline stock solution, 5000μg/ml.*** This solution is prepared after distillation of aniline (BDH) by diluting 0.49 ml (0.5g) to 100 ml with distilled water.

***Aniline working solution, 50μg/ml.*** This solution is prepared by diluting 1ml of aniline stock solution to 100 ml with distilled water.

***Sodium nitrite solution, 1% (w/v).*** This solution is prepared by dissolving 1g of sodium nitrite (BDH) in 100 ml distilled water.

***Sulphamic acid solution, 3% (w/v).*** This solution is prepared by dissolving 3g of sulphamic acid in 100 ml distilled water.

***Hydrochloric acid solution, 1M.*** This solution is prepared by diluting 8.5 ml of concentrated acid (11.76 M) to 100 ml with distilled water.

***2,6-Dihydroxybenzoic acid solution, 1% (w/v).*** This solution is prepared by dissolving 1 g of 2,6-dihydroxybenzoic acid (BDH) in distilled water and the volume is completed to the mark with distilled water in a 100- ml volumetric flask.
***Sodium carbonate solution, 1N.*** This solution is prepared by dissolving 26.5 g of $Na_2CO_3$ in 500 ml distilled water in a volumetric flask, and then the solution is transferred to a plastic bottle.
***Waste water.*** A waste water sample (taken from the Kornish street in Mosul city) is filtered to remove the suspended solids, A 1, 3, 5 ml and 10 ml of filtered waste water is treated with 2 ml of 0.2% formaldehyde added after addition of 25, 50, 70 μg aniline, respectively to eliminate the interferences of sulphide, sulphite and thiosulphate(20) and then the recommended procedure is applied.
***River water.*** This water which is taken from the middle of Tigris river in Mosul city is filtered and then 25, 50 and 70 μg aniline are added to different volumes of water (1, 3, 5 and 10 ml) and the recommended procedure is applied without any further treatment.

**Recommended procedure and calibration graph**

To a series of 25-ml calibrated flasks, aliquots of aqueous solution containing 5-120μg aniline are transferred , then 1.5 ml of 1N hydrochloric acid and 0.5 ml of 1% (w/v) sodium nitrite solution are added and the mixture is allowed to stand for 1 minute and then 1 ml of 3% (w/v) sulphamic acid solution is added with occasional shaking for 1 minute. After that a 2 ml of 1% (w/v) 2,6-dihydroxybenzoic acid solution and 3 ml of 1N sodium carbonate are added . After the volumes are completed to the mark with distilled water, the absorbance is read at 432nm against a reagent blank. A linear calibration graph is obtained over the concentration range of 5-80μg/25ml aniline and a concentration above 80 μg/25ml gives a negative deviation (Fig. 1).The sensitivity of the method, expressed as the molar absorptivity has been found to be $4.2\times10^4$l.mol$^{-1}$.cm$^{-1}$.

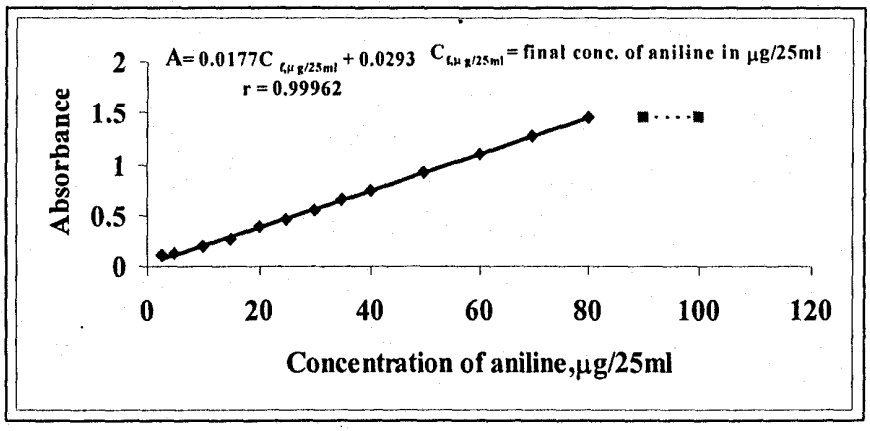



Fig. 1. Calibration graph of aniline determination

## RESULTS AND DISCUSSION

The method involves the diazo-coupling reaction of aniline with 2,6-dihydroxybenzoic acid in an alkaline (carbonate) medium to give a yellow azo-dye.

The factors effecting the colour development, reproducibility, sensitivity, and adherence to Beer's law have been investigated and are reported below:

### Effect of diazotisation acid

Different amount and type of acids have been used in diazotisation of aniline, the results show that 1M hydrochloric acid solution gives the best results, when added in a volume of 1.5 ml.

### Effect of sodium nitrite amount and time

The maximum absorbance reading is obtained by adding 0.5 ml of 1% sodium nitrite with 1 minute reaction time.

### Effect of sulphamic acid amount and time

The excess of nitrite can be removed by the addition of sulphamic acid solution. The results indicate that 1 ml of sulphamic acid solution (3% w/v) with 1 minute as standing time for the reaction give the most suitable effect on the intensity of the dye with corresponding low reagent blank absorbance.

### Effect of 2,6-dihydroxybenzoic acid amount

The effect of the amount of 2, 6-dihydroxybenzoic acid solution (1%) has been studied on the dye absorbance at different amounts 2.5-150 μg/25ml of aniline with varying amounts from 1-5 ml of 2,6-dihydroxybenzoic acid solution. A 2 ml of 2,6-dihydroxybenzoic acid solution(1%) in a total volume of 25ml has been found to be sufficient from linearity and sensitivity points of view (Table 1).

Table 1. The effect of coupling agent amount on absorbance

| Ml of 2,6-Dihydroxy-benzoic acid(1%) | Absorbance /μg aniline | | | | | | | | | Correlation coefficient (r) |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2.5 | 5 | 10 | 25 | 50 | 75 | 100 | 125 | 150 | |
| 1 | 0.031 | 0.169 | 0.188 | 0.424 | 0.845 | 1.168 | 1.521 | 1.770 | 1.949 | 0.993918 |
| 2 | 0.054 | 0.138 | 0.198 | 0.444 | 0.867 | 1.077 | 1.426 | 1.676 | 1.888 | 0.994801 |
| 3 | 0.062 | 0.124 | 0.201 | 0.423 | 0.838 | 1.167 | 1.403 | 1.665 | 1.859 | 0.993378 |
| 4 | 0.069 | 0.081 | 0.194 | 0.415 | 0.447 | 1.142 | 1.137 | 1.318 | 1.386 | 0.969582 |
| 5 | 0.011 | 0.172 | 0.109 | 0.410 | 0.318 | 0.435 | 0.522 | 0.614 | 0.735 | 0.945935 |

**Effect of base**

To develop a quantitative method based on this reaction, a study has been conducted to determine the most effective alkalis and the optimum alkali amount to be used. Sodium carbonate is found to be the most effective base compared to sodium or potassium hydroxide, the strong bases give low intensity but high colour contrast and the yellow azo-dye is unstable. Hence, a volume of 3 ml of 1N sodium carbonate (final reaction mixture pH=10.36) is used in the subsequence experiments. (Table 2).

Table 2. The effect of base on absorbance and colour contrast

| IN base used | Absorbance and colour contrast / ml base added | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 1.5 | | 2 | | 3 | | 4 | |
| | A | Δλ | A | Δλ | A | Δλ | A | Δλ | A | Δλ |
| NaOH | 0.211 | 71 | 0.293 | 70 | 0.415 | 74 | 0.622 | 142 | 0.472 | 150 |
| KOH | 0.199 | 65 | 0.305 | 76 | 0.397 | 86 | 0.642 | 140 | 0.622 | 157 |
| $Na_2CO_3$ | 0.139 | 64 | 0.666 | 127 | 0.816 | 129 | 0.855 | 128 | 0.855 | 129 |
| $NaHCO_3$ | 0.086 | 1 | 0.120 | 68 | 0.214 | 73 | 0.472 | 120 | 0.65 | 124 |
| $CH_3COONa$ | 0.074 | 40 | 0.070 | 45 | 0.079 | 47 | 0.077 | 45 | 0.082 | 45 |

$\Delta\lambda=\lambda_{max}^{S}-\lambda_{max}^{B}$ S= the azo-dye B= the blank

**Effect of time**

The coloured azo-dye developed rapidly after addition of base and immediately attained maximum intensity at room temperature. The colour is stable for at least 1 hour and the results are given in Table 3.

Table 3. The effect of time and aniline amount on absorbance

| μg Aniline/ 25ml | Absorbance / minute standing time | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | 30 | 40 | 50 | 60 |
| 15 | 0.277 | 0.276 | 0.278 | 0.281 | 0.283 | 0.288 | 0.284 | 0.284 |
| 30 | 0.550 | 0.545 | 0.547 | 0.547 | 0.546 | 0.547 | 0.547 | 0.547 |
| 40 | 0.711 | 0.708 | 0.906 | 0.706 | 0.707 | 0.710 | 0.712 | 0.712 |

The above stability period is sufficient to allow several measurements to performed sequentially.

**Final absorption spectrum**

The absorption spectrum of the yellow azo dye formed from coupling of diazotized aniline with 2,6-dihydroxybenzoic acid in basic medium shows a maximum absorpion at 432 nm. The reagent blank has practically negligible absorption at this wavelength (Fig. 2).

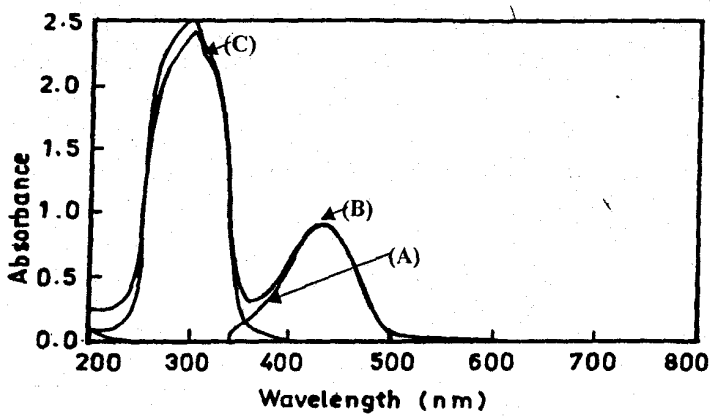



Fig. 2. Absorption spectra of 50µg aniline/25ml treated according to the recommended procedure and measured against (A)reagent blank, (B) distilled water and (C) reagent blank measured against distilled water

**Nature of the dye**

The stoichiometry of the formed azo dye between diazotized aniline and 2,6-dihydroxybenzoic acid is investigated by applying the continuous variations method(21) (Fig. 3).

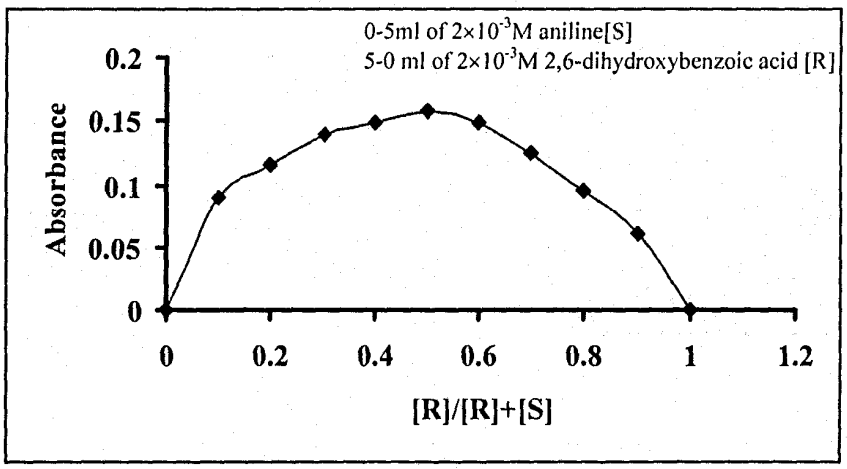
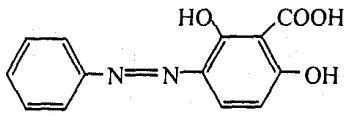



Fig. 3: Continues variations plot for diazotized aniline-2,6-dihydroxybenzoic acid dye

The results indicate that the azo-dye has been found in the ratio of 1:1(diazotised aniline : reagent), and the azo dye may have the following suggested structure.




Yellow azo-dye

**Accuracy and precision**

To check the accuracy and precision of the calibration graph, three different concentrations of aniline are determined. The results shown in Table 4 indicate that the method is satisfactory.

Table 4. Accuracy and precision

| µg aniline/25ml | Relative error, %* | Relative standard deviation, %* |
|---|---|---|
| 20 | 1.139 | ±3.602 |
| 40 | 2.16 | ±0.76214 |
| 50 | 4.40 | ±1.382939 |

* Average for five determinations

**Analytical application**

**Waste water**

The method has been applied to the determination of aniline in waste water using the recommended procedure without further treatment or treated with 2 ml of 0.2% formaldehyde solution (Table 5).

Table 5. Determination of aniline in waste water

| Aniline added (µg) | Sample | Recovery % of aniline/ml of waste water | | |
|---|---|---|---|---|
| | | 1 | 3 | 5 |
| 25 | A | 112.12 | 117.23 | 125.38 |
| | B | 98.42 | 97.81 | 106.10 |
| 50 | A | 108.81 | 109.98 | 111.23 |
| | B | 97.7 | 99.09 | 94.03 |
| 70 | A | 110.90 | 106.31 | 107.87 |
| | B | 97.5 | 96.4 | 95.3 |

A = Sample without further treatment
B = Sample with 2ml of 0.2% formaldehyde solution

The results in the above table show that the recovery of aniline is improved by adding 2 ml of 0.2% formaldehyde solution(20).

**River water**

Table 6 shows the results obtained by present method for the assay of aniline in river water.

Table 6. Determination of aniline in river water

| Aniline added µg/25ml | Recovery % of aniline/ml of river water | | |
|---|---|---|---|
| | 1 | 3 | 5 |
| 25 | 97.71 | 103.45 | 102.57 |
| 50 | 104.72 | 97.97 | 98.01 |
| 70 | 96.23 | 97.44 | 97.25 |

The above results in Table 6 indicate a satisfactory recovery for aniline in river water without any treatment.

## Comparison of the method

Table 7 shows the comparison between the analytical parameters of the present method with those of other spectrophotometric methods

Table 7. Comparison of the methods for aniline determination

| **Analytical parameters** | **Present method** | **Literature method[13]** | **Literature method[18]** |
|---|---|---|---|
| pH | 10.36 | Basic medium | 2.7 and 4.8 |
| Temperature (C°) | Room temp. | Room temp. | Room temp. |
| Development time (minutes) | Immediately | 5 | 15 |
| Stability time (minutes) | 1 hour | - | 20-75 |
| $\lambda_{max}$, nm | 432 | 420 | 520 and 590 |
| Reagent | 2,6-Dihydroxyben-zoic acid | Pyrogallol | Promethazine |
| Beer's law range (ppm) | 0.2-3.2 | 0.2-10 | 0.4-6.4 |
| Molar absorptivity ($l.mol^{-1}.cm^{-1}$) | $4.2\times10^4$ | $0.72\times10^4$ | $1.36\times10^4$ |
| Relative error% | 1.13 to 4.40 | <3.5 | <2.5 |
| RSD% | ±0.3 to ±0.76 | <3 | <2.1 |
| Colour of the dye | Yellow | Yellow | Bluish-green to red |
| Nature of dye | 1:1 | 1:1 | - |
| Type of reaction | Azo-dye formation | Azo-dye formation | Oxidative coupling |
| Application of the method | Determination of aniline in river and waste water | Has not been applied to any extent | Has not been applied to any extent |

The results in Table 7 indicated that the present method is more sensitive and has an applicable part.

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