# Colorimetric Determination of Indole using *p*-hydroxybenzaldehyde

**Wafaa M. Yousef, Saad A.Al-Tamrah, Basmah H. Al-Shammari**

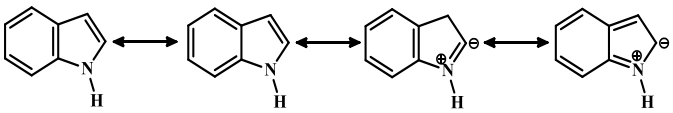
***Abstract*— Indoles are very important compounds and found in many alkaloids of physiological importance. They are also found in seeds and plants such as orange and lemon. Para-hydroxybenzaldehyde dehydrogenase is an enzyme found in carrots, it can be found in the orchid.**

**Indole reacts with *p*-hydroxybenzaldehyde in the presence of concentrated hydrochloric acid to form an orange color complex which has a maximum absorbance at 475 nm. The parameters affecting this reaction were studied in order to find the suitable conditions for the reaction to complete. The effect of acid concentration, reaction temperature and the amount of the reagent on the determination of indole- *p*-hydroxybenzaldehyde complex were investigated. The system requirements and the best conditions for complete determination are sequently studied. At the optimal conditions, 0.02% w/v *p*-hydroxybenzaldehyde, 3MHCl at30$^{o}$C, was used for the determination of indole. Calibration graph was over the range from 0.5-5.00 µg ml$^{-1}$. The detection limit was 0.08 µg ml$^{-1}$ and the correlation coefficient was 0.999. Linearity was obtained with slope equals 0.119 and the intercept equals zero.**

***Index Terms*— Indole; p-hydroxybenzaldehyde; Hydrochloric acid; Colorimetric method**

## I. INTRODUCTION

Indole or benzopyrrole is a heterocyclic compound which has a nitrogen in the five membered ring [pyrrole ring]. The word indole is derived from the word India, a blue dye imported from India was known as indigo. Chemical degradation of this dye gave indoxyl. Indole is a pleasant solid used as a perfume base. It is a planar aromatic molecule with conjugated 10πelectrons contributed by eight carbon atoms and a lone pair contributed by nitrogen. Because of the involvement of lone pair of nitrogen, charge separated canonical forms can be written for indole [1] as shown below:



Indole Canonical

Indole is a natural product of pancreatic digestion, bacterial action and putrefaction decomposition. The properties of indole are similar to that of naphthalene. Indole acetic acid is found in seeds and in some plants such as orange, lemon and catharanthus which produce indole alkaloids. Indole based novel small molecules were designed as potential anticancer agents. Multi step synthesis of these compounds was carried out by using Pd/C–Cu mediated coupling–cyclization strategy as a key step. The single crystal X-ray diffraction study was used to confirm the molecular structure of a representative compound unambiguously. Many of these compounds were evaluated for their anti-proliferative properties in vitro against six cancer cell lines as well as noncancerous cells. All these compounds showed selective growth inhibition of cancer cells and several of them were found to be promising with IC50 values in the range of 0.1–1.2M, comparable to the known anticancer drug doxorubicin [2]. Indole and its derivatives were determined titrimetrically using reaction of indole with N-chlorosuccinamide, potassium iodide was added and the liberated iodine was titrated with sodium thiosulphate. Indole and its derivatives have also been determined using brominating agents [3]. *High Performance Liquid Chromatography* (HPLC) with fluorescence detection was applied for the determination of indoles. Two step pre-column derivatization method was employed for the simultaneous determination of catechol and 5- hydroxy indole amine in urine samples. The detection limit was 0.11–21 nM using fluorescence detection [4].

The electrochemical behavior of 5-methoxy-1-indole-3-acetic acid has been studied by Osteryoung square wave voltammetry in the $NH_3$–$NH_4Cl$ buffer solution [5].

A flow injection chemiluminescence method has been developed for the determination of indole derivatives, it is based on the increased chemiluminescence reaction of potassium permanganate-formaldehyde system in acidic medium, strong chemiluminescence was observed [6].

Spectroscopic methods of analysis are the most important methods which have been used for chemical analysis mainly in the visible region. They can be used for the determination of microgram, nanogram and picogram amounts of many organic and inorganic compounds **[7]**.

A spectrophotometeric method has been developed for the determination of indole derivatives. The method is based on the reaction with Folin reagent (1,2-naphthoquinone-4 sulphonate) with indole. The method was applied for the determination of sumatriptan succinate in commercially available tablets by measuring the absorbance at 455.6 nm [8].

Colorimetric methods for the estimation of indole have been developed [9]; the method depends on the reaction of acidified Ehrlich reagent with certain indoles to produce colored complexes with absorption maxima from 530–580 nm. The sample containing 1–50 µg ml$^{-1}$ of indole can be

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determined after 30 minutes. Another method was described in which indole solution was heated with *p*–dimethylaminobenzaldehyde in 95% ethanol in a boiling water bath for 10 minutes, after cooling to room temperature; the absorbance was measured at 590 nm. The calibration graph was linear from 1.0–12 µg $ml^{-1}$ of indole [10]. Recently, a color sensor for indole vapor has been developed based on Ehrlich reaction in solid polymer film. Exposure of this film to the air containing 5-100 ppb of indole gave a pink or magenta color which can be observed by the naked eye [11].

Para-hydroxybenzaldehyde dehydrogenase is an enzyme found in carrots [12], it can be found in the orchid [13].

Our work is extended to modify and establish a simple, rapid , sensitive and inexpensive method for the determination of indole using *p*-hydroxybenzaldehyde in HCl. The method used could be applied for the determination of samples containing indole.

## II. EXPERIMENTAL

### 2.1. Chemicals and Solutions

Unless otherwise stated all chemical reagents used were of analytical grade with high purity. A stock solution of indole 100.0 µg $ml^{-1}$ was prepared by dissolving 0.01 g of indole (Winlab) in 50 ml absolute ethanol (BDH) and completing the volume to 100 ml with bidistilled water. A stock solution of 10 M HCl (AnalaR, BDH, 11.96 M) was prepared by taking 83.3 ml and diluting to 100 ml with bidistilled water. 96% Ethanol (BDH) was used as a solvent for p-hydroxybenzaldehyde and indole. A stock solution 1% (w/v) of *p*-hydroxybenzaldehyde (BDH, Laboratory Reagent) was prepared by dissolving 1.00 g in 50 ml absolute ethanol and completing the volume to 100 ml with bidistilled water.

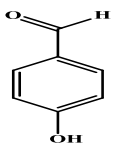
### 2.2. Instrumentation

UV-Vis Ultrascope 2000, Pharmacia Biotech Spectrophotometer was used for all absorbance measurements, with Labomed Inc. Q-4, Quartz cells.

## III. RESULTS AND DISCUSSION

Indole derivatives have recently been studied because of their pharmaceutical and biological importance. The antimicrobial, inflammatory and antitumor activities of indoles encouraged the analysis for developing various methods for their determinations.

### 3.1 Preliminary Investigation




***p*-Hydroxybenzaldehyde**

When *p*-hydroxybenzaldehyde is added to indole, no color was absorbed as shown in Fig 1. However in the presence of concentrated hydrochloric acid an orange color complex was formed which has a maximum absorbance at 475(Fig. 2). The parameters affecting this reaction were studied in order to find the suitable conditions for the reaction to complete.

### 3.2 Optimization of conditions

A suitable concentration of *p*-hydroxybenzaldehyde(0.02% w/v) was added to 5µg $ml^{-1}$indole solution in the presence of 3M HCl. The mixture was shaken well and the absorbance of the formed complex was measured at different time intervals from 2-24 minutes. It was noticed that the absorbance increases with increasing time of the reaction with a maximum absorbance through 15 min, after that, the curve be steady stable (Fig.3).

### 3.3 Effect of temperature:

The effect of temperature on the reaction of indole with *p*-hydroxybenzaldehydewas studied in the range from 25$^{o}$C to 100$^{o}$C. Keeping other conditions constant (5µg $ml^{-1}$indole + 0.02%w/v *p*-hydroxybenzaldehyde in 3M HCl. It was found that the absorbance increases with increasing the reaction temperature up to 30$^{o}$C and then decrease due to the formation of some precipitates as a result of decomposition of indole- *p*-hydroxybenzaldehyde complex (Fig. 4).

### 3.4 effect of concentration of *p*-hydroxybenzaldehyde:

The effect of *p*-hydroxybenzaldehyde concentration was studied using variable concentration of this reagent from 0.005-0.05 % (w/v) in the presence of hydrochloric acid while applying optimized conditions of heat and time. The absorbance was increasing with increasing the concentration of *p*-hydroxybenzaldehyde up to 0.02% (w/v), and then remains constant as illustrated in Fig. 5.

### 3.5 Effect of HCl concentration

At the optimized conditions, the absorbance was found to increase with increasing the concentration of hydrochloric acid up to 3M and then decrease due to decomposition of indole- *p*-hydroxybenzaldehyde complex with increasing concentration of HCl higher than 3M as shown in Fig 6.

### 3.6 Effect of heating time

As mentioned before about 15 minutes are needed for the reaction to complete at room temperature. The study of this parameter was achieved by heating the reaction mixture up to 30$^{o}$C with different time intervals from 2-14 minutes to see the effect of heating time at all other previously optimized conditions. It was found the color began to appear after five minutes and then became more intense with time with a maximum absorbance after 8 minutes which means that heat accelerates the reaction to complete and after that the reaction start to decrease because of the formation of precipitate as illustrated in Fig .7.

### 3.7 General Procedure

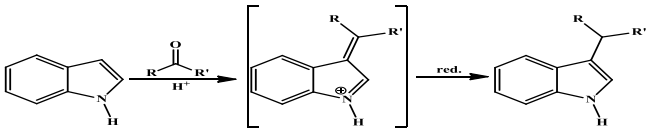
The successful study of the previous parameters, helped us to introduce a simple and accurate procedure for the determination of Indole as follows: Into a 50 ml volumetric flask, transfer 1.0 ml of the sample containing 0.5-10 µg $ml^{-1}$

of indole, add 15 ml of 10 M hydrochloric acid followed by 1.0 ml of 1 % w/v *p*-hydroxybenzaldehyde. Shake well and complete the volume to the mark with bidistilled water. Warm the mixture in a water bath at 30°C for eight minutes. Cool to room temperature, then adjust to volume with bidistilled water and measure the absorbance of the orange complex formed at 475 nm.

### 3.8 Calibration graph

At the optimum conditions, it was found that the absorbance is directly related to the concentration of indole. After reaching the optimum conditions for the reaction of indole and *para*-hydroxybenzaldehyde , a linear calibration graph was obtained in the range from 0.5-5.00 µg $ml^{-1}$ of indole. The detection limit (signal: noise 3:1) was 0.08 µg $ml^{-1}$ and the correlation coefficient was 0.999, the slope equals 0.119 and the intercept equals 0.000, as shown in Fig. 8.

In general, aldehydes and ketones react with indole in acidic media to form C-3 substitutes according to the following equation:




Unlike most amines, indole is not basic and the bonding situation is completely analogous to that in pyrrole. Very strong acids such as hydrochloric acid are required to protonate indole. The protonated form is more sensitive to react with benzaldehyde derivatives.

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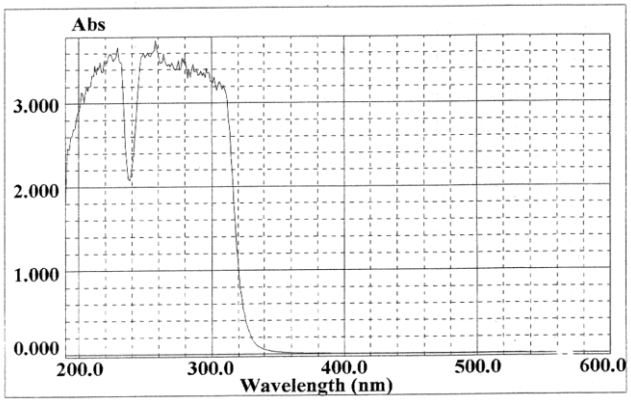


**Fig. 1: Spectrum of *p*-hydroxybenzaldehyde + indole 5 µg $ml^{-1}$ at room temperature.**

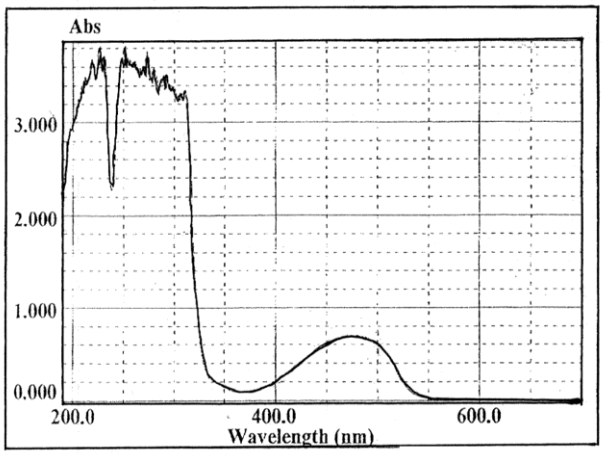


**Fig. 2: Spectrum of *p*-hydroxybenzaldehyde + 5 µg $ml^{-1}$ indole in HCl**

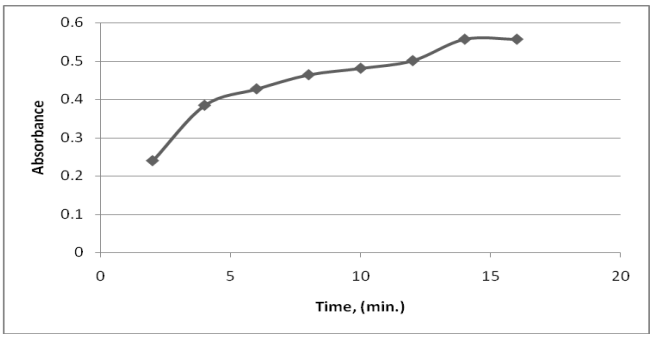


**Fig. 3: Effect of time on the reaction of *p*-hydroxybenzaldehyde with 5 µg $ml^{-1}$indole in the presence of HCl.**

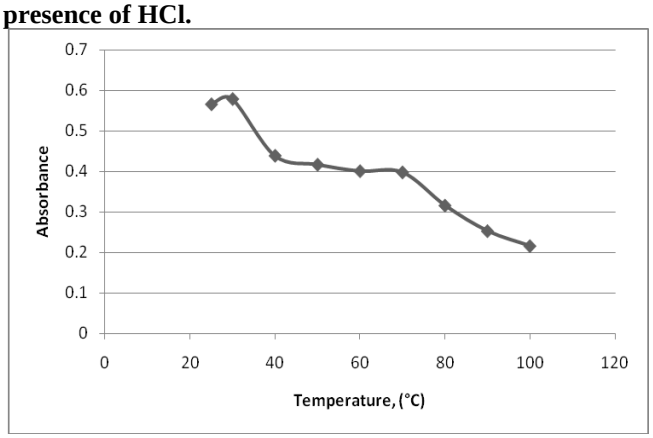


**Fig. 4: Effect of temperature on the reaction of 5µg $ml^{-1}$indole with *p*-hydroxybenzaldehyde**

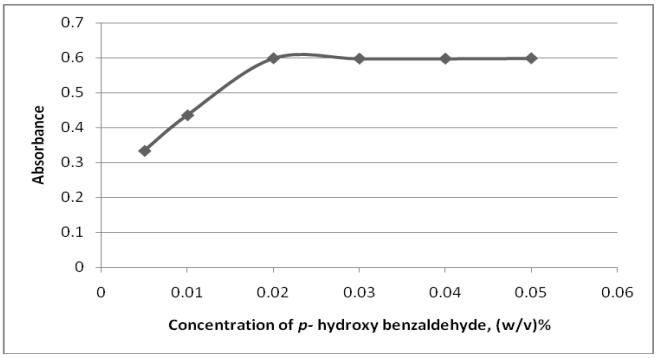


**Fig. 5: Effect of concentration of *p*-hydroxybenzaldehyde on the reaction of 5µg ml$^{-1}$ of indole.**

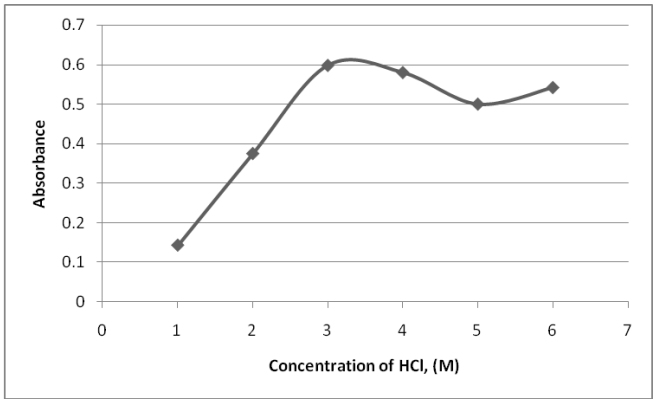


**Fig. 6: Effect of HCl concentration on the reaction of 5µg ml$^{-1}$ of indole with *p*-hydroxybenzaldehyde.**

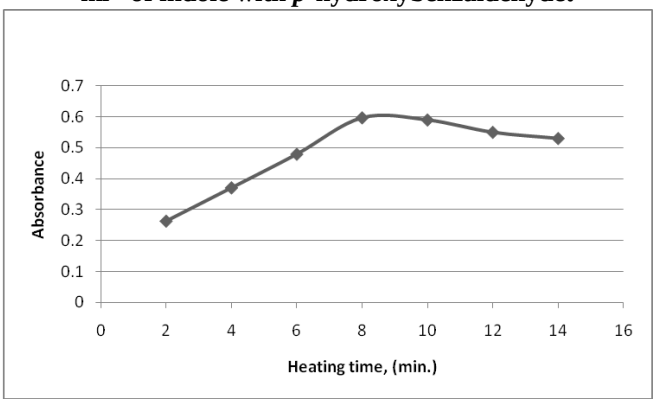


**Fig. 7: Effect of heating time on the reaction of 5µg ml$^{-1}$indole and *p*-hydroxybenzaldehyde in HCl.**

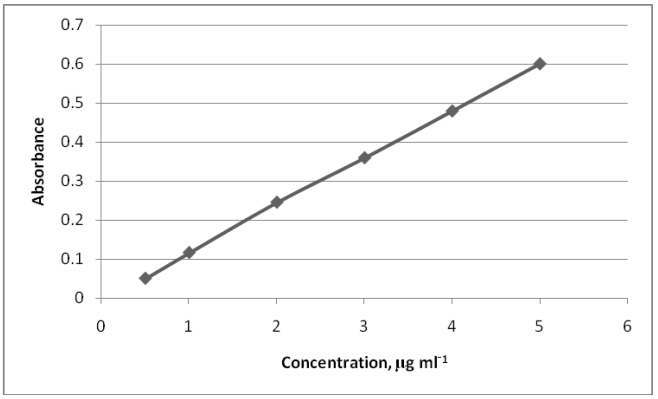


**Fig.8: Calibration graph for indole-*p*-hydroxylbenzaldehydesystem in HCl.**