

# Colorimetric Determination of Indole using 2,4,6-trimethoxybenzaldehyde

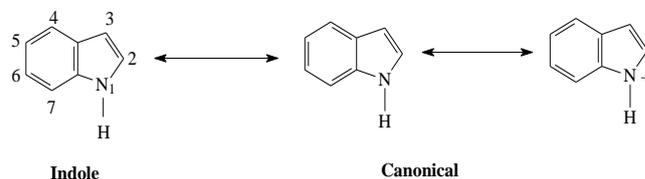
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**Abstract**— Indole is an aromatic heterocyclic organic compound. The indole nucleus is very important for many natural and synthetic molecules with significant biological activity. Compounds that contain an indole ring are called indoles. Indoles are an important class of heterocycles not only because they are among the most ubiquitous compounds in nature, but also because they have a wide range of biological activities. 2,4,6-Trimethoxybenzaldehyde can be used as antibacterial synergist. When indole was mixed with 2,4,6-trimethoxybenzaldehyde at room temperature, no color was observed. However in the presence of concentrated hydrochloric acid, a brown red complex is formed which has a maximum absorbance at 488 nm. The parameters affecting this reaction were studied in order to find the suitable conditions for the reaction to complete. The effect of the acid concentration, the reaction temperature and the amount of the reagent on the determination of indole- 2,4,6-trimethoxybenzaldehyde complex were investigated. The system requirements and the best conditions for complete determination are sequentially studied. At the optimal conditions, 0.02% w/v 2,4,6-trimethoxybenzaldehyde, 4M HCl at 60°C, were used for the determination of indole, At these conditions, it was found that the absorbance is directly related to the concentration of indole. Different concentrations of indole over the range from 0.25-1 µg ml<sup>-1</sup> were reacted with the reagent. The detection limit (signal : noise 3:1) was 0.02 µg ml<sup>-1</sup> and the correlation coefficient was 0.995, Linearity was obtained with slope equals 0.608 and the intercept equals 0.000..

**Index Terms**— Indole; 2,4,6-Trimethoxybenzaldehyde; Colorimetric method; heterocyclic compound.

## I. INTRODUCTION

Indole is an aromatic heterocyclic organic compound. It has a bicyclic structure, consisting of a six-membered benzene ring fused to a five-membered nitrogen-containing pyrrole ring. It is a planar aromatic molecule with conjugated 10π electrons 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; Canonical representation shows electron density between N-1 and C-3 in the pyrrole unit [1], as shown below:



Indole, a secondary amine, is a natural product of pancreatic digestion, bacterial action and putrefaction decomposition. Indole is the precursor to many pharmaceuticals. The name indole is portmanteau of the words indigo and oleum, since indole was first isolated by treatment of the indigo dye with oleum. Indole chemistry began with the study of the dye indigo. The indole ring is also found in many natural products such as the vinca alkaloids, fungal metabolites and marine natural products [2]. Although indole moiety is very small but is fascinated by scientists because of the diverse biological activities by not only indole but its various substituted derivatives as well. Indole derivatives constitute an important class of therapeutic agents in medicinal chemistry including antihypertensive, antiproliferative, antiviral, antitumor, analgesic, anti-inflammatory, antimicrobial, antifungal activities, etc. Due to its wider applications in pharmaceutical industries, they will replace many existing heterocyclic based pharmaceuticals [3]. 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. All these compounds showed selective growth inhibition of cancer cells [4]. 2,4,6-Trimethoxybenzaldehyde can be used as antibacterial synergist. It exhibits significant anti-candida activity, *Candida albicans* is a ubiquitous organism in humans and causes serious disseminated infections in the immunocompromised population [5].

Many methods have been applied for the determination of indoles. Alzheimer's disease (AD) is a fast growing neurodegenerative disorder of the central nervous system and anti-oxidants can be used to help suppress the oxidative stress caused by the free radicals that are responsible for AD. A series of selected synthetic indole derivatives were biologically evaluated to identify potent new antioxidants. Most of the evaluated compounds showed significant to modest antioxidant properties (IC<sub>50</sub> value 399.07 140.0±50 µM). Density Functional Theory (DFT) studies were carried out on the compounds and their corresponding free radicals. Differences in the energy of the parent compounds and their corresponding free radicals provided a good justification for the trend found in their IC<sub>50</sub> values. In silico, docking of compounds into the proteins acetylcholinesterase (AChE) and butyrylcholinesterase

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(BChE), which are well known for contributing in AD disease, was also performed to predict anti-AD potential [6]. 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 [7]. Chromatographic methods were applied for the separation and determination of indole and its derivatives. 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 method is based on the derivatization of the analytes with a poly (methacrylic acid-co-ethylene glycol dimethacrylate), monolithic capillary column was used. Using the optimal operating conditions, the detection limit was 0.11–21 nM using fluorescence detection [8].

Spectroscopic methods of analysis are the most important methods which have been used for chemical analysis mainly in the visible region because of precision, accuracy and simplicity. Protonation of indole-2-carboxylic acid, 3-methylindole, 3-acetylindole and D-tryptophan in perchloric acid media was studied by UV spectroscopic methods in the 400–190 nm region. Absorbance values were measured at four selected wavelengths and the molar absorptivity was calculated. From these values, the position of additional protons in protonated compounds was discussed. [9]. Most of the spectroscopic methods depend on the absorption or emission of part of the electromagnetic radiation by the analyte being determined. A novel flow injection chemiluminescence method for the determination of indole-3-acetic acid (IAA) in biological samples by using trivalent silver. The chemiluminescence signal from the reaction of an Ag (III) complex and a sulfuric acid system was enhanced in the presence of IAA. The conditions of the CL system were investigated and optimized. Under the optimal conditions, the relative chemiluminescence intensity was linear with the IAA concentration in the range of  $1.0 \times 10^{-10}$  g mL<sup>-1</sup> to  $1 \times 10^{-8}$  g mL<sup>-1</sup>. The detection limit for IAA was  $7.7 \times 10^{-11}$  g mL<sup>-1</sup>, and the relative standard deviation ( $n = 11$ ) was 0.5%. The proposed method was applied to the analysis of IAA in human urine, mung bean sprouts and soil samples with recoveries of 103.5–117.1%, 87.0–98.4% and 94.1–118.8%, respectively, and the relative standard deviations was 0.6–2.7%. The chemiluminescence mechanism is discussed by comparison of fluorescence spectra and the UV-vis absorption spectra [10].

A spectrophotometric method has been developed for the determination of indole derivatives. The method is based on the reaction with Folin reagent (1,2-naphthoquinone-4 suphanate) with indole. The method was successfully applied for the determination of sumatriptan succinate in commercially available tablets by measuring the absorbance at 455.6 nm [11].

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. Calibration graph was over the range from 0.5-5.00 µg ml<sup>-1</sup>.

The detection limit was 0.08 µg ml<sup>-1</sup> and the correlation coefficient was 0.999. Linearity was obtained with slope equals 0.119[12].

Our work modifies and establishes a simple, rapid and sensitive 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<sup>-1</sup> 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 2,4,6-trimethoxybenzaldehyde and indole. A stock solution 1% (w/v) of 2,4,6-trimethoxybenzaldehyde (Analar, BDH) was prepared by dissolving 1.0 g in 50 ml absolute ethanol and completing the volume to 100 ml with bidistilled water.

### 2.2. Instrumentation

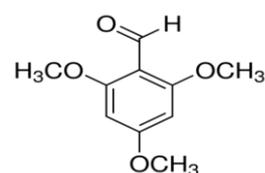
UV-Vis Ultrascope 2000, Pharmacia Biotech Spectrophotometer was used for all absorbance measurements, with Labomed Inc. Q-4, Quartz cells.

## III. RESULTS AND DISCUSSION

Indole derivatives are very important compounds because of their antimicrobial, inflammatory and antitumor activities which encouraged the analysis for developing various methods for their determinations.

### 3.1 Preliminary Investigation

When 2,4,6-Trimethoxybenzaldehyde is added to indole, no color was observed as shown in Fig1. However in the presence of concentrated hydrochloric acid a brown red complex is formed which has a maximum absorbance 488 nm (Fig. 2). The parameters affecting this reaction were studied in order to find the suitable conditions for the reaction to complete.



2,4,6-Trimethoxybenzaldehyde

### 3.2 Optimization of conditions

Many parameters affect the reaction and must therefore be optimized in order to achieve better sensitivity, these parameters include:

#### 3.2.1 Effect of time::

To study the effect of time on the reaction between indole and 2,4,6-trimethoxybenzaldehyde, 1 ml of 0.02% (w/v) of the reagent was added to 1.0 µg ml<sup>-1</sup> of indole and the medium was acidified with 4M hydrochloric acid. The absorbance was measured at 488 nm after different time

intervals (0.5, 10, 15, 20, 25, 30, 35, 40 minutes). It was found that absorbance increases with increasing time to give stable complex up to 30 minutes. The reaction needs about half an hour to complete at room temperature, the results obtained are shown in Fig 3.

### 3.2.2 Effect of temperature::

As mentioned before, the reaction needs about 30 minutes at room temperature to complete, temperature usually enhances most of the reactions. The effect of temperature was studied using the same reaction mixture (0.02% w/v of 2,4,6-trimethoxybenzaldehyde, 4M hydrochloric acid and 1  $\mu\text{g ml}^{-1}$  of indole). The reaction temperature was varied from 25oC to 100oC. The results obtained showed that the absorbance increases with increasing temperature up to 60oC and then start to decrease because of formation a precipitate probably due to the decomposition of the brown red complex. Results are summarized in Fig. 4.

### 3.2.3 Effect of 2,4,6-trimethoxybenzaldehyde Concentration:

The effect of the concentration of the reagent 2,4,6-trimethoxy benzaldehyde was studied keeping other conditions constant by adding various concentrations of the reagent from 0.01-0.25% w/v to 1  $\mu\text{g ml}^{-1}$  of indole using 4M hydrochloric acid and the recommended conditions of time and temperature. It was observed that the absorbance increases with increasing the concentration of 2,4,6-trimethoxybenzaldehyde up to 0.02% w/v and remain stable above this concentrations shown in Fig.5.

### 3.2.4 Effect of HCl concentration

As mentioned before, the addition of hydrochloric acid is necessary for the reaction to take place, at the optimized conditions of time, temperature and the concentration of 2,4,6-trimethoxybenzaldehyde previously studied. The effect of hydrochloric acid was investigated by adding different concentrations of hydrochloric acid from 1-6 M. It was found that the absorbance increases with increasing the acid concentration up to 4M and then started to decrease after that because higher acidity cause decomposition of the complex and formation of some precipitate as shown in in Fig. 6.

### 3.2.5 Effect of heating time

As mentioned before about 30 minutes are necessary to complete the reaction at room temperature. In this study, we use the optimum reaction conditions found earlier. The reaction mixture was heated for different time intervals from 2-10 minutes keeping the reaction temperature constant at 60oC. It was noticed that the deep brown red color gives the highest absorbance after 6 minutes which means the complete formation of the complex. Results obtained are summarized in in Fig. 7.

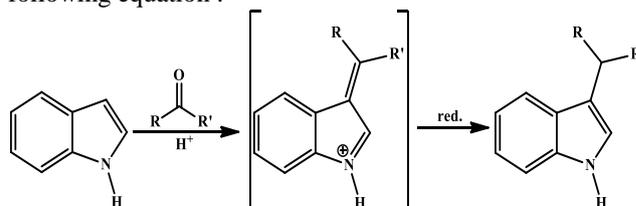
### 3.2.6 General Procedure

Into 50 ml volumetric flask, transfer 1 ml of the sample containing 0.25-2  $\mu\text{g ml}^{-1}$  of indole, add 20 ml of 10 M hydrochloric acid followed by 1 ml of 1% w/v 2,4,6-trimethoxybenzaldehyde . Shake well and complete to the mark with bidistilled water. Heat the mixture in water bath at 60oC for 6 minutes, cool to room temperature and adjust the volume with distilled water. Measure the absorbance of the brown red color complex at 488 nm.

### 3.2.7 Calibration graph

After reaching the optimum reaction conditions for the reaction between indole and 2,4,6-trimethoxybenzaldehyde, it was found that the absorbance is directly related to the concentration of indole. At all other conditions constant, linear relation was achieved between the concentration of indole and the absorbance over the range 0.25-1  $\mu\text{g ml}^{-1}$  of indole. The slope of the curve was 0.608, the intercept was 0.00 and the correlation coefficient ( $r^2$ ) was 0.995 and the limit of detection signal: noise (3:1) was 0.02  $\mu\text{g ml}^{-1}$  of indole. Results obtained are summarized in 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 sensitivity to react with benzaldehyde derivatives

## REFERENCES

- [1] J.M. Khurana, Chemistry of Heterocyclic Compounds, University of Delhi (2006).
- [2] Srivastava, Anupam, Pandeya SN. Indole: A versatile nucleus in pharmaceutical field. *Int J Curr Pharma Rev and Res*; 1(3), 1-17(2011).
- [3] Biswal S, Sahoo U, Setty S, Kumar H.KS and Banerjee M. Indole: The molecule of diverse biological activities. *Asian J Pharm Clin Res*; 5(1), 1-6(2012).
- [4] B.Dulla, E. Sailaja, U. Reddy, M.Aeluri, A. M. Kalle, S. Bhavani, D. Rambabu, M. V. B. Rao, Manojit, *Tetrahedron Letters*, 55, 921-926(2014)
- [5] Kim J, Sudbery P. *Candida albicans*, a major human fungal pathogen. *The J Microbiol*; 49,171-172(2011).
- [6] Muhammad Yar<sup>1, \*</sup>, Muhammad Arshad<sup>3</sup>, Ariba Farooq<sup>1</sup>, Mazhar Amjad Gilani<sup>2</sup>, Khurshid Ayub<sup>3, 4</sup>, Asma Ejaz<sup>5</sup>, Anupriya Kumar<sup>6</sup>, Ichiya Ninomiya<sup>5</sup>, Synthesis and DPPH scavenging assay of reserpine analogues, computational studies and in silico docking studies in AChE and BChE responsible for Alzheimer's disease, *Braz. J. Pharm. Sci.*; 5(1) (2015).
- [7] I. Islam, D. Misra, R. Singh and J. Sharma, *Talanta*, 31(8), 642 (1984).
- [8] Y.B. Wu, J.H. Wu, Z.G. Shi and Y.Q. Feng, *J. Chromatogr. B, Anal. Tech. Biomed., Life Science*, 1, 877 (2009).
- [9] Blagoja S. Andonovski\*, UV Study of the Protonation of Indole-2-carboxylic Acid, 3-Methylindole, 3-Acetylindole and D-Tryptophan in Perchloric Acid Solutions, *CROATICA CHEMICA ACTA (CCACAA)* 72 (4), 711-726 (1999).
- [10] Zhaofu Fu,<sup>a</sup> Gongke Li<sup>\*a</sup> and Yufei Hu<sup>\*a</sup>, A novel flow injection chemiluminescence method for the determination of indole-3-acetic acid in biological samples by using trivalent silver, *7*, 4590-4595(2015).
- [11] B.K. Ramu and K. Raghun, *Int. J. Pharm. Biomed. Sci.*, 1(3), 49 (2010).
- [12] Wafaa M. Yousef, Saad A.Al-Tamrah, Basmah H. Al-Shammari, Colorimetric Determination of Indole using p-hydroxybenzaldehyde, *International Journal of Engineering and Applied Sciences (IJEAS)* 2(1),23- 26 (2015).

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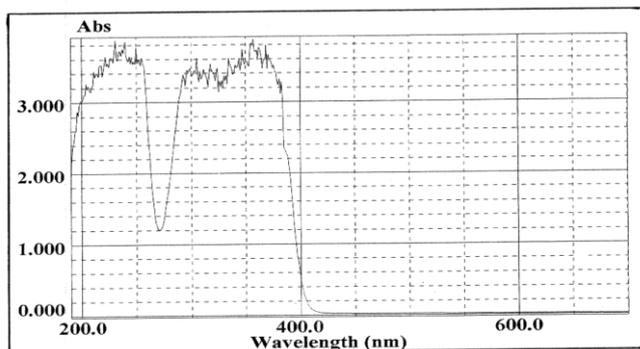


Fig. 1: The spectrum of  $2 \mu\text{g ml}^{-1}$  indole + 2,4,6-trimethoxybenzaldehyde without hydrochloric acid at room temperature

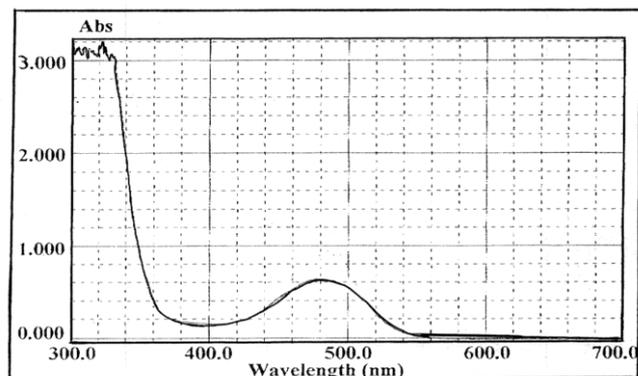


Fig. 2: The spectrum of  $2 \mu\text{g ml}^{-1}$  indole + 2,4,6-trimethoxybenzaldehyde in the presence of hydrochloric acid.

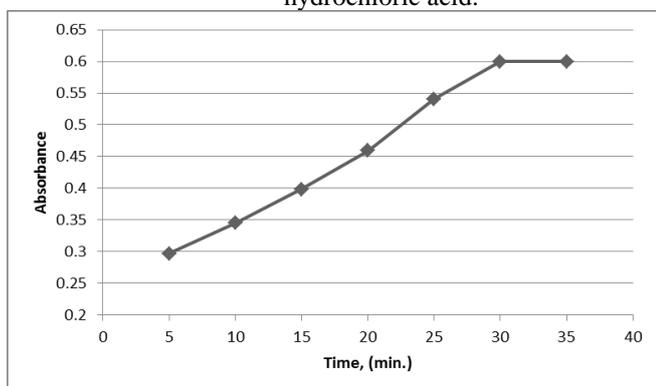


Fig. 3: Effect of time on the reaction of  $1 \mu\text{g ml}^{-1}$  indole with 2,4,6-trimethoxybenzaldehyde in the presence of hydrochloric acid

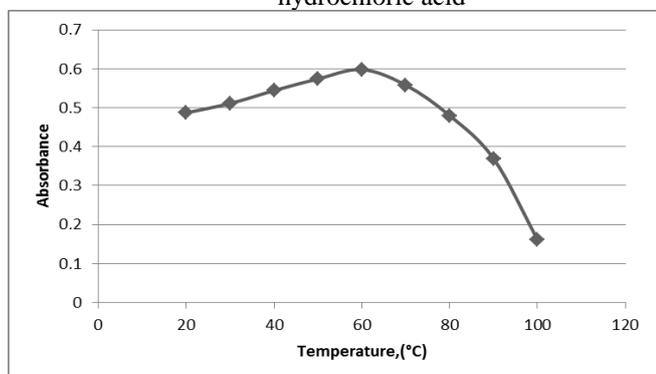


Fig. 4: Effect of temperature on the reaction of  $1 \mu\text{g ml}^{-1}$  indole with 2,4,6-trimethoxybenzaldehyde in the presence of hydrochloric acid.

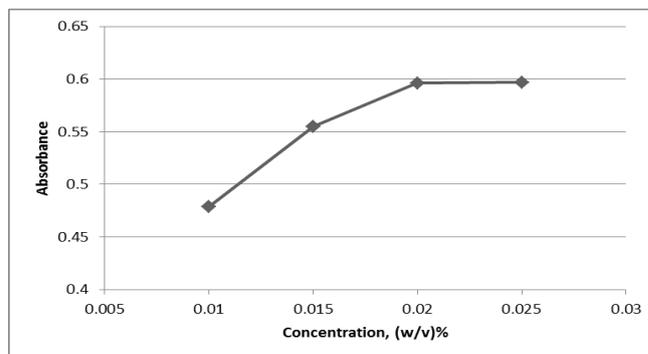


Fig.5: Effect of concentration of 2,4,6-trimethoxybenzaldehyde on the reaction between  $1 \mu\text{g ml}^{-1}$  indole and 2,4,6-trimethoxybenzaldehyde.

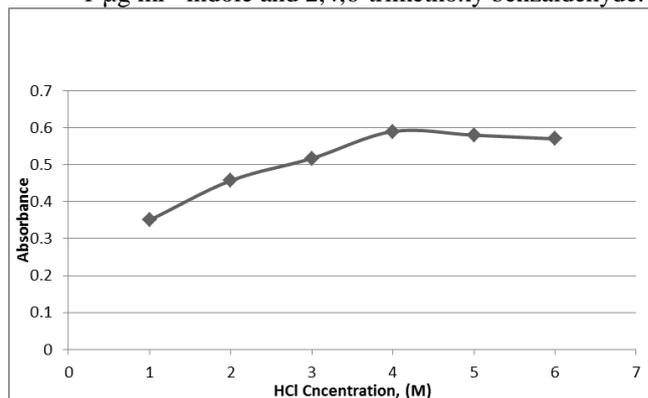


Fig. 6: Effect of concentration of hydrochloric acid on the reaction between  $1 \mu\text{g ml}^{-1}$  indole and 2,4,6-trimethoxybenzaldehyde.

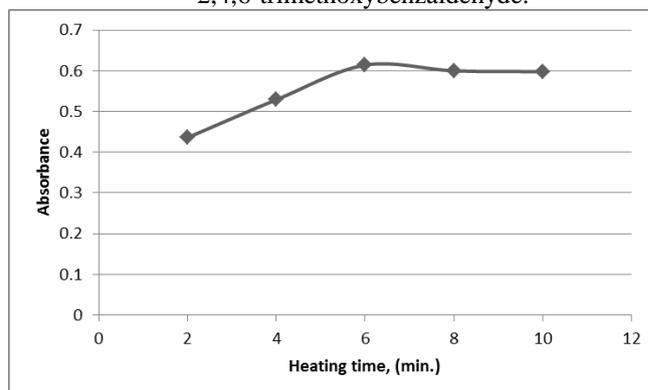


Fig. 7: Effect of heating time on the reaction of  $1 \mu\text{g ml}^{-1}$  indole and 2,4,6-trimethoxybenzaldehyde in the presence of HCl.

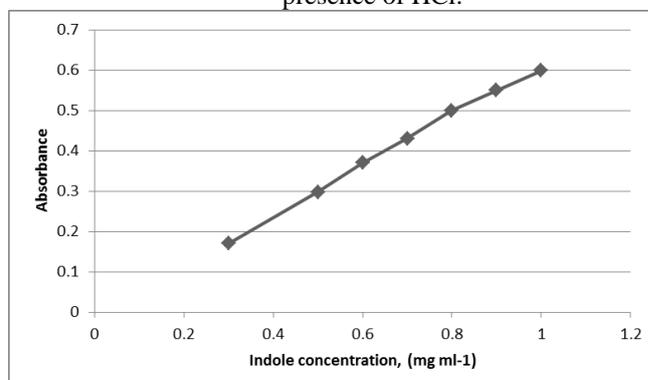


Fig. 8: Calibration graph for indole and 2,4,6-trimethoxybenzaldehyde in the presence of HCl.