



Research Paper

Chromogenic Reagent System for Complexation of Nitrobenzene and its Application to Real Sample

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Abstract- A new sensitive analytical method for the determination of nitrobenzene is proposed. In this method, nitrobenzene is reduced in presence of Zn dust in acidic medium. The reduced nitrobenzene is then oxidized and subsequently coupled with phloroglucinol in alkaline medium. The red-purple color dye formed, shows an absorbance maxima at 450 nm. The reagent blank shows negligible absorbance in similar conditions. The color system obeyed Beer's law in the range of 120 to 480 μg (12 to 48 mg L^{-1}) nitrobenzene in 10 mL of final volume. . Molar Absorptivity and Sandell'S Sensitivity were found to be $2.1 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$ and 0.06 cm respectively. The Standard Deviation and Relative Standard Deviation were found to be ± 0.003 and 1.41% respectively. Various reaction parameters like time, temperature, alkalinity and reagent concentration etc. also studied and have been optimized for the complete color reaction. To assess the applicability of the method, it has been applied successfully for the analysis of nitrobenzene in polluted water, vegetables and grains.

Keywords: Nitrobenzene, Indophenol dye, Spectrophotometry, Waters, Vegetables and Grains.

Introduction

Nitrobenzene is a poisonous organic compound, either bright yellow crystals or an oily liquid, having the odor of almonds. It is sometimes called oil of mirbane or nitrobenzol. It is only slightly soluble in water, but is readily soluble in ethanol, ether, and benzene^[1, 2]. Nitrogen with other compounds i.e. Nitro-compounds has wide interest in industry where it is used as solvent and intermediate in the preparation of pharmaceutical, insecticides, shoe-polish, hair dyes, textiles and explosives^[3-6]. Nitrobenzene absorbed through intact skin and inhalation causes methemoglobinemia in warm blooded animal and man. The threshold limit value recommended by OSHA is 5 mg m^{-3} ^[4, 7] and odor threshold for nitrobenzene is 0.0047 mg L^{-1} ^[8].

Nitrobenzene and nitro aromatic compounds are environmental pollutants discharged through wastewater from nitro aromatic manufacturing plants. Nitrobenzene and other aromatics are toxic to several forms of aquatic life^[9, 10]. In the pharmaceutical industry nitrobenzene is used in the production of the analgesic acetaminophen, or paracetamol. As oil of mirbane, nitrobenzene was used as an inexpensive perfume for soaps and cosmetics but is now

considered too toxic for such applications^[1, 2]. Various methods have been reported, for its determination/detection based on various techniques^[8, 11-13] like GC and H-NMR Spectroscopy^[14, 15], Differential Pulse Voltametry^[16], Hanging Mercury Drop Electrode^[17], Cyclic Steam Extraction^[18], Voltametric Determination^[19], GC-Mass Spectrometry^[20] and Differential Pulse Voltametry and Chemometrics^[21]. In some colorimetric methods either it is converted into m-dinitro benzene and subsequent color development with butane or reduced to aniline and subsequent coupling has been made with hypochlorite^[6], Chicago acid^[8] or disodium 1, 2 naphthoquinone-4-sulphonate^[13].

Material and Methods

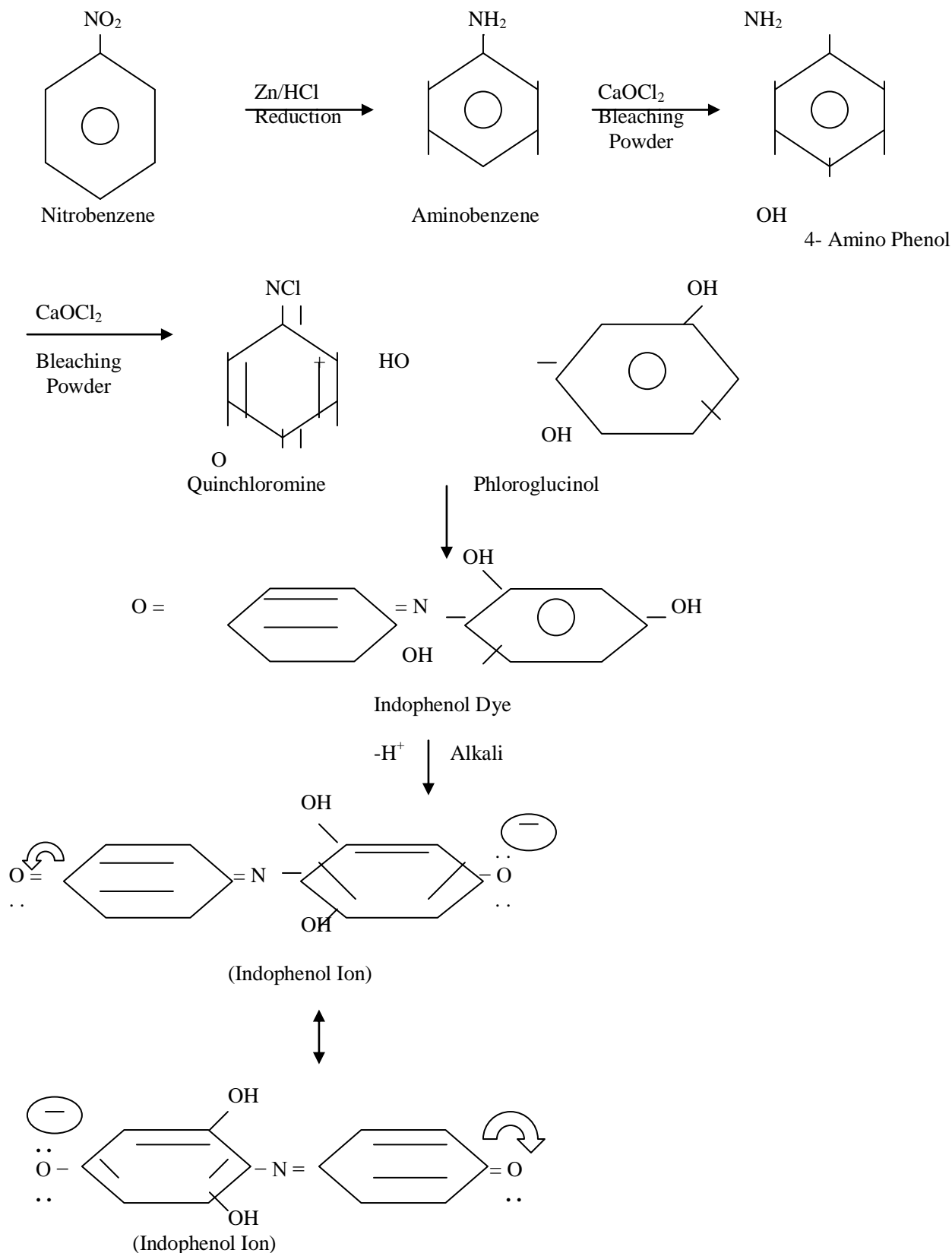
Apparatus: A systronics spectrophotometer 166 with matched 1-cm cells and systronics digital spectrophotometer model BSM-13 was used for all spectral measurements.

Reagents: All chemicals used were of analytical reagent grade and all solutions were prepared in distilled water. All chemicals used were analytical reagent grade E-Merk supplied by local Supplier V. K. Traders.

Nitrobenzene: A stock solution of 1 mg mL^{-1} was prepared by dissolving 100 mg Nitrobenzene in 10 mL ethanol and 90 mL distilled water. A working standard of $120\ \mu\text{g mL}^{-1}$ was prepared fresh daily by the appropriate dilution of the stock.

Zn-dust: Finely divided zinc of AR grade has been used.

Hydrochloric Acid: A 1.5 M hydrochloric acid solution by appropriate dilution of conc. HCl with distilled water was used.



EDTA: 5 g sodium salt of EDTA was dissolved in 100 mL distilled water.

Bleaching powder: A 1.0 g bleaching powder dissolved in 100 mL distilled water contains 1mL Conc. HCl.

Phloroglucinol: A 1.0 g phloroglucinol was dissolved in 100 mL distilled water.

Sodium Hydroxide: A 3 M aqueous solution was prepared fresh daily and standardized by standard procedure^[22].

Results and Discussion

The red-purple colored dye exhibits the maximum absorbance at 450 nm (Figure 1). The reagent blank has negligible absorbance at this wavelength (Table 1). It was found that a pinch (~0.1 gm) of Zn dust is sufficient in presence of hydrochloric acid to reduce nitrobenzene into aniline. It was found that 55-60% nitrobenzene was reduced to aniline which is in agreement with that obtained by earlier reported method^[23]. An increase in amount of Zn dust decreases the absorbance values. The 1.5 M HCl was sufficient for complete reduction. Higher concentration of HCl shows less absorbance values or turbidity formed.

Table 1

A. Calibration data for determination of Nitrobenzene

B.

Prepared from standard aniline solution		
Sample No.	Amount of Aniline (µg/10 mL)	Absorbance 450 nm*
1.	10	0.160
2.	20	0.319
3.	30	0.480
4.	40	0.638
5.	60	0.960
6.	80	1.270
7.	90	1.440

B. Prepared from standard nitrobenzene solution

Sample No.	Amount of Nitrobenzene (µg/10 mL)	Absorbance 450 nm*
1	10	0.099
2	20	0.197
3	40	0.394
4	60	0.590
5	90	0.888
6	120	1.180
7	180	1.772

*Mean of three repetitive analyses

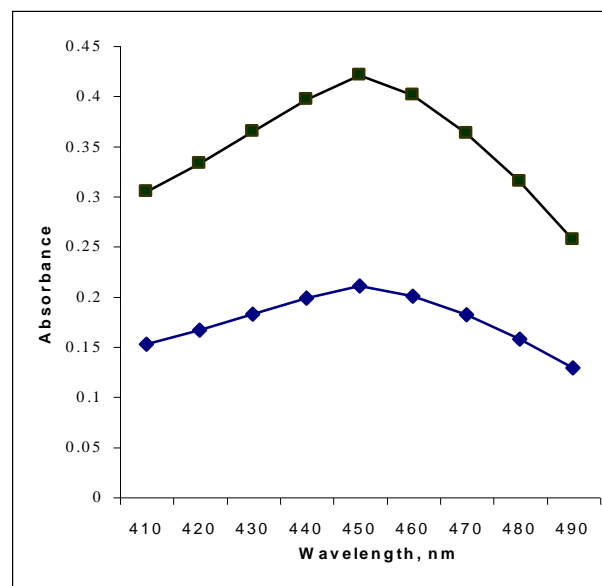


Figure 1: Absorption Spectra of the Dye and Reagent Blank

- A. Concentration of Nitrobenzene = 240 µg/10mL.
- B. Concentration of Nitrobenzene = 120 µg/10mL.
- C. Reagent Blank

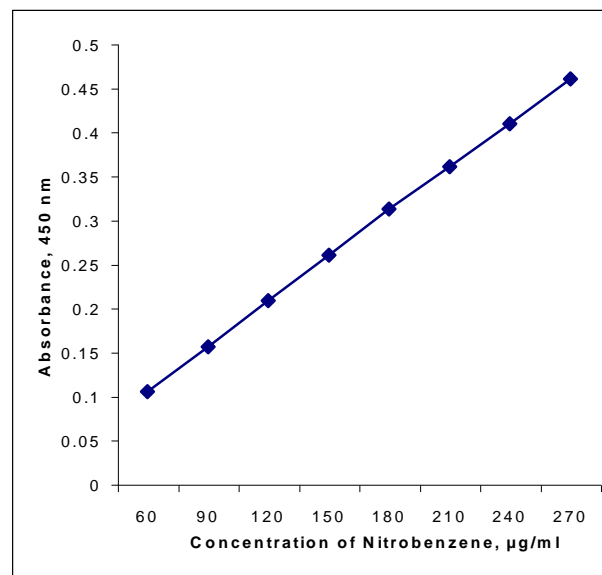


Figure 2: Calibration Curve for Spectrophotometric Determination of Nitrobenzene with Phloroglucinol

A minimum of 1 mL of 1 % phloroglucinol was required to obtain maximum absorbance values. Use of more or less than the concentration and amount of phloroglucinol shows decrease in absorbance values (Figure 3). The 3 M sodium hydroxide solution was sufficient to make alkaline the medium. At high sodium hydroxide concentrations i.e. above 3M gives rise to turbidity or negligible color development, whereas less than 3 M concentration sodium hydroxide solution shows decreases in absorbance values (Figure 4).

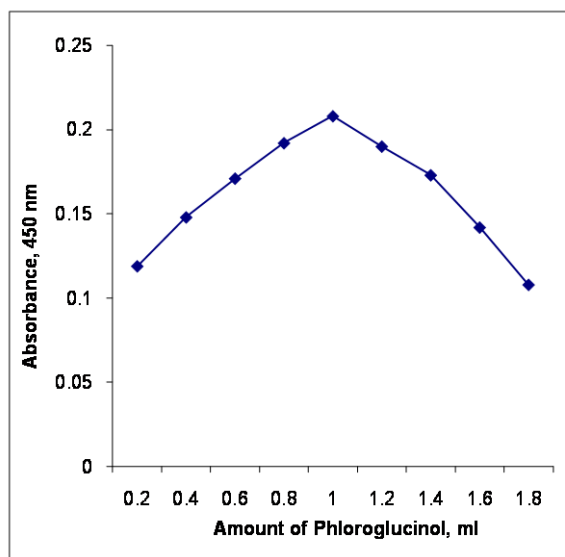


Figure 3: Effect of Amount of Phloroglucinol on the Colour Reaction

Concentration of Nitrobenzene = 120 µg /10mL

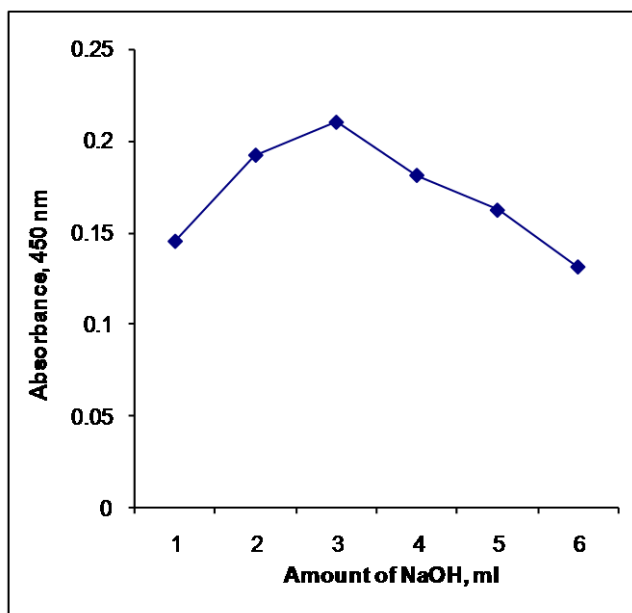


Figure 4: Effect of Amount of NaOH on the Colour Reaction

Concentration of Nitrobenzene = 100 µg /10mL

It was found that 1 mL of 1 % bleaching powder is sufficient for oxidation. Higher or lower amount of it caused increase in absorbance values of reagent blank. The red-purple color of the dye appeared immediately after the addition of sodium hydroxide but it reached at the maximum after 5 minutes when we make up fully with alkali. The dye was found to be stable for 10 minutes in the temperature range of 10 to 45 °C.

The color system was found to obey Beer's law in the range of 120 to 480 µg nitrobenzene in 10mL of the

final volume. The Molar Absorptivity and Sandell's Sensitivity were found to be 2.1×10^3 litres $\text{mol}^{-1} \text{cm}^{-1}$ and $0.06 \mu\text{g cm}^{-2}$, respectively. The reproducibility of the method was checked by replicate analyses over a period of 7 days (Table 2).

Table 2
Reproducibility of the Method
Concentration of Nitrobenzene= 120 µg/ 10ml

No. of Days	Absorbance* at 450 nm
1	0.198
2	0.194
3	0.2
4	0.198
5	0.196
6	0.202
7	0.198
	Mean = 0.198

* Mean of three repetitive analyses

Interday Precision	
Concentration of Nitrobenzene= 120 µg/ 10ml	
No. of Days	Absorbance* at 450 nm
1	0.197
2	0.195
3	0.199
4	0.198
5	0.197
6	0.201
7	0.199
	Mean = 0.198

Intraday Precision	
Concentration of Nitrobenzene= 120 µg/ 10ml	
No. of Days	Absorbance* at 450 nm
1	0.198
2	0.196
3	0.199
4	0.198
5	0.197
6	0.202
7	0.198
	Mean = 0.198

The Standard Deviation and Relative Standard Deviation were found to be ± 0.003 and 1.41 % respectively. To assess the validity of the method the effect of various possible interferences has been studied by adding known amounts of interferences to 24 µg of nitrobenzene. The tolerable limit for various interfering species are shown in Table 3. The tolerance limits of various interfering

species in alkaline medium were improved after masking with EDTA prior to analysis. The method was found to free from interferences of various organic and inorganic species including metal ions. Primary aromatic amines show positive interference. Copper (II) and Calcium (II) do not interfere with the determination of nitrobenzene.

Table 3
Effect of Foreign species

Foreign Species	Tolerance limit in mg mL ⁻¹
Acetone, hydrazine	1000
Pyridine	1250
Phenol	1280
P-nitroaniline	20,000
Nitrite	2000
Benzidine	1100
Salicylic acid	25,000
Cu ²⁺ , Ca ²⁺	1000
SO ₄ ²⁻ , Cl ⁻	1000
K ⁺	1, 25,000
Zn ⁺⁺	20
Fe ⁺⁺	10, 00,000
I ⁻	1, 25,000
Ammonia	12,000
Cr ₂ O ₇ ⁻	50,000
Pesticide (Methyl Parathion)	1100
Pesticide (Bronopol)	1000

Note. Concentration of nitrobenzene was 24 µg/10mL (2.4 mg L⁻¹)

Amount that may vary by ±2%, Masked with 1mL of 5% EDTA.

Application

The method has been applied for the analysis of nitrobenzene in polluted water, vegetables and cosmetics.

(1) **Polluted Waters:** The proposed method has been checked by applying for the analysis of nitrobenzene in waste water. Since real samples were not available, synthetic samples were prepared by spiking the polluted water with nitrobenzene prior to analysis. These samples were analyzed by the proposed as well as earlier recommended method. The results obtained are in a good agreement (Table 4).

(2) **Vegetables and Grains:** Different samples of vegetables and grains were collected from the market. These samples were blended by means of a mixer. Known amounts of nitrobenzene were added to these blended samples and kept for some time. The blended pulp was washed with 2x5 mL of distilled water. These extracted samples were then filtered and 1mL of each extracted sample was analyzed by the proposed method (Table 5).

Conclusion

The proposed method is simple, highly sensitive and free from most of the interfering species. The method is also compared with other spectrophotometric methods reported for its determination (Table 6). The results are in agreement with them. It can be applied for plant material and also for industrial hygienic works.

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Table 4
Analysis of Nitrobenzene in Polluted Water

Amount of nitrobenzene in µg added per 10 mL of water sample	Set No.	Recovery by proposed method	by Reference method[24]
25	1	23.95	23.75
	2	23.91	23.69
	3	23.93	23.72
50	1	48.85	46.95
	2	48.84	46.90
	3	48.88	46.93
100	1	98.15	94.82
	2	98.11	94.80
	3	98.16	94.84

Table 5
Recovery of Nitrobenzene from spray residues

Sample	Nitrobenzene (μg) Added	Found	% Recovery
Tomato	24	18.4	76.6
Brinjal	24	21.0	87.5
Chilly	24	21.7	90.4
Beans	24	20.4	85.0
Rice	24	19.6	81.7

Note: Mean of three replicate analyses

Table 6
Comparison of Other Spectrophotometric Methods Reported for the Determination of Nitrobenzene

Set No.	Reagents (Ref)	λ_{max} (nm)	Range of Determination (mg L^{-1})	Remarks
1	Sodium 1, 2-naphthoquinone-4-sulphonate [13]	450	0.8-1.6	Extractive, aliphatic amines interfere, pH(7.0-9.0) sensitive method
2	Guaiacol N-Chlorosuccinimide[25]	615	20-120	Less sensitive
3	Phloroglucinol	450	12-48	Sensitive, stable and free from interference

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