CHAPTER EIGHT

Spectrophotometric Determination
Of Phenol With DPAAP
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Summary

A spectrophotometric determination of phenol based on the coupling with diazotised p-amino acetophenone in alkaline medium is described. The dye formed is measured at 475 nm against the reagent blank. The Beer's law is obeyed over the concentration range of 0.6 - 6.0 µg per 25 ml of final solution (0.024 - 0.24 ppm). The molar absorptivity and Sandell's sensitivity were found to be $3.13 \times 10^4$ l mol$^{-1}$ cm$^{-1}$ and 0.0003 µg cm$^{-2}$ respectively. The method has been applied for the determination of phenol and phenol liberating pesticides i.e. carbaryl, propoxur, carbofuran and fenthion after their TLC separation in environmental and biological samples. The method is free from the interference of various organic compounds and inorganic ions.

Introduction:

Phenols also called "tar acids" an important constituent of coal tar are highly toxic. These are extensively used in the production or manufacture of a large variety of aromatic compounds including explosives, fertilizers, coke, illuminating gas, lampblack paints, paint remover, rubber, textiles, drug preparations, perfumes and other plastics etc. Phenols find their way into environment through industrial discharges, municipal sewage, bunker fuel, oil industries and coking plant effluents. Phenol is also used in the petroleum, leather, paper, soap, toys, tanning dye and agricultural industries (1-5).

Phenol traces are contained in automobile exhaust, tobacco smoke and occasionally in the hospital air (6). It is also used as mosquito repellent, insecticide, weedicide and herbicide etc. (7). It is a frequent pollutant in industrial waste and occurs in drinking water supplies. Phenol is a key component of important pesticides. It is produced as a by-product of the petroleum refining industry (8).

Phenol exerts a strong corrosive action on the skin and serious burns results from skin contact. Exposure to phenol shows several symptoms like convulsions, dizziness, dimness of vision, irregular respiration and muscular weakness. Chronosis i.e. pigmentation of cartilage and tendons occurs due to acute exposure of atmospheric phenol. Phenol is reported to be carcinogenic and chronic phenol poisoning may cause digestive disturbance, diarrhea, headache, ptyalism anorexia, vomiting apetite, salivation, mental disturbance, nausea etc. It also causes damage to bone marrow. Phenol poisoning can even lead to death due to respiratory failure. The absorbed phenol is partly oxidised to hydroquinone which may be excreted in urine (1, 9-12).

Phenols are also reported to be toxic in aquatic environment. The toxicity related to aquatic organisms, fishes, mammals etc. are available in literature. Phenols react with chlorine used for chlorination of water and forms chlorophenol which imparts colour and causes objectional taste in water and can also cause several types of disease if used for drinking purpose (13-16).

OSHA has set 5 ppm as the permissible exposure limit value for phenol in air. The USA Environmental Protection Agency recommends rejection of water for drinking purpose which contains more than 1 μg of phenol per litre. The odour recognition of phenol is 0.05 ppm (7, 17).

Due to its wide use and extremely toxic effects various analytical techniques have been developed for the determination of phenol like gas chromatography (18-19), liquid chromatography (20), high performance liquid chromatography (21-22), capillary gas chromatography (23), GC - MS (24-25), gas chromatography - microbiosensor (26), GC/Ion trap MS (27), flow injection analysis (28-29), flow injection chemiluminescent (30),
The spectrophotometric methods with the use of reagents like 4-aminoantipyrine (39-41), p-aminophenol (42), Gibb's reagent or 2, 6-dibromoquinone chlorimide borate buffer (43), 2,6-diphenyl p-quinone 4-chlorimide (44), peroxidase (45), 2, 4, 6-p-trimethyl aniline (46), diazotised p-nitroaniline (7, 47-48), benzidine (49), N-chlorosuccinamide p-anisidine (50), 2-cyano-4-nitroaniline (51) etc. are available in literature for its determination.

Some of these reagents are either expensive, toxic, not easily procurable, unstable in solution form or less sensitive or less selective or time consuming.

In the present communication a simple and sensitive method is proposed for the spectrophotometric determination of phenol. The reaction is based on the coupling of phenol with diazotized p-aminoacetophenone in alkaline medium. The method is applied for the determination of several phenol based pesticides such as carbaryl, propoxur, carbofuran and fenthion after their separation on chromatographic plate.

**Experimental:**

**Apparatus** - A Systronics UV-VIS Spectrophotometer 108 with matched silica cells and a PIMCO make calibrated rotameter with 35 ml fritted midget impingers were used for all spectral measurement and air sampling respectively.

**Reagents** - All chemicals used were of Analar grade and double distilled water was used throughout the experiment.

**Standard phenol solution** - A stock solution containing 1 mg ml⁻¹ of phenol was prepared in 20% ethanol. Working standard was prepared by appropriate dilution of stock. The solution was kept in amber coloured flask to avoid decomposition.

**Sodium nitrite** - 2% aqueous solution.

**Hydrochloric acid** - 0.5 M aqueous solution.

**p-Aminoacetophenone (PAAP)** - A 1% solution was prepared in 20% ethanol.

**Sulphamic acid** - 3% aqueous solution.

**Diazotised p-aminoacetophenone (DPAAP)** - To 10 ml of PAAP, 1 ml of 0.2% sodium nitrite and 1 ml of 0.5 M hydrochloric acid were added with continuous shaking in an ice bath. The excess of nitrite was removed by adding 1 ml of 3% sulphamic acid. The solution was stable for 4 hours when kept in cold in amber coloured bottles.

**Pesticides standard solution** - 1 mg ml⁻¹ solution of carbaryl (Union Carbide, Bhopal), propoxur (Bayer, India), carbofuran (Rallies, India) and fenthion (Bayer, India) were prepared.
separately in methanol. Working standards was prepared by appropriate dilution of the stock.

Silica gel - Standard glass plates (20 x 45 cm) were coated with a slurry of silica gel and water (1+2) to a thickness of 0.25 mm. The plates were air dried and then activated by heating in an oven at 110° for ~ 1 hr.

Solvent system for TLC - Chloroform-methanol (4+1 v/v) was used as developing solvent.

Procedure:

(a) Preparation of Calibration Curve - An aliquot containing 0.6 µg - 6 µg of phenol solution was taken in graduated tubes and 2 ml of p-aminoacetophenone that was diazotised by adding sodium nitrite and hydrochloric acid with continuous shaking in an ice bath was added followed by ~ 2 ml of 2 M sodium hydroxide solution. A orange red coloured dye was formed. The solution was made up to the mark and kept for 15 min for full colour development. The absorbance was measured at 475 nm against the reagent blank.

(b) Determination of Phenol in Polluted Water and Effluent - Samples were collected from near by river, coke oven of an integrated plant in plastic containers. 50. ml aliquots of samples were made alkaline (pH 10 - 11) with sodium hydroxide, transferred to 125 ml separatory funnel and shaken with 10 ml chloroform. The organic phase was discarded and the aqueous phase was warmed for 2 min in a boiling water bath. The solution was cooled and filtered through a Whatman filter paper no. 41 (7). An aliquot of the filtrate was then analysed as recommended in the procedure. Table 1.

(c) Determination of Phenol in Air - As the laboratory air was free from phenol synthetic samples were prepared by evaporating standard solution of phenol in a fuming chamber. Then the air was sucked through two impingers (connected in series) each containing 10 ml solution of sodium hydride (0.1M) at the flow rate of 1 l min⁻¹ for the collection of phenol. (6). After sampling the aliquots of sample were analysed as described above. Table 1.

(d) Determination of Phenol in Biological Samples - Phenol is found to be present in detectable concentration in urine samples of steel plant workers. The amount of phenol in urine is also reported to increase with benzene vapour absorption (6). Known amount of phenol solution was added to the urine samples of steel plant workers after adding 1 ml each of trichloroacetic acid and EDTA for deproteination and masking respectively. The aliquots of the sample was then analysed by the recommended method. Table 1.

(e) TLC Detection of Pesticides - The method has been applied for the TLC separation of various phenol liberating pesticides such as carbaryl, propoxur, carbofuran and fenthion. These pesticides were first hydrolysed with aqueous sodium hydroxide (2M) and the corresponding phenols thus formed were applied on the TLC plates with a fine capillary or micro pipette. The chromatogram was developed in the TLC chamber using chloroform -
methanol (4+1 v/v) as eluent (52).

The pesticides were detected on TLC plates by spraying first with DPAAP and then with sodium hydroxide. A purple spot, a red violet spot, an orange spot and a wine red spot were observed with Rf value of 0.71, 0.52, 0.57 and 0.61 for carbaryl, propoxur, carbofuran and fenthion respectively.

The spots obtained were scraped separately from the plates and eluted with 2 ml of 2 M sodium hydroxide and centrifuged. The supernatant was taken in a calibrated tube and the residue was again washed with 2 ml of 2M sodium hydroxide and the coloured solution obtained were made up to 10 ml with deionised water. The coloured solutions had their λ max 555 nm, 580 nm, 505 nm and 530 nm for carbaryl, propoxur, carbofuran and fenthion respectively. It may be possible to determine quantitatively carbaryl, propoxur, carbofuran and fenthion at these corresponding wavelength using respective compounds for preparing calibration curve.

Results and Discussion:

Spectral characteristics - The absorption maxima of the dye is shown in fig 1. The spectra of phenol shows a maximum absorption at 475 nm in aqueous solution. The reagent blank has negligible absorbance at this wavelength.

Adherence to Beer’s law, Molar absorptivity and Sandell’s sensitivity - The response was linear between concentration range of 0.6-6 μg of phenol per 25 ml final solution (0.024-0.24 ppm) (fig 2). The molar absorptivity and sandell’s sensitivity were found to be 3.31 x 10⁵ l mol⁻¹ cm⁻¹ and 0.0003 μg cm⁻² respectively.

Effect of acidity - The effect of acidity for the diazotisation reaction was studied ranging from 0.1-5.0 M hydrochloric acid. It was found that at least 0.5 M hydrochloric acid was necessary for complete diazotisation and upto 5 M hydrochloric acid gives constant and maximum absorbance values.

Effect of nitrite concentration - It was found that 10 ml of 1% p - aminoacetophenone and 1 ml of 0.2 % sodium nitrite was needed for constant and maximum absorbance. Excess of nitrite was removed by addition of sulphanamic acid.

Effect of alkalinity - It was found that a minimum of 2 ml of 2 M sodium hydroxide was needed for full colour development. The addition of more than 3 ml of alkali causes decrease in the absorbance. (fig 3)

Effect of diazotised PAAP - It was found that 2 ml DPAAP was needed for constant and maximum absorbance values. (fig 3)

Effect of time and temperature - Diazotisation of p-aminoacetophenone was completed in 2 min and upto a period of 10 min no change in the absorbance values were observed. After coupling with phenol the reaction mixture was allowed to stand for different intervals of time and it was found that 5 min were required for full colour development and the colour was stable for several days. (fig 4)
The optimum temperature of diazotisation is 0-5°C and coupling reaction takes place at room temperature. At higher temperature absorbance values as well as stability of dye both decreases. (fig 4)

**Effect of pH** - It was found that the maximum absorbance and stability of the dye was obtained was at pH 10-12. At pH lower and higher than this the absorbance values decreased. (fig 5).

**Reproducibility** - Reproducibility of the method was checked by the replicate analysis of the solution containing 3 μg per 25 ml of phenol over a period of seven days. The standard deviation and relative standard deviation were found to be ±0.013 and 2.03% respectively.

**Effect of different eluents** - For the TLC separation of pesticides different eluents were used. Hexane-acetone (4+1 v/v), butane-glacial acetic acid-water (3+1+6 v/v), chloroform-methanol (4+1 v/v) were tested for the separation of various pesticides. It was found that the separation was poor and tailing of the spots were severe with hexane-acetone (4+1 v/v), butane-glacial acetic acid-water (3+1+6 v/v). Chloroform-methanol (4+1 v/v) was the best eluent for the chromatographic separation of pesticides by TLC.

**Colour stability** - The colour of dye formed by TLC separation of pesticides was found to be stable for ~ 12 hours.

**Effect of foreign species** - The validity of the method was assessed by investigating the effect of foreign species and other common ions in the analysis of phenol. The tolerance limit for various metal ions were increased by masking them with EDTA and sodium potassium tartrate solution. It was found that the method is free from the interference of most of the organic compounds and several inorganic ions. The tolerance limit values of different species in solution containing 3 μg per 25 ml of phenol are shown in Table 2.

**Colour Reaction**:

The colour reaction involves the following steps (Scheme A) -

1. Diazotisation of p-aminoacetophenone with sodium nitrite in the presence of hydrochloric acid to form diazonium salt.
2. The coupling of resulting diazotised salt with phenol to form a orange reddish coloured dye in alkaline medium.

**Application**:

The proposed method has been satisfactorily applied for the determination of phenol in polluted water, environmental and biological samples. The results are found to be in good agreement with standard method (40). Table 1. The method has also been applied for the TLC separation and detection of pesticides liberating phenol on decomposition or hydrolysis.

**Conclusion**:

The proposed method for phenol is compared with other spectrophotometric methods, Table 3, and found to be more sensitive and free from most of the common interferants. The method does not require extraction of dye and the dye formed is stable for several days. It is applied for the TLC separation and detection of several phenol based pesticides.
Scheme A - Colour Reaction of Phenol

(1) \[ \text{CH}_3\text{C}=\text{C}=\text{C}=\text{N}\text{H}_2 + \text{NO}_2^- + \text{H}^+ \xrightarrow{\text{HCl}} \text{CH}_3\text{CO}=\text{C}=\text{C}=\text{N}=\text{NCl} \]

\text{p-aminoacetophenone} \quad \text{Diazonium salt}

(2) \[ \text{CH}_3\text{CO}=\text{C}=\text{C}=\text{N}=\text{NCl} + \text{OH}^- \xrightarrow{\text{NaOH}} \text{CH}_3\text{CO}=\text{C}=\text{C}=\text{N}=\text{N}\text{OH} \]

\text{Diazonium salt} \quad \text{Phenol} \quad \text{Orange red colour dye} \quad \lambda_{\text{max}} 475 \text{ nm.}
A : CONCENTRATION OF PHENOL : 4 μg/25 ml
B : CONCENTRATION OF PHENOL : 2 μg/25 ml
C : REAGENT BLANK

fig 1. - ABSORPTION SPECTRA OF THE DYE AND REAGENT BLANK

fig 2. - CALIBRATION CURVE FOR DETERMINATION OF PHENOL
fig 3. - EFFECT OF AMOUNT OF DIAZOTISED p-AMINOACETOPHENONE AND SODIUM HYDROXIDE ON COLOUR REACTION

fig 4. - EFFECT OF TEMPERATURE AND TIME ON COLOUR REACTION
CONCENTRATION OF PHENOL: 4 μg/25 ml

fig 5. - EFFECT OF pH COLOUR REACTION
Table 1. Determination of phenol in various samples.

<table>
<thead>
<tr>
<th>Sample mass/volume**</th>
<th>Phenol added (µg)</th>
<th>Phenol initially found (µg)</th>
<th>Phenol total found* (µg)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>R&lt;sup&gt;90&lt;/sup&gt;</td>
<td>P</td>
</tr>
<tr>
<td>1. Polluted water &lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1.00</td>
<td>0.156 (±0.0000)</td>
<td>1.117 (±0.0011)</td>
<td>1.074</td>
</tr>
<tr>
<td>B</td>
<td>2.00</td>
<td>0.020 (±0.0010)</td>
<td>1.983 (±0.0015)</td>
<td>1.966</td>
</tr>
<tr>
<td>C</td>
<td>5.00</td>
<td>0.101 (±0.0011)</td>
<td>4.878 (±0.0000)</td>
<td>4.815</td>
</tr>
<tr>
<td>2. Coke oven effluent &lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>2.00</td>
<td>2.500 (±0.0010)</td>
<td>4.440 (±0.0020)</td>
<td>4.370</td>
</tr>
<tr>
<td>B</td>
<td>2.00</td>
<td>1.600 (±0.0010)</td>
<td>3.558 (±0.0010)</td>
<td>3.444</td>
</tr>
<tr>
<td>C</td>
<td>2.00</td>
<td>2.349 (±0.0013)</td>
<td>4.249 (±0.0013)</td>
<td>4.185</td>
</tr>
<tr>
<td>3. Air &lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>2.00</td>
<td>-</td>
<td>1.960 (±0.0000)</td>
<td>1.926</td>
</tr>
<tr>
<td>B</td>
<td>4.00</td>
<td>-</td>
<td>3.848 (±0.0015)</td>
<td>3.822</td>
</tr>
<tr>
<td>C</td>
<td>5.00</td>
<td>-</td>
<td>4.920 (±0.0000)</td>
<td>4.880</td>
</tr>
<tr>
<td>4. Urine &lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1.00</td>
<td>2.592 (±0.0010)</td>
<td>3.554 (±0.0010)</td>
<td>3.518</td>
</tr>
<tr>
<td>B</td>
<td>2.00</td>
<td>1.110 (±0.0000)</td>
<td>3.039 (±0.0011)</td>
<td>2.928</td>
</tr>
<tr>
<td>C</td>
<td>3.00</td>
<td>0.059 (±0.0000)</td>
<td>3.481 (±0.0000)</td>
<td>3.407</td>
</tr>
</tbody>
</table>

* Mean of three replicate analysis.
** a, b, c, d = 50 ml sample, after treatment described in procedure, 1 ml of aliquot was analysed.

c = 10 l air, d = 5ml. P= Proposed method, R = Reported method.
Table 2. Effect of foreign species
Phenol Concentration 3μg/25 ml (0.12 ppm)

<table>
<thead>
<tr>
<th>Foreign species</th>
<th>Tolerance limit*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol, Methanol</td>
<td>550</td>
</tr>
<tr>
<td>Aniline, Formaldehyde, Pyridine, Benzene</td>
<td>425</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>350</td>
</tr>
<tr>
<td>Nitro toluene</td>
<td>250</td>
</tr>
<tr>
<td>p-Nitrophenol, o-Aminophenol</td>
<td>150</td>
</tr>
<tr>
<td>p-Chlorophenol</td>
<td>100</td>
</tr>
<tr>
<td>p-Aminophenol, o-Aminophenol</td>
<td>50</td>
</tr>
<tr>
<td>o-Cresol, m-Cresol</td>
<td>25</td>
</tr>
<tr>
<td>Hg(^{2+}), Ca(^{2+}), Mg(^{2+}), Pb(^{2+})</td>
<td>550</td>
</tr>
<tr>
<td>CH(_3)COO(^{-}), PO(_4)(^{3-}), SO(_4)(^{2-})</td>
<td>500</td>
</tr>
<tr>
<td>NO(_2)(^{-}), NO(_3)(^{-}), SiO(_3)(^{2-})</td>
<td>500</td>
</tr>
<tr>
<td>Co(^{2+}), Cr(^{3+}), Cu(^{2+}), Ni(^{2+})</td>
<td>250</td>
</tr>
<tr>
<td>I(^{-}), Br(^{-}), F(^{-})</td>
<td>80</td>
</tr>
</tbody>
</table>

* Causing an error of ±2% in absorbance value.
<table>
<thead>
<tr>
<th>Method / Reagent / Ref.</th>
<th>λ max (nm)</th>
<th>Beer's law/detection limit (ppm)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Aminoantipyrine⁴⁰</td>
<td>510</td>
<td>0.1 - 2</td>
<td>Sulphur compound and metallic ions interfere, long waiting time required.</td>
</tr>
<tr>
<td>Dibromoquinone chloromide⁴³</td>
<td>670</td>
<td>1 - 10</td>
<td>Sulphur and all reducing agent interfere.</td>
</tr>
<tr>
<td>p-Nitroaniline⁷</td>
<td>530</td>
<td>0.1 - 0.4</td>
<td>The reagent is carcinogenic.</td>
</tr>
<tr>
<td>p-Anisidine-NCS-catalyst mixture⁵⁰</td>
<td>650</td>
<td>0.05 - 0.4</td>
<td>Coloured reagent blank, higher temperature required.</td>
</tr>
<tr>
<td>Bulk acoustic waves³⁷</td>
<td>-</td>
<td>0.05</td>
<td>Though sensitive but non-spectrophotometric.</td>
</tr>
<tr>
<td>Flow injection chemiluminescent quench analysis³⁶</td>
<td>-</td>
<td>0.6</td>
<td>Non-spectrophotometric, less sensitive.</td>
</tr>
<tr>
<td>p-Aminoacetophenone (Proposed method)</td>
<td>475</td>
<td>0.024 - 0.24</td>
<td>Non extractive, sensitive, higher colour stability, easily available, non toxic reagent.</td>
</tr>
</tbody>
</table>
References: