A New Reagent System For The Determination Of Formic Acid In Biological Samples
A New Reagent System for the Determination of Formic Acid in Biological Samples

Summary

A spectrophotometric method using phloroglucinol is described for the determination of formic acid at the ppm level. The method involves the reduction of formic acid by hydrochloric acid and magnesium powder. The formaldehyde so formed is treated with phloroglucinol to give an orange red coloured dye with maximum absorbance at 475 nm. The Beer's law is obeyed over the concentration range of 2 - 15.0 µg (0.08 - 0.6 ppm) of formic acid per 25ml of the final solution. The molar absorptivity of the colour solution was found to be 6.1 x 10⁴ l mol⁻¹ cm⁻¹. The analytical parameters were optimised and the method has been applied for the determination of formic acid in biological samples.

Part Accepted in J. Indian Chem. Soc., MSS No. 290 / 97.
Introduction:

Formic acid, also known as methanoic acid, aminic acid, formylic acid, hydrogen carboxylic acid is a corrosive and highly toxic chemical having considerable significance in forensic science. Its corrosive actions are very similar to strong mineral acids (1-3). It is produced by wooden building materials such as roof supports, boarding and adhesives, particularly when conditions are damp. It is aggressive towards lead and can lead to the corrosion and failure of lead roofs on historic buildings (4-6). It is formed in the body due to direct oxidation of denatured alcohol containing methanol and some toxic drugs (1-3). In India many deaths and physical disorder occur due to inhalation or ingestion of methanol added for denaturing alcohols. Methanol is eliminated by the breath but a large portion of it is slowly oxidised into formic acid and formaldehyde and thus causes acidosis. Formic acid is extracted in urine. Its concentration in blood (serum) and urine are directly related to the quantity of methanol taken (7).

Formic acid is also widely used as preservatives for fruit juices, jams and jellies, cloves, corn, alfalfa, vegetables, cereals etc (8). Some countries have prohibited the addition of formic acid to food stuff as a preservative due to its toxic effect (9-10). Small amount of formic acid may also occur naturally in some foods due to fermentative action of bacteria and yeast on carbohydrate (11). It is also formed by sugars decomposition through heating especially in the presence of alkali (12). It is commonly used in the reducing agent of formyl esters and as a reducing agent in wool, leather, rubber industries and electroplating (13-15).

It is also used as tanning agent in preparation of plasticiser for epoxy resins, pharmaceutical (vitamin and sulphamines), salts (nickel formation), building materials, fungicides, perfumes and as solvent (16). It is also used as decalcifier (17). Looking to its toxic effect the ACGIH TWA value is 5 ppm (9mg/m^3). The IDLH level is 100 ppm. A maximum contaminant level (MCL) in drinking water has been set at 124 µg/l based on health effects (17). Its threshold limit value (TLV) has been set at 5 ppm (13) by ACGIH and OSHA.

It mainly affects the respiratory system, skin, kidney, liver and eyes. It also effects the body through ingestion, inhalation and contact. The decalcifier formulations ingested by children may suffer lesions in mouth and oesophageal tissue (2, 3, 18). Toxicity by ingestion is moderate and by inhalation is mild. It is also suspected to cause conjunctivitis, keratosis, dermatitis, bronchitis, cough, dyspnea, vomiting, nausea, diarrhoea, anuria, hematuria and albuminura (3, 13).

Due to its wide use toxicity to human beings and its corrosive action various methods have been reported for its determination. Some of them are gas chromatography (19-22), high performance liquid chromatography (23-24), ion chromatography (25-26), ion exchange chromatography (5), potentiometric titration (27), flow injection analysis (28),
Various spectrophotometric methods using reagents like chromotropic acid (38), fuschin sulphite (39), ethyl glycol (40), phenyl hydrazine (41), disodium chromotropate (42), ferric perchlorate (43) and potassium iodide (44) are available in literature (39-44). While some of these methods are less sensitive and time consuming others require costly reagents for conversion of formic acid to formaldehyde.

Here a method based on the conversion of formic acid to formaldehyde using magnesium powder in hydrochloric acid medium (45) which is subsequently reacted with phloroglucinol to form orange red colour dye showing maximum absorbance at 475 is proposed. The method is applied for the determination of formic acid in biological samples.

Experimental:

Apparatus - A Systronics UV-VIS Spectrophotometer model 108 with matched silica cells was used for all spectral measurement. pH meter model 331 was used for pH measurements. PIMCO make calibrated rotameter and midget impingers of 35 ml capacity were used for air sampling.

Reagents - All chemicals used were of AnalR grade. Double distilled water was used throughout the experiment.

Standard formic acid solution (E.MERCK Ind. Ltd.) - 1 mg ml⁻¹ solution was prepared in double distilled water. Working standard was prepared by appropriate dilution of the stock.

Sodium hydroxide - 1.5 M aqueous solution.

Phloroglucinol (Loba Chemie) - 0.05 M aqueous solution.

Absorbing solution - Phloroglucinol and sodium hydroxide were taken in the ratio of 3:1.

Reducing agent - A pinch of magnesium powder with a 0.5 ml of 2 M hydrochloric acid was used as a reducing agent.

Hydrochloric acid - 2 M aqueous solution.

Procedure:

Preparation of Calibration Curve - An aliquot of sample containing 2.0 - 15.0 μg of formic acid was taken in an impinger. A pinch of magnesium powder and 0.5 ml of 2 M hydrochloric acid were added and kept in ice bath for 10 min and connected to two more impingers each containing 4 ml of absorbing solution, connected to an air sampling train sucking air at a flow rate of 1.0 l min⁻¹. The colourless absorbing solution becomes orange red after 15 min. The air was passed for another 35 min. The solution was cooled to room temperature and the volume was made upto 25 ml with double distilled water. The absorbance of the orange red colour was measured at 475 nm against reagent blank.
Results and Discussion:

**Spectral characteristics** - The orange red dye showed maximum absorbance at 475 nm. As the reagent blank has negligible absorbance at this wavelength. All the measurements were made against reagent blank. (fig 1)

**Adherence to Beer's law, Molar absorptivity and Sandell's sensitivity** - The Beer's law was obeyed over the concentration range of 2.0 - 15.0 µg of formic acid in 25 ml of final solution (0.08 - 0.6 ppm) (fig 2). The molar absorptivity and Sandell's sensitivity were found to be $6.1 \times 10^4$ l mol$^{-1}$ cm$^{-1}$ and 0.0007 µg cm$^{-2}$ respectively.

**Effect of reducing agent** - A pinch of magnesium powder and 0.5 ml of 2 M hydrochloric acid were found to be sufficient to reduce formic acid to formaldehyde. Excess of magnesium powder leads to the formation of turbid solution.

**Effect of time on reduction** - Reduction of formic acid to formaldehyde was completed in 5 min. More time for reduction did not have any adverse effect on absorbance. (fig 3)

**Effect of temperature on reduction** - $0^\circ$C temperature during reduction of formic acid gave the desired result. Higher temperature i.e. $10^\circ$C or more resulted in a turbid solution at the later stage. (fig 3)

**Effect of absorbing solution** - Constant and maximum absorbance values were obtained when 0.05 M phloroglucinol and 1.5 M sodium hydroxide were taken in the ratio of 1:3. Below and above this concentration absorbance values decreases. (fig 4)

**Effect of pH** - Effect of pH on the colour reaction was studied and constant absorbance values were found in the pH range 9-10. Above and below this pH absorbance values decreased. (fig. 5).

**Reproducibility** - The reproducibility of the method was checked by seven replicate analysis of the solution containing 10 µg of formic acid per 25 ml of solution. The standard deviation and relative standard deviation were found to be ±0.011 and 2.11% respectively.

**Interference of foreign species** - The effect of foreign species was studied by addition of various interferents to 10 µg of formic acid before reduction to formaldehyde. The tolerance limit values are given in Table 1. Other aliphatic acid like propanoic acid, butanoic acid, oxalic acid, succinic acid did not interfere with the proposed method under optimum condition. Other compounds which can be reduced or oxidised to formaldehyde under the proposed condition interfere in the method.

**Colour Reaction**:

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

1. Reduction of formic acid to formaldehyde.
2. Condensation of resulting formaldehyde with two molecules of phloroglucinol in alkaline medium.

127
Application:

It has been reported that the formic acid present in blood (serum) and urine (1) is formed due to oxidation of methanol into formic acid within the body. So the estimation of formic acid in blood and urine also indicates the intoxication of methanol (7). As the blood and urine samples analysed did not contain formic acid, synthetic samples were prepared. About 1 ml of blood (serum) and urine sample were spiked with known amount of formic acid and kept aside for 2 hours. The aliquots were analysed by the above procedure. The recoveries were found to be in the range of 95.2 - 99% and the results are shown in Table 2.

Conclusion:

The method has been compared with the other reported spectrophotometric methods, Table 3, and it was found that the proposed method is simple, sensitive, selective and free from the interference of various copollutant. The reagents used are comparatively cheaper and easily available. The method can be applied for the analysis of formic acid in biological samples.
**STEP 1: Reduction of formic acid**

1. \[ \text{HCOOH} \xrightarrow{\text{Mg powder}} \xrightarrow{+ 0.5 \text{ ml HCl}} \text{HCHO} \]

   - Formic acid
   - Formaldehyde

**STEP 2: Condensation**

2. \[
\begin{align*}
\text{Phloroglucinol} & \quad \text{HCHO} \quad \text{Phloroglucinol} \\
\text{OH} & \quad \text{OH} & \quad \text{OH} & \quad \text{OH} & \quad \text{OH}
\end{align*}
\]

   - Orange red dye
   - \( \lambda_{\text{max}} 475 \text{ nm} \)

---

Scheme A - Colour Reaction of Formic Acid
**fig 1.** - Absorption spectra of the dye and reagent blank

- **A:** Concentration of formic acid, 10 μg/25 ml
- **B:** Concentration C = formic acid, 5 μg/25 ml
- **C:** Reagent blank

**fig 2.** - Calibration curve for the determination of formic acid
TIME IN MINS. B

TEMPERATURE °C, A

CONCENTRATION OF FORMIC ACID. 10 µg/25 ml

fig 3. - EFFECT OF TIME AND TEMPERATURE ON REDUCTION

AMOUNT OF ALKALINE 1,3,5-TRIHYDROXY BENZENE, ml

CONCENTRATION OF FORMIC ACID. 10 µg/25 ml

fig 4. - EFFECT OF AMOUNT OF ABSORBING SOLUTION ON COLOUR REACTION
CONCENTRATION OF FORMIC ACID, 10 μg/25 ml

fig 5. - EFFECT OF pH ON COLOUR REACTION
Table 1. Effect of foreign species
Concentration of formic acid 10 μg/25 ml (0.4 ppm)

<table>
<thead>
<tr>
<th>Foreign species</th>
<th>Tolerance limit value* (μg ml⁻¹, ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol, Benzene, Aniline</td>
<td>2600</td>
</tr>
<tr>
<td>Nitrophenol, Trichloroacetic acid, Ether</td>
<td>2300</td>
</tr>
<tr>
<td>Pyridine, isoamyl alcohol</td>
<td>1900</td>
</tr>
<tr>
<td>Ammonia, K⁺, Na⁺, Ca²⁺, Fe³⁺</td>
<td>1200</td>
</tr>
<tr>
<td>Cl⁻, Br⁻, SO₄²⁻, Zn²⁺</td>
<td>1000</td>
</tr>
<tr>
<td>CH₃COO⁻, HCO⁻, As³⁺, Pb²⁺, PO₄³⁻</td>
<td>900</td>
</tr>
<tr>
<td>Cu²⁺, Cd²⁺, Hg²⁺</td>
<td>500</td>
</tr>
</tbody>
</table>

* Amount of foreign species causing an error of ±2% in absorbance value.

Table 2. Application of the method for the determination of formic acid in biological samples.

<table>
<thead>
<tr>
<th>Sample mass / volume*</th>
<th>Amount added (μg)</th>
<th>Amount found** (μg)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood (serum)</td>
<td>5</td>
<td>4.75</td>
<td>95.20</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9.52</td>
<td>95.20</td>
</tr>
<tr>
<td>Urine</td>
<td>5</td>
<td>4.95</td>
<td>99.00</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9.71</td>
<td>97.10</td>
</tr>
</tbody>
</table>

* Size of sample = 1 ml.

** Mean of three replicate analysis.
<table>
<thead>
<tr>
<th>Reagent: Reference</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>Colour</th>
<th>Beer's law range (ppm)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromotropic acid / HCl</td>
<td>570</td>
<td>Orange</td>
<td>0.5 - 10</td>
<td>Less sensitive</td>
</tr>
<tr>
<td>Phloroglucinol / NaOH (Proposed method)</td>
<td>475</td>
<td>Orange red</td>
<td>0.08 - 0.6</td>
<td>Selective, highly sensitive and selective</td>
</tr>
<tr>
<td>$\text{H}_2\text{SO}_4$, Silver p-sulphamido benzoate</td>
<td>400</td>
<td>Yellow</td>
<td>0.5 - 100</td>
<td>Costly reagent, high temperature, complicated process</td>
</tr>
<tr>
<td>Disodium chromotrope</td>
<td>585</td>
<td>Pink</td>
<td>0.30</td>
<td>Time consuming, phenol &amp; m-cresol interfere</td>
</tr>
<tr>
<td>Mercuric chloride</td>
<td>550</td>
<td>Black</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Disodium chromotropate</td>
<td>425</td>
<td>Orange</td>
<td>0.30</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Comparison of the proposed method with other extractive spectrophotometric method of formic acid.
References: