Spectrophotometric Determination of Thymol in Lastarine Antiseptic by Diazotization of 4-Aminoantipyrine in the Presence of TritonX-100.

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Abstract— The conditions for the diazitization coupling reaction of thymol with 4-aminoantipyrine at pH in the presence of Triton X-100 were studied. The reaction gave an intense water soluble yellow product that has a maximum absorption at 454 nm and \( \varepsilon_{\text{max}} \times 10^4 \) L.mole\(^{-1}\).cm\(^{-1}\). A linear correlation (1-14µg ml\(^{-1}\)) was found between absorbance at \( \varepsilon_{\text{max}} \) and concentration. The results obtained are both precise (RSD was better than 1.9) and accurate (relative error was better than -2.5). The colored product was found to be 1:1 thymol : 4-aminoantipyrine. The stability constant of the product at room temperature were 0.75 x10\(^4\) L.mole\(^{-1}\). The proposed method was successfully applied to the determination of thymol in synthetic samples and in lastarine antiseptic pharmaceutical formulation.

Index Term— Thymol, Diazotization coupling reaction, Spectrophotometric determination, Triton X-100

INTRODUCTION
Thymol is a phenolic compound, \( \text{C}_9\text{H}_8\text{O} \), resembles phenol in its action, but its use is limited due to its low solubility in water [1]. Thymol is a widely known anti-microbial agent, Anti-inflammatory and cicatrizing activities [2-5]. The properties of thymol decreased peroxidation of phospholipid liposomes in the presence of iron(III) and ascorbate. Thymol possess useful antioxidant properties and may become important in the search for ‘natural’ replacements for ‘synthetic’ antioxidant food additive [6,7]. At present, several techniques such as high performance liquid chromatography[8], gas chromatography[9-11], reverse liquid chromatography [12] and differential pulse voltammetry [13,14], show good sensitivity but is limited because of expensive instrumentation and high cost for routine analysis. According to the best of our knowledge, this reagent in the presence of surfactant has not been reported in the literature as being used for any sample of thymol determination. The present study describes the development method based on diazotization and coupling reaction between thymol and diazotized 4-aminoantipyrine as a chromogenic reagent in alkaline medium and in the presence of TritonX-100. It has been applied to the determination of thymol in synthetic samples and in lastarine antiseptic pharmaceutical formulation.

EXPERIMENTAL

Apparatus
Spectral and absorbance measurements were made with UV-visible – 1700 double beam spectrophotometer using 1 cm quartz cells.

Reagents
All chemical used were of analytical grade.

Thymol stick solution (200 µg.ml\(^{-1}\)): a 0.0400 g amount of pure thymol (BDH) was dissolve in 20 ml of ethanol then complete to 200 ml in a volumetric flask with distilled water. Working standard of thymol solutions were prepared by simple dilution of the appropriate of the compound in distilled water completing the volume in a volumetric flask.

4-Aminoantipyrine reagent solution (10 mM): 0.4065 g of reagent was dissolved in 25 ml of ethanol then complete to 200 ml in a volumetric flask with ethanol.

Triton X-100 solution (5% w/v): 5 ml of Triton X-100 solution was dissolved in 25 ml of distilled water then complete to 100 ml in a volumetric flask. Working solutions were prepared by simple dilution of the appropriate of the compound in distilled water completing the volume in a volumetric flask.

Hydrochloric acid solution (1M): This solution was prepared by diluting suitable amount of 11.64 M HCl (BDH) with distilled water in 200 ml volumetric flask.

Sodium nitrite solution (1% w/v): 1 g of sodium nitrite was dissolved in 25 ml of distilled water then complete to 100 ml in a volumetric flask.

Sodium hydroxide solution (5%): 5 g of sodium hydroxide was dissolved in 25 ml of distilled water then complete to 100 ml in a volumetric flask.

Procedure for determination of Thymol in the presence of Triton X-100
Into a series of 25 ml calibrated flask, an aliquot of sample containing 25-350 µg of pure thymol was transfer. The 1 ml of thymol standard solution and 4 ml of 5% of sodium hydroxide were added to 3 ml of 10 mM of 4-aminoantipyrine, 2 ml of 1M HCl and 2 ml of 1% sodium nitrite. They were mixed and completed with 2% TritonX-100 to the mark, mixed well and left for ten min at room temperature, the absorbance of the yellow dye formed was measured at 454 nm against a reagent blank containing all materials except thymol.
Results and Discussion

The result of this investigation indicated that the reaction of diazotization of 4-aminoantipyrine and coupling with thymol in the presence of triton x-100 yield highly soluble coloured product which can be utilized as a suitable assay procedure for thymol. This yellow coloured product has a maximum absorption at 454nm, the blank at this wavelength shows zero absorbance (Fig 1), was adopted in all subsequent experiments.

![Absorption spectrum of the azo dye against reagent blank (A) and blank against all of addition except Thymol (B).](image1)

The influence of various reaction variable on the colour development was tested to establish the most favorable conditions and these are:

Effect of reagent of concentration

When various concentration of 4-aminoantipyrine was added to affixed concentration of thymol, 3 ml of 10 mM reagent solution was sufficient to develop the colour to its full intensity and gave minimum blank value, above 3 ml, the absorbance of the blank value was increased causing a decrease in the absorbance of the sample. Therefore, 3 ml of 10 mM of 4-aminoantipyrine was used in all subsequent experiments (Fig 2).

![Effect of the concentration of 4-aminoantipyrine reagent in mM](image2)

To establish the optimum conditions (stability of the dye resulting from the reaction of thymol with the reagent intensity of the dye formed, minimum blank value and relatively rapid reaction rate), the effect of different acids were studied. Only HCl with concentration 1M was found to be optimum. The $\text{H}_2\text{SO}_4$, $\text{HClO}_4$ and $\text{CH}_3\text{COOH}$ results in low sensitivity of the diazotization of 4-aminoantipyrine and was not stable. The effect of the amount of HCl used was also investigated and 2 ml was found to be optimal (Fig 3,4).
Effect of Sodium nitrite concentration

To obtain the optimum results, the amount of NaNO₂ was studied. Various concentration of NaNO₂ was added to fixed of 4-aminoantipyrine 10mM and HCl 1M, 2 ml of 1% NaNO₂ was used in all subsequent experiments (Fig 5).
Effect of Sodium hydroxide concentration

The dye formation reached maximum with about 4 ml of 5% NaOH solution and remain at this maximum 2-4.5 ml of the sodium hydroxide concentration was added. 4 ml volume of NaOH solution was therefore, used in the procedure since it gives high sensitivity, a minimum blank value and ensures a quantitative determination at the upper limit of the calibration graph (Fig 6).

Effect of Surfactants concentration

To establish the optimum conditions (stability of the dye resulting from the reaction of thymol with the reagent intensity of the dye formed, minimum blank value and relatively rapid reaction rate), the effect of different surfactants were studied. Nonionic micelles are often preferred to anionic micelles for the determination of reaction due to attraction forces between the negative head of micelles and the positive charge of ion in azo reaction causes lower apparent formation constant between metal ion and ligand. Faster Formation and high stability for reaction was observed in Triton X-100 Therefore , Triton X-100 was selected as the micellizing agents further studies[15].

Only Triton X-100 with concentration 2% was found to be optimum. The aqueous solution and Tween-80 results in low sensitivity of the colour and was not stable. The effect of the concentration of TritonX-100 used was also investigated and was found to be optimal (Fig 7,8).
Development Time and Stability period

The colour intensity reached maximum after thymol solution had been reacted with dizonium salt of 4-aminoantipyrine in alkaline medium for more than 2 hr. The colour obtained was stable for at least 2 hr and this stability period was sufficient to allow several measurements to be performed sequentially.

Order of addition of Reagents

To obtain the optimum results, the order of addition of reagents should be followed as given by the procedure, otherwise, a loss in colour intensity and stability are observed.

Calibration Graph

Employing the condition described under procedure a linear calibration graph for thymol is obtained which shows that Beer’s law is obeyed over the concentration range of 25-350 µg per 25 ml 1-14 ppm (Fig 9). The molar absorptivity of the coloured product with reference to thymol was $1.966 \times 10^4$ \text{ Lmol}^{-1}\text{cm}^{-1}$.
The stoichiometry of the reaction between thymol and diazotized 4-aminoantipyrine in the presence of Triton X-100 was investigated using the continuous variation method [16]. The results obtained (Fig 10) show a 1:1 diazotization coupling formed between thymol and 4-aminoantipyrine at 454 nm. Therefore, the formation of the dye probably occurs according to the following equations:

\[
\begin{align*}
&\text{4-aminoantipyrine} \quad \text{NaNO}_2 \quad \text{HCl} \\
&\text{Diazotised 4-aminoantipyrine} \\
&\text{Diazotised 4-aminoantipyrine} \quad \text{Thymol} \quad \text{NaOH} \\
&\text{Yellow azo dye}
\end{align*}
\]
The apparent stability constant was calculated by comparing the absorbance of a solution containing stoichiometric amounts of thymol and 4-aminoantipyrine with that of a solution containing a five-fold excess of 4-aminoantipyrine reagent. The average conditional stability constant of the dye in water, under the described experimental conditions is $0.7 \times 10^4 \text{L mole}^{-1}$.

**Effect of Interferences**

In order to assess the possible analytical application of the proposed method, the effect of some foreign ions often accompany this drug in pharmaceutical products were studied by adding different amounts of foreign ions to 8 µg.ml$^{-1}$ of thymol. Thymol can be determined without any interference in the presence of a 5-fold excess of the foreign ions Table I.

### Table I

<table>
<thead>
<tr>
<th>Interference</th>
<th>% Error</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0.09%</td>
<td>99.91%</td>
</tr>
<tr>
<td>lactose</td>
<td>1.11%</td>
<td>101.11%</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.83%</td>
<td>100.83%</td>
</tr>
<tr>
<td>Ni$_{2+}$</td>
<td>-0.09%</td>
<td>99.91%</td>
</tr>
<tr>
<td>Fe$_{3+}$</td>
<td>1.6%</td>
<td>101.6%</td>
</tr>
</tbody>
</table>

### Applications

**1-Accuracy and Precision**

To determine the accuracy and precision of the method, thymol was determined at three different concentrations. The results are shown in (Table II) indicate that satisfactory precision and accuracy could be attained with the proposed method.

### Table II

<table>
<thead>
<tr>
<th>Thymol taken (µg)</th>
<th>Error %</th>
<th>Relative Standard Deviation %*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-2.5</td>
<td>1.9</td>
</tr>
<tr>
<td>10</td>
<td>-0.0857</td>
<td>0.38</td>
</tr>
</tbody>
</table>

*Results of five determination

**2- Determination Thymol in lastarine antiseptic for Mouth wash[17]**

Transfer 10 ml of the lastarine antiseptic to a 25 ml volumetric flask and added 3ml of ethanol. The volume was diluted to the mark with distilled water and mixed well. The absorbance of solution was measured at 454 nm against a reagent blank Table III.

### Table III

<table>
<thead>
<tr>
<th>Application of proposed method for the determination of Thymol in mouth Wash.</th>
<th>Mouth wash</th>
<th>Lastarine antiseptic</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.S.D % *</td>
<td>-0.59</td>
<td>1.3</td>
</tr>
</tbody>
</table>

*For five determination

### REFERENCES


