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Flow Injection Spectrophotometric Determination of Thymol using 4-Aminoantipyrine and Copper(II) Nitrate

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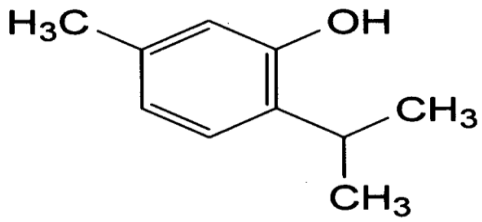
Abstract:

Flow-injection (FI) spectrophotometric method has been developed for the analysis of thymol in pharmaceutical preparations. The method is based on organic coupling reaction between thymol and 4-amino antipyrine in the presence of alkaline medium to form an intense stable red color complex with copper nitrate that has a maximum absorption at 490 nm. Optimum conditions for determination of the drug was investigated .The calibration graph was linear over the range of 5-500 $\mu g.ml^{-1}$ of thymol . The limit of detection (LOD) and limit of quantification (LOQ) were 1.81 $\mu g\ mL^{-1}$ and 3.60 $\mu g\ mL^{-1}$ respectively .The proposed method was applied satisfactorily to the determination of thymol in mouth wash preparations. The procedure is characterized by its simplicity, accuracy and precision.

Key words: Thymol, Flow-injection Spectrophotometric determination, 4-Aminoantipyrine, copper nitrate.

Introduction:

Thymol is a 5-methyl-2-(methylethyl)phenol, $C_{10}H_{14}O$, whereas its chemical structure is shown in scheme (1) [1]:



Scheme(1): Structure of Thymol.

Thymol resembles phenol in its action, but owing to its insolubility in the fluids of the body it is absorbed much more slowly; it is also less irritant to wounds, while its germicidal action is greater than that of phenol, though less than that of naphthol . In alcoholic solution it penetrates the skin and produces local anaesthesia [1]. It is used as an antiseptic lotion and mouth wash (1 in 1000) [1]. A number of analytical methods have been reported for the determination of Thymol, these included high pressure liquid chromatography [2-4], liquid chromatography with electrochemical detection [5], gas chromatography [6-9], differential-pulse voltammetry [10], ultraviolet spectrometry [11] and colorimetric analysis [12].

The present study describes the development of FIA method based on a complexation reaction between Thymol, 4-Aminoantipyrine and copper(II)nitrate in ammonium hydroxide medium. The red complex product was spectrophotometrically measured at 490 nm. The method has been satisfactorily applied for the determination of Thymol in pure and mouth wash preparations, the reaction can be carried out in FIA method.

Materials and Methods:

Flow manifolds. Flow injection system. . A two channels manifold was employed for continuous flow

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injection spectrophotometric determination of thymol (Figure 1). Channel A was used to transport 4-aminoantipyrine and ammonium hydroxide .While copper(II) nitrate was transported via channel B.The sample was injected into the stream of channel A through the injection valve (Rheodyne, Altex 210, supelco-USA) .Finally ,the solutions were propelled by the peristaltic pump (Ismatec, labortechnik-Analytic, Glatbrugg-Zurich, Switzerland) which had individual flow rate of 0.75 ml/min using flexible vinyl tubing of 0.5 mm internal diameter , reaction coil (RC) with length of 100 cm and injection loop of 100 µl. The absorbance has been measured in quartz flow cell with 50 µl internal volume and 1 cm bath length at 490 nm using Shimadzu UV-Visble-260 digital double-beam recording spectrophotometer (Tokyo-Japan) .

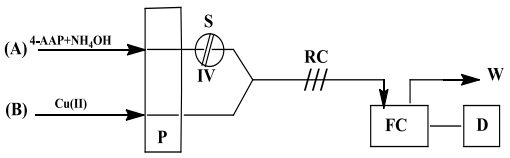


Fig.(1) : Aschematic diagram of FIA manifold where (A) and (B) ,solution of amixture of 4-AAP + NH_4OH and copper nitrate respectively ; P= peristaltic pump ; S= injection sample of thymol ; IV= injection valve, RC=reaction Coil ; FC=flow cell ; W=waste ; D=detector.

Reagents and materials:

Distilled water and analytical grade reagents were used throughout without further purification.

- A stock solution of Cu(II) nitrate(BDH, UK): 0.01M was prepared by dissolving the required amount (0.2416gm) of Cu(II) nitrate in distilled water in a 100-ml volumetric flask .Working solutions were prepared by appropriate dilution of the stock solution with distilled water.
- 4- Amino anti-pyrine 4-AAP (BDH, UK): 0.1 M was prepared by dissolving 2.0324gm of 4-AAP in 0.1M ammonium hydroxide in a 100-ml volumetric flask .Working solutions were prepared by appropriate dilution of the stock solution with 0.1M ammonium hydroxide.
- Ammonium hydroxide (BDH, UK): 0.1 M was prepared by appropriate dilution of the concentrated solution (3M) with distilled water in a volumetric flask.
- Standard thymol solution (1000 $\mu g.ml^{-1}$): was prepared by dissolving of 0.1 g of the pure compound (provided from BDH in 5 ml of ethanol and diluted to 100 ml in a volumetric flask with distilled water. More dilute solutions were prepared by simple dilution with distilled water.

Pharmaceutical preparation of Thymol.

Pharmaceutical preparations were obtained from commercial sources:-

1- Listerine – antiseptic (USA): containing 0.063% Thymol .

2- Breath Rx (mouth rinse – anti bacterial – USA) containing 0.060% Thymol.

Procedure for Mouth wash.

An aliquot of 20 ml of the above mouth wash preparation (Breath, Rx or Listerine) was dissolved in 5 ml of ethanol and was diluted to 50 ml with distilled water in a volumetric flask to obtain 240 $\mu g.ml^{-1}$ and 252 $\mu g.ml^{-1}$ of thymol in Breath, Rx and Listerine mouth wash respectively .Further appropriate solutions of mouth wash were made by simple dilution with distilled water.

General FIA procedure.

Working solutions of thymol in the range cited in Table (1) were prepared from the stock solution. A 200µl

portion of drug solutions were injected into the stream of channel A ($5x10^{-3}$ M of 4-AAP and 0.1 M of NH_4OH solution) and was then combined with stream of channel B ($5x10^{-3}$ M of copper nitrate solution) with flow rate of 0.75 $ml.min^{-1}$ in each channels (Fig.1). The red product absorbance was measured at 490 nm. A 100 $\mu g.ml^{-1}$ of thymol solution was used for optimization of conditions .

Results and Discussion:

Thymol react with 4-AAP and copper nitrate in the presence of ammonium hydroxide at room temperature to give a red colored complex. The absorption spectra of the colored product against reagent blank in the range of 300-700 nm are illustrated in Figure(2). The absorption shows a maximum at490 nm for Thymol complex whereas the reagent blank gives no absorption at this wavelength and was used in all subsequent experiments.

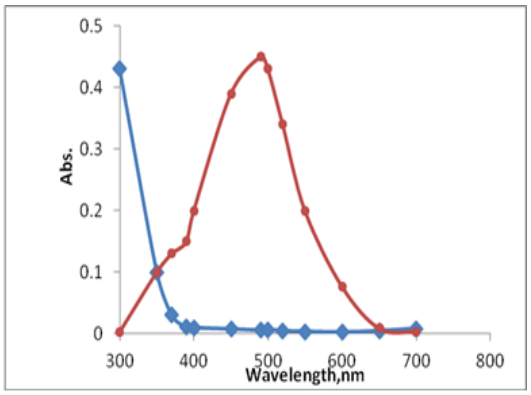


Fig. (2): Absorption spectra of the colored dye formed against blank, and blank against distilled water Configuration designs.

The FIA configuration used to determine Thymol was designed to provide different reactions condition for magnifying the absorbance signal generated by the reaction of Thymol with 4-amino anti-pyrine and copper (II) nitrate in the presence of ammonium hydroxide. Maximum absorbance intensity was obtained when the sample was injected into a stream of a mixture of $5x10^{-3}$ M of 4-AAP and 0.1 M of NH_4OH solution and then combined with a solution of $5x10^{-3}$ M of copper nitrate.

The effect of chemical parameters.

Concentration of NH_4OH .

The effect of different concentrations of ammonium hydroxide were studied in the range of 0.01–0.5M. and in the presence of $5x10^{-3}$M 4-AAP and $5x10^{-3}$M solution of copper nitrate . A concentration of 0.1M gave a highest absorbance and was chosen for further use (Figure 3).

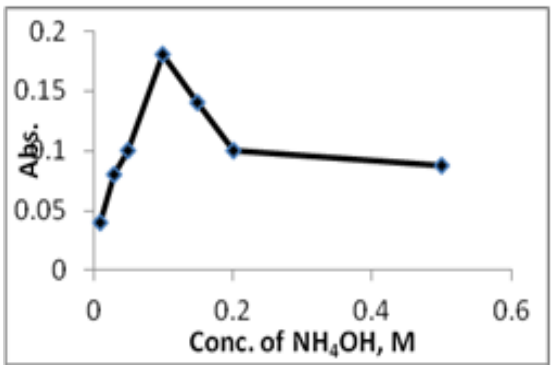


Fig. (3): The effect of NH_4OH concentration

Concentration of 4-AAP.

The effect of various concentration of 4-AAP solution was investigated in the range of $1x10^{-3}$ – $1x10^{-2}$ M and in the presence of 0.1 M ammonium hydroxide and $5x10^{-3}$M of copper nitrate solution .A concentration of $5x10^{-3}$M of 4-AAP gave a highest absorbance and was chosen for further use . The results are shown in Figure (4).

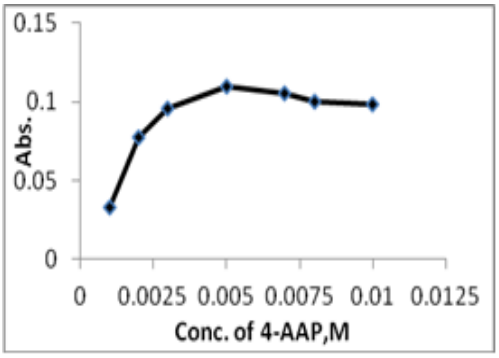


Fig. (4): The effect of 4-AAP concentration

The concentration of Cu (II) nitrate*:* The effect of different concentrations of copper (II) nitrate solution was studied in the range of $1x10^{-3}$ – $9x10^{-3}$ M . A concentration of $5x10^{-3}$M gave a highest absorbance and was chosen for further use (Figure 5).

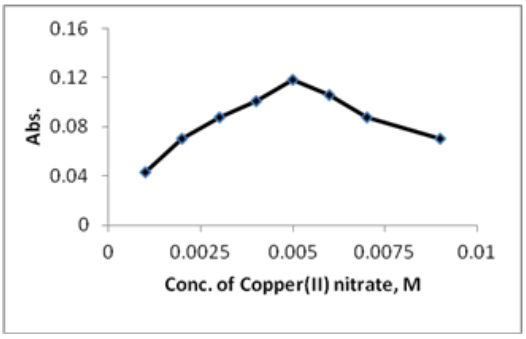


Fig.(5): The effect of concentration of Copper (II) nitrate

The effect of manifold parameters.

The physical parameters which are studied under the optimized reagent concentrations were the flow rate, the injected sample volume and the reaction coil length. Coil length is an essential parameter that affected on the sensitivity of the colored reaction product and was investigated in the range of 25–250 cm. The result obtained showed that a coil length of 100 cm was adequate to create an efficient mixing of both streams and gave highest absorbance as shown in (Figure 6) and were used in all subsequent experiments.

The effect of flow rate on the absorbance of the red product was investigated in the range of 0.5-2 ml min^{-1}. The results obtained (Figure 7) indicated that a total flow rate of 1.5ml.min^{-1} (0.75 ml min^{-1} in each channel) gave the highest sensitivity.

The results of investigating the effect of the injected sample volume revealed that an injected sample of 200µl was optimum and gave the highest absorbance (Figure 8).

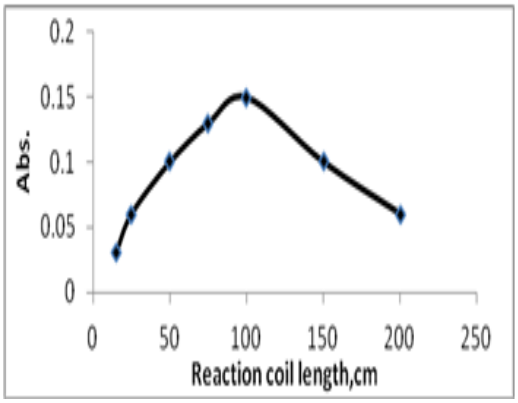


Fig.(6):The effect of the length of the reaction coil in cm .

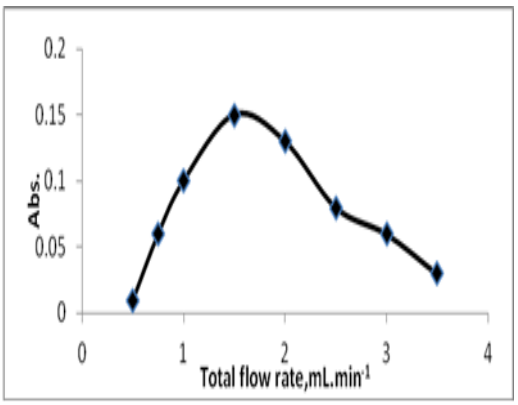


Fig.(7):The effect of the total flow rate (ml min^{-1}).

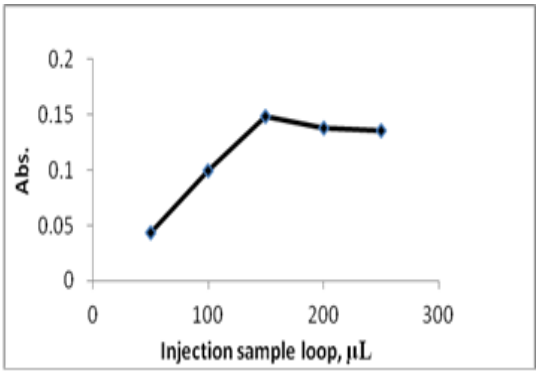


Fig.(8): The effect of the injection volume in µL.

Stoichiometry of the reaction:

It is apparent from the literature [13] that a mole ratio of phenolic drug : 4AAP was 1:1 forming a new ligands having low absorbance . The absorbance sensitivity has been increased by its reaction with Cu(II) to give an intense colored complex .The stoichiometry of the reaction of thymol with 4-AAP and Cu(II) was performed by mole ratio method [14] .The results obtained (Figure(9) and Figure (10)) indicated that the colored complex with stoichiometry ratio of 2:1 [4-AAP-thymol] ligand : Cu(II) and were in agreement with those obtained recently by the reaction of similar phenolic drugs such as sulbutanol, amoxicillin and pyridoxine hydrochloride [15].

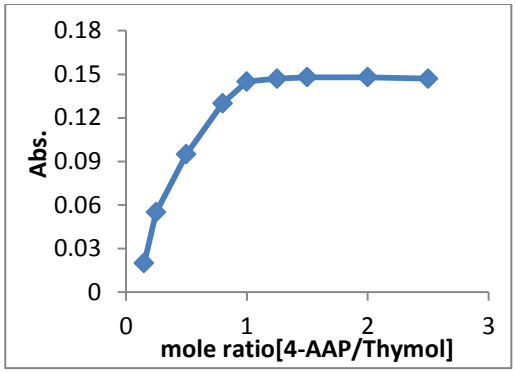


Fig.(9): Molar ratio of Thymol and 4-AAP

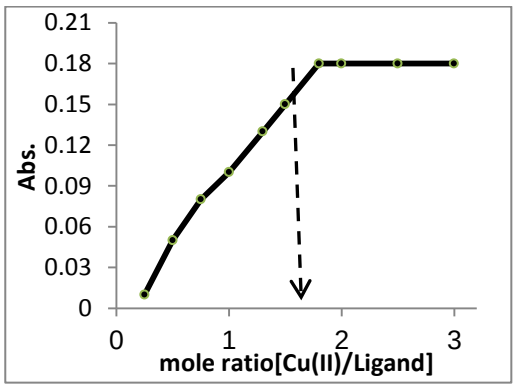
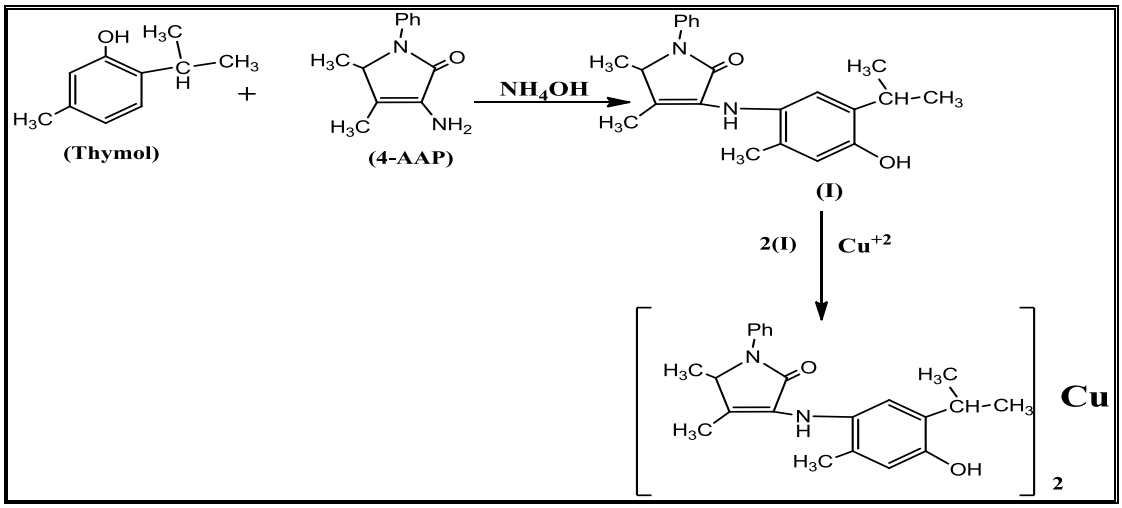


Fig.(10): Molar ratio of Ligand and Cu(II).

The proposed reaction sequence based on the above results and literature [13,15] is shown in scheme (2)



Scheme(2): Proposed mechanism of the complex formation between Thymol ,4-AAP and copper nitrate.

Analytical characteristics of the proposed spectrophotometric method:

For the proposed method, a calibration graph was obtained by the procedure described previously and a series of standard solutions were analyzed in triplicate to test the linearity. The slope (a) and the intercept (b) were determined and are included in Table(1) . The accuracy and precision of the proposed method was tested by analyzing five replicate of Thymol using the proposed method for three different concentration of Thymol . The values of relative standard deviation RSD% and relative error E_{rel} % are summarized in the same table .These values indicated a high accuracy and precision of the proposed method. The limit of detection (LOD) was determined by using the ratio of the standard deviation (SD) of the blank with respect to water and the slope of the calibration graph multiplied by a factor of three.

Table(1): Analytical characteristics of the proposed FIA spectrophotometric method

Parameter	Value
λmax (nm)	490
color	red
Linear range (µg mL^{-1})	5-500
Correlation coefficient	0.9975
Regression equation	y=0.0014x+0.0618
Limit of detection (LOD)	1.81
LOQ(s/n=3) µg mL^{-1}	3.60
Limit of quantation	5.67
Relative standard deviatin RSD%	< 0.87
Average recovery	99.85
Sample frequency per hour	65

Table (2): Application of the proposed for the determination of Thymol in mouth wash

drug	Flow injection method			
	Conc. (µg.mL^{-1})	E%	Rec.%	RSD%
Listarine	40	+0.24	100.24	0.901
	80	-0.15	99.85	0.749
	100	+0.06	100.06	0.337
Breath,Rx	50	-0.55	99.45	1.201
	100	+0.25	100.25	0.807
	200	+0.65	100.65	0.920

* for five determinations

Table(3): Comparison of the proposed and official methods for the determination of Thymol

Mouth wash sampls	Proposed FIA method		Official method
	Recovery%*	RSD %*	Recovery%
Listarine	100.05	0.662	100.86
Breath,Rx	100.11	0.976	99.48

* for five determinations

Conclusion:

The results obtained confirm that the proposed method is simple, economical with reasonable precision and accuracy for the determination of Thymol. The optical parameters and statistical comparison justify this method for application in routine drug estimation in pure and dosage form. Also, the procedure does not involve any critical reaction conditions or tedious sample preparation steps. So, the recommended method is well suited for the assay and evaluation of Thymol in mouth wash preparation and can also be considered as a general method for the quantification of Thymol. In comparison of the other methods [2-12] with FIA procedure, the later is more convenient than the former method because of its speed (sample through-put of 65 injection h^{-1}) and wider linear range of the calibration graph. F- and t- test at 95% confidence level[16] showed that there was no significant difference between the proposed method and the standard

Bp method during its application to the analysis of mouth washes samples Table (2 and 3).

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التقدير الطيفي بتقنية الحقن الجرياني للثايمول باستخدام 4- امينوانتي بايرين ونترات النحاس(II)

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الخلاصة:

يتضمن البحث تطوير طريقة طيفية جديدة وبسيطة للتقدير الكمي لمقادير ضئيلة من الثايمول في المحاليل المائية وغسول الفم باستخدام تقنية الحقن الجرياني. تعتمد الطريقة على تفاعل الازدواج للثايمول مع كاشف 4-امينوانتي بايرين في وسط قاعدي حيث يتكون معقد ذائب في الماء ذو صبغة حمراء بوجود فلز النحاس واعطى اعلى قمة امتصاص عند طول موجي 490 نانوميتر. تشير منحنيات الامتصاص مقابل التركيز بان قانون بير ينطبق ضمن مدى التراكيز 5-500 مايكروغرام.مل$^{-1}$ من الثايمول، وكانت قيم حد الكشف وحد الكمية1.81 و 3.60 مايكروغرام.مل$^{-1}$ من الثايمول على التوالي وبمعدل نمذجة 65 نموذج في الساعة وتم دراسة الظروف المثلى للتفاعل وجميع المتغيرات الكيميائية والفيزيائية بدقة، طبقت الطريقة بنجاح على غسول الفم الحاوية على الثايمول.

الكلمات المفتاحية: ثيمول، الحقن الجرياني، تقدير طيفي، 4- امينوانتي بايرين، نترات النحاس.