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DETERMINATION OF TRIMETHOPRIM BY CHARGE-TRANSFER COMPLEX FORMATION

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ABSTRACT

A simple and accurate charge-transfer complex (CT) method for the determination of trimethoprim is presented. The method involved the use of p-chloranil in dioxane: methanol (1:2) medium. The absorbance of the violet colour of the charge-transfer complex was measured at 535 nm against reagent blank. The method permits determination of about 60-480 μg ml^{-1} of trimethoprim in the final solution mixture with mean percentage recovery 98.84 ± 1.23%.

INTRODUCTION

Chemically, trimethoprim is 5-(3, 4,5-trimethoxybenzyl) - pyrimidine- 2,4-diamine widly used as an antibacterial agent either alone, or in conjunction with sulphonamides[1]. Mechanistically, trimethoprim is a dihydrofolate reductase inhibitor which affects the nucleoprotein metabolism of micro-organisms by interference in the folic acid systems[2].

Different methods have been reported for trimethoprim determination including TLC[3-5], GLC[6], HPLC[7,10], differential pulse polarography[11,12], NMR[13], titrimetric[14-16], IR spectrophotometric[17] spectrofluorimetric[18,19], UV-spectrophotometric[20,21], colorimetric methods[22-26] and differential spectrophotometry[27, 28].

Amines are well known compounds with excellent electron donation property. Charge-transfer complexes of these compounds with halogen and pseudohalogens have been reported[29-31]. Chloranil a π-acceptor, is known to form charge-transfer complexes and radical ions with a variety of electron donors, including amines. Feigl et al[32] reported that chloranil forms coloured condensation products with primary and secondary arylamines, aminoacids, phenols and naphthalene. The utility of chloranil as a reagent for the spectrophotometric analysis of various amino-acids has been studied by many workers[33-35]. These investigations revealed that the spectra produced were due to n-π charge-transfer complexes. Al-Ghobashy et al[36], investigated the reaction of chloranil with a wide range of amines and described a method for their determination. Korany and Wahbi[37] used chloranil for the spectrophotometric determination of some primary and secondary amines.

This paper describes the spectrophotometric determination of trimethoprim in pharmaceutical preparation through the formation of charage-transfer complexes with chloranil. In addition, the work involves the study of the effect of different organic solvents, effect of temperature on the complex formed.

EXPERIMENTAL

Instrument :-

A UVIDEC-320 spectrophotometer and a pair of two matched 1 cm. cells were used.

Reagents :- All the reagents used were analytical-reagent grade.

1- **p-Chloranil solution:** p-chloranil 0.2 g was dissolved in 10 ml of dioxane-absolute methyl alcohol 1:4.

2- **Trimethoprim solution:** 60 mg were dissolved in 50 ml methyl alcohol. The raw material was provided kindly by the Nile Company for pharmaceuticals and chemical industries, Egypt.

The purity of materials is tested by the pharmacopoeial testing according to B.P[2] by using TLC method (silicagel GF 245 as coating substance and mixture of 85 ethyl acetate, 10 methanol, 5 water and 2 anhydrous formic acid as mobile phase).

3- **Solvent :** dioxane: absolute methyl alcohol (1:2).

4- **Pharmaceutical Formulations.**

a- Triprim capsules: each capsule contains 300 mg of trimethoprim, Memphis Chemical Company, Cairo.

b- Theraprim tablets: each tablet contains 100 mg trimethoprim, Nile Company, Cairo.

c- Septazole tablets: each tablet contains 80 mg trimethoprim, 400 mg sulphamethoxazole Alexandria Company for Pharmaceuticals and Chemical, Industry, Alexandira.

d- Sutrim suspension: each 1 ml contains 5 mg trimethoprim, 40 mg sulphamethoxazol (Memphis Chemical Company-Cairo.

e- Septazole suspension: each 1 ml contains 5 mg trimethoprim, 40 mg sulphamethoxazole (Alexandria Company for Pharmaceuticlas and Chemical, Industry, Alexandria.

Procedures :-

Preparation of standard graph :-

Into a volumetric flask 10 ml, 0.5, 1.-, 1.5, 4 ml of trimethoprim solution equivalent to 0.6, 1.2, 1.8, 2.4, 3.0, 3.6, 4.2, 4.8 mg of trimethoprim were transferred. The volume was complete to 5 ml with absolute methyl alcohol, then 2 ml of p-chloranil solution, were added. The volume was completed with the dilution mixture then allowed to stand at room temperature for 40 min. The absorbance of the violet colour was measured at 535 nm against a reagent blank prepared similarly using 5 ml methyl alcohol in absence of trimethoprim. The concentration of trimethoprim was then calculated from a properly constructed calibration graph (Fig. 5).

Pharmaceutical formulation :-

Triprim capsule and theraprim tablet: The content of 20 capsules or tablets were well mixed and the powdered weight of one capsule or tablet transfered to 250-ml or 100 ml volumetric flask. Absolute methyl alcohol was added, and then the mixture was warmed gently in a water bath, until the contents were dissolved. The volume was completed with methyl alcohol. The flask was shaked thoroughtly, and the contents, were filtered. The first 50 ml of the filterate were discarded. The subsequent portion was collected of which 1 ml equivalent to 1.2 mg and 1 mg trimethoprim.

Septazole tablets: The presence of sulphamethoxazole interfers with the reaction of trimethoprim with chloranil. Therefore, trimethoprim was isolated from the tablets before its determination. The powder of 20 tablets were well mixed. Finely powdered tablets equivalent to about 120 mg of trimethoprim were transfered into a seperating funnel

containing 30 ml of 0.1 M sodium hydroxide. Trimethoprim was extracted with chloroform (4 x 50 ml). The chloroform was evaporated to dryness and the residue was dissolved in absolut methyl alcohol, transfered into 100 ml volumetric flask and the volume was completed with methanol.

Then, it was proceeded as described under preparation of standard graph. The trimethoprim content is determined from the standard graph.

Septazole and sutrim suspensions: Sulpamethoxazole was removed from the suspensions of the pharmaceutical preparations and trimethoprim was extracted. The suspension equivalent to 129 mg of trimethoprim, was shaked well in a separating funnel containing 30 ml of 0.1 M sodium hydroxide. Trimethoprim was extracted with chloroform (4 x 50 ml). The chlorform was evaporated to dryness and the residue was dissolved in absolute methyl alcohol. The contents was transfered to 100 ml volumetric flask and complete the volume was completed with methanol. Results are shwon in Table (1).

RESULTS AND DISCUSSION

Different solvents were tried such as chloroform, carbon-tetrachloride, ethanol, methanol, and 50% ethanol methanol as media for the reaction. They all failed to show complete formation of the charge-transfer complex.

It was found that chloranil (dissolved in dioxane: Absolute methyl alcohol 1:4) reacts with trimethoprim (dissolved in methyl alcoho) produced a violet colour. The violet colour is a result of charge-transfer complex. The absorption bands appear as single asymmetric bands with exceptional large half bandwidths. The slope of the curve at the shorter wavelength is greater than that at the longer wavelength. This indications that chloranil acts as π acceptor to the electron donner trimethoprim[38].

The spectrum of the complex exhibits maximum absorption at 535 nm as shown in Fig. (1).

Because the reaction with chloranil at room temperature was slow, (Fig. 2) the absorbance was measured after 40 min. Trials were made to accelerate the reaction by heating at different temperatures, but decay of the absorbance was observed at 60°C (Fig. 3).

The molar ratio determined according to Job's method of continuous variation[39], indicated a donor to acceptor ratio of 1:1 for trimethoprim and chloranil (Fig. 4).

Hence, for quantitative formation of the 1:1 complex an excess of chloranil solution was needed. Two ml of 0.2% chloranil in 1:2 v/v dioxane methanol was added gives maximal complex formation with trimethoprim in the concentration range, 60-480 µg/ml. Completness of the reaction was checked by TLC, using benzene: diethyl ether, methanol: NH_4OH (50: 50: 12: 1) as mobile phase.

The use of dioxane: methyl alcohol (1:4) as a solvent for chloranil and trimethoprim produced maximum colour formation. Also, the use of dioxane: methanol 1:2 for dilution stabilized the violet colour and gave a good medium for the charge-transfer complex formation.

Regression analysis of Beer's plot at 535 nm using chloranil revealed an excellent correlation (r = 0.9997). A linear relationship existed in the range 60-480 mg trimethoprim per ml in the final assay solution.

The results obtained by the proposed method were compared with the official British Pharmacopoeia[2]. There was no significant difference between the two methods indicating that excipients with tablets and capsules did not interfere in the determination of trimethoprim (Table 1).

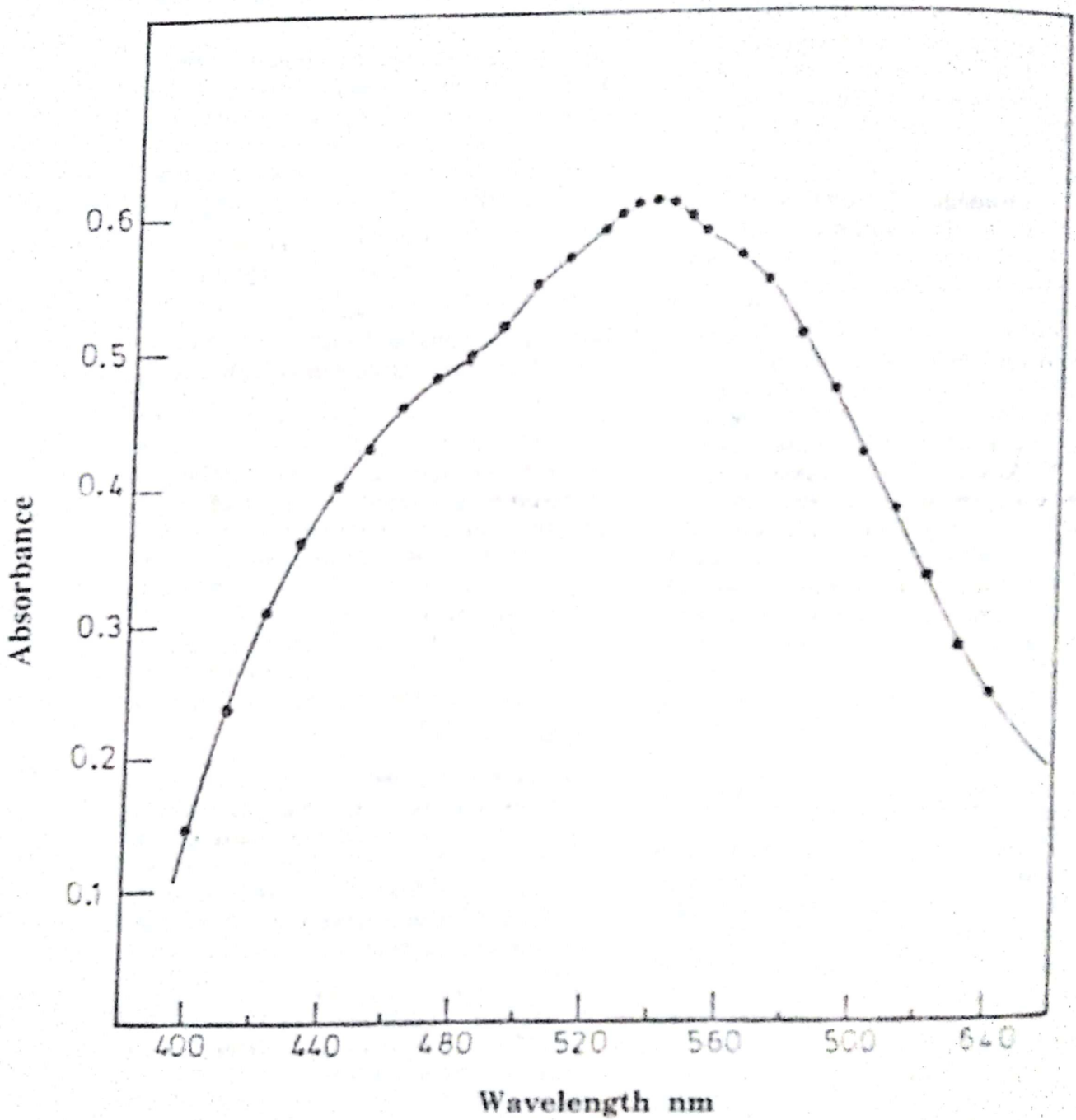

Fig. (1): Absorption spectrum of chloranil-trimethoprim complex.

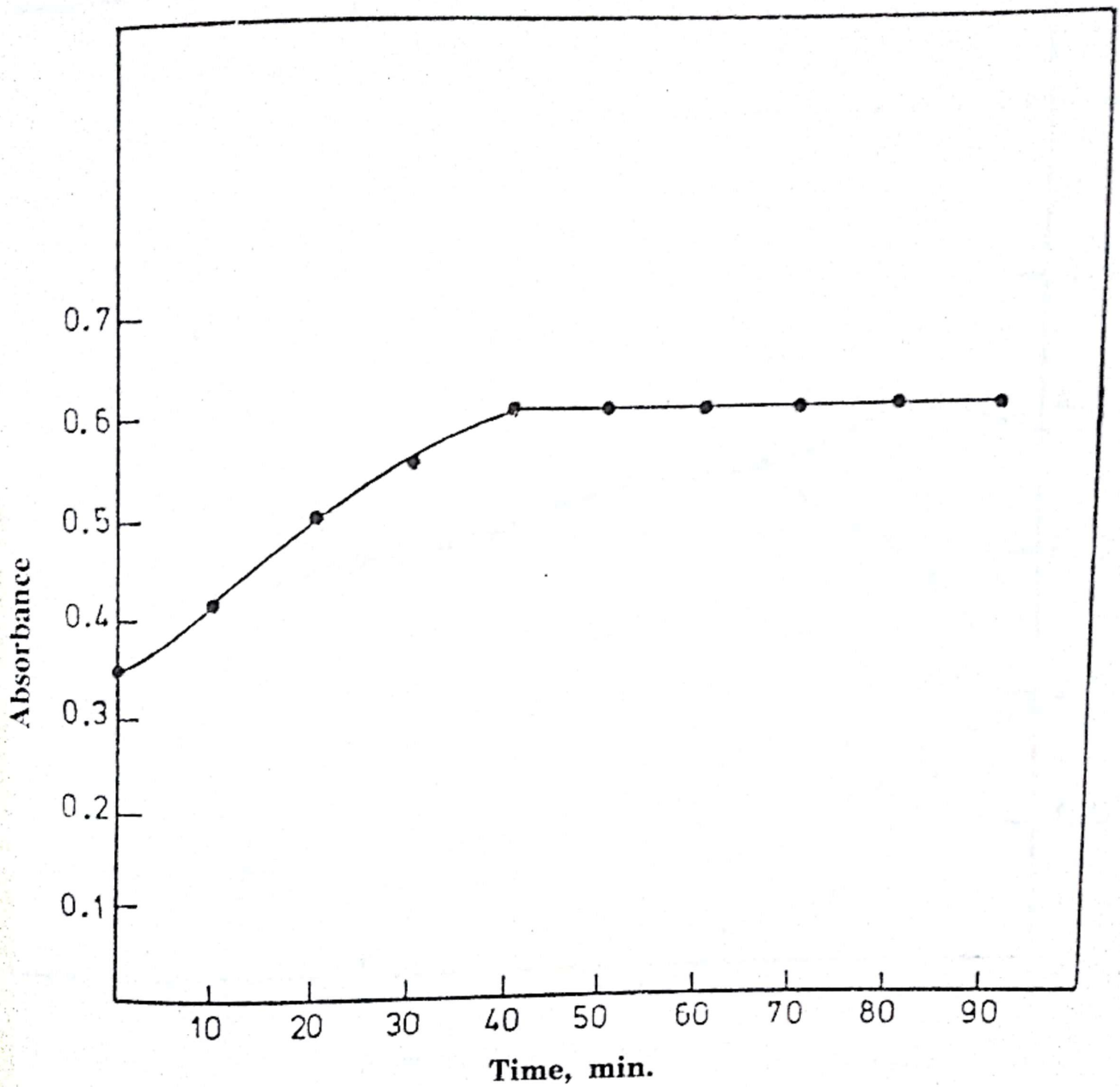

Fig. (2): Effect of time (at room temp.) on the stability of chloranil-trimethoprim complex.

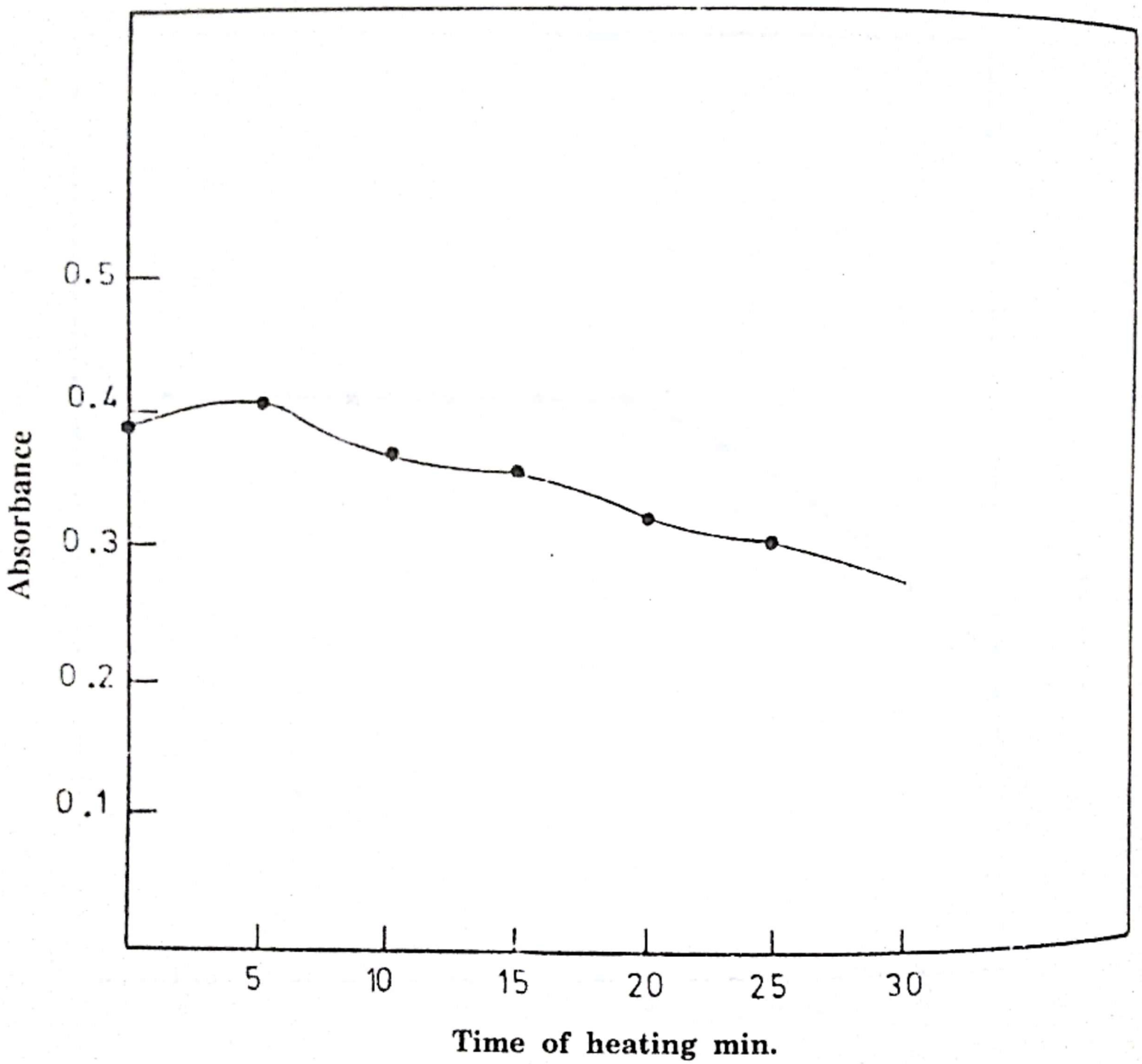

Fig. (3): Effect of heating time at 60°C on the absorption of chloranil-trimethoprim complex.

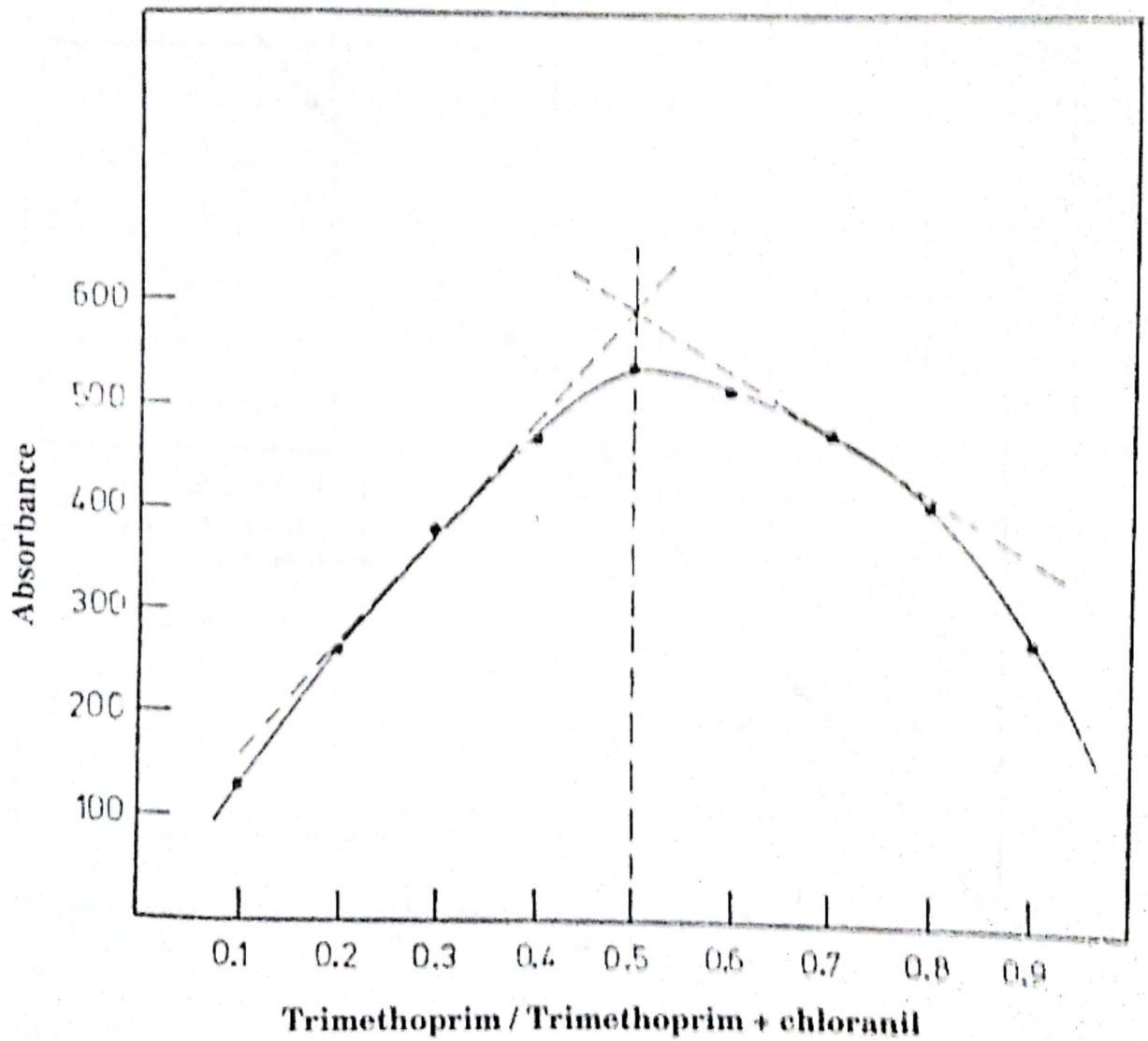

Fig. (4): Continuous variation plot of trimethoprim-chloranil complex in dioxane-methanol medium.

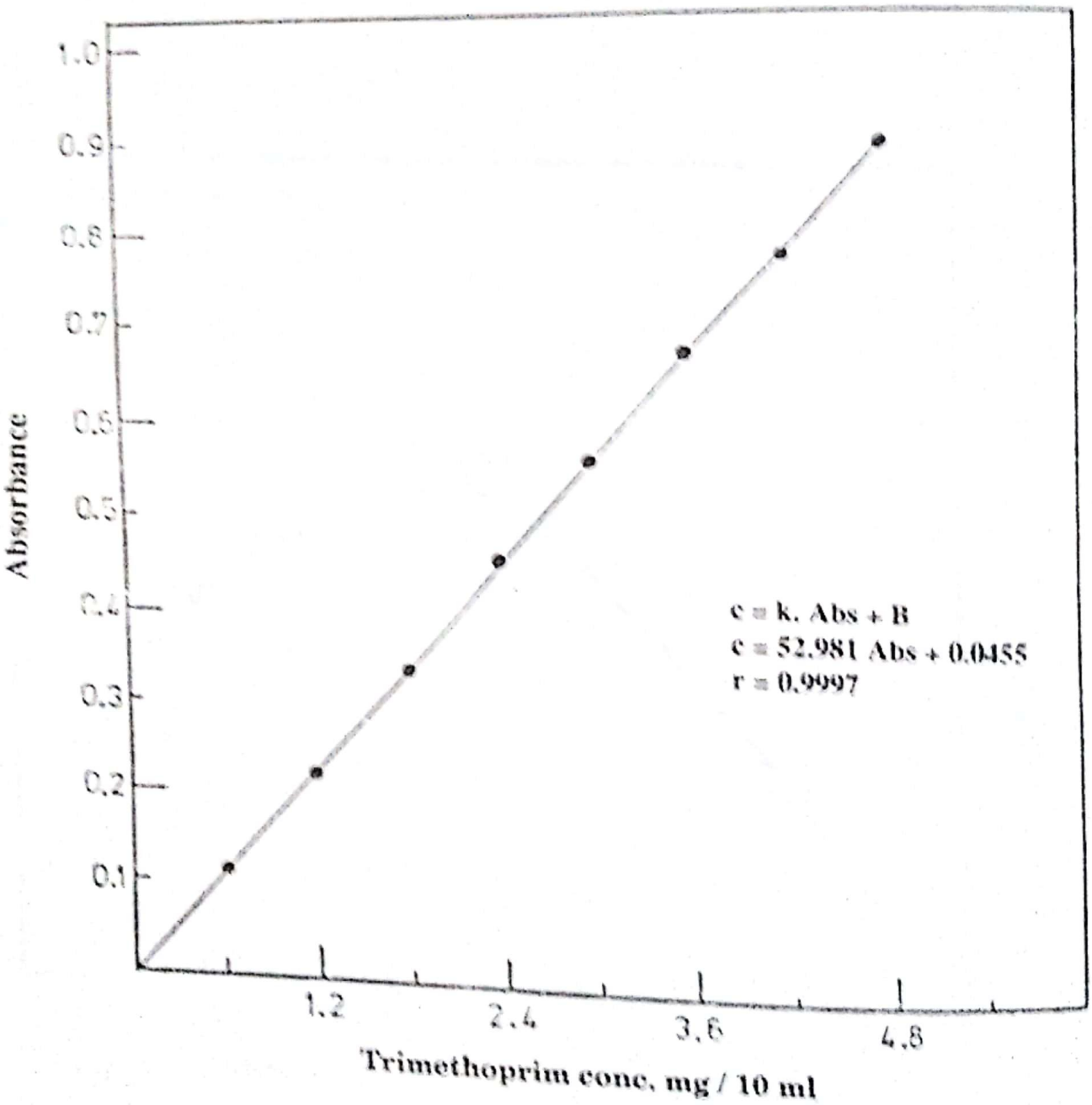

Fig. (5): Calibration curve for the charge-transfere complex formed between trimethoprim and chloranil.

Table (1): Comparison of the results obtained by the proposed method and that obtained by the official method.

Mean recovery % ± SD			t**	F*
Sample	Proposed method	Official method[2]		
Pure trimethoprim.	99.47 ± 0.60	99.68 ± 0.70	0.535	1.124
Triprim capsules.	99.67 ± 1.02	100.33 ± 1.10	1.077	1.173
Theraprim tablets.	100.13 ± 1.03	99.63 ± 1.03	0.824	1.011
Septazole tablets.	100.76 ± 1.36	99.73 ± 0.83	1.543	2.691
Septazole suspension.	98.45 ± 1.21	98.92 ± 1.10	1.235	1.845
Sutrim suspension.	101.40 ± 0.51	99.40 ± 1.23	2.122	2.890

* Mean of six determinations (n = 6). ** Tabulated t at P=.05 = (3.58).

* Tabulated F at P=.05 = (4.28).

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تعيين ماده الترايميثوبريم بواسطة نقل الشحنات

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فى هذا البحث تم تحليل ماده الترايميثوبريم كماده منفصلة وكذلك فى وجودها بالمستحضرات الصيدليه سواء كانت منفرده أو مخلوطه مع مركبات السلفا وقد تم التحليل بطريقة تكوين نقل الشحنات بواسطه ماده الكلورانيل المستقبلة وقد تم تعيين نقطه الامتصاص بالتحليل الطيفى عند ٥٣٥ نانوميتر.

وقد أوضحت النتائج المطبقة على المستحضرات الصيدليه (أقراص - ومعلقات) أن الطريقة المقترحة بسيطة وعالية الدقة ومتكرره بمقارنتها بالطرق الدستورية.