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Spectrophotometric Determination of Chlorocresol via Nitrosation Reaction – Application to Pharmaceutical Preparations (Creams)*

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ABSTRACT

A spectrophotometric method for the determination of pure chlorocresol and in a number of its pharmaceutical preparations (creams) has been developed that offers advantages of simplicity, sensitivity and stability. The proposed method was based on the formation of a nitroso derivative of chlorocresol. The colored product have maximum absorption at 424 nm. Beer's law is obeyed over the concentration range of (2-26) ppm, with a molar absorptivity of 0.417×10^4 $l.mol^{-1}.cm^{-1}$. Relative standard deviation of the method was better than ±1.8% and the average recovery was 99.2%. The proposed method has been successfully applied to the determination of chlorocresol in pure form and in pharmaceutical preparations (creams).

Keywords: Chlorocresol, Spectrophotometry; Nitrous acid.

التقدير الطيفي للكلوروكريزول عن طريق تطبيق تفاعل النترزة في المستحضرات الدوائية (الكريمات)

الملخص

يعتمد البحث على تطوير طريقة طيفية جديدة لتقدير الكلوروكريزول بحالته النقية وفي عدد من المستحضرات الدوائية (الكريمات) وتمتاز الطريقة بالبساطة و الحساسية والاستقرارية والطريقة المقترحة تعتمد على تفاعل الكلوروكريزول مع حامض النتروز لتكوين مركب ملون له أقصى امتصاص عند الطول الموجي 424 نانوميتر وقد لوحظ أن قانون بير يسري على الكميات التي تتراوح بين 2-26 جزء/مليون وبامتصاصية مولارية 0.417×10^4 لتر مول$^{-1}$.سم$^{-1}$. إن الانحراف القياسي النسبي للطريقة أحسن ± 1,8% وبمعدل استرجاعية 99,2% وطبقت الطريقة بنجاح في تقدير الكلوروكريزول في حالته النقية وفي بعض مستحضراته الصيدلانية (الكريمات).

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INTRODUCTION

Chlorocresol (4-chloro-3-methylphenol) is widely used as a preservative and germicide in creams and other preparations for external use which contain water, but its effectiveness was reduced if oils, fats, or non-ionic surface active agents were present (The pharmaceutical codex,1979). Also, it was used as external germicide, as preservative of glues, gums, paints, inks, textile and leather goods (Wawley,1981). Chlorocresol is generally determined by chromatographic method (Tripathi *et al.*,1990 and Paul *et al.*,1999, Marengo *et al.,* 2001 and Gatti *et al.*,1997) and the official indirect titrimetric method (British pharmacopoeia, 2005and The United States Pharmacopoeia, 2003). Other methods are polarographic method (Rao *et al*.,1990) and enzyme-amperometric which compared favorably with those found by using classical HPLC or spectrophotometric method (Bloofidel and Prebble,1992).

Very few spectrophotometric methods have been reported for chlorocresol determination(Ahmed and Ibrahim, 2004 and Campanella *et al.*, 1992). In general, spectrophotometric methods require less expensive instrumentation than polarographic and HPLC methods (Paul *et al.*,1999 and Rao *et al*.,1990) which are widely used for the determination of chlorocresol. Therefore the research work in this paper is being directed to an approach in the spectrophotometric method for the determination of chlorocresol in pure form and a number of its pharmaceutical preparations.

EXPERIMENTAL

Apparatus

Spectro-scan 50 uv-visible spectrophotometer with 1- cm quartz cells was used for absorption measurements.

Reagents

All chemicals used were of analytical grade.

Chlorocresol standard solution (100 ppm or 7×10^{-4}M**):** This solution was prepared by dissolving (0.1 g) of chlorocresol (Merck) in 1 L distilled water.

Sodium nitrite solution (3%). This solution was prepared by dissolving 3 g of sodium nitrite in 100 ml of distilled water.

Sodium hydroxide solution (2 N). This solution was prepared by dissolving 8 g of sodium hydroxide in 100 ml of distilled water. from the above solution (1 N) NaOH solution was prepared.

Nitric acid solution (5 N and 1 N)

Recommended procedure

An aliquot of standard solution of chlorocresol (50-650 µg) were transferred into a serious of boiling test tubes. To each test tube, 1 ml of 1 N HNO_3 and 1ml of 3% $NaNO_2$ were added, mixed well and placed on a water bath maintained at 80 ±5 C° for 10 min. The solutions were cooled to room temperature and transferred to 25- ml standard volumetric flasks, then 3 ml of 1 N NaOH was added. The contents were diluted to the volume with distilled water. The absorbances were measured at 424 nm against a reagent blank.

Procedure for the determination of chlorocresol in pharmaceutical preparations (creams)

A quantity of creams equivalent to 0.01 g of chlorocresol was accurately weighed into 100 ml flask, 50 ml of 2M NaOH solution was added and shaken until the solid dispersed. The content was mixed well and filtered using a Wnatman No. 42 filter paper. The filtrate neutralized by 5N HNO_3. The volume was finally adjusted to 100 ml in a volumetric flask by distilled water. A 6 ml of this solution was treated as mentioned under the recommended procedure**.**

RESULTS AND DISCUSSION

Absorption spectra and effect of experimental variables

The absorption spectra of colored product shown in Fig.(1) is made on a solution containing 300 µg of chlorocresol, 1 ml of HNO_3, 1 ml of $NaNO_2$. The flask was immersed on a water bath maintained at 80 ± 5 C° for 10 min, the solution was cooled to room temperature, then 3 ml of 1 N NaOH was added and transferred to 25 ml volumetric flask. The content were diluted to volume with distilled water. The absorption spectra of the product against blank was recorded. The colored product showed maximum absorption at 424 nm and this wavelength was recommended for determination.

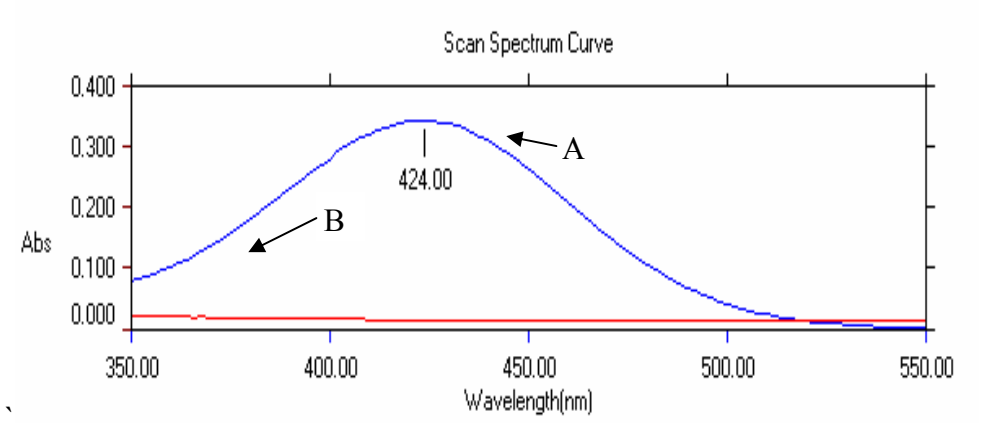


Fig. 1: (A) Absorption spectra of colored product against reagent blank, (B) blank against distilled water

Study of the optimum reaction conditions

The effect of various parameters on the absorption of the product formed was studied and the reaction conditions are optimized.

Effect of acid

The effect of different acids on the absorbance of the colored product is shown in Table (1), which shows that maximum intensity was reached when using 1 ml of 1 N nitric acid solution. This amount was selected for the subsequent experiments.

Table 1: Effect of acids on the absorbance

Acids (1N) Solutions	Absorbance / ml of acid added				
	0.1	**0.5**	**1**	**2**	**5**
HCL	0.059	0.115	0.142	0.071	0.051
H_2SO_4	0.070	0.142	0.168	0.072	0.063
HNO_3	0.117	0.204	0.248	0.163	0.058
H_3PO_4	0.067	0.087	0.120	0.167	0.117

Effect of sodium nitrite reagent

The effect of the amount of $NaNO_2$ solution on the absorbance of the colored product was studied. It was observed that the addition of 1 ml of 3 % $NaNO_2$ solution was required to obtain a maximum absorbance. This amount was selected for subsequent experiments.

Effect of temperature and heating time

The effect of heating time on the color intensity was studied, in practice the absorbance of the color increases with increasing temp erature and reached maximum at temperature greater than 70 C^0,80±5C˚ was selected. However, the reaction is complete within 10min. The absorbance was then stable for at least 6 hr. the effect of heating time and temperature is shown in Table(2).

Table 2 : Effect of temperature and heating time

Temp. C˚	40			60			70			80		
Time(min)	5	10	15	5	10	15	5	10	15	5	10	15
Absorbance	0.138	0.140	0.142	0.258	0.260	0.264	0.320	0.324	0.324	0.336	0.340	0.340

Effect of NaOH concentration

The amount of NaOH solution for maximum color intensity was examined. The maximum constant color intensity was reached when using 3 ml of 1 N NaOH solution. This amount was selected for subsequent experiments.

Effect of order of addition

To test the effect of order of the addition of the reagents on the absorbance of the product, different orders were tested. The selected order was chlorocresol solution, HNO_3 solution followed by $NaNO_2$ solution then heating and cooling then NaOH solution is added. This order gives the highest absorbance value.

[Chlorocresol + HNO_3 solution + $NaNO_2$ heating then cooling + NaOH]

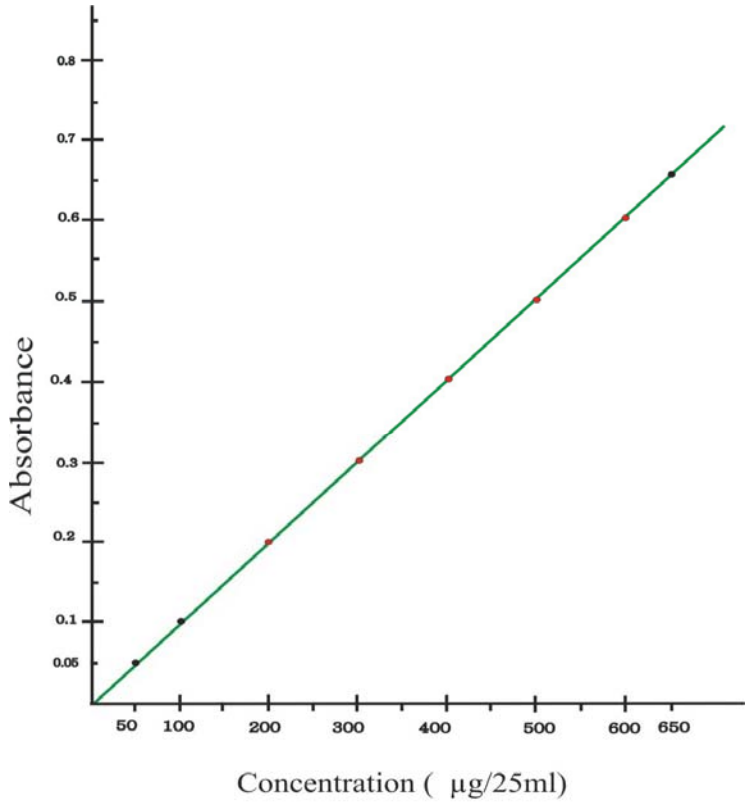
Beer's Law

Under the recommended conditions described above, a linear calibration graph was obtained for chlorocresol within concentration range of 50 – 650 µg/25ml. Linear regression equation : A= 0.033 + 0.097C (r=0.9998, n=7) where A is the absorbance and C is the concentration in µg/ml. The molar absorptivity was 0.417×10^4 $l.mol^{-1}.cm^{-1}$ and the limit of detection was evaluated as (Bassaviah, and Smoashekar, 2007 and International Conference……) :

$$LOD = 3.3 \frac{\sigma}{S}$$

Where " S is the slope and σ is the standard deviation of the regression line. The limit of detection was 0.042 µg/ml

Calibration graph of chlorocresol is shown in Fig (2).



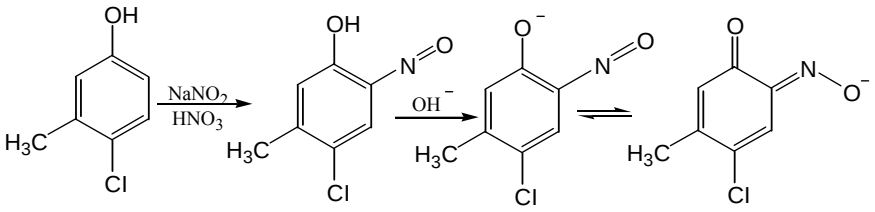
Fig[2]Calibration graph of chlorocresol

Accuracy and precision

The five independent analysis of chlorocresol samples at three different concentration levels (100, 200, and 400 µg/ 25 ml) were performed. The relative standard deviation was found better than 1.8% and the accuracy (average recovery) was found 99.2%. These results were quite satisfactory.

Composition of the colored product

The stoichiometry of reactants was investigated by the mole- ratio method (Bauer *et al.*, 1978). The results obtained indicated that the existence of 1:1 chlorocresol – $NaNO_2$ at 424 nm. Thus the suggested reaction might be written as (Belal *et al.*, 1979).



Chlorocresol

Apparent stability constant of the product

The conditional stability constant of the product is estimated by using Vargas method (Gareia *et al.*, 1981) with the following equation:

$$K = \frac{a - (\Delta A / \epsilon)}{n^n (\Delta A / \epsilon)^{n+1}}$$

Where : a = chlorcresol total concentration.

ΔA = sample absorbance in reagent excess minus the sample absorbance in stoichiometric reagent amount.

ϵ = molar absorptivity at the measured wavelength.

and n = number of ligand.

The stability constant (mean of five values) is found to be 4.3×10^5 $l.mol^{-1}$, indicating that the product is stable.

Effect of interferences

In order to assess the possible application of the proposed method, the effect of substances that often accompany chlorocresol in various creams was studied by adding different amounts of substance to 300 µg/ 25 ml of chlorocresol. An attractive feature of the method is its relative freedom from interference by the usual diluents and excipients in amount far in excess of their normal occurrence in pharmaceutical preparations. The results are given in Table (3).

Table 3 : Determination of chlorocresol in presence of Interferences

Interferences substance	Amount taken* (mg)	Average recovery %
Betamethasone 17 valerate	1	100.05
Clobetasol propionate	5	100.00
Clotrimazole	1	100.03

*Average of three replicate analyses

Analytical Application

Three types of creams containing chlorocresol (Betnosam, Dermodin, and Fugidin) were analyzed. The results were compared statistically by student t-test and by the variance ratio F-test with those obtained by official method at 95% confidence level. The calculated t- and F- values did not exceed the theoretical values indicating that there was no significant differences between the precision of the proposed and official methods as cited in Table (4)

Table 4: Determination of chlorocresol in pharmaceutical preparations (cream)

Type of creams supplied by NDI	**Amount of chlorocresol**		
	Present method	**B.P Official method**	**Certified value**
Betnosam	0.098% t =1.34, F= 1.32	0.099%	0.100 %
Dermodin	0.148% t = 1.84,F=1.09	0.15%	0.150 %
Fugidin	0.097% t = 1.55,F=1.42	0.095%	0.100 %

- Mean of ten determinations**.**

t = 1.34 , F= 1.32
t = 1.84 , F= 1.09
t = 1.55 , F= 1.42

t values (n = 10, at 95% confidence level tabulated value 2.262
F values (n_1 and n_2 = 10, at 95% confidence level tabulated value 3.18

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