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Spectrophotomtric Determination Phenothiazine Via Oxidative Cupling Reaction

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Abstract:
The general research is a new spectrophotometric method for the determination of phenothiazine by oxidation and coupling reaction, using potassium chromate as an oxidizing agent for phenothiazine, which is then combined with in an phenothiazines acidic medium to form a stable product with an orange color, with the highest absorption at 450 nm. The molar absorption coefficient is 1474.598 $l.mol^{-1}.cm^{-1}$. Beer limits are (2-170) µg/ml.
Keywords: Spectrophotometry, phenothiazine , Oxidative coupling

طريقة طيفية لتقدير الفينوثيازين

هديل مصطفى عبد القادر

الملخص
يتضمن البحث طريقة طيفية جديدة لتقدير الفينوثيازين بتفاعل الأكسدة والاقتران ،باستخدام كرومات البوتاسيوم كعامل مؤكسد للكاتيكول الذي يقترن بعد ذلك مع الفينوثيازين في الوسط الحامضي ليكون ناتج مستقر ذو لون برتقالي ، له اعلى امتصاص عند 450 نانوميتر. معامل الامتصاص المولاري 1474.598 لتر.مول$^{-1}$.سم$^{-1}$. وكانت حدود قانون بير (2-170) مايكروغرام / مل .

Introduction.

Phenothiazine. As has been described above, methylene blue was the first compound synthesized with a phenothiazine skeleton. Only afterwards was phenothiazine synthesized by heating diphenylamine and sulfur by Bernthsen. Currently, the reaction is mostly performed by the cyclization of 2- substituted diphenyl sulfides (14) (Fig(1).

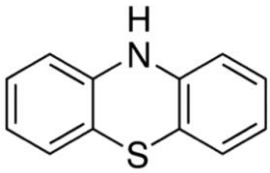

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Fig. (1) phenothiazine.

phenothiazines are basic compounds in pharmacology. The history of these compounds goes back to the second half of the 19th century, when a German chemist, Heinrich August Bernthsen began to study the structure of methylene blue, which was first synthesized in 1876 by Heinrich Caro. In 1885, two years after Bernthsen first managed to produce phenothiazine, he also succeeded in describing the structure of methylene blue (1)., methylene blue was often prescribed for patients with malaria in the subsequent period (3). Research conducted in the 1930s and ‘40s proved the potential wide use of phenothiazines. The insecticidal (4), and antibacterial (7), antihelmintic (5, 6), another novel phenothiazine derivative, namely

promethazine was brought to the forefront of attention; it was investigated by Paul Charpentier at the Rhone-Poulenc laboratory in Paris (8). It was shown that promethazine had an antihistamine effect (9), which was later used in the therapy of allergic diseases and in anesthesia (10, 11). chlorpromazine was discovered, which has strong anxiolytic and antipsychotic effect along with its antihistaminic effect (12). Psychiatry fully exploited this feature in psychotic patients (13).

Experiments

***Apparatus*:**

A Shimadzu UV-VIS 1800 digital double-beam recording spectrophotometer (Kyoto, Japan) is used for all spectral and absorbance measurements with matched 1cm quartz cells.

Reagents

All Chemicals used are of the highest purity available.

1-Catechol solution: (100 µg/ml): This solution is prepared by dissolving 0.01 g of Catechol in distilled water in a 100ml volumetric vial.

2- Potassium dichromate solution (5 x 10^{-3} M): This solution is prepared by dissolving 0.07 g of dichromate in 2.5 ml of sulfuric acid at a concentration of 1 M and fill the volume with distilled water in a volumetric bottle of 50 ml.

3- sulfuric acid solution: A diluted (1M) was used.

4- Phenothiazine (100 µg/ml): Phenothiazine is prepared by dissolving 0.01 g of phenothiazine in 100 ml distilled water.

***5*-Pharmaceutical solution:** The contents of ten tablets of the medicinal preparation Neurazine tablets is weighed, crushed and mixed well, then weighed the equivalent of one tablet (25 mg of Phenothiazines). This is hydrolyzed and then processed in the same manner as described in the tablet analysis.

Results and discussion:

1 ml of phenothiazine concentration (100 µg/ml) is added to a series of 25 ml titrated flask and 0.5 ml of potassium chromate (5 x 10^{-3} M) followed by the addition of increasing volumes of catechol solution (100 µg/ml) and 1ml sulfuric acid solution(1M) and. This is completed to the mark with distilled water and the reaction mixture was left for 5 min. The absorbance of each solution was measured at 450 nm against the vacuum prepared in the same way but without phenothiazine.

Study of the Optimum Reaction Conditions

The effects of different parameters in addition to its relation with the mentioned product is studied and presented in this section. Moreover, the optimum conditions are provided.

Effect of the potassium chromat

The effect of potassium chromate was studied and the absorbance was measured at 450 nm versus blank.

Table (1): Effect of the potassium chromate

of K_2CrO_4 $\times10^{-3}$ M)	sorbance/min.						ank
5	18	17	15	15	13	12	52
	02	07	08	07	07	07	68
	48	53	50	51	51	52	19
	65	71	72	73	71	72	04
	23	23	29	25	21	21	75

The result shows that the dye formation reached the maximum with 0.5 ml of potassium chromate.

***Effect of reagent concentration*:**

Different volumes of catechol (100 ppm) are added into a series of 25ml calibrated flask to present the effect of reagent concentration. The absorbance was measured at 450 nm versus blank. The results included in Table (2) indicate that the use of 0.5 ml of (100 ppm) catechol reagent gives the maximum color intensity.

Table (2): Effect of the concentration of reagent on absorbance.

agent conc.(ml)	sorbance
5	84
	14
	34
	95
	52

The result shows that the dye formation reached the maximum with 0.5 ml of Catechol **.**

Effect of acid

The effect of different amounts of different types of strong and weak acids was studied due to the acid being one of the components of the reaction, and the results were as follows**.**

Table (3) Effect of the amount of different acids on absorption

id used (1M)	sorbance / ml of acid used					
Cl	10	02	94	83	67	48
SO_4	10	17	05	96	71	49
NO_3	10	06	91	73	51	34

It was found from the results listed in the above table that the use of 0.5 ml of sulfuric acid (1 M) gives the highest absorption intensity, so this volume was fixed in subsequentexperiments.

Effect of Surfactant:

Table (4): Effect of Surfactant on absorbance of colored product.

rfactant	sorbance / ml of surfactant used				
S (1×10^{-3}M)	17	13	14	15	14
ton X(100) (1%)	17	14	14	13	15
C (1×10^{-3}M)	17	14	13	12	12

* Cetyl trimethyl ammonium bromide (CTAB)
*Sodium deodecyl sulphate (SDS)
* *iso*-octylphenyl polyethoxy ethanol (Triton X-100)

It is noted from the table that the addition of surfactants did not improve the dye specifications, and therefore none of them were used in the subsequent experiments.

Effect of temperature

This study provides the effect of temperature on the absorbance of the coloured product. This was implemented by placing three 25ml calibrated flasks, 2ml of (100 μgml^{-1}) phenothiazine solution , 0.5 ml of (5×10^{-3}M) potassium chromate, followed by 0.5ml of ($100\mu gml^{-1}$) catechol solution followed by0.5 ml of sulfuric acid (1 M), The solution was diluted to the mark with distilled water and the first flask was allowed to stand for increasing time at room temperature, the second was at 5°C and the third in water bath at 45°C. The absorbance was measured at 450nm at different periods versus blank prepared in the same way but containing no phenthiazine. The results included in Table (3) indicate that the absorbance of the coloured product was decreased when the reaction was carried out at 5°C or 45°C. Therefore, it is recommended that the reaction mixture should be carried out at room temperature.

Table (5): Effect of temperature on absorbance of colored product.

Temp. ºC	Absorbance/minutes							
	0.054	0.067	0.072	0.085	0.091	0.098	0.108	0.105
R.T.	0.610	0.614	0.617	0.615	0.615	0.615	0.614	0.598
	0.504	0.485	0.469	0.453	0.435	0.409	0.352	0.318

*R.T.=Room temperature=25 C

Order of addition of reagents.

The reagent 0.5ml of(100ppm) Catechol (R), the oxidant 0.5ml of (5×10^{-3}M) potassium chromate (ox) ,the sample 2ml (100µgml^{-1}) solution of phenothiazine, and sulfuric acid solution (1M), were mixed in various orders as shown in Table (4).

Table (6): Effect of order of addition on the absorbance of the coloured product.

Reaction components	Order number	Absorbance at 450nm
R+OX+S+H	I	0.617
R+S+OX+H	II	0.352
S+R+OX+H	III	0.458
OX+S+R+H	IV	0. 386

The results indicate that order (I) gives the highest absorbance of the product. Therefore, this is considered in all subsequent experiments.

Stability of the product.

The stability of the product was studied by placing 2ml of (100ppm) phenothiazine , into a series of 25ml calibrated flasks, followed by 0.5 ml of (5×10^{-3}M) potassium chromate and 0.5ml of (100µgml^{-1}) Catechol solution followed by0.5 ml of sulfuric acid . The solution was diluted to the mark with distilled water and the absorbance was measured at 450nm at different periods versus reagent blank. The results included in Table (4) show that the product needs 2 minutes to attain maximum absorbance and it remains stable for about 40 minutes.

Table (7): Rate of reaction and stability of product.

Time (min)	1	3	5	10	15	20	25	30	40	35
8µgml^{-1} Absorbance	0.612	0.617	0.615	0.616	0.617	0.617	0.615	0.614	0.610	0.599

Final absorption spectra.

Phenothizine complex is formed based on the optimum conditions described above. An absorption spectrum is ranging between 400and 650 nm with a maximum absorption at 450nm in contrast to the reagent blank which shows small absorption at λ max. Therefore, the maximum absorption at 450nm is considered in the subsequent experiments.

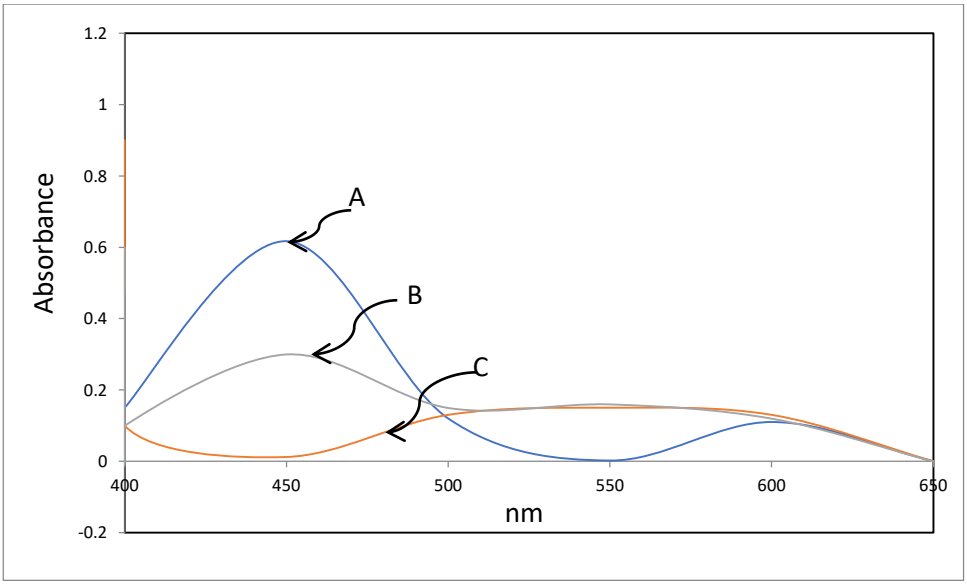

Fig. (2): Absorption spectra of 8µgml^{-1} Phenothiazine measured, (A) sample against blank, (B) sample against distilled water, (C) Blank against distilled water.

Quantification

A calibration graph is constructed by plotting absorbance versus concentration. Beer's law is obeyed over the range (1-170) µg /ml of the solution as shown in Fig (3).

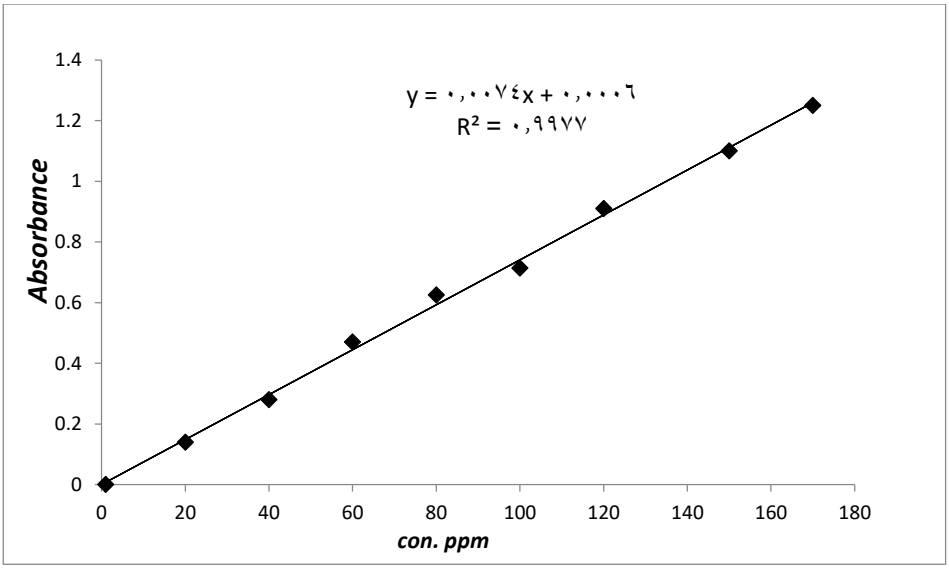

Fig. (3): Calibration graph for the determination of Phenothiazines

nount of tak /ml Phenothiaz	nountof enothiazine nd, µg/ml	lative ror % *	lative standard viation, % *	covery (%)
	.32	6	.829	1.6
	.80	5	.1	.75
0	0.17	141	.086	0.14

Accuracy and precision of the method

phenothiazine is determined at three concentrations in order to present the accuracy and precision of the method. The results included in Table (8) indicate that the method is performing well

* Rate 6 replicates

Table (8): Accuracy and precision of the method

* Rate 6 replicate

Nature of the product

The stoichiometry of the reaction between phenothiazine and Catechol in the presence of potassium chromate is investigated based on the mole–ratio method. In this experiment, 0.5 ml of potassium chromate (5×10^{-3}M) was added into a series of 25ml calibrated flask followed by increasing volumes of phenothiazine (100ppm) and 2 ml of Catechol. The solutions are diluted to the mark with distilled water and the reaction mixture was allowed to stand for 5 minutes. The absorbance of each solution was measured at 450nm versus blank. The results shown in Fig. (4) exhibit the existence of a 1:1 Catechol.: phenothiazine.

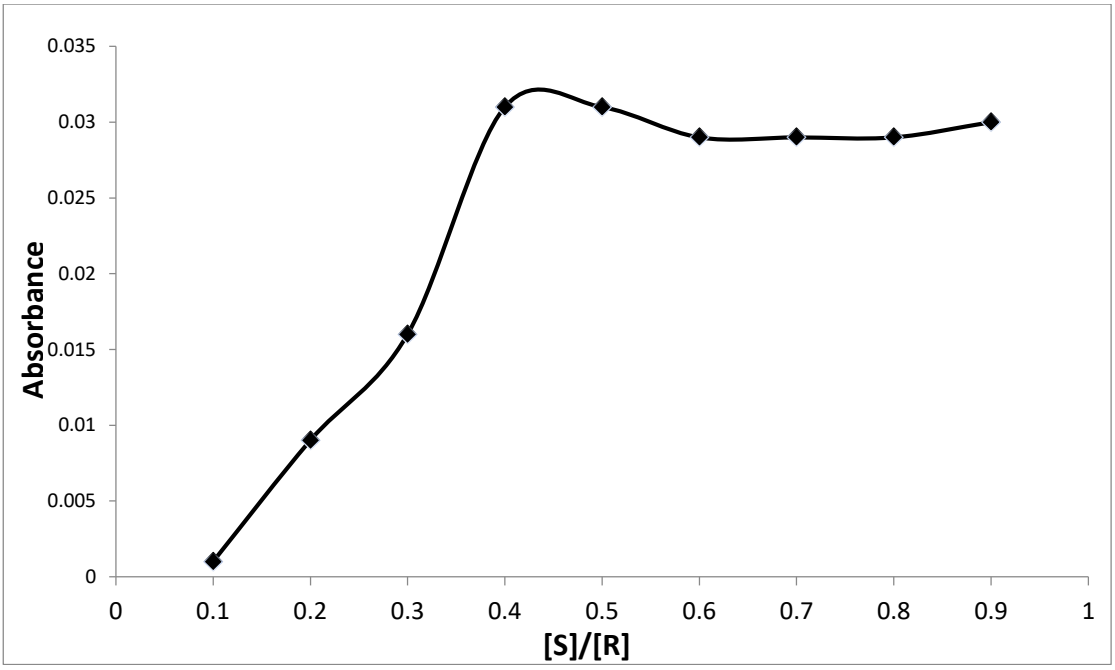

Fig. (4): Mole–ratio plot for Catechol : phenothiazine reagent in the presence of potassium chromate.

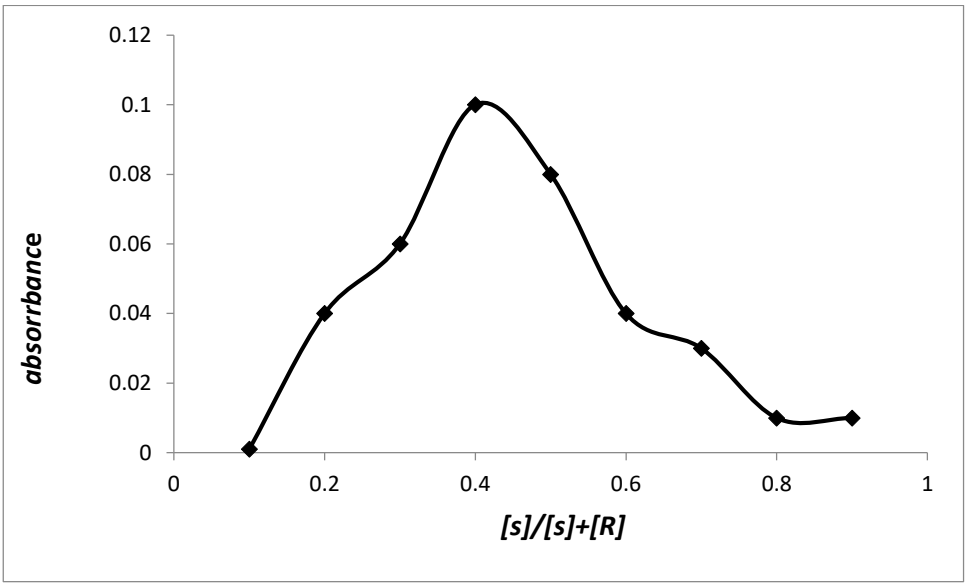

Fig (5) Jops method for catechol.: phenothiazine reagent in the presence of potassium chromate.

Application of the method:

Table (9): Accuracy and precision of application of the method Pharmaceutical drug

armaceutical mulation	hount urazine ken, µg	unt of Neuraz d, µg/ml	lative error, %	lative standar viation, % *	covery (%)
urazine tablet		04		.239	1
		.45.	75	.128	0.75
	0	0.04	1	.097	0.04

* Rate 6 replicates

Conclusion

A new spectrophotometric method was proposed to determine in phenothiazine solution. The method was based on coupling of with Catechol reagent in the presence of potassium chromate in an acidic medium to form a colored dye which presents maximum absorption at 450 nm. The molar absorptivity is 1474.598 $l.mol^{-1}cm^{-1}$. The proposed method was applied for the determination of phenothiazine in synthetic pharmaceuticals.

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