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التقدير الطيفي لبعض المركبات الفينولية بواسطة تفاعل الاقتران التأكسدي باستخدام ٤-امينوانتي بايرين

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ملخص البحث:

طُـورت طريقة طيفية لتـــقدير بعض المركبات الفينولية وتتضمن (الكاتيكول، الريسورسينول والهيدروكوينون)، تعتمد الطريقة على تفاعل الاقتران التأكسدي لهذه المركبات مع كاشف ٤-امينوانتي بايرين (4-AAP) بوجود كبريتات النحاس كعامل مؤكسد في وسط قاعدي لتكوين نواتج ملونة. بلغت تلك النواتج اقصى امتصاص عند الاطوال الموجية ٤٠٤ و ٤٧٤ و ٣٩٢ نانوميتر لكل من الكاتيكول، الريسورسينول الهيدروكوينون على التوالي. وكانت الامتـــصاصية المـــولارية 12133.02 و 7695.99 و ١٦٥٧٠ لتر/ مول. سم لتراكيز اتبعت قانون بير بحدود ٠,٢ – ١٤ و ٠,٠٤ – ١٨ و٠,٢ – 4 مايكروغرام/مللتر للمركبات اعلاه على التوالي. اذ تراوحت دقة الطريقة بين ٩٧,٧٢٪ و ١٠٠,٠٥٪ في حين كانت التوافقية اقل من ٣,٤ لجميع المركبات المدروسة، لقد وجد ان المركبات الفينولية المدروسة تكّون نواتج مع 4-AAP بنسبة ٢:١ (المركب الفينولي : ٤-أمينو انتي بايرين) بأستثناء المعقد كاتيكول:٤-أمينو انتي بايرين يكون بنسبة ١:١، وكان معدل ثابت الاستقرار للمعقدات المتكونة 1.8369×10^4 لتر/مول و 2.82×10^{10} و 1.285×10^{10} لتر^2/مول^2 لكل من نواتج الكاتيكول، الريسورسينول والهيدروكوينون على التوالي مما يدل على الاستقرار الجيد لتلك النواتج، كما تم دراسة تأثير عدد مـن الـمتداخلات والمتمثلة بالكحولات الالـيفاتية الاولية والثانوية والامينات الاليفاتية ومركبـات عـضوية اخرى وتشير النتائج التي تم الحصول عليها الى انـتقائية الـطريقة لعدم حدوث تداخل من قبل المواد المضافة.

Spectrophotometric Determination of Some Phenolic Compounds Via Oxidative Coupling Reaction Using 4-Aminoantipyrine

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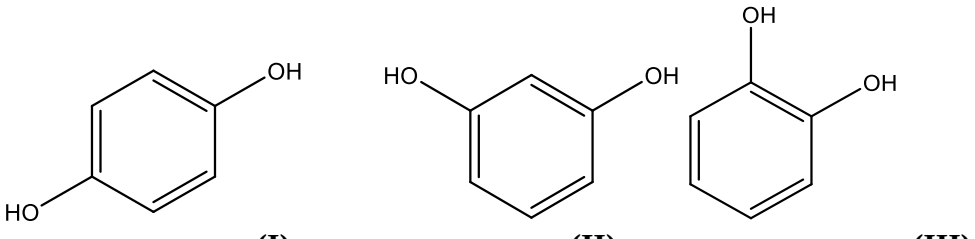
Abstract

A spectrophotometric method has been developed for the determination of some phenolic compounds (Catechol, Resorcinol and Quinol). The method is based on oxidative coupling reaction of these compounds with 4-aminoantipyrine (4-AAP) in the presence of cupper sulphate as oxidizing agent in alkaine medium forming a brown colour. The products show maximum absorption at 404 nm, 474 nm and 392 nm for Catechol, Resorcinol and Quinol respectively. The molar absorptivities are 12133, 7695 and 16570 l/mol.cm for concentrations obeyed Beer's law in the range 0.2 - 14, 0.04 - 18 and 0.2 - 4 $\mu g.ml^{-1}$ for the above compounds respectively. The average recovery was ranged between 97.72% and 100.05% with relative standard deviation less than 3.4 for all the studied compounds, The 4-AAP products were formed in the ratio of 1:2 phenolic compound: 4-AAP With the exception of the complex catechol:4-amino antipyrine, it is in a 1:1 ratio. The stability constant of the products was 1.8369×10^4 $(l.mol^{-1})$, 2.82×10^{10} and 1.285×10^{10} $(l^2.mol^{-2})$ for Catechol, Resorcinol and Quinol products respectively indicating the good stability of these products, The effect of a number of interfering substances represented by primary and secondary aliphatic alcohols, aliphatic amines and other organic compounds was also studied. Finally, the results obtained indicate the selectivity of the method as there was no interference from the additives.

Key words: Oxidative coupling; Catechol; Resorciol; Quinol, 4-aminoantipyrine

Introduction

Phenols are used in many fields, including industrial ones, as raw materials that enter many chemical industries, such as the iron and steel industry and phenol–formaldehyde resins, as well as in the manufacture of dyes, plastics, and petrochemical industries[1] and in the manufacture of pesticides. Because of their high toxicity and presence in industrial wastewater, they are a major source of pollution[2]. Phenolic compounds are considered the main active substance in medicines used to treat skin diseases and sedative and antipyretic drugs. They are also described as preservatives such as insulin[3] and as antiseptics to sterilize wounds, as they are added to mouthwashes due to the effectiveness of anti–fungal and antibacterial phenols[4]. Oxidative coupling reactions are one of the important reactions in analytical applications as they are highly sensitive methods. 4–Aminoantipyrine is one of the important reagents, as it was used for the determination of some phenolic compounds[5–7] and pharmaceuticals[8–11]. In view of the importance of the studied phenols, several analytical methods were used to estimate these compounds, including spectroscopic methods[12–16], chromatography[17–19] and electrical methods[21]. In this paper, some phenolic compounds (Catechol I, Resorcinol II, Quinol III) fig. (1) were determined in combination with 4–AAP and in the presence of aqueous copper sulfate as an oxidizing agent in an alkaline medium.



(I) **(II)** **(III)**

$C_6H_6O_2$
M.wt =110.1 (g/mol)

The Studied Phenolic Compounds Fig. (1)

Experimental part

Apparatus used: The measurements were made with a two-beam photometric spectrometer "Shimadzu UV-1800 Double-beam spectrophotometry" Glass cells were used, with a width of 1 cm, and the weight was carried out using a sensitive scale (ae ADAM), Heating was carried out using a type of water bath (elektro.mag)", The acidity was measured by a pH-function device of type (inolab pH 7110).

Chemical solutions: All chemicals used were of a high degree of purity.

The phenolic compounds ($100\ \mu g.ml^{-1}$) were prepared from dissolving 0.010 g of the pure substance in distilled water and diluted to 100 ml with distilled water.

4-Aminoantipyrine (1%) was prepared by dissolving 1.0 gm of the pure substance in distilled water and diluted to 100 ml with distilled water.

Copper sulfate ($CuSO_4.5H_2O$) (0.1%) was prepared by dissolving 0.10 g of the pure substance in distilled water and diluted to 100 ml.

Sodium hydroxide (5×10^{-2} M) was prepared by dissolving 0.50 g of the pure substance in distilled water and then diluted to 250 ml with distilled water.

Ammonium hydroxide (5×10^{-2} M) was prepared by diluting 1.93 ml of concentrated ammonia (6.45 M) with distilled water to 250 ml.

Results and discussion

Initial Tests:

1 ml of 4-aminoantipyrine at a concentration of 1% to 1.0 ml of each of catechol, resorcinol and quinol at a concentration of $100\ \mu g.ml^{-1}$ were added separately, and 1.0 ml of aqueous copper sulfate and 1.0 ml of sodium hydroxide

was added to the sodium mercodipoxide for each one. The volume was completed to 25 milliliters with distilled water and waited for 10 minutes at laboratory temperature (30° C), then the absorption of the resulting brown solution was measured and it was found that the absorption intensity of the studied compounds reached at wavelength 404, 430 and 392 nm for the above compounds.

Study of Optimum Reaction Conditions:

The following studies were performed using 1 μg.ml^{-1} catechol, 4 μg.ml^{-1} resorcinol, and 2 μg.ml^{-1} quinol in a final volume of 25 mL.

1- Quantitative Study of Coupling Reagent: The effect of the amount of 4-Aminoantipyrine on the product of the studied compounds was studied by adding different amounts of 4-AAP ranging between (0.0-٣.٥) milliliters with a concentration of 1% of catechol, resorcinol and quinol alone and 1.0 ml of aqueous copper sulfate (0.1%) concentration. and 1.0 mL of sodium hydroxide at a concentration of 5 x 10^{-2} M and fill the volume with distilled water to the mark and wait for 10 minutes at laboratory temperature, The absorbances were measured at wavelengths 404, 430 and 392 nm for the above compounds, respectively, and Table (1) shows the best size of the detector for the studied compounds.

Table (1) Study of The Quantity of The Reagent

Ml of 4-AAP (1%)	Absorbance		
	Catechol	Resorcinol	Quinol
Without	0.019	0.001	0.023
0.25	٠٩٥0.	0.012	0.150
0.5	١٦٩0.	0.038	0.237
1.0	٢٣٦0.	0.142	0.287
1.5	٢٠٥0.	0.188	0.269
2.0	١٧٤0.	0.252	0.250
2.5	١٥٩0.	0.307	0.234
3.0	٠,١٤٣	0.241	0.176

٣,٥	Turbid	0.153	Turbid

2- Study of The Oxidizing Agent: The effect of the oxidizing agent on the adsorption of the product was studied by using several oxidizing agents such as copper sulfate (CuSO4.5H2O), N-bromosuccinimide (NBS), potassium dichromate ($K_2Cr_2O_7$), potassium iodate (KIO_3), sodium nitroprusside, potassium pyriodrate (IO_4) and cyanide Potassium iron (III) $K_3[Fe(CN)_6]$ at a concentration of 0.1% for each of them, where 1.0 ml of solutions of these substances were added to the series of bottles that contain the studied compounds and the optimum amount of reagent for each compound separately, The medium was made basic by adding 1.0 ml of sodium hydroxide, then diluted with distilled water to 25 ml, and the absorption was measured at appropriate wavelengths against their sham solutions. The study showed the best oxidizing agent is copper sulfate (CuSO4.5H2O) as shown in Fig. (2).

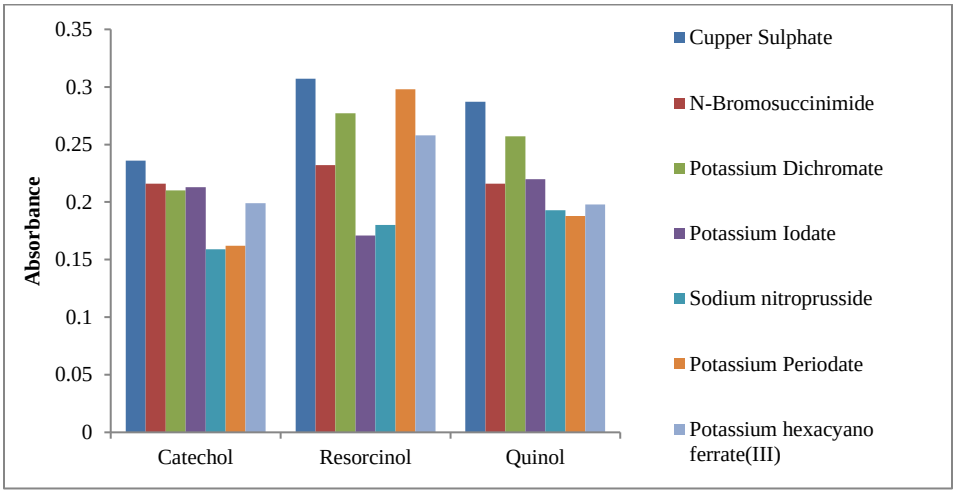


Fig. (2) Study of The Type of Oxidizing Agent

3- Study The Size of The Oxidizing Agent: The best volume of the oxidizing agent copper sulfate was studied using different volumes (0.0-3.0) ml, Table (2) shows the optimum volume for each compound, which was adopted later.

Table (2) Study of The Size of The Oxidizing Agent

Ml of 0.1% $CuSO_4.5H_2O$	Absorbance		
	Catechol	Resorcinol	Quinol
Without	0.24	0.121	0.098
0.25	0.256	0.248	0.195
0.5	0.275	0.289	0.272
1.0	0.252	0.307	0.287
1.5	6٣0.2	0.356	0.258
2.0	6٢0.2	0.390	0.243
2.5	Turbid	0.444	Turbid
3.0	---	0.412	---

4- The Study of The Type of Base: To choose the best base, several different bases were studied, such as sodium hydroxide (NaOH), potassium hydroxide (KOH), ammonium hydroxide (NH_4OH) and sodium carbonate (Na_2CO_3) at a concentration of (0.05 M), as (1 ml) was added to the volumetric bottles and it was found that the use of NH_4OH shows The maximum absorption is for catechol and resorcinol, while the use of NaOH is the highest with quinol, as in Fig. (3).

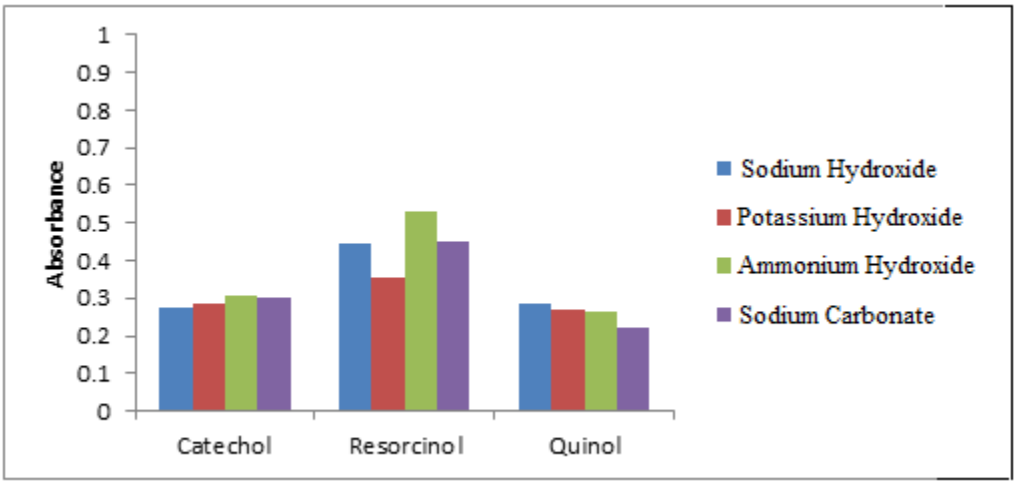


Fig. (3) Study of The Base Type

5- Effect of Base Size and pH: The best size of the base was studied at a concentration (0.05 M) and the pH of the solutions was measured, and Table (3) shows the optimum size in the estimation and it was used in subsequent experiments.

Table (3) Study of The Amount of Base

Volume of 0.05M Base (ml)	Catechol		Resorcinol		Quinol	
	Absorbance	pH	Absorbance	pH	Absorbance	pH
Without	٠,١٠٤	7.2	0.370	5.7	0.017	6.3
0.5	٢١٩0.	7.5	0.437	7.4	0.214	7.4
1.0	0.307	7.9	0.529	8.0	0.287	8.5
1.5	87٣0.	8.3	0.565	8.5	0.336	9.2
2.0	٦٢0.4	8.5	0.603	8.7	0.362	9.6
2.5	٨٠0.4	8.7	0.591	8.9	0.313	9.9
٣,٠	٠,٣٦٢	8.9	0.575	9.0	0.295	10.1

6- Effect of acid and regulating solution: The effect of HCl acid at a concentration (0.05 M) was studied, as well as the effect of solutions with an acidity function of 8.7 (for Catechol and Resorcinol solutions) and 9.6 (for Quinol solution), as a decrease in the absorption value was observed, so the addition of acid and buffer solution was excluded.

7- The Effect of Temperature and Time: The effect of different temperatures (30-60°C) was studied in the presence of (1 μg.ml^{-1} of catechol, 2 μg.ml^{-1} of quinol and 4 μg.ml^{-1} of resorcinol) and the optimum amounts of reagent, oxidizing agent and base for each compound separately and then completed the volume to the mark with distilled water. The absorption was measured at wavelengths 404, 430, and 392 nm for catechol, resorcinol and quinol, respectively. Fig. (4) shows that complexes are formed with the highest sensitivity after 10 minutes of additions at 30° C (room temperature) and remain stable for more than 60 minutes.

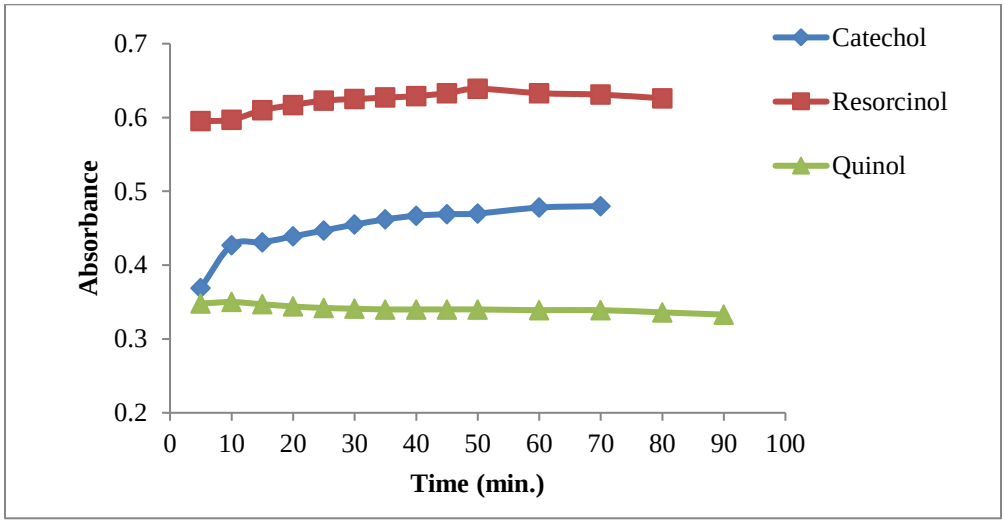
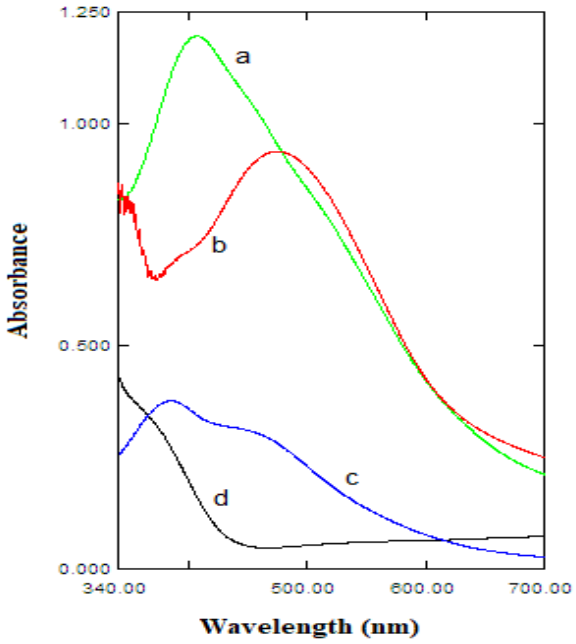


Fig. (4) Study of Temperature and Stability Time

8- Study of The Effect of Surface Tensile Materials: For the purpose of increasing sensitivity, a number of surfactants were studied and it was found that they have a negative effect on the absorption values in the case of catechol and quinol. With resorcinol, 1.0 ml of CTAB increases the absorption and shifts the maxλ to 474 nm.

Final Spectrum

The final spectrum of phenolic compounds was plotted with 4-AAP at 340-700 nm. It was noted that the λmax of the products was at 404, 474 and 392 nm for catechol, resorcinol and quinol, respectively. Fig. (5) shows the final drawing of the studied compounds.



a: Catechol ($10\ \mu g.ml^{-1}$) b : Resorcinol ($8\ \mu g.ml^{-1}$)

c: Quinol ($2\ \mu g.ml^{-1}$) d: Blank solution vs. distilled water

Fig. (5) Final Spectrum

Standard Curve

Standard curves following Beer's law were obtained in a range of concentrations (0.2−14), (0.04−18) and (0.2−4) μg.ml^{-1} for catechol, resorcinol and quinol, respectively. Fig. (6) indicates that there is a negative deviation after the limits The highest estimate, £ $_{max}$ 12133, 7695 and 16570 l/mol.cm, detection limits 0.15, 0.138 and 0.17 μg.ml^{-1}, and quantitative limits 0.5, 0.463 and 0.567 μg.ml^{-1} for the above compounds, respectively.

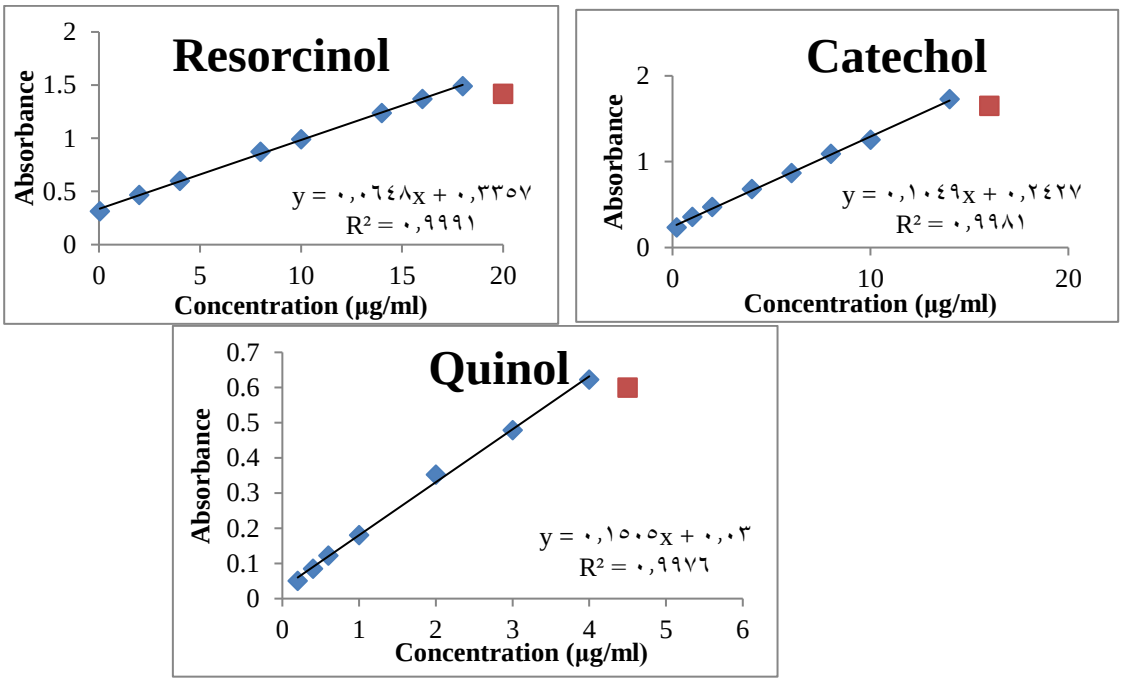


Fig. (6) Standard Curves of The Studied Compounds

Accuracy and Precision of the Method

Recovery and RSD values were calculated using five readings for three different concentrations of each compound, and Table (4) shows the good accuracy and precision, as the accuracy reached 98.55%, 97.71% and 100.05% for catechol,

resorcinol and quinol, respectively. The RSD is less than 2.5% for catechol, less than 1.5% for resorcinol, and less than 3.4% for quinol, which indicates the precision of the method.

Table (4) The Accuracy and Precision of The Method

Compound	"Amount added" (ppm)	"Recovery*" (%)	"Average recovery" (%)	"RSD*" (%)
Catechol	2 4 6	98.71 98.37 98.59	98.55	2.45 2.18 1.82
Resorcinol	2 4 6	97.32 97.24 98.59	97.71	1.46 0.53 1.46
Quinol	0.2 0.6 2	95.75 100.37 104.03	100.05	3.36 2.20 1.35

*Average of Five Determination.

The Effect of The Nature of The Complex and Its Stability

Job's Method[21] was studied to clarify the structural ratios of the studied phenol products with 4-AAP reagent using dilute solutions of the reagent and the studied compounds at a concentration of 1.0×10^{-3} M of catechol and 1.0×10^{-4} M for each of resorcinol and quinol, The results obtained in Fig. (7) showed that the complexes are in a ratio of 2:1 (the phenolic compound: 4-AAP), except for the complex catechol- 4-AAP in the ratio of 1:1. The stability constant of the products formed in a ratio of 2:1 for the above compounds was also calculated separately by applying the following law:

$$K_{st} = \frac{1-\alpha}{4\alpha^3 c^2}$$

For the product formed in a ratio of 1:1 (Catechol: 4−AAP) by applying the following law:

$$K_{st} = \frac{1-\alpha}{\alpha^2 c}$$

Where C: the complex concentration in units (mol/L), α: the degree of dissociation and Kst: the stability constant and the results are shown in Table (5).

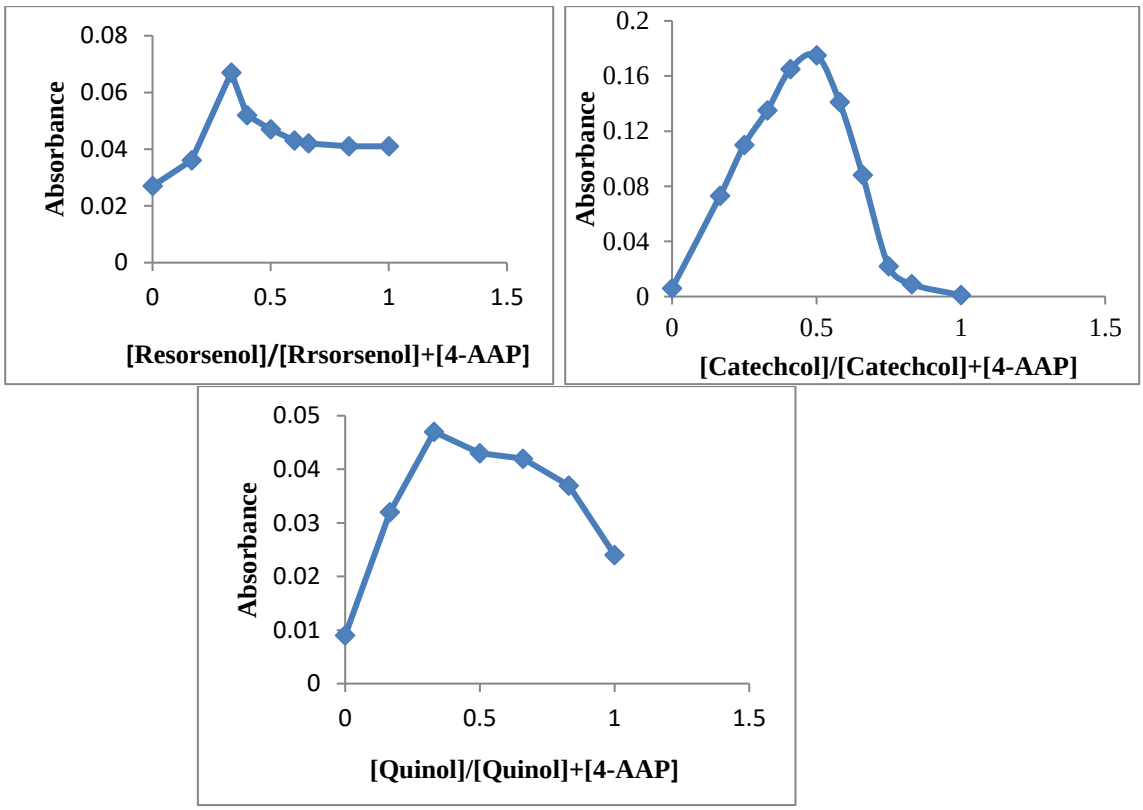


Fig. (7) Continuous Changes of The Products of Phenolic Compounds and 4−AAP

Table (5) Stability Constant of Complexes

Compound	Conc. ($mol.l^{-1}$)	Absorbance		α	Average K_{st} ($l^2.mol^{-2}$)
		As	Am		
Catechol	2×10^{-5}	0.112	0.461	0.757	1.8369×10^{4} ($l.mol^{-1}$)
	4×10^{-5}	0.152	0.524	0.7099	
	6×10^{-5}	0.227	0.555	0.591	
Resorcinol	2×10^{-6}	0.074	0.241	0.693	2.82×10^{10}
	4×10^{-6}	0.089	0.256	0.652	
	6×10^{-6}	0.095	0.292	0.675	
Quinol	2×10^{-6}	0.008	0.033	0.757	1.285×10^{10}
	4×10^{-6}	0.021	0.184	0.886	
	6×10^{-6}	0.031	0.304	0.898	

Study of Interferences

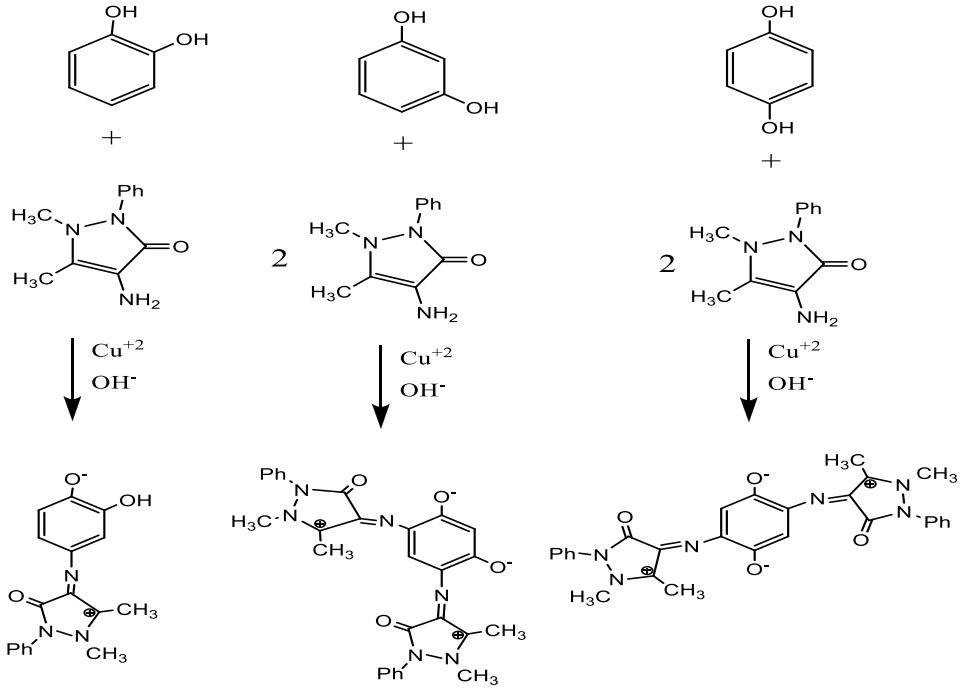
The effect of a number of interfering substances, represented by primary and secondary aliphatic alcohols, aliphatic amines, and other organic compounds, on the absorption of ($4\ \mu g.ml^{-1}$) of the studied phenols separately, as different amounts of the interfering were added. Absorption was measured at optimal conditions for each complex at 404, 474 and 392 nm for catechol, resorcinol and hydroquinone, respectively. The values below indicate the selectivity of the method to the absence of interference by additives.

Table (6) Effect of Interferons

Foreign compound	Recovery (%) of 4µg/ml of aminophenol isomer per µg/ml Foreign added											
	Catechol				Resorcinol				Quinol			
	100	500	750	1000	100	500	750	1000	100	500	750	1000
Butanol	98.2	98.2	97.9	97.7	102.7	103.6	103.7	104.0	98.8	97.5	97.4	95.9
Isobutyl alcohol	97.1	99.3	97.1	98.44	100.3	100.8	98.2	100.0	101.2	104.0	102.9	100.3
Cyclohexanol	97.7	97.1	98.6	98.4	98.5	100.1	100.9	101.0	98.9	97.3	97.1	95.7
Glucose	95.8	96.6	97.4	95.6	102.9	103.0	104.0	104.0	102.75	100.0	104.1	102.2
Lactose	98.2	104.7	101.0	100.7	96.2	101.0	97.6	101.0	102.2	104.8	103.03	96.3
Sucrose	101.0	97.2	97.4	98.9	98.8	95.9	97.3	100.0	95.6	103.03	104.2	104.21
Ethylene diamine	95.4	95.08	95.0	97.5	101.6	97.25	97.25	96.53	97.1	98.8	98.5	97.6
Propylamine	98.6	96.5	98.4	99.7	103.7	104.8	102.6	103.5	97.4	97.8	99.3	102.0

Suggested Reaction

It was observed through the chemical composition of the studied phenolic compounds that they contain the active hydroxyl group in two different locations, and by studying the nature of the product formed by the JOB method, the result proved that the correlation is 1:1 (Catechol: 4-AAP) and 2:1 (Resorcinol: 4-AAP).) and (quinol: 4-AAP) in the presence of the oxidizing agent and in an alkaline medium, and this indicates that the linkage is with an ortho group in relation to the active group of quinol, as for catechol and resorcinol, the binding to the Para site is due to the steric and mechanical impediment proposed below.



Conclusion

A spectrophotometric method was developed for the determination of some phenolic compounds in aqueous solution with 4-amino antipyrine in the presence of ammonium hydroxide, except for quinol in the presence of sodium hydroxide, depending on the oxidative coupling reaction. It was possible to estimate infinitesimal quantities of the studied phenols in the range of (0.2-14) ppm for catechol, (0.04-18) ppm for resorcinol and (0.2-4.0) ppm for quinol, with good accuracy and compatibility, and the method was characterized by sensitivity, simplicity and lack of need to a pre-extraction process.

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