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Spectrophotometric determination of phenol, catechol and resorcinol using denitrification reactions and coupling with benzidine reagent.

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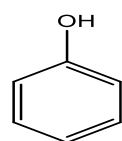[a,b] *Department of chemistry ,College of Education for Girls ,University of Al Mosul.Mosul, Iraq*

Abstract

A spectrophotometric method was developed for the determination of phenol, catechol and resorcinol. The method based on the reaction of diazonium salt with diazotized benzene as a reagent in the alkaline medium. The products show the maximum absorption at 456, 359 and 497 nm for phenol, catechol and resorcinol respectively. The method showed linearity over concentration ranging between (0.25-15), (0.25-5) and (0.25-7.5) µg/ml for the mentioned compounds, respectively, The molar absorptivity value were 1.26×10^4, 3.72×10^4 and 2.86×10^4 L .mol $^{-1}$.cm^{-1} respectively. The limits of detection (LOD) were 0.0963, 0.0430 and 0.0496 µg/ml, and the limit of quantitation (LOQ) were 0.3211, 0.1440 and 0.1655 µg/ml for phenol, catechol and resorcinol. The accuracy ranged between 99.58% -100.41% with a relative standard deviation ≤ 0.38 for all compounds It was found that these compounds formed a color product with benzidine at a ratio of 1:2. The rate constant of stability was 4.29×10^{11}, 4.28×10^{10} and 8.74×10^{10} liters ^{2}.mol^{-2} for the products phenol, catechol and resorcinol respectively. This indicates the good stability of these products.

Keywords: diazonium salt , spectrophotometry, benzidine; phenol, catechol, Resorcinol.
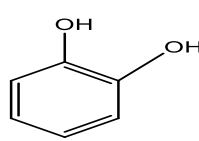
1. Introduction

Catechol and resorcinol are phenolic compounds are commonly employed in tanning cosmetics, pharmaceuticals, and photo-graphic development [1,2].These compounds are harmful to humans and animals even in very low concentration. Therefore it is very important to develop a highly sensitive and selective analytical method for the determination of catechol and resorcinol in food[3,4].As environmental pollutants in the ecological system , it Phenols have the following chemical structure:
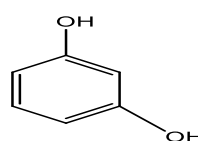
is very important to develop simple and rapid analytical methods for determination of ,CC and RC. Because of their similar chemical structures and the difficulties in simultaneously separating and detecting them, like that trace dihydroxy benzene isomers. Up to now[5,6]. Several methods have been described for the determination of phenols such as spectrometry [7-15], gas chromatography [12-16] and other methods [17-18].

C₆H₆O
M.wt=94.11g/mol

C₆H₆O₂
M.wt=110.11g/mol

C₆H₆O₂
M.wt=110.11g/mol

As for the benzidine reagent that used in this study, it is a gray crystalline powder when exposed to air and light turns dark and melts at 128°C [19]. used in the past in the paper and leather industries and the production of dyes for fabrics [20], it is considered

highly toxic to animals and affects the blood Liver, urinary system and skin, resulting in bladder cancer [21-22]. It was used as a reagent [23-24] and was used in other interactions as a substance to be evaluated[25-26].
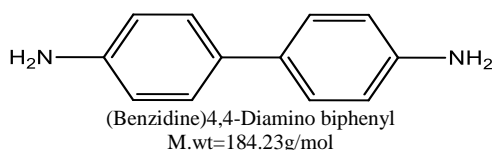
As benzidine has the following chemical structure:

$$H_2N - \text{(benzene ring)} - \text{(benzene ring)} - NH_2$$

(Benzidine)4,4-Diamino biphenyl
M.wt=184.23g/mol

2. Experimental

Devices used:

1 –All Spectral and absorbance measurements are carried out on a Shimadzu UV-1800 PC, UV-Visible double_beam –Spectrophotometer.

2 - Heating of the solutions using a water bath (type elektro.mag).

3- Weight using a sensitive scale, type (ae ADAM).

Chemical Solutions

Phenol, catechol and resorcinol (50 μg/ml): prepared by dissolving 0.0125 g of the pure substance in a volumetric flask of 250 ml of distilled water.

Benzidine (1×10^{-3}M): The solution was prepared by dissolving 0.0184 g benzidine powder in a volumetric flask of 100 ml of ethanol.

Sodium nitrite (2%): Prepare the solution by dissolving 2 g of sodium nitrite in a volumetric flask of 100 ml of distilled water. After that, 1% of this percentage was prepared by dilution.

Nitric acid (1M): The solution was prepared by diluting 6.13 ml of concentrated nitric acid (16.29M) in a volumetric flask of 100 ml distilled water. Lower concentrations were prepared from this concentration by the law of dilution.

Hydrochloric acid (1M): The solution was prepared by diluting 8.5 ml of concentrated hydrochloric acid (11.7M) in a volumetric flask of 100 ml of distilled water.

Urea solution (1%): The solution was prepared by dissolving 1.0 g of the pure substance in a volumetric flask of 100 ml distilled water.

Sodium hydroxide solution (1 M): Prepared by dissolving 4.0 g of the substance in a volumetric flask of 100 ml distilled water[27].

3. Results And Discussion

An alkaline medium, the compounds were reacted with the diazotized reagent in a volumetric flask of 10 ml and after 10 minutes the absorption was at wavelengths 456, 359 and 497 nm for phenol, catechol and resorcinol respectively.

Optimum Conditions
Effect of solvents

The solvents of the studied compounds, were it has been diluted with various solvents to the mark limit and it was found that the solvents do not affect the absorbency. water was chosen as the best solvent (1).

Table 1: Effect of solvents on the studied compounds.

Solvent	Phenol		Catechol		Resorcinol	
	Abs	λmax (nm)	Abs	λmax (nm)	Abs	λmax (nm)
Water	0.312	456	0.422	359.0	0.650	497.0
Ethanol	0.102	451	0.249	358.1	0.598	496.2
Acetone	0.164	452	0.171	357.9	0.621	496.8
Acetonitrile	0.081	455	0.137	358.0	0.508	496.0

Effect of benzidine concentration

various concentrations of benzidine reagent were taken on the studied compounds, and the best concentration for all compounds was 1×10^{-3} M, as shown in Table (2).

Table 2: Reagent concentration Effect on the studied compounds.

Concentration of Benzidine	Phenol Abs at (456nm)	Catechol Abs at (359nm)	Resorcinol Abs at (497nm)
1×10^{-2}	0.282	0.393	0.542
1×10^{-3}	0.313	0.422	0.652
1×10^{-4}	0.202	0.362	0.568
1×10^{-5}	0.166	0.281	0.411

Effect of the reagent volume (1×10^{-3}m)

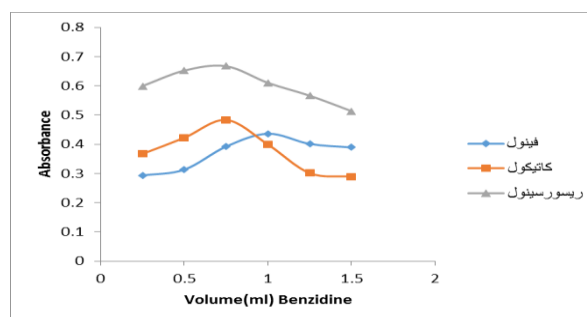Various volumes of benzidine reagent were studied for compounds and the optimal quantities are shown in Figure (1).

Fig.1 : Effect of benzidine quantities (1×10^{-3}M) on the studied compounds.

Effect volume of sodium nitrite

This study was carried out by taking increased volumes of sodium nitrite for the studied compounds, and table (3) shows that

Table 3: Effect volume of sodium nitrite on the studied compounds.

Xml of NaNO$_2$	Phenol*	Catechol**	Resorcinol*
0.25	0.380	0.339	0.582
0.5	0.436	0.483	0.667
0.75	0.439	0.512	0.574
1.0	0.444	0.420	0.486
1.25	0.421	0.311	0.406
1.5	0.399	0.279	0.381

* 1% NaNO$_2$ **2% NaNO$_2$

Effect of acid type

Various types of acids were taken at a concentration of 1 M. The table (4) shows the best acid for each studied compound.

Table 4: Effect of acid type on the studied compounds.

Different of acids	Phenol	Catechol	Resorcinol
HCl	0.444	0.512	0.667
H2SO4	Turbid	Turbid	Turbid
HNO3	0.479	0.421	0.591
CH3COOH	0.370	0.332	0.264

Effect of acid concentration

After selected the appropriate type of acid that gave the highest absorption of the studied compounds, various concentrations of the best acid were studied, as shown in Figure (2).
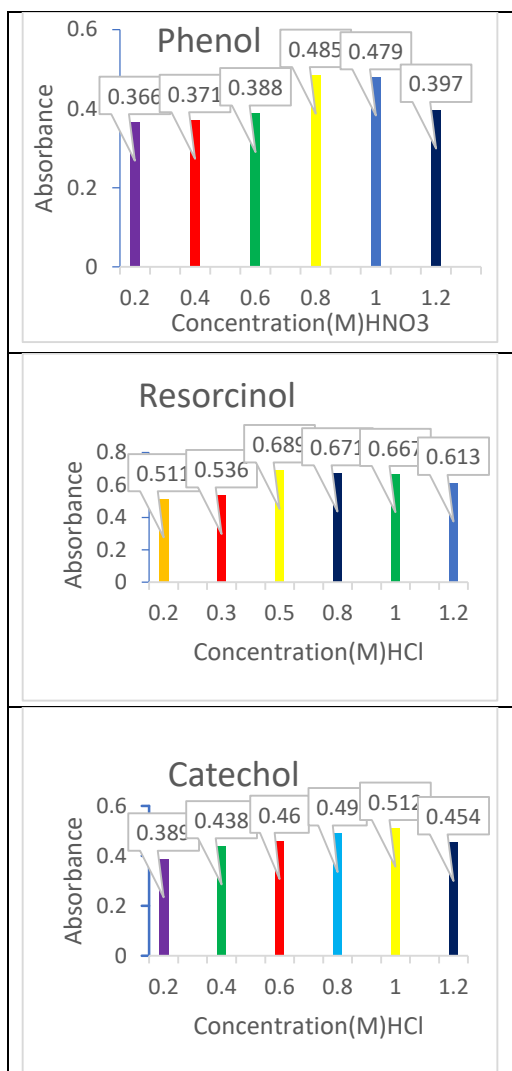

Fig.2 : Effect of acid concentration on the studied compounds

Effect of the amount of acid

Increased volumes of the best acid were taken for the studied compounds and Figure (3) shows that 0.5 ml is the optimal amount for all.
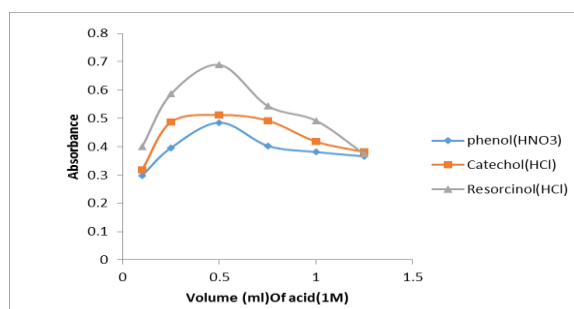

Fig.3 : Effect of the amount of acid on the studied compounds.

Effect of 1% urea

Increasing volumes of 1% urea solution were added to the studied compounds and table (5) shows the optimal volume for each studied compound

Table 5: Effect of 1% urea volume on the studied compounds.

X ml of Urea(1%)	Phenol	Catechol	Resorcinol
0.1	0.362	0322	0.565
0.25	0.399	0.532	0.702
0.5	0.485	0.512	0.689
0.75	0.431	0.410	0.600
1.0	0.410	0.399	0.542

Effect of the base type

After the optimal conditions were established for the previous studies, the type of base utilized for each chemical was investigated using several bases at a concentration of 1.0 M, and Figure (4) reveals that sodium hydroxide is the optimum base for the compounds tested.
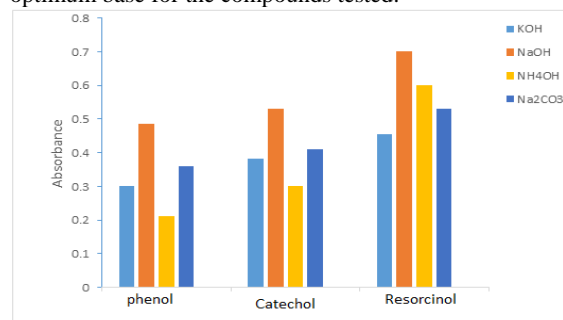

Fig.4 : Effect of the base type on the studied compounds.

Effect of sodium hydroxide concentration

various concentrations of sodium hydroxide were taken for all studied compounds and it was found that 1 M is the best concentration for all as shown in Table (6)

Table 6: Effect of base concentration on the absorption of the studied compounds[28].

The Concentration of Base(M)	Phenol	Catechol	Resorcinol
0.5	0.398	0.441	0.679
1.0	0.484	0.533	0.701
2.0	0.356	0.410	0.645
3.0	0.288	0.339	0.541

Effect of the volume of sodium hydroxide (1 M)

This study was conducted to choose the best volume of sodium hydroxide for the studied compounds, as it shows that 0.5 ml of all compounds gave the highest absorption as in Figure (5)
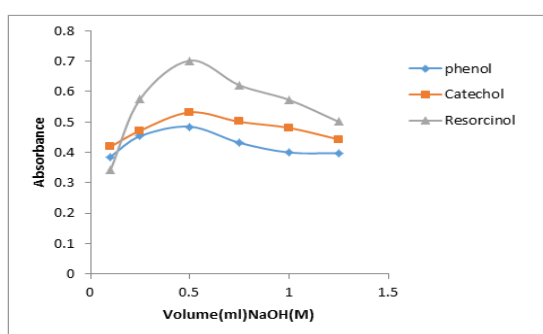

Fig.5 : Effect volume of base (1M) on the studied compounds.

Study of the formation time of diazonium salts

Several solutions were prepared by adding the optimum volume of reagent, sodium nitrite and acid, then placing the solutions in ice for various periods (0-10) minutes, then adding the organic compound and the optimum volume of the base, completing the volume to the mark and leaving them for 10 minutes at the laboratory temperature and the table (7) Shows the best time for each compound

Table 7: The time of formation of diazonium salts on the studied compounds[29].

Time(min)	Phenol	Catechol	Resorcinol
1	0.466	0.413	0.244
3	0.521	0.549	0.718
5	0.485	0.532	0.702
10	0.433	0.398	0.469

Effect of temperature

The effect of different temperatures ranging between (0-35) °C was studied, and their effect on the formation and stability of the formed product was studied. Figure (6) shows the studied compounds[30].
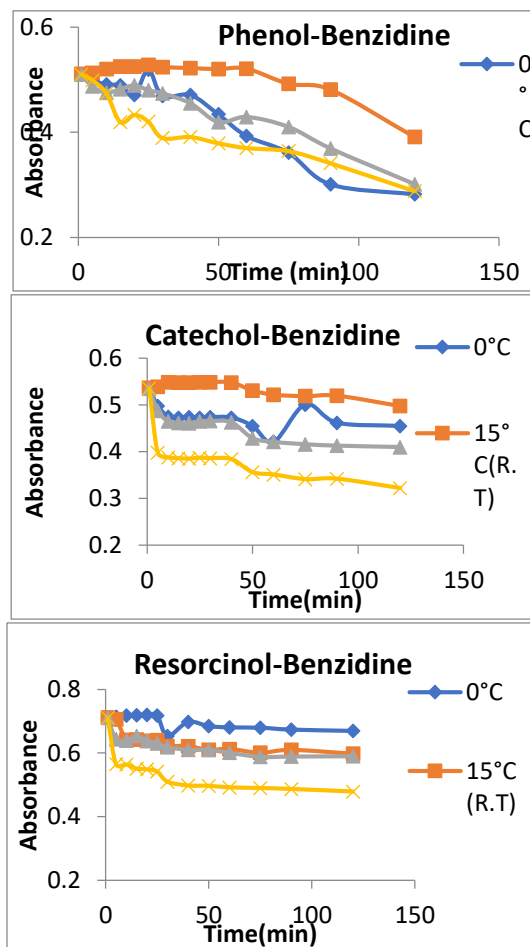

Fig.6 : Effect of temperature.

Absorption spectrum

After selected the optimal conditions for the studied compounds with the diazotized benzidine reagent, the absorption spectrum for each compound was drawn as shown in Figure (7).
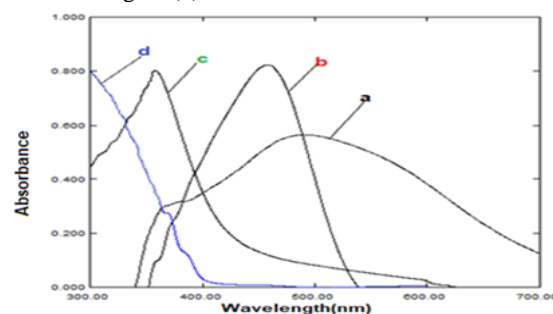

Fig. 7: Absorption spectrum of the studied compounds
a-Resorcinol (2μg/ml) versus blank
b-phenol (5 μg/ml) versus blank
c-Catechol (2 μg/ml) versus blank
 d- Blank (Catechol) versus water
Calibration graph

Volumetric flasks of 10 ml were taken and volume was added according to the optimal conditions for each phenol,

catechol and resorcinol with benzidine reagent according to the temperature and the curves were as shown in Figure (8).
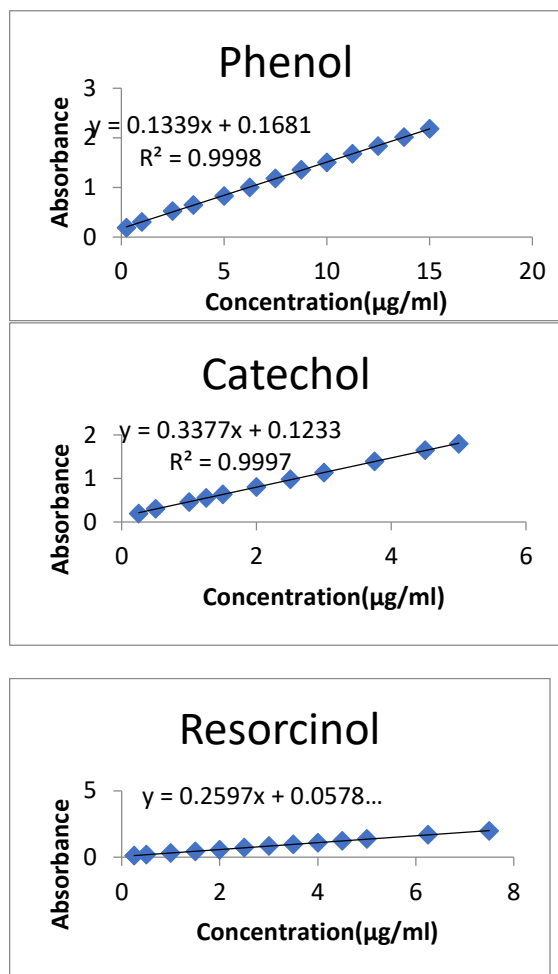
Phenol

$y = 0.1339x + 0.1681$
$R^2 = 0.9998$

Catechol

$y = 0.3377x + 0.1233$
$R^2 = 0.9997$

Resorcinol

$y = 0.2597x + 0.0578...$

Fig. 8: Calibration graph of the studied compounds.

Accuracy and precision

Three concentrations of each studied compound were taken and the readings were repeated five times as shown in Table (8).

Table 8: Accuracy and precision.

Compound	Amount added(µg.ml^{-1})		Recovery (%)	Average Recovery (%)	RSD (%)
	Taken	Found			
Phenol	3.50	3.53	100.85		0.30
	7.50	7.55	100.66	100.41	0.29
	11.25	11.22	99.73		0.18
Catechol	1.00	0.98	98.00		0.36
	2.00	2.01	100.50	99.58	0.23
	3.75	3.76	100.26		0.15
Resorcinol	1.00	0.98	98.00		0.38
	2.50	2.54	101.60	99.70	0.17
	4.00	3.98	99.50		0.10

Studying the nature of the resulting product

The methods of continuous changes and molar ratios were used to determine the molar structural ratio of the products of the examined compounds with the diazotized benzene reagent in the alkaline medium[31].

Continuous changes method (Job's method)

Different quantities of equal concentration of all studied compounds and benzidine were taken, where the concentration of phenol and benzidine in this method was 5 x 10^{-4} M, and the concentration of catechol, resorcinol and benzidine were 4.5 x 10^{-4} M to a final volume of 10 ml and in the presence of the optimal amounts of sodium nitrite, acid urea and base at the wavelength mentioned previously for each compound, as it was found through this method that the ratio of the complex is(1:2) (Benzidine: the studied compound) As shown in Figure (9).
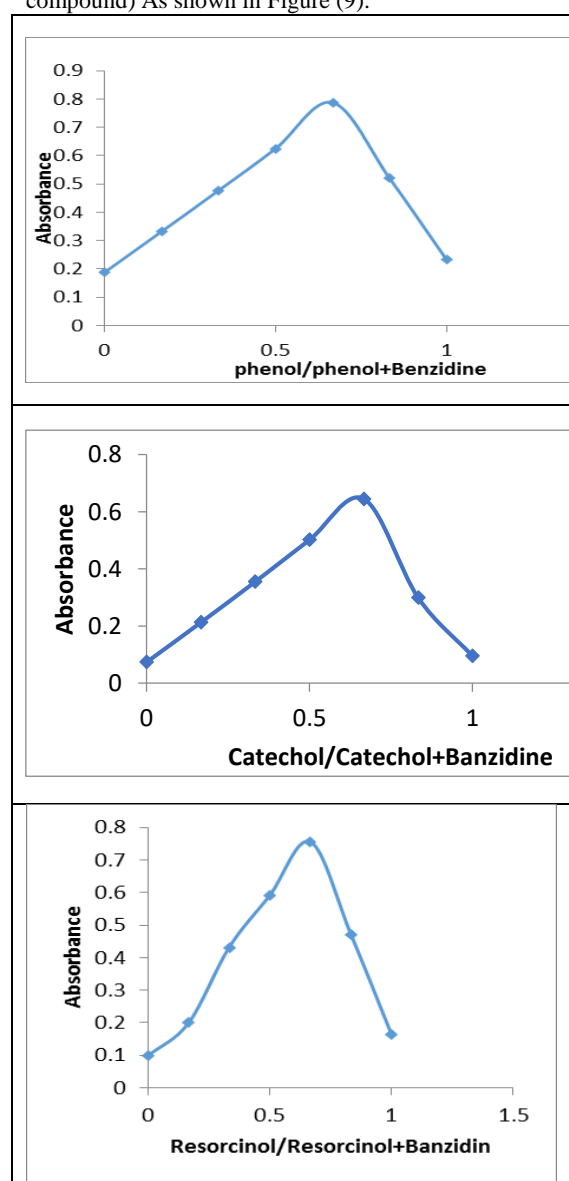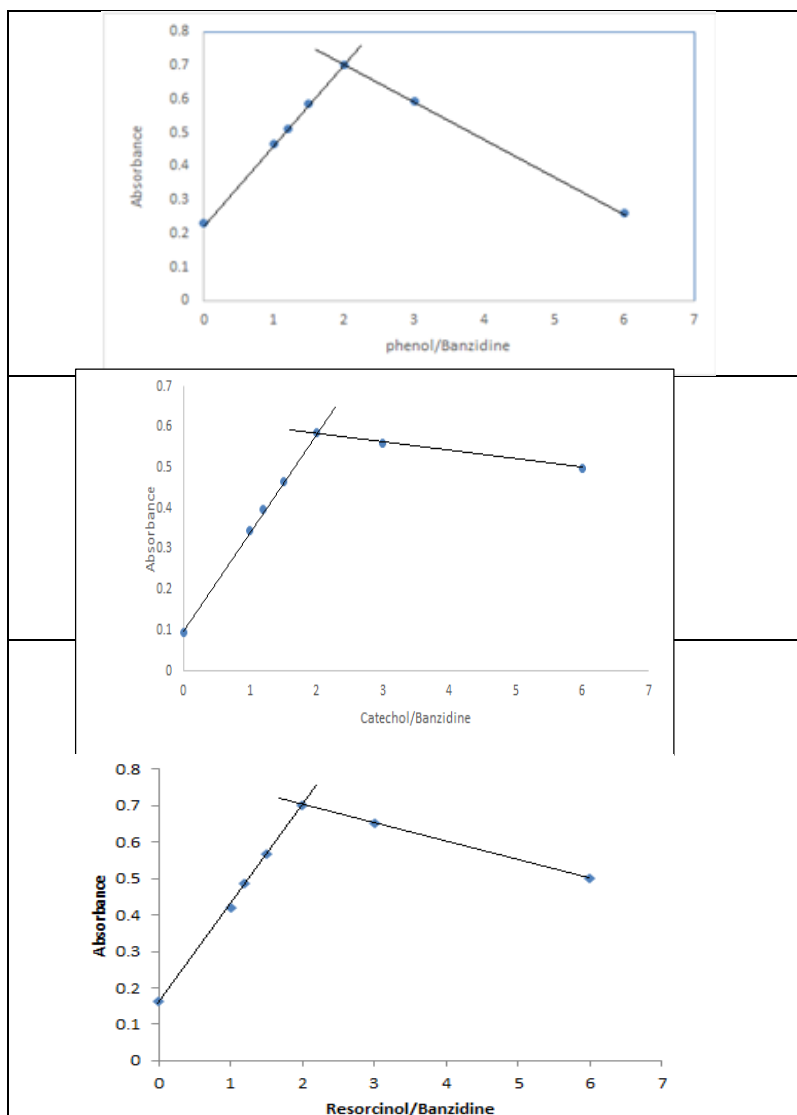
Fig..9: The method of continuous changes for the studied compounds

molar ratio method to verify the method job's, this method was applied, as shown in Figure (10).

Fig.10: The method of molar ratios for the studied compounds

The Stability Constant
The stability constant was calculated for the products of the studied compounds formed in ratios of (1:2) (benzidine: the studied compound) as in Table (10).

Effect of interference
Some interfering substances have been studied, including aromatic and aliphatic amines and some alcohols. The conclusion is that the method is selective for phenolic compounds[32].

4.   Conclusion
A rapid, selectivity and sensitivity spectrophotometric method was developed for the determination of phenolic compounds by applying diazonium salt and coupling between the studied compounds and the diazotized benzidine reagent in the base medium.

Suggested chemical reaction
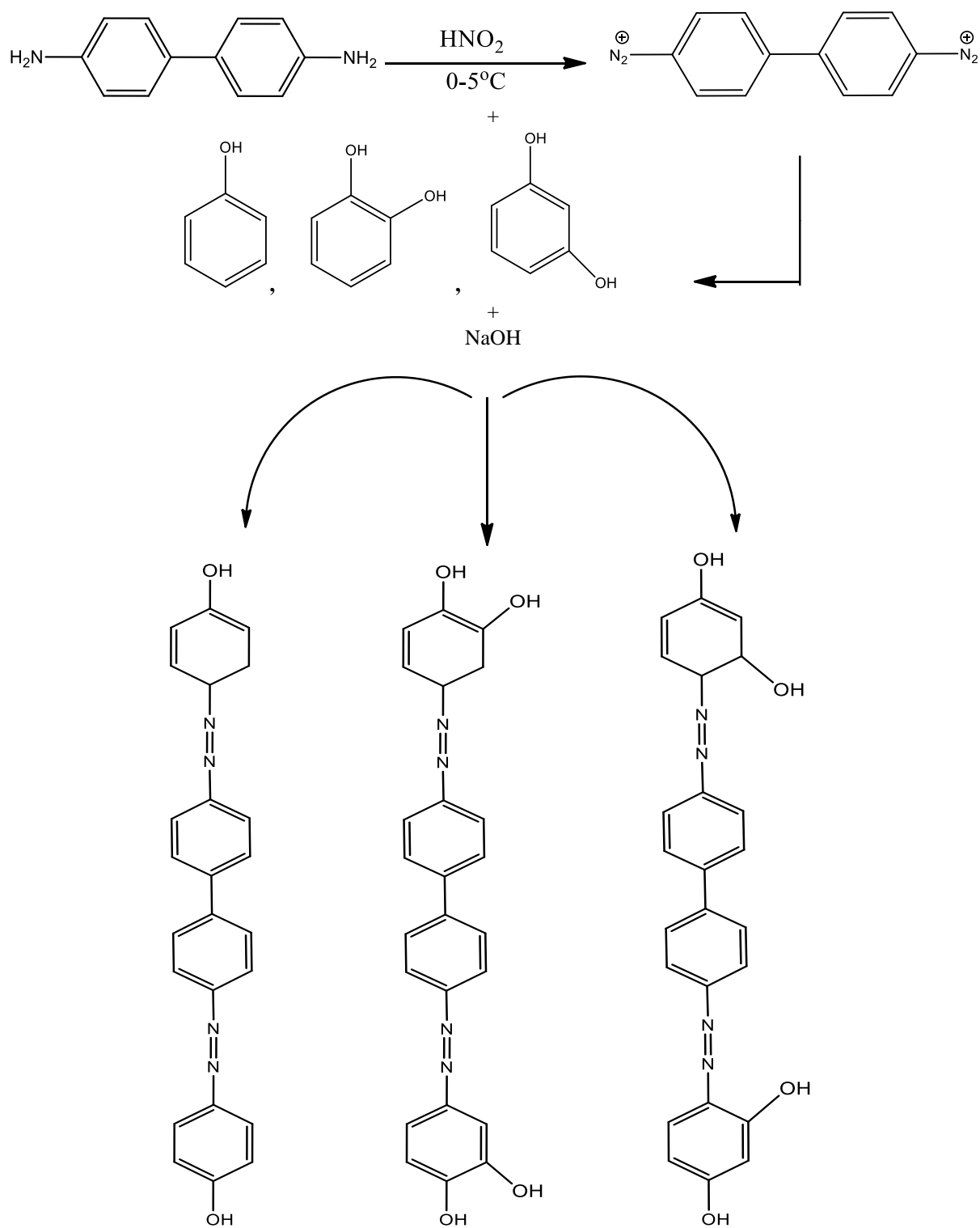
Table 10: Stability Constant.

Compound	Conc (mol.l^{-1})	Absorbance		α	Average K$_{st}$ (l^2.mol^{-2})
		As	Am		
Phenol	2.5×10^{-5}	0.356	0.521	0.3166	
	5×10^{-5}	0.580	0.824	0.2961	4.29×10^{11}
	7.5×10^{-5}	0.859	1.182	0.2732	
Catechol	9×10^{-6}	0.321	0.456	0.2960	
	18×10^{-6}	0.534	0.804	0.3358	4.28×10^{10}
	27×10^{-6}	0.899	1.132	0.2058	
Resorcinol	18×10^{-6}	0.398	0.565	0.2955	8.74×10^{10}
	0.1772	27×10^{-6}	0.701	0.852	
	0.1280	36×10^{-6}	0.953	1.093	

It is proved by the above table that the stability of the products formed for the studied compounds is of high stability.

Table 11: Effect of Interferons.

Compound	Foreign compound	Recovery % of 2 µg/ml of compounds per µg/ml Foreign added			
		100	500	750	1000
Phenol	Aniline	99.01	102.65	104.71	101.11
	Chloroaniline	100.30	97.77	103.14	100.23
	Butylamine	99.28	98.95	100.18	96.41
	Butanol	100.14	101.20	96.77	98.42
	Cyclohexanol	96.73	98.80	103.03	102.22
	Glucose	97.21	102.43	103.22	100.97
Catechol	Aniline	99.01	103.10	104.89	99.95
	Chloroaniline	96.01	100.02	99.67	99.96
	Butylamine	96.46	101.84	100.99	100.20
	Butanol	98.68	98.02	99.91	104.54
	Cyclohexanol	101.01	97.01	96.79	101.29
	Glucose	97.10	95.99	98.71	99.65
Resorcinol	Aniline	101.20	97.35	103.49	101.56
	Chloroaniline	97.85	98.52	101.03	99.71
	Butylamine	100.01	100.75	103.24	104.21
	Butanol	100.21	103.86	99.21	98.74
	Cyclohexanol	99.97	101.12	99.25	95.97
	Glucose	96.45	100.64	104.79	103.25

5.   References

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