

## **Quantity determination of O-nitro phenol compound by coupling reaction with diazotized**

**Of**

### **P-amino benzoic acid**

**التقدير الكمي لمركب اورثو - نايتروفينول بتفاعل الازدواج مع المحلول المؤزوت لمركب بارا امينو حامض البنزويك**

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#### **ABSTRACT**

Development of an analytical method was used to determine O-nitro phenol compound by coupling reaction for diazotized solution of p-amino benzoic acid in the presence of buffer solution  $\text{Na}_2\text{HPO}_4$  (PH= 10.8) to give a compound with a single azo dye salt having orange color soluble in water with high absorptivity at a wave length 449 nm , an early study to have a perfect optimum condition was made for the determination.

A calibration curve for a range of concentration (5-75)pm was taken and the value of molar absorptivity was  $1.5 \times 10^3 \text{ L.mol}^{-1}.\text{cm}^{-1}$  with a relative standard divation more than 1.26% and a recovery 98.77% .

As we study the nature of the azo dye by mole fraction method, from the practical value we found that the mole fraction of the dye compound is 1:1(phenol:p-amino benzoic acid) and the stability factor reach to  $1.4 \times 10^6 \text{ L.mol}^{-1}$  .

#### **الخلاصة**

تطوير طريقة تحليلية لتقدير اورثو- نايتروفينول عن طريق تفاعل الازدواج مركب بارا امينو حامض البنزويك المؤزوت بوجود منظم  $\text{Na}_2\text{Hpo}_4$  (PH= 10.8) لاعطاء مركب ذو صبغة املاح ازو احادية برتقالية اللون و ذائبة في الماء لها اقصى امتصاصية عند طول موجي 449 نانو ميتر , و تم عمل الدراسات الاولية للحصول على الظروف المثالية للتقدير . و تم الحصول على منحنى المعايرة لمدى من التراكيز من (5-75)ppm و كانت قيمة الممتصية المولارية  $1.5 \times 10^3$  و الانحراف القياسي النسبي افضل من 1.126 % و نسبة الاستعدادية 98.77 % و درسنا طبيعة صبغة الازو بطريقة النسبة المولية للنتائج الملون هي 1:1 (اورثو- نايتروفينول: بارا -امينو حامض البنزويك) و بلغت قيمة ثابت الاستقرار  $1.4 \times 10^6$  لتر.مول<sup>-1</sup> .

#### **Introduction:**

The diazotizing and coupling reactions is considered one of the good, easy, fast and useful method for analytical applications for samples<sup>(1)</sup> espically the samples which is difficult to measure by original methods due to the interference that effect the determination operation. This method depends on the formation of a coloured azo dye<sup>(2)</sup> which absorbed light at a specific wave length which is considered on of the sensitive method for the determination with a wide applications as a diazotizing p-amino benzoic acid compound used in the determination of many compound like using it in determine little amount of pyrol in dilute solution this method depends on the reaction of pyrol with diazotized compound for p-amino benzoic acid in the present of sodium acetate to produce a single stable azo dye soluble in water with a dark yellow colour that can determine the dye absorbance at a wave length about 420 nm<sup>(3)</sup>. there is additional method for the determination

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of sulfonamide medicine, and its coupling reaction with O-nitro phenol in basic media. To form a yellow azo dye which have a maximum absorbance at 425 nm. As there is a colorful method to determine phenols in drink water<sup>(4)</sup> which depend on the reaction of nitric acid with phenol to give nitrophenol, the last compound couples with phenol to give a coloured product. This method have a specific properties as its easy and fast also can be applicable in a substituted phenol in p-position there for we can determine some of the pharmaceutical cosmetics which have a substituted phenols. An idometric method<sup>(5)</sup> was used for the determination also the optical phosphorylation and cerimetry method by dissolving the samples in ethanol and after acidation of the solution we added additional of serem quntary solution<sup>(6)</sup> then we measure the external volume by titrating with sodium thio sulfate, this method have a high recovery also there is another method like ion-exchange, atomic adsorption and oxidation reaction with potassium dichromate compound<sup>(7)</sup>. There is another way to determine the phenol which depends on the appearance of colour, when O-nitro phenol react with phosphat buffer solution with PH=4.5 after that we filter the solution to measure the intense of the final solution at a wave length 450 nm it will give a good recovery<sup>(7)</sup>

Zhang develop a method for the determination which depends on the reaction of phenol with 15% of potassium bromid solution with little drops of sulfuric acid then the final compound which is succinimide titrate, the error percentage was less than  $\pm 0.87$ <sup>(10)</sup>.

The science Norman shane developed an absorbance method to determine phenol depending on preparing of an extract then dilute it with standard amount of water then we measure the absorbance of the final solution to know the intensity of absorbance at a specific wave length and the resulting error was less than  $\pm 0.82$ <sup>(11)</sup>.

There is a an ion-exchang method which is used in some drugs compound<sup>(12)</sup>, after we extract O-nitro phenol with dichloroforme then pass the product through an ion-exchanger also an oxidation method was used<sup>(13)</sup> by using dichromate solution and a catalyts with noticing the intensity of absorbance of the final solution, error percentage was less than  $\pm 0.75$ . and nuclear magnetic resonance method was used to determine the O-nitro phenol<sup>(14)</sup>. And the thin layer chromatography method was used by using silica jel product and the percentage error was less than  $\pm 0.81$ .

Choir developed a determination method which depend on the analysis by using a high performance liquid chromatography method by taking a sample that we want to determine phenol<sup>(15)</sup> and mixe it with phosphoric acid separate it by using a centrifuge, this method was succesful and the error percentage was less than  $\pm 0.61$  also a gel filtration was used to separate some of the drugs compound with pharmicitacl uses which have a low molecular weight, here we use specific colums in separation and this method have a high recovery.

### **Material and expermental part:**

a. Standard solutions

- O-nitro phenol solution (500 ppm)

It prepare by dissolving a 0.50 gm of phenol (from BDH ANALAR company) in 50 ml absolute ethanol then complete it with distle water to 250 ml in volumetric flask.

-diazotizing solution for 4-aminobenzoic acid (1000 ppm)

Which prepare by weighting the compound from (BDH ANALAR) then dissolve it in 40 ml of distilled water, then heat the solution to increase the solubility then added 4 ml of 0.7  $\mu$  standard hydrochloric acid with cooling to zero degree by using an ice bath then added 0.0077 gm sodium nitrate with mixing. After 5 minute pour the dizonium final solution to 250 ml volumetric flask, and complete the volume by using cooling distil water to (4c) and keep the solution in the freezer. The final solution will be stable after 1 hr in room temperature which is (22°C).

-hydrochloric acid (0.7  $\mu$ ) from (FLUKA A.G company).

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We prepare it with an approximate concentration by dilution of concentrated hydrochloric acid then titrate it with standard sodium carbonate to fix the concentration to (0.7  $\mu$  ).

-sodium carbonate  $\text{Na}_2\text{CO}_3$  (0.7  $\mu$  ) from (BDH company)

By dried sodium carbonate for an hour in (115°C) in watch glass then cool it and weight 3.32449 gm from it then we dissolve it in distl water and complete to the final volume which is 250 ml in volumetric flask.

-solution 8  $\mu$  of sodium hydroxide (from Fluka A.G company)

Prepared from standard amonia solution, to determine the exact molarity for amonia we prepare 8  $\mu$  of  $\text{NH}_4\text{OH}$  according to dilution equation.

### B. Buffer solution

-To prepare a buffer solution with PH=3.2 and 6.4 respectively.

Prepare a (0.1  $\mu$  ) solution of  $\text{Na}_2\text{HPO}_4$  and (0.2  $\mu$  ) solution of citric acid.

The table below shows the preparation of buffer solution (buffer citrate):

**Table (1) prepare buffer solution (buffer citrate)**

PH	0.1 M $\text{Na}_2\text{HPO}_4$	0.2M citric acid
2.3	2.13 ml	19.65 ml
7.4	18.45 ml	1.59 ml

-To prepare a buffer solution with PH=9.8 and 11.10 respectively Prepare a (0.2  $\mu$ ) solution of sodium carbonate and a (0.2  $\mu$ ) solution of sodium bicarbonate, the table (2) represent the preparation of the buffer solution (carbonate and bicarbonate buffer).

**Table (2) prepare the buffer solution (carbonate buffer)**

PH	0.2 M $\text{Na}_2\text{CO}_3$	0.2M $\text{NaHCO}_3$
9.8	4 ml	6 ml
11.10	10 ml	2 ml

-to prepare phosphate buffer solution with PH=11.6 take 70 ml of (0.3 )of  $\text{Na}_2\text{HPO}_4$  solution and added to it 5.5 ml of (0.2 ) sodium hydroxid solution then dilute to 250ml by using distle water.

### C. experimental method

#### 1. Determination of O-nitro phenol by coupling reaction

The coupling operation done by using (500 ppm) of phenol with (1000 ppm) of p-amino benzoic acid in present of phosphate buffer solution (PH=10.80) as we added 3ml of diazotized compound to 6ml of buffer solution then added different volume of O-nitro phenol then added to it 500ml distle water after that measure the absortivity (A) for this solution against the blank solution at a wave length 449nm, as we draw calibration curve between the absorbance and the concentration we have a straight line.

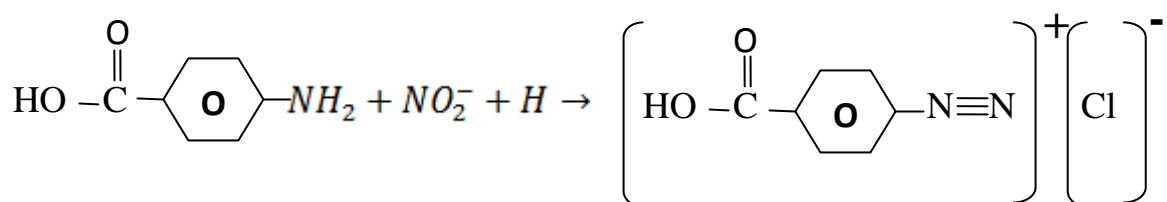
## Result and discussion:

### Primary test

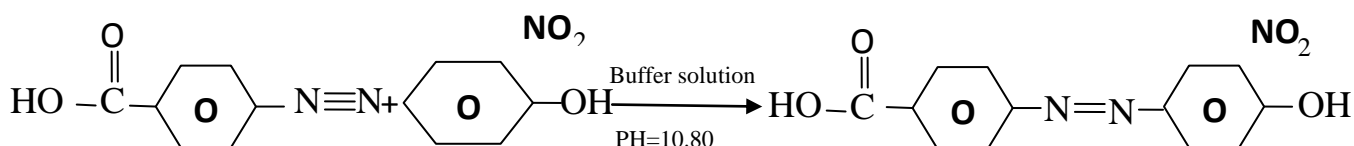
We notice that in mixing of p-aminobenzoic acid compound with phenol in basic media, a single azo dye with orange colour soluble in water formed, the reaction involve two step which is :

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- a. P-aminobenzoic acid compound react in acidic media in 0°C with equal amount of sodium nitrate to give diazonium salt as in:



- b. when added phenol to the diazotizing compound in basic media a single azo dye with orange colour formed which give a high absorptivity at a wave length 499nm according to the equation below:



### 1. The effect of PH

To reach to the optimum condition (high sensitivity, fast reaction and low absorbance) for the blank solution a study of O-nitro phenol with diazotizing compound p- amino benzoic acid in neutral, acidic and basic media was mad as we notice that no azo dye is formed in neutral and acidic media and the best media for the coupling reaction is the basic media which PH=10.80 as table (3) represent the effect of PH on coupling reaction.

**Table (3) represents the effect of PH on coupling reaction.**

Kind of buffer	PH	$\lambda$ Max.	Abs.
Na <sub>2</sub> HPO <sub>4</sub>	2.6	449nm	0.18
Na <sub>2</sub> HPO <sub>4</sub>	7.00	449nm	0.29
Na <sub>2</sub> CO <sub>3</sub> - NaHCO <sub>3</sub>	9.3	449nm	0.328
Na <sub>2</sub> CO <sub>3</sub> - NaHCO <sub>3</sub>	10.4	449nm	0.368
Phosphate buffer	10.8	449nm	0.661
Hydroxide – chloride buffer	13.8	449nm	0.285

A 5ml of buffer solution was used for every 25 ml of the final solution, the table (3) shows that the best buffer solution for coupling reaction is phosphate buffer.

2. the effect of buffer solution kind after the perfect value for the PH was know. Which is equal to (10.80) to know the effect of the buffer solution on obsertivity which is phosphate buffer NaOH – Na<sub>2</sub> HPO<sub>4</sub> and KCL- NaOH buffer as the table (4) represent the kind of the buffer solution

**Table (4) represents the kind of the buffer solution**

**Kind of buffer**

Kind of buffer	PH	$\lambda$ Max.	Abs.
Na <sub>2</sub> HPO <sub>4</sub> - NaOH	10.80	449 nm	0.661
KCL- NaOH	10.80	449 nm	0.357

3. The effect of the buffer solution on the intensity of absorbance

After fixing the optimum condition for the reaction. An experiment was made to give the perfect volume for the buffer solution which give a high sensitivity as table (5) shows that 4ml is the perfect volume for the buffer solution which give a high sensitivity as table (5) shows that 4 ml is the perfect volume for the buffer solution.

**Table (5) represents the volume of the buffer solution**

volume of the buffer solution	A
2ml	0.189
2ml	0.271
3ml	0.321
4ml	0.661
5ml	0.521
6ml	0.439

4. Measure the amount of **diazotizing** compound for p-aminobenzoic acid compound an experiment made to show the size effect of diazotizing solution on the absorptivity. The final result shown in table (6) as the perfect size for the **diazotizing** agent is 3 ml

**Table (6) shows the effect of diazotizing solution size on the absorptivity**

Size of diazotizing solution	A
1ml	0.349
3ml	0.661
5ml	0.414
6ml	0.215

5. measure the order addition after fixing the diazotizing agent for p-amino benzoic acid compound and using aphosphate buffer solution , as for the important of order addition measurement for the solution and its effect on the intensity color of the formed azo day compound so we study order addition .The table (7) represent the result .

**Table (7) shows the effect of order addition on the absorptivity**

Order number	Reaction components	A
I	A+D+W	0.661
II	W+D+A	0.438
III	W+A+D	0.259

(D=buffer solution)(W=phenol)(A=diazotizing agent)

As we consider I as number one because it a high sensitivity

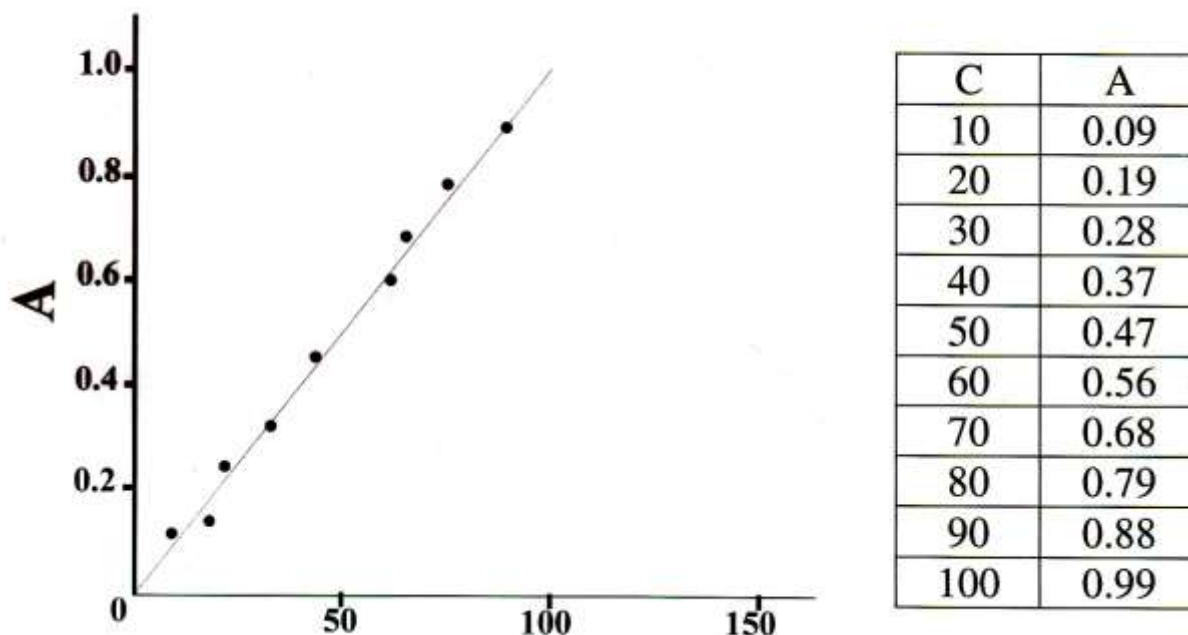
6. Study the colour stability for the formed complex from the reaction An experiment to explain the stability of the colour of the complex formed due to the reaction between the phenol and p-amino benzoic acid compound in present of phosphate buffer with optimum condition as shown in table (8) the stability time for the colour of the complex is ½ hr

**Table (8) shows the effect of the time of the stability of the complex**

A	T (min.)
0.188	5
0.291	10
0.457	15
0.347	20
0.481	25
0.661	30
0.598	1 hr.
0.499	2 hr
0.399	24 hr.

7. Measurement of the calibration curve to a series of volumetric flask (250 ml) added 2ml of diazotizing agent solution , 4ml of phosphate buffer solution then pour to it (2-12 ml) of (500 ppm) O-nitro phenol solution after that complete with distle water until reach the mark then we measure the absorbance against the blank solution at 449 nm after  $\frac{3}{4}$  hr from reaching the final solution (250ml) and the figure (1) show the standard curve.

**Figure (1) the titration curve of O-nitro phenol**



**8. study of accuracy and compatibility of method**

A study for the accuracy and compatibility for the method was mad as we take a different concentration of O-nitro phenol (80-25-5) ppm by using an optimum condition, the table (9) represent the result.

**Table (9) represents the result**

Represent (ppm)	Found (ppm)	Error %	Recovery %	R.S.D %
5	10.55	-0.70	95.5	±1.18
25	60.18	+5.55	101.44	±1.22
80	97.80	-0.66	98.25	±1.33

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