



Research Paper

## Using spectrophotometric methods for determination the of ionization constant of Ruthenium Red and Phenol Red indicators

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### Abstract

The ionization constant of Ruthenium Red and Phenol Red indicators by spectrophotometric method .The  $P^{ka}$  of the phenol red was measured according to the half method , where the absorbance was plotted versus the PH values , the resulting curve .The data indicated that there is increase in absorbance in weak acidic media where the high value of absorbance was recorded at PH = 4&5 , then the absorbance decreased for the above PH values .The Pka value of phenol red was calculated in this study by half method .So the data recorded that the Pka = 6.7 . On the other side the data showed increase the absorbance at basic media for the ruthenium red, and the Pka value was calculated by half method , the recorded that the Pka = 7.2.

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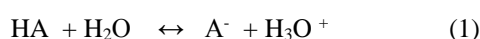
### I. Introduction

The value of the acid dissociation constant (pKa) is an important parameter that indicates the degree of ionization of molecules in solution at different pH values. Many chemical, physical and biological properties of natural and synthetic compounds are governed by the interactions of acidic and basic groups. In such compounds, the pKa controls many aspects of metabolism and even transport across membranes; therefore, its study is of significant interest in biology, pharmaceuticals, medicine, and numerous other scientific fields. [1,2] Determined of the pKa of indicators and complexes and their relationship to atomic orbital's. Several groups [3-6] have investigated the behaviors of different drugs, including those used against cancer, and the dependence of their behaviors on their pKa values. Novel pKa determination methods that use pH colorimetric indicators have also been reported [7-9]. Two main methods exist for determining the pKa of a compound: potentiometric titration and spectrophotometric titration. The main advantage of the second method is the ability to obtain a titration curve, which allows for estimation at any point without necessitating an experiment. In contrast, potentiometric titration requires knowledge of the equilibrium concentrations of the reagents, which are not necessary in spectrophotometric titration because the ratio of the concentrations of the basic and acidic parts is obtained from the results of absorbance measurements.

When the mixing of a solution and an indicator is investigated spectrophotometrically, the mixture absorbs in the UV region (250 - 380 nm), whereas the indicator, depending on the pH of the solution, absorbs in the visible region (380 - 700 nm). The pKa can be determined from the spectrophotometric data using different methods. Multi wavelength analysis and nonlinear least squares regression methods. Computational methods, and some new methods have also been developed [12 - 15].

### Theoretical Foundations :

When an acid HA(Indicators) is dissolved in water, equilibrium is established:



The acid HA transfers a proton to water, and it becomes the anion  $A^-$ . This anion tends to retrieve the proton and behave as a base;  $A^-$  is therefore referred to as the "conjugate base" of acid HA, and HA and  $A^-$  are referred to as a "conjugate pair" [16][17].

A shift of the equilibrium in Equation (1) to the right or left depends on the relative strengths of the HA and H<sub>3</sub>O<sup>+</sup> acids.

The “strength” of an acid refers its tendency to transfer protons, and one method of standardizing its force is to compare the protonation state when it interacts with water. The result of this comparison is expressed as the “acid dissociation constant,” K<sub>a</sub>, as follows:

$$K_a = [HA] / ([H^+] \cdot [A^-]) \quad (2)$$

K<sub>a</sub> is a constant of the stoichiometric equilibrium defined in terms of the concentration ratio [A<sup>-</sup>] / [HA], which can be determined spectrophotometrically<sup>[18]</sup>.

If a solution with a total concentration of indicator CT becomes very acidic, all indicator exists as HA. The absorbance of the solution at a given wavelength λ is given by :

$$A_{HA} = \epsilon_{HA} \cdot b \cdot CT \quad (3)$$

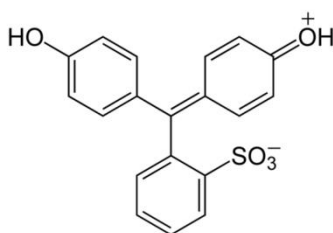
Where(  $\epsilon_{HA}$  ) is the molar absorptivity of HA at wavelength λ and ( **b** ) is the width of the cell containing the solution.

If the solution is too basic, the same concentration of indicator is converted entirely into A<sup>-</sup> and the absorbance at the same wavelength is given by :

$$A_{A^-} = \epsilon_{A^-} \cdot b \cdot CT$$

### Phenol Red :

Phenol red (also known as phenolsulfonphthalein or PSP) is a pH indicator frequently used in cell biology laboratories<sup>[19]</sup>.



**Chemical structure of phenol red .**

### Chemical structure and physical properties:

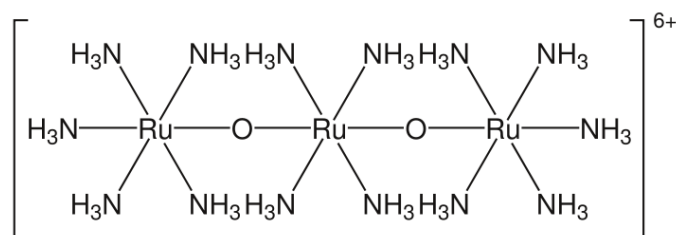
Phenol red exists as a red crystal that is stable in air. Its solubility is 0.77 grams per liter (g/l) in water and 2.9 g/l in ethanol.<sup>[19]</sup> A solution of phenol red is used as a pH indicator, often in cell culture. In crystalline form, and in solution under very acidic conditions (low pH), the compound exists as a zwitterion as in the structure shown above, with the sulfate group negatively charged, and the keton group carrying an additional proton. This form is sometimes symbolically written as H<sup>+</sup> 2PS<sup>-</sup> and is orange-red<sup>[20 and 21]</sup>. If the pH is increased (pK<sub>a</sub> = 1.2), the proton from the ketone group is lost, resulting in the yellow, negatively charged ion denoted as HPS<sup>-</sup>. At still higher pH (pK<sub>a</sub> = 7.7), the phenol's hydroxide group loses its proton, resulting in the red ion denoted as PS<sup>2-</sup>.<sup>[22]</sup> In several sources, the structure of phenol red is shown with the sulfur atom being part of a cyclic group, similar to the structure of phenolphthalein<sup>[22]</sup> However, this cyclic structure could not be confirmed by X-ray crystallography. Several indicators share a similar structure to phenol red, including bromothymol blue, thymol blue, bromocresol purple, thymolphthalein, and phenolphthalein.

### Ruthenium Red (RR) :

The inorganic dye **ammoniated ruthenium oxychloride**, also known as **ruthenium red**, is used in the chemical analysis as indicator for different types of titrations . Ruthenium red (**RR**) has also been used as a pharmacological tool to study specific cellular mechanisms. Selectivity is a significant issue in such studies as RR is known to interact with a large number of proteins.<sup>[1]</sup> Ruthenium red displays nano molar potency against several of its binding partners . RR has been used on plant material since 1890 for staining pectins, mucilages, and gums. RR is a stereo selective stain for pectic acid, insofar as the staining site occurs between each monomer unit and the next adjacent neighbor.

### Chemical Properties :

The most formula is Ammoniated ruthenium oxy chloride , and the chemical structure are shown below:



### The chemical structure of ammoniated ruthenium oxychloride

**Aim of Study** Two different dyes( indicators ) were selected in this study including (phenol red and ruthenium red indicators) ,The main aim of this study is determining the P<sup>ka</sup> values of the studied indicators by spectrophotometric methods in addition to study the effect of PH values on λ max of indicators absorbance.

### Experimental Part

#### Equipments

Pipette.. Standard 100 ml bottles. Standard 10 ml bottles.

#### Instruments : -

Computerized spectrophotometer (type DU 800). PH meter.

#### Chemicals :-

Phenol red . Ruthenium red . Distilled water, H<sub>3</sub>BO<sub>4</sub> , H<sub>3</sub>PO<sub>4</sub> , CH<sub>3</sub>COOH and NaOH.

### Preparation of Buffer solutions:-

The universal buffer type (99) was prepared by taking 100ml of an acid mixture containing 0.04 M of H<sub>3</sub>BO<sub>4</sub> ,H<sub>3</sub>PO<sub>4</sub> and acids and adding the required volume of 0.2 N NaOH gave the desired pH as follows :-

No	PH
1	2.3
2	3.5
3	4.8
4	5.8
5	7.8
6	9.7
7	10.89

### Preparation of indicators

The stock solution of the studied indicators (Phenol red and ruthenium red ) were prepared by taken 0.01 gram of the dye and placed in a standard 100 ml flasks and supplemented to the mark with distilled water.

#### The standard solutions :

The standard solutions were prepared by dilution the stock solution by taken ( 1, 2 , 3 , 4 and 5 ml ) of stock solution and transferred to measuring flask , then the volume completed to 10 ml . The absorbance of the standard solution was scanning by computerized spectrophotometer (Type DU 800) .**Determination of P<sup>ka</sup> :** The ionization constant (PKa) of the studied dyes was estimated by half method .

## II. RESULTS &DISCUSSION

### Results :

The λ max of the studied indicators were estimated by scanning the standard solutions and the spectra was shown in Figure (1) of phenol red and Figure (2) of ruthenium red .The results showed that the maximum absorbance of phenol red was obtained at ( 428 nm ) , Figure (1) . While the maximum absorbance of ruthenium red was recorded at ( 529 nm ) Figure ( 2 ) .

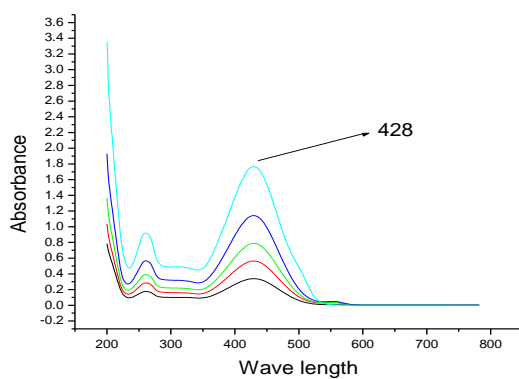


Figure ( 1 ) : The maximum absorption ( $\lambda_{max}$ ) of phenol red .

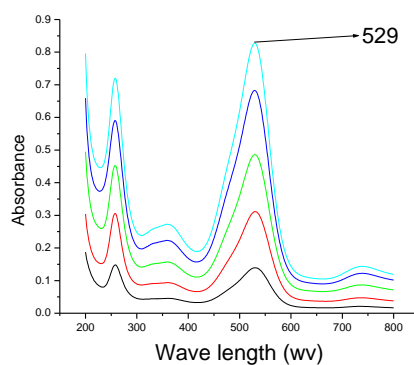


Figure ( 2 ) : The maximum absorption of ruthenium red .

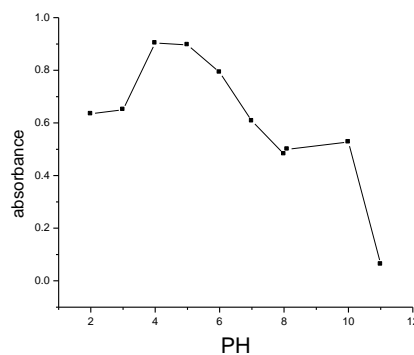


Figure ( 3 ) : The relationship between PH and Absorbance of Phenol red.

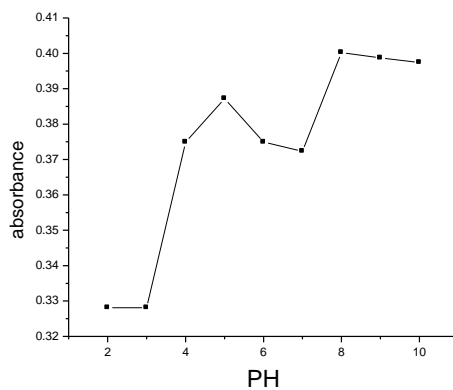


Figure ( 4 ) : The relationship between PH and Absorbance of Ruthenium red.

### Discussion:

The absorbance of the studied indicators solutions was measured at different PH values , The resulting Figures were shown in Figure ( 3 &4 ) .The Results indicated that there are different absorbance and varied from PH to another . The effect of PH values on the absorbance was carried out comparing the original solution (without any addition of PH solution ) with the absorbance at different PH solution additions . The results recorded that the high absorbance was recorded after addition at (PH =3&4) acidic media for the phenol red . While the high absorbance of ruthenium red was recorded at PH ( 8& 9 ) , basic media . The other PH additions not recorded direct effect on the absorbance comparing with the original solution .

### The effect of PH values on the absorbance at $\lambda$ max (Determination of pKa) :

The P<sup>ka</sup> of the phenol red was measured according to the half method , where the absorbance was plotted versus the PH values , the resulting curve was shown in Figure (3).The data indicated that there is increase in absorbance in weak acidic media where the high value of absorbance was recorded at PH =4&5 , then the absorbance decreased for the above PH values .The Pka value of phenol red was calculated in this study by half method .So the data recorded that the Pka = 6.7 . On the other side the data showed increase the absorbance at basic media for the ruthenium red (Figure ,4) , and the Pka value was calculated by half method , the recorded that the Pka = 7.2.

### III. Conclusion

This study showed that the PH values were affecting on the absorbance of the selected indicators for this study ( Phenol red and ruthenium red ) ,also the study showed that the pka values can measuring by the spectrophotometric method . Where the study recorded that , the absorbance was varied from media to media , For the phenol red the high absorbance was recorded in acidic media , on the other side the high absorbance of ruthenium red was recorded in basic media.

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