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Study the effect of some solvents on the λmax and absorbance of bromo phenol blue and phenol red indicators

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Abstract

This study which carried out on some indicators including (Phenol red and Bromo phenol Blue). Different solvents were used including (Methanol, propanol, DMF and DMSO). The effect of solvents on the λ max and absorbance values were investigated. The results recorded that the λ_{max} values were effected and changed according to the polarities of the applied solvents. Also, the obtained results showed changes in the absorbance values after applied solvents compared with the original absorbance values.

Keywords: Bromophenol blue; Phenol red; Effect of solvents on electronic transitions

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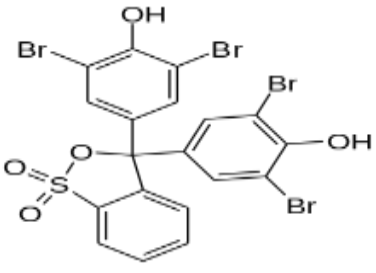
Introduction

Bromophenol blue (3′,3″,5′,5″-tetra bromo phenol sulfonphthalein, BPB, [1,2] albutest [3]) is used as a pH indicator, a color marker, and a dye. It can be prepared by slowly adding excess bromine to a hot solution of phenol sulfonphthalein in glacial acetic acid [4]. The chemical structure of it can be shown in following structure:



The chemical and physical properties are summarized in following Table (1):

Table 1: The properties of Bromophenol bl

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Chemical formula	$C_{19}H_{10}Br_4O^5S$
Molar mass	669.96 g·mol^{-1}
Odor	odorless
Density	2.2 g/mL
Melting point	273 °C (523 °F; 546 I
Boiling point	279 °C (534 °F; 552 I

As an acid–base indicator, its useful range lies between pH 3.0 and 4.6. It changes from yellow at pH 3.0 to blue at pH 4.6; this reaction is reversible [5]. Bromophenol blue is structurally related to phenolphthalein (a popular indicator).

Color marker

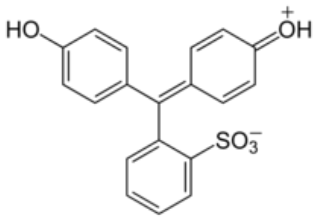
Bromophenol blue is also used as a color marker to monitor the process of agarose gel electrophoresis and polyacrylamide gel electrophoresis. Since bromophenol blue carries a slight negative charge at moderate pH, it will migrate in the same direction as DNA or protein in a gel; the rate at which it migrates varies according to gel density and buffer composition, but in a typical 1% agarose gel in a 1X TAE buffer or TBE buffer, bromophenol blue migrates at the same rate as a DNA fragment of about 300 base pairs, in 2% agarose as 150 bp. Xylene cyanol and orange G may also be used for this purpose [6].

Dye

Bromophenol blue is also used as a dye. At neutral pH, the dye absorbs red light most strongly and transmits blue light. Solutions of the dye, therefore, are blue. At low pH, the dye absorbs ultraviolet and blue light most strongly and appears yellow in solution. In solution at pH 3.6 (in the middle of the transition range of this pH indicator) obtained by dissolution in water without any pH adjustment, bromophenol blue has a characteristic green red color, where the apparent color shifts depending on the concentration and/or path length through which the solution is observed. This phenomenon is called dichromatic color [7]. Bromophenol blue is the substance with the highest known value of Kreft's dichromaticity index. This means it has the largest change in color hue, when the thickness or concentration of observed sample increases or decreases.

Phenol red

Phenol red (also known as phenolsulfonphthalein or PSP) is a pH indicator frequently used in cell biology laboratories. 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.[1] It is a weak acid with pKa = 8.00 at 20 °C (68 °F). A solution of phenol red is used as a pH indicator, often in cell culture. Its color exhibits a gradual transition from yellow (λmax = 443 nm) to red (λmax = 570 nm) over the pH range 6.8 to 8.2. Above pH 8.2, phenol red turns a bright pink (fuchsia) color [4,5].



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.[1][7] 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.

Effect of solvents on electronic transitions

Whenever a substituent (either acidic and basic) such as -OH, -SH and $–NH_2$ and etc is present in nitrogen heterocyclic acid base and tautomeric equilibria are likely to occur in dilute solution which may cause a shift in the n-π and π-π transitions. The polar solvents and those which can form hydrogen bonds tend to interact electro statically with various chromophores [6]. This changes is due to the charge distribution in the molecule and result in increased delocalization. For π-π transitions both the ground and excited states are established and absorption towards longer wavelengths [7].

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For n-π* transitions, the ground state in hydrogen bonding involves only one electron of lone pair, the other having been promoted to an upper energy level. The hypsochromic shift with increasing solvent polarity i.e. ethanol→ methanol →water is a useful means of recognizing n-π transitions .

During the present study also n-π transition and π-π transition both are blue shifted with increasing solvent polarity .In the title compound hydrogen bonding by the solvent is formed. The fluorine acts as a proton acceptor and electronic interactions between the lone pair of electrons of the fluorine group and electrons of the ring is decreased, leading to a hypsochromic shift. This possibility is arise due to the decrease in electron density on the atom. The greater effective size of the group may result in twisting it out of plane of the aromatic ring [8]. It is evident that the greater the possibility of hydrogen bonding by the solvent, the greater the hypsochromic shift or vice versa.

Thus increasing dielectric constant of solvent, which increases the ionising potentiality [8] of the solute molecules. However, solvation may also be partly due to hydrogen bonding of the solvent [7]. This results that hypsochromic shift observed in the title compound on going from ethanol to water. After correlation of experimental and theoretical data we find that theoretical calculated wavelengths are approximately as same as experimental values .Two different dyes (indicators) were selected in this study including (phenol red and Bromo phenol blue indicators), The main aim of this study is estimate the effect of different solvents on the λ_{max} and the absorbance values of the studied indicators by spectrophotometric methods on λ_{max} of the studied indicators.

Experimental

Preparation of indicators

The stock solution of the studied indicators (bromo phenol blue and phenol red) were prepared by taken 0.01 gram of the dye transferred into 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).

Results and Discussions

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 Bromophenol blue. The results showed that the maximum absorbance of phenol red was obtained at (428 nm) Figure (1) . While the λ max value of Bromophenol Blue was obtained at (580 nm) Figure (2) .

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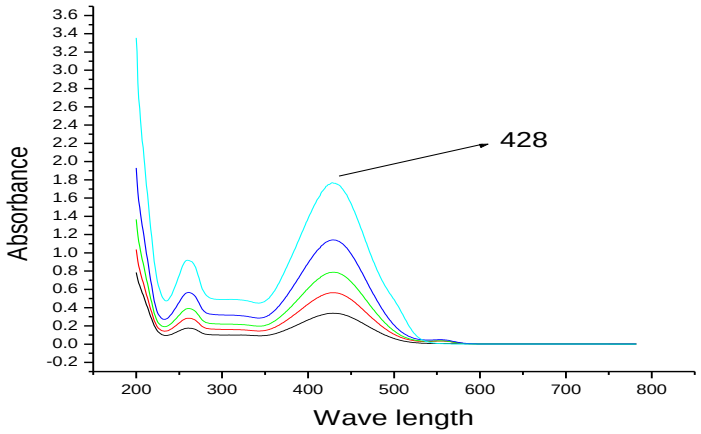

Figure 1: The maximum absorption (λ_{max}) of phenol red.

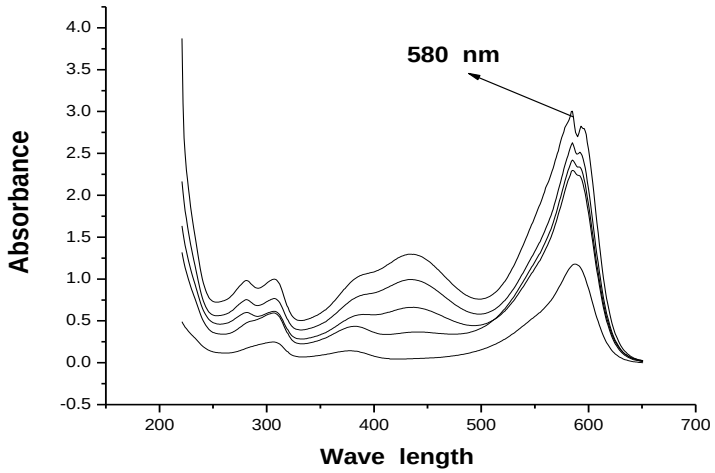

Figure 2: The maximum absorption (λ_{max}) of Bromophenol Blue.

Effect of the used solvents on the λ max and absorbance values:

Different solvents were applied on the studied dyes (Indicators) to estimate the effect of each one on the the λ max and absorbance values, those solvents included (Methanol, Propanol, Di methyl formamide (DMF) and Dimethylsulphoxide (DMSO). The spectra of the studied dyes were shown in the following Figures (3-6):

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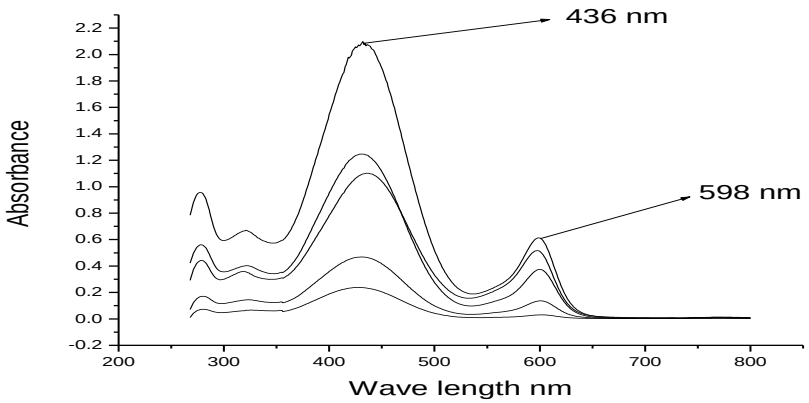

Figure 3: Effect the DMSO on the phenol Red.

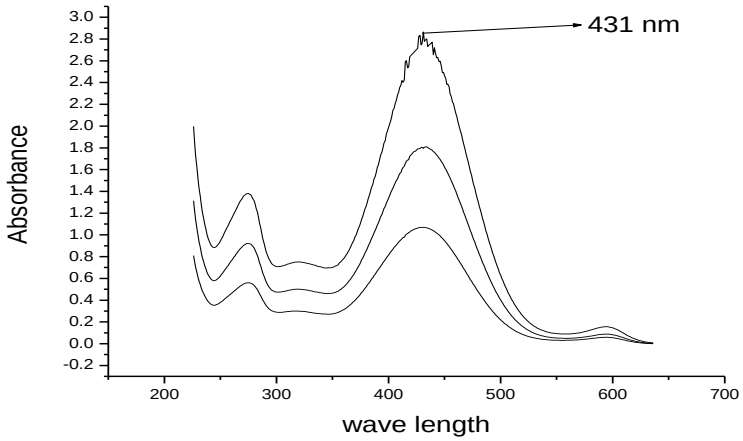

Figure 4: Effect the Methanol on the phenol Red.

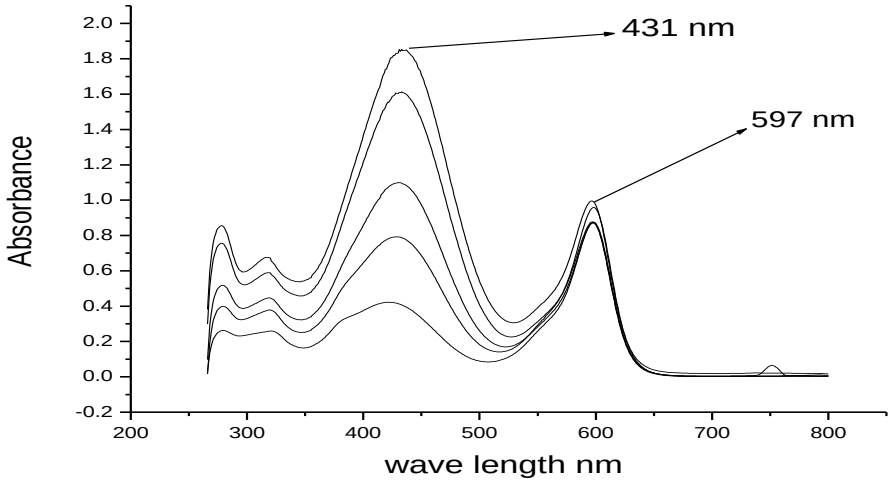

Figure 5: Effect the DMF on the phenol Red.

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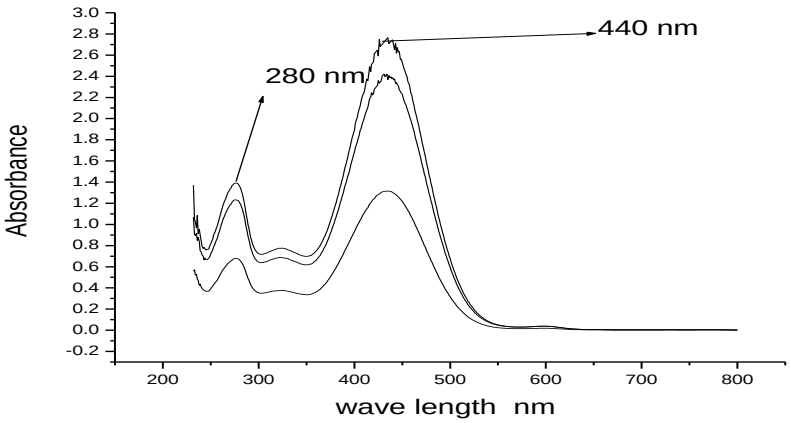

Figure 6: Effect the propanol solvent on the phenol Red.

On the other side the effect of the applied solvents on the bromoPhenol blue were shown in the following Figures (7-9):

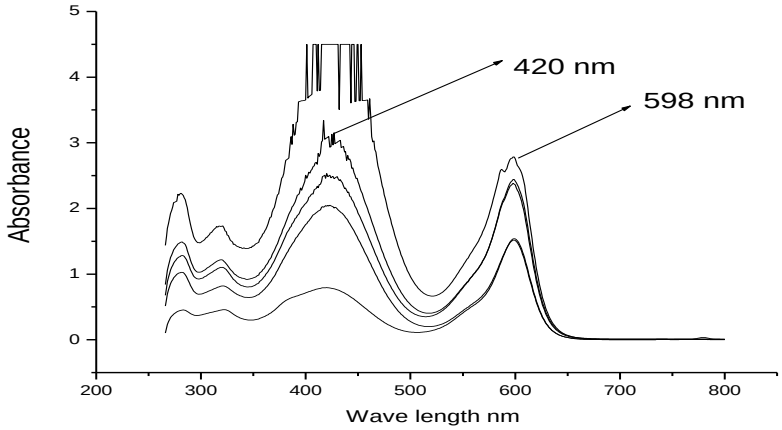

Figure 7: The effect of DMSO solvent on the spectra of Bromophenol Blue.

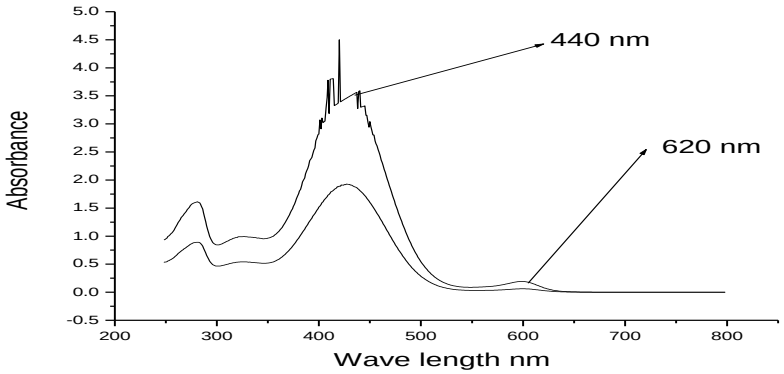

Figure 8: The effect of DMF solvent on the spectra of Bromophenol Blue.

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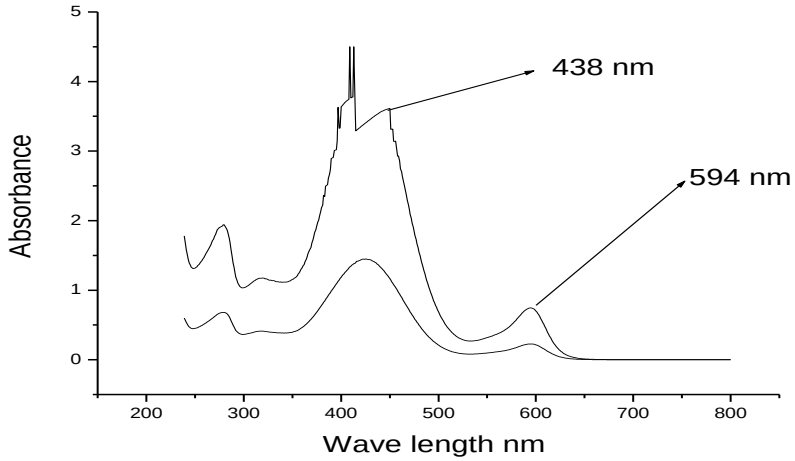

Figure 9: The effect of propanol solvent on the spectra of Bromophenol Blue.

Discussion

The Factors which effecting on the absorbance and electronic transfer:
Different factors mainly effecting on the electronic transfer of the compounds, one of these factors is concerning to the solvents.

Effect of solvent

The effect on the λ max of a compound when diluted in a solvent will vary depending on the chemical structures involved. Generally, non-polar solvents and non-polar molecules show least effect. However, polar molecules exhibit quite dramatic differences when interacted with a polar solvent. Interaction between solute and solvent leads to absorption band broadening and a consequent reduction in structural resolution and λ max. Ionic forms may also be created in acidic or basic conditions. Thus, care must be taken to avoid an interaction between the solute and the solvent [8,9].

In this study the effect of solvents on the electronic transfer was studied on the phenol red and bromo phenol blue indicators, where the results recorded that: By using the selected solvents on the different concentrations of phenol red, the λ_{max} values were shifted to higher values of all the studied concentrations, also some showed appear new bands as: (propanol, DMF and DMSO), The values of the λ_{max} were given in Table (2).

Table 2: The effect of the applied solvents on the λ_{max} of phenol red.

Solvent	$\lambda_{max\ 1}$	$\lambda_{max\ 2}$
Water	428	-
DMF	431	597
DMSO	436	598
Propanol	280	440
Methanol	431	-

On the other side the effect of the studied solvents on the bromo phenol blue was shown in Table (3):

Table 3: The values λ_{max} of bromo phenol blue.

Solvent	λ max 1	λ max 2
Water		580
DMF	440	620
DMSO	420	598
Propanol	438	620

The results indicated that the effect of the used solvents on the showed increased to high values , where the new values were 620 , 598 and 620 nm by applied the solvents of DMF , DMSO and Propanol , respectively .Also new bands were appeared at 440 , 420 and 438 when used the solvents of DMF , DMSO and Propanol , respectively , those bands were not

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recorded in the original spectra of bromophenol blue (in water) , the new bands and the higher λ_{max} values are attributed to the effect of polarity of the solvents on the electronic transfer and interaction with the electrons of different types of bonds. Usually, the spectral shifts are attributed to specific solute - solute and solute-solvent interaction in form of hydrogen bonding or bulk solvent properties[9]. Apart from these interactions, there are several other factors that may influence the spectra such as acid- base chemistry or charge transfer interactions. The magnitude of the spectral shifts in various solvents (with different polarities) mainly depends on the strength of the intermolecular hydrogen bond(s) between the substituent groups of the spectrally active molecule and the – OH or – NH or S –H or other functional groups of the solvent molecules and whether the intra molecular hydrogen bonds are present or not. For the molecular systems without intra molecular hydrogen bond, the spectral shifts are sensitive to the solvent polarity. Thus, in many molecules the bands (π-π*) are shifted bathochromically when the solvent polarity increases. These changes were attributed to hydrogen-bonding interaction between the solute molecule (Dyes) and the solvent molecule. On the other hand, the spectral shifts in the molecular systems with intramolecular hydrogen bonds are very small. In the absence of the intermolecular hydrogen bonds in all the intramolecular hydrogen bonded systems, the solvent shifts are well interpreted by the solvent polarity function.

Therefore, some of bonds which presence in bromo phenol blue as C – OH, C –Br, C-S, - S =O, S – O, beside presence the π electrons in benzene rings, on the other side the types of bonds which presence in phenol red indicator including: C –OH, C- SO_3, C=C and benzene ring, this is mainly explain the changes of wave length shifts after used the solvents.

Conclusion

This study which carried out on some indicators including (Phenol red and Bromo phenol Blue). Different solvents were used including (Methanol, propanol, DMF and DMSO). The effect of solvents on the λ max and absorbance were investigate. The results recorded that the λmax values were effected and changed according to the polarities of the applied solvents.

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