Preparation, analytical Studies and application of a New Azodye Derived from Pharmaceutical Materials (Procaine Hydrochloride & Salicylic acid)

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Abstract. Procaine hydrochloride was used as an aromatic amine, and salicylic acid (5-[2-(diethylamino)-4-benzoateazo]-2-hydroxybenzoic acid) was used to make a new azodye derived from pharmaceutical materials (PS). C.H.N., H1-NMR, I.R., and visible spectroscopic techniques have also been used to characterize dye. At different pH values (2 - 12), the electronic spectra of this azo dye was investigated in terms of acid-base properties, which included defining isobestic points and determining protonation and ionization constants. The effect of different polarities of solvents on the electronic spectra was the subject of another study. The dye has been used in a variety of applications, including as an indicator for strong acid with strong base and for nitrite determination.

1. Introduction
Azo compounds are commonly used as dyes because of their numerous uses in fields such as synthetic fabrics, material coloring, biomedical research, and organic synthesis [1-2]. Azo compounds are often used as markers of biomedical research. Furthermore, azo dyes have been documented to have a wide range of biological applications, including antineoplastics, antidiabetics, antiseptics, anti-inflammatory, and other chemotherapeutic agents [3-6]. Since most azo dyes have acid-base properties and have fixed isobestic points (which reflect the number of equilibriums in such an azo dye), they are used as acid-base indicators [7] and some can also be used as metallochromic indicators. Azo compounds are commonly used as dyes because of their numerous uses in fields such as synthetic fabrics, material coloring, biomedical research, and organic synthesis [8-12]. Azo compounds are often used as markers of biomedical research. Furthermore, azo dyes have been documented to have a wide range of biological applications, including antineoplastics, antidiabetics, antiseptics, anti-inflammatory, and other chemotherapeutic agents [13-17]. Since most azo dyes have acid-base properties and have fixed isobestic points (which reflect the number of equilibriums in such an azo dye), they are used as acid-base indicators [18] and some can also be used as metallochromic indicators. To the spectrophotometric determination of nitrite, the azo dye was used by forming daizonium salt. Such as p-nitroaniline in acidic medium with frusemide at λmax.680nm [19], and also the azo dye formed form coupling of 2-thioparbutric acid with diazonium salt of p-aminobenzoic acid at λmax.420nm [20] and the diazonium salt of luminal with 4-hydroxycumarine [21].

2. Experimental
The reagents and solvents were all of the reagent-grade variety. TLC was used to monitor the reaction's progress on a silica gel-coated plate, and spots were visible under UV light. The FT.IR-
8400S shimadzu was used to record infrared spectra (in KBr pellets). Melting points were measured using a melting point apparatus, element analysis (C.H.N.) was performed using a Perkin element 2400 element analyzer, and UV/V absorption spectra were reported using a Perkin Elmer Lampda EZ 210 UV/V Spectrophotometer. PH-Meter was used to take the readings (H. Jurgons Co. Bremen, L. Puls Munchen 15).

A sequence of acetate and universal buffer solutions with different pH values (2-12) were prepared to measure the ionization and protonation constants for hydroxyl and nitrogen groups [22].

2.1. Preparation of azodye

The azo dyes reagents PS were made by dissolving 0.004 mole of procaine hydrochloride in 1.4 ml concentrated HCl, 20 ml distilled water, and then adding a drop-by-drop solution of (0.304 g sodium nitrite in 20 ml distilled water) to form diazonium salt at a temperature of less than 5 o C. To the above diazonium salt, add an alkaline solution containing 0.004 mole of salicylic acid in 1.2 percent v/w NaOH. Adding dilute HCl to the prepared dye to convert it from sodium salt to hydrogen type. Recrystallization from metanol was used to purify the dye [23]. Figure (1).

![PS dye synthesis](image)

Figure 1. PS 5-(Procaine Azo) - Salicylic acid = 5-[2-(diethylamino)-4-benzoateazo]-2-hydroxybenzoic acid

2.2. Solutions

* 1 x 10^{-3} M stuck solution of azo dye PS
* Universal buffer solutions (pH 2-13) [24]
* 0.1 M solution of each sodium carbonate, sodium hydroxide and hydrochloric acid. The solutions were prepared and standardized by recommended methods [24]
* For nitrite solution
  1-100 ml Acidic solution of 8 x 10^{-3} M Procaine hydrochloride (P) (0.2183 g of P + 0.28 ml conc. HCl).
  2-100 ml Basic solution of 8 x 10^{-3} M Salicylic acid (S) (0.1105 g of S + 0.25 g NaOH)
  3-100 ml solution of 8.8 x 10^{-3} M NaN02 (N)

2.3 Procedures

- For acid-base, the absorbance of PS dye solutions at concentrations of 6x10^{-5} M were calculated in the range of 350-560 nm at different pH buffer solution values of (2-12).
- The absorbance of PS dye solutions with a concentration of 4x10^{-5} M in different solvents with different polarities was calculated in the range of 320-600nm for the solvent effect.
- To investigate the best conditions for determining nitrate.
Mix equal volumes of cold P and N solutions at 5° C, then add an equal volume of S solution and calculate the absorbance in the 320–600 nm range.

3. Results and discussion
The compounds were prepared in good yield. The compounds are stable in air and light. Elemental Analysis data and physical properties of compounds are summarized in table (1)

3.1. Infrared radiation
Table 1 shows the spectral data of azo dye PS. The (N-H) and (O-H) bands in the IR spectrum of free ligand are responsible for the medium band about 3234 cm⁻¹. There is a high intensity band in the range of 1300-1270 cm⁻¹ due to (C=N) in the novel azo dye. In the azo spectrum, the azo (-N=N-) group can be seen at 1427.32 cm⁻¹, the bands at 1664 cm⁻¹ for (C=O), the bands around 1309 cm⁻¹ observed due to C-N stretching. Figure. (2) [22]

![Figure 2. IR-Spectrum of azo dye PS](image)

3.2. Spectra of ¹H-NMR
In deuterated dimethyl sulfoxide solutions with tetramethylsaline as an internal norm, ¹H-NMR spectra of the prepared azo dye PS were obtained. The DMSO solvent caused a peak at 2.5 ppm in the spectra. Figures (3) show the ¹H NMR data.

![Figure 3. NMR-Spectrum of azo dye PS](image)
Table 1. Chemical analysis (CHN, IR and NMR) of azo dye PS

|                  | CHN |          |          |          |          |          |          |
|------------------|-----|----------|----------|----------|----------|----------|----------|
|                  | C%  | H%       | N%       |          |          |          |          |
|                  | Calculated | Found | Calculated | Found | Calculated | Found |          |
| C%               | 62.34 | 62.66 | 5.97 | 6.01 | 10.91 | 11.01 |          |
| IR               | Wavenumber (cm⁻¹) |          |          |          |          |          |          |
| OH&NH            | 3234.62w | 1664.57s |          |          |          |          |          |
| C=O              | 1427.32s | 1309.67 |          |          |          |          |          |
| N=N              | 1219.01s | 1579.70s |          |          |          |          |          |
| C-N              |          |          |          |          |          |          |          |
| C-O              |          |          |          |          |          |          |          |
| C=C              |          |          |          |          |          |          |          |
| H¹NMR            | Chemical shift (ppm) |          |          |          |          |          |          |
| S,3H,CH₃        | 1.2 |          |          |          |          |          |          |
| S,CH₂,          | 3.5 |          |          |          |          |          |          |
| S,6H,DMso       | 2.5 |          |          |          |          |          |          |
| m,7H,Ar-H       | 7.3-8.2 |          |          |          |          |          |          |
| S,HOH           | 11-12 |          |          |          |          |          |          |
| S NH             | 7.2 |          |          |          |          |          |          |

3.1 Some of spectrophotometric studies of azo dye PS

3.1.1 Ionization constants

The absorption spectra of 6 x 10⁻⁵ M solution of dye in the range of wavelength (350- 560 nm) of varying PH values (2 – 12) were represented graphically (Figure 4).

Figure 4: Electronic spectrum of dye PS at different value of PH.

The spectrum had three bands, the first of which was at 360 nm and was in the pH range (3-8) due to dye protonation. In the pH range, the second and third bands (440 and 460 nm), of high intensity (8-12). At 390 nm, the spectrum also revealed an isobestic point (a wavelength with constant absorbance despite changes in pH value medium).
At pH 2, the spectrum revealed no and band. To determine the protonation and ionization constants of azo dye PS, the wavelength 440nm is chosen as best wavelength (from Figure 4) to draw a curve between pH values as x-axis with corresponding their absorbance (Figure 5).

![Absorbance – pH curve of the azo dye PS](image)

Figure 5. Absorbance – pH curve of the azo dye PS

From (Fig.5) the ionization and protonation constants were calculated (Table 2). From Absorbance–PH curve and by the aid of half-height method [25], the PK values were obtained by this relation:

\[ PK = PH \text{ ( at } A_{1/2} \text{ ) where } A_{1/2} = \frac{A_{I} + A_{min.}}{2} \]

And \( A_{I} \) and \( A_{min} \) are limiting and minimum absorbances respectively.

Table (2): Protonation and ionization constants of azo dye PS

| \( A_{m} \) | \( A_{I} \) | \( A_{1/2} \) | \( PK_{p} \) | \( A_{m} \) | \( A_{I} \) | \( A_{1/2} \) | \( PK_{a1} \) | \( A_{m} \) | \( A_{I} \) | \( A_{1/2} \) | \( PK_{a2} \) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ---- | 0.25 | ---- | < 3 | 0.3 | 1.7 | 1 | 8.8 | 1.8 | ---- | ---- | > 11 |

\( P \) = protonation of H of \(-COOH\) group, \( a_{1} \) = ionization of H of \(-COOH\) group, \\
\( a_{2} \) = ionization of H of \(-OH\) group

The suggested chemical mechanism of protonation and ionization was shown in Figure (6).

![Suggested mechanism of protonation and ionization of azo dye PS](image)

Figure 6. Suggested mechanism of protonation and ionization of azo dye PS
3.2. Effect of solvents of different polarities

Figure 7 depicts the spectrum of the azo dye PS (concentration $4 \times 10^{-5}$ M) in various polarity solvents (Acetone, chloroform, ether, DMF, DMSO, ethyl acetate, water, ethanol, methanol and 1,4-dioxane). In all solvents, the dye has only one band, as shown in Fig.7. When polar solvents (ether, methanol, chloroform, and ethanol) are compared to non-polar solvents (benzene) at 350 nm, there is a small blue change at 340 nm. The broad red shift at 380 nm in comparison to solvents (DMF and DMSO) was due to hydrogen bonding between dye molecules (solute) and solvent molecules, as shown in Fig.8. The dielectric function of solvents and the maximum wavelength of dye PS in various solvents are shown in Table 3.

![Figure 7: Absorbance spectra of dye PS at different solvents with different polarities](image)

Table (3): values of dielectric function of solvents and maximum wavelength of dye PS at different solvents

| Symbol | Solvent      | Dielectricco (nst.D) | $(D - 1) / (D + 1)$ | $\lambda_{max}$ nm | $\varepsilon_{max} \times 10^4$ | mol$^{-1}$ \cdot cm$^{-1}$ |
|--------|--------------|-----------------------|---------------------|--------------------|---------------------|--------------------------|
| A      | Acetone      | 20.60                 | 0.907               | 360                | 2.875               |
| B      | Benzen       | 2.30                  | 0.394               | 350                | 4.083               |
| C      | Chloroform   | 4.80                  | 0.655               | 340                | 4.433               |
| DF     | DMF          | 36.71                 | 0.923               | 390                | 2.833               |
| DO     | DMSO         | 47.00                 | 0.958               | 390                | 3.100               |
| Et     | Ethanol      | 24.30                 | 0.921               | 345                | 3.350               |
| E      | Ether        | 4.33                  | 0.625               | 340                | 3.503               |
| Ea     | Ethyl acetate| 6.02                  | 0.715               | 340                | 3.520               |
| M      | Methanol     | 32.70                 | 0.941               | 360                | 2.588               |
| W      | Water        | 78.40                 | 0.975               | 360                | 3.270               |
By plotting a curve between dielectric function \((D-1)/(D+1)\) and \(\lambda_{\text{max}}\) of dye at different solvents (Figure. 8), the linearity of all solvents which means the dielectric constant that affected except for the DMF and DMSO gave deviation from the linearity because of hydrogen bonding between dye molecules (solute) and solvent molecules.

Figure 8: The relation between \(\lambda_{\text{max}}\) and \((D-1)/(D+1)\) for of dye PS at different solvents

3.2. Some applications of the azo dye PS

Determination of nitrite

To select the maximum wavelength of azo dye that formed, a curve of absorbance and wavelength was plotted (Figure. 9).

Figure 9. Absorption curve of dye PS ([P] = [S] = 1.6 \times 10^{-3} \text{ M} \text{ and [N]} = 1.76 \times 10^{-3} \text{ M})

The optimum time for forming the dye PS was studied at \(\lambda_{\text{max}} = 420\) nm, which found to be in the range of (20 – 25 sec) (Table 4 and Figure. 10).

Figure 10. Time effect for forming stable azo dye PS
By using of calibration curve (Beer’s law) for calculating precession, sensitivity of the method and limits of Beer's law were calculated (Figure 11).

Figure 11. Calibration curve for determination nitrite ([PS] = 4.8x10^-4 M)

Table (4): Beer's law of the dye PS.

| ε x10^4 l.mol^-1.cm^-1 | a  | S µg.cm^-2 | R^2   | S.D.  | D.L x10^-5 µg.cm^-1 | Beer’s law up to ppm |
|-------------------------|----|------------|-------|-------|---------------------|----------------------|
| 0.053                   | 0.012 | 0.083     | 0.9800 | 0.0085 | 0.048               | 40                   |

Where ε, a, S, R^2, S.D. and DL are molar absorptivity coefficient, specific absorptive, Sandel sensitivity, standard deviation and detected limit respectively.

Titration of strong acid (HCl) with strong base (NaOH) uses the azo dye as an acid–base indicator. The base was titrated spectrophotometrically with standard HCl using the new azo dye PS as an indicator (Fig. 12) after HCl and NaOH were standardized to be 0.0917 N and 0.0961 N, respectively. The relative error for obtaining the best PS concentration was discovered (Table 5).

Figure 12: The titration curves of (HCl and NaOH) at different concn. Of PS
Table (5): Relative error for different concn. of dye PS

| [PS] M | (e.p.) ml NaOH | [NaOH] M Found | [NaOH] M True | Jump / 0.9 ml | ±R.E% |
|--------|----------------|----------------|----------------|----------------|--------|
| 1 x 10^{-4} | 7.65 | 0.0959 | 0.0961 | 0.072 | 0.21 |
| 2 x 10^{-4} | 7.60 | 0.0965 | 1.262 | 0.42 |
| 3 x 10^{-4} | 7.65 | 0.0959 | 1.483 | 0.21 |

Using 8 mL of 0.0917 M HCl Table (5) and Figure. (12) Revealed that the concen.of PS in the range of (0.00001- 0.00003 M) The best concn. Was 0.00003M of PS, which resulted in more jumbling (that means more sensitivity) When the concn. Is greater than 0.00003, the titration fails due to minor dissuasions of dye PS at high concentrations.

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