Preparation and characterization azo dyes derived from 4-hydroxycoumarin and studying their analytical Applications

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ABSTRACT
The preparation of azo dyes is the subject of this research. 4-hydroxycoumarin with each ofo-amino benzoic acid (3-[o-Amino benzoic acidazo]-4-hydroxycoumarin} (B 1 ) and with Sulfa guanidine (3-[Sulfa guanidine azo]-4-hydroxycoumarin} (B 2 ) were syntheses. Azo compounds were characterized by I.R, CHN, 1 H-NMR Electronic and Mass spectroscopy. At various pH levels, the electronic spectra of these azo dyes were investigated in terms of acid-base properties, which includes the determination of protonation and ionization constants as well as the establishment of isobestic points. On the electronic spectra, the solvent effect was also investigated at various polarities.

Keyword: dyes, 1 H-NMR Acid, effect of solvents and constant of protonation

1. Introduction
Azo dyes are the largest group of organic dyes for their widespread application in many areas of textile and medicine (1). Azo metal chelates have also grown in popularity as a result of their intriguing electronic and geometrical activities in regard to their applications. (2). Because of their acid and base sensitivity, some of them can be used as metallochromic indicators, antipyretic reagents, and corrosion inhibitors. The absorption spectra of – conjugated compounds can be influenced by solvents of various polarities, which show (n*) and (s*) transitions, triggering blue or red changes (3). In the present investigation concerned with preparation five new mercerized azo dyes by reaction of paracetamol (4-acetamido phenol) with 2-mercury chloride -4-X- aniline(4). Azodyes are a very interesting class of chemical compounds that contain heterocyclic moieties and have drawn the attention of many researchers in recent years. Azodyes are weak acids or bases of a very important class of chemical compounds containing heterocyclic moieties that have piqued the interest of many researchers in recent years. Azodyes are weak acids or bases of a very important class of chemical compounds containing heterocyclic moieties that have piqued the interest of many researchers in recent years. (5) Sulfa drugs have many uses in the field of medicine, and sulfa compounds are considered the first compounds used as antibiotics, which have a great effect on many types of bacteria. Another study, the sulfa compounds (sulfadiazine, sulfa miraazine, and sulfa methazine) were identified after combining the sulfa compounds with 8-hydroxyquinoline and forming azo dyes, as well as these compounds were identified in medicines (7). Many azo dyes were prepared from sulfa compounds with acid 1- hydroxy 2-naphthenic acid (8), these azodyes were also used for determination of aluminum (9). Two new azo dyes were synthesized and derived from 4,5-diphenylimidazole and predidine sulfa and imadazoleto form these dyes 2-(sulfapyridineazo)-4,5-diphenyl imidazole and 2- (sulfapyridineazo)-imidazole at
wavelengths of 490 and 370 nm, respectively\(^\text{(10)}\). The study of these two azo dyes were used to form complexes with cadmium and mercury(II) at 460 and 490 nm respectively\(^\text{(11)}\), as well as its complexes with cobalt (II), copper(II) and nickel with the study of optimal conditions\(^\text{(12)}\). Two Azo dyes were prepared from pyridine and sulfamethazine with N-(4-hydroxyphenyl) acetamide, their complexes were studied with both dioxide cobalt, nickel and copper (II)\(^\text{(13)}\), as well as its complexes with cobalt (II), copper (II), and trichromide\(^\text{(14)}\). Mercury azo dyes were prepared between the precursor compounds (sulfaguanidine, sulfamid, and sulfadiazine) through the formation of dyes corresponding to oxidation\(^\text{(15)}\). Azo dyes derived from chromotropic acid were prepared with sulfamide, sulfamethaxol and sulfadazene\(^\text{(16)}\). Two Azo dyes derived from ortho-vanillin were also prepared with both sulfadiazine and sulfaguanidine, withstudtng their acid-base properties and effect of solvents at different polarities\(^\text{(17)}\). New azodye derived from Metoclopramide was prepared with study of acid-base properties at different pH values and solvent effect of polar and non-polar solvents\(^\text{(18)}\). New azo dyes derived from luminol with 4-hydroxycoumarin[3-(luminolazo)-4-hydroxycoumarin] and with 2-vanillin [5-(luminolazo)-2-vanillin] were ready to go\(^\text{(19,20)}\).

2. EXPERIMENTAL

The reagents and solvents were all of the reagent-grade class, and were manufactured by Merck and Fluka. TLC was used to monitor the progress of the reaction using a silica gel plate, and spots were test by UV radiation. I.R were captured using a Shimadzu FT-IR-8400S in Japan. The dyes’ UV/V absorption spectra were recorded. using Spectrophotometer Type:SPV-72S,made in Germany. The pH measurements were made with pH-Meter (H. Jurgons Co. Bremen,L.Puls Munchen15), And use Heidolph MR Series Magnetic stirrer Hotplates, And Drying oven made by (KARL KO/Germany).

3. Preparation of azodyes

Azodyes were made by dissolving 0.006 mole of (o-aminobenzoic acid)Sulfaguanidine in 2.1 ml Drop by drop into a stirring solution of (0.456g sodium nitrite in 10 ml distilled water) to form diazonium salt at -5°C with concentrated HCl and 10 ml distilled water. An alkaline solution of 0.006 mole (4-hydroxycoumarin) in (1.2 percent w/v. NaOH) was applied to the above diazonium. Using CHN, 1H-NMR, and mass spectrometry, the suggested molecular structures of azo compounds were shown in Scheme-1. using CHN, 1H-NMR, Mass spectrometry, and IR.
Solutions
Both of the azo dyes B1 and B2 in a concentration of 1 x 10^{-3} M, pH 2-12) universal buffer solutions \(^{(21)}\) (recommended approach for standardization) were prepared.

4. Methods
At different pH levels, the acid-base properties are different. For total dye concentration of dyes 110-4 M (for B1) and 110-4 M (for B2), a series of universal buffer solutions (universal) with different pH values (2–12) were prepared using universal buffer (for B2). The absorbance of these solutions was calculated over a pH range of (320–600 nm) (using the pH value as a blank solution).

5. Solvent effect of different polarities
Ethanol, Methanol, H2O, Cyclohexanol, Acetone, DMF, Chloroform, Ether, Ethyl acetate, Benzen, 1,4- Dioxan, and DMSO were used to complete a series of dye solutions with concentration of 110-4 M (for B1) and 110-4 M (for B2). The absorbance of these solution was recorded at 320–600 nm using the solvent as a blank solution.

6. Results and discussion
Prepared compounds are stable in the presence of air and soluble in water in acetone, ethanol, methanol, DMSO, and DMF at room temperature.

6-1. Analysis of the elements
Table-1 lists the physical properties and analytical details for the prepared azo dyes (B1 and B2).

Table-1 Physical properties and elemental analysis of novel azo dyes

| Comp. | Molecular formula | m.p °C | Color   | C % Cal. (found) | H % Cal. (found) | N % Cal. (found) |
|------|------------------|--------|---------|-----------------|-----------------|-----------------|
| B1   | C_{16}H_{10}N_{2}O_{5} | 296    | orange  | 61.88 (60.54)   | 3.22 (4.00)     | 9.02 (8.24)     |
**6-2 IR-Analysis**

The two prepared azo dyes (B₁ and B₂) were diagnosed by infrared spectroscopy all within the range (400-4000 cm⁻¹). (Figs. 1 and 2) illustrate the infrared spectrum of the two dyes, Table (2) illustrates the most important frequencies of dyes. It was observed from the spectra that the most important expected bands. In Table (2), the azodyes with the most IR absorption bands are mentioned. The bands in the range (3421.71-3446.79 cm⁻¹) are due to ν (O-H).

Table 2. B1 and B2 selected infrared data

| Compound | ν(OH) cm⁻¹ | ν(N-H) cm⁻¹ | ν(C-H) cm⁻¹ -Ar | ν(C=O) cm⁻¹ | ν(N=N) cm⁻¹ | ν(C=O) cm⁻¹ | ν(C-N) cm⁻¹ |
|----------|-------------|--------------|-----------------|-------------|-------------|-------------|-------------|
| B₁       | 3421.71w    | ----         | 3066.82m        | 1749.44s    | 1465.90s    | 1600.92s    | 1394.53s    |
| B₂       | 3446.79w    | 3358.07m     | 3226.91m        | 1741.72s    | 1481.33s    | 1631.78s    | 1398.39s    |

w=Weak, m=Medium, s=Strong

Figure. (1): Fourier-transform infrared spectroscopy of B₁
Figure. (2): Fourier-transform infrared spectroscopy of $\text{B}_2$.

6-3. NMR spectra of compounds

The azodyes' $^1\text{H}$-NMR spectra (Figs. 3 and 4) were determined in DMSO as a solvent. The phenyl ring has peaks at (6.82-8.12 ppm) in the spectral results of the azodyes. The OH groups in azodyes induce a single peak at (=15.46 ppm). Due to s,2H,NH$_2$, the signal peaks at 1.05-1.1 ppm. The result is shown in Table (3).
Table 3. The azo dyes' NMR data

| Dye | \(\delta\) (ppm) |
|-----|------------------|
| \(\text{B}_1\) | 2.5 (s, 6H, DMSO), 3.3 (s, H, HOD), 1.1 (m, H, OH), 3.49 (m, H, COOH), 7.31-8.12 (m, 8H, Ar-H) |
| \(\text{B}_2\) | 2.5 (s, 6H, DMSO), 3.37 (s, H, HOD), 13.85 (s, 2H, NH), 6.82-8.03 (m, 8H, Ar-H), 15.46 (s, H, OH), 1.05-1.1 (s, 2H, NH\(_2\)) |

\(s=\text{Singlet}, m=\text{Multiplet}\)

6-4 Mass spectrometry of azodyes

Mass spectroscopy is one of the important diagnostic tools used in determining the molecular formulas of chemical compounds and studying their dissociation pathways. The mass spectrum of \(\text{B}_1\) ligand (Fig. 5) indicates the appearance of the peak of the molecular ion at 310.2 m/z. The mass spectrum of the \(\text{B}_2\) ligand (Fig. 6) indicates the appearance of the peak of the molecular ion at 345.2 m/z, and the spectrum indicates the appearance of the peak of the base ion at 92.1 m/z.
Figure. (5): Mass spectrometry of B₁
7. Acid-Base properties

The absorption spectra (Fig. 7) of \(1 \times 10^{-4}\) M solution of azo dye (B\(_1\)), show at alkaline medium range (10-12) the \(\lambda_{\text{max}}\) is 350 nm. At pH 9, there was two \(\lambda_{\text{max}}\) the first at 360 nm while the second at 380 nm. At pH range (5-7) the \(\lambda_{\text{max}}\) is 410 nm. But in acidic medium (pH 2-5) the spectra show weak broad bands at 400 nm. The Fig. shows also three isobestic points at 370, 375 and 388 nm. and max. absorbance at pH 7.

From Fig. (7), the wavelength 410 nm was chosen to plot a curve between the pH value and corresponding absorbance (Fig. 8), to establish \(A_{\text{min}}\) and \(A_i\) in order to determine the protonation and ionization constants (pK).
The absorption spectra (Fig.9) of $1\times10^{-4}$ M solution of azo dye ($B_2$), show all pH range (6-12) the $\lambda_{\text{max}}$ at 360 nm. and with very weak band at pH 5 ($\lambda_{\text{max}}$). While no bands at very acidic solutions pH (2-4). The Fig. shows also no any observed isobestic point and max. absorbance at pH 10.
FIG. 9. Absorption spectra of \( B_2 \) at different pH values

From Fig. (9), the wavelength 360 nm. was chosen to plot a curve between the pH value and corresponding absorbance (Fig. 10), to establish \( A_{\text{min}} \) and \( A_l \) in order to determine the protonation and ionization constants (pK).

Fig. 10. absorbance–pH curves of B2

To obtain the pK values, this relationship was derived from Absorbance–pH curves and the half-height method.
\[ p_k = \text{pH (at } A_{1/2} \text{)} \quad \text{where} \quad A_{1/2} = (A_{L1} + A_{\text{min}})/2 \]

and \( A_L \) and \( A_{\text{min}} \) are limiting and minimum absorbance’s respectively.

Table (4) shows values of \( A_{\text{min}}, A_L, A_{1/2}, \text{and } p_k \) values

| \( A_{\text{min}} \) | \( A_L \) | \( A_{1/2} \) | \( \text{pK} \) | \( \text{pK}^* \) |
|------------------|---------|-------------|----------|-----------|
| 0.56             | 1.64    | 1.10        | 4.52 (p) | 0.15      |
| 0.41             | 1.84    | 1.13        | 8.47 (a) | 0.78      |

\( \text{p=} \text{protonation, a=} \text{ionization} \)

The protonation constant for the oxygen atom in the molecule of O-amino benzoic acid is \( p_{Kp} \).

The hydroxyl group of 4-hydroxycumarine has an ionization constant of \( p_{Ka} \).

\( p_{Kpis} \) protonation constant for the oxygen atom in the 4-hydroxycumarine aldehyde group.

\( p_{Kaionization} \) constant of 4-hydroxycumarine's hydroxyl group.

### 8. Effect of solvents

Figures 11 and 12 depict the spectra of azo dyes (B1 and B2) in various solvents. (Ethanol, Methanol, H\(_2\)O, Cyclohexanol, Acetone, DMF, Chloroform, Ether, Ethyl acetate, Benzen, 1,4- Dioxan and DMSO). For azo dye B\(_1\) (Fig.11) of non polar solvent (like 1,4-dioxan, benzene and Chloroform) give \( \lambda_{\text{max}} \) at 415 nm, while all solvents give blue shift like ether at \( \lambda_{\text{max}} \) at 395 nm, (methanol, ethanol, DMSO and acetone) give \( \lambda_{\text{max}} \) at 405 nm, and (water, Cyclohexanol, ethyl acetateand DMF) give slight blue shift at \( \lambda_{\text{max}} \) of 410 nm. DMF and Water having highest absorbance due to high dielectric constants of them (D).

For azo dye B\(_2\) (Fig.12) of non polar solvent (like 1,4- dioxan and benzene) give \( \lambda_{\text{max}} \) at 420 nm, while all solvents give blue shift at 410 nm, except for DMF at 380 nm. Acetone having highest absorbance due to high dielectric constants also.

The absorption spectra of different solvents are affected by solvent solvation and/or dielectric effects. To see whether the band shift (\( \nu \)) is due to solvation energy changes or pure dielectric. The functions \((D-1)/(D+1)\) of each solvent, as well as the maximum and molar absorptivity coefficients of (max) azo dyes B1 and B2, are shown in Table(5).
Fig. 11 - Visible spectra of B₁ at different solvents

Fig. 12 - Visible spectra of B₂ at different solvents
Table 5. The solvent's (D-1)/(D+1) ratio and $\lambda_{\text{max}}$ of B1 and B2

| Symbol No. | Solvents    | D $^{[21]}$ | (D-1)/(D+1) | $\lambda_{\text{max}}$ nm. | $\varepsilon_{\text{max}} \times 10^4$ | $\lambda_{\text{max}}$ nm. | $\varepsilon_{\text{max}} \times 10^4$ |
|------------|-------------|-------------|-------------|-----------------------------|--------------------------------|-----------------------------|--------------------------------|
| 1          | Ethanol     | 24.30       | 0.921       | 405                         | 1.48                          | 410                         | 0.62                          |
| 2          | Methanol    | 32.70       | 0.940       | 405                         | 1.50                          | 410                         | 1.48                          |
| 3          | Water       | 78.40       | 0.975       | 410                         | 1.68                          | 410                         | 0.44                          |
| 4          | Cyclohexanol| 15.00       | 0.875       | 410                         | 1.18                          | 420                         | 0.35                          |
| 5          | Acetone     | 20.60       | 0.907       | 405                         | 1.46                          | 410                         | 1.76                          |
| 6          | DMF         | 36.71       | 0.946       | 410                         | 1.68                          | 380                         | 1.64                          |
| 7          | Chloroform  | 4.81        | 0.655       | 405                         | 1.52                          | 410                         | 1.10                          |
| 8          | Ether       | 4.30        | 0.622       | 395                         | 0.98                          | 420                         | 0.30                          |
| 9          | Ethyl acetate| 6.02       | 0.715       | 410                         | 1.38                          | 410                         | 1.22                          |
| 10         | Benzene     | 2.30        | 0.393       | 415                         | 1.46                          | 420                         | 0.49                          |
| 11         | 1,4-Dioxane | 2.20        | 0.375       | 415                         | 1.38                          | 420                         | 0.76                          |
| 12         | DMSO        | 47.00       | 0.958       | 405                         | 1.44                          | 410                         | 0.95                          |

$\varepsilon_{\text{max}}$ is the molar absorptivity coefficient in $\text{l.mol}^{-1}.\text{cm}^{-1}$

With mild polarity solvents, plotting (D-1)/(D+1) against the max of azo dye B1 yields a more or less high linear relationship (Fig.13). Except for ether, which is slightly deviated due to hydrogen bonding between the solute (dye) and the solvent, this indicates that the medium's dielectric constant is the key factor regulating the band change in such solvents. Although all solvents in azo dye B2 have the same effect of linearity, except for the solvent DMF, which is out of linearity, this may be due to hydrogen bonding. (Fig.14).
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